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BOOK OF PROCEEDINGS

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MINISTRY OD CONSTRUCTION, URBAN DEVELOPMENT AND ENVIRONMENTAL PROTECTION (UNA-SANA CANTON)



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Bihać, June 2022.

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1. "SYNTHESIS OF BIO - COMPOSITE SUSTAINABLE MATERIALS AND THEIR APPLICATIONS IN ENVIRONMENTAL TECHNOLOGY"

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3. "THE DARK SIDE OF ARTIFICIAL LIGHT"

Tahir Sofilić, PhD - University of Zagreb, Croatia

4. "FOLIAR DISEASES OF BARLEY AND WILD BARLEYS FROM THE FERTILE CRESCENT"

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5. "NATURE BASED SOLUTIONS (NBS) AS A TOOL FOR SUSTAINABLE DEVELOPMENT"

Mirjana Bartula, PhD - Faculty for Applied Ecology - Futura, Belgrade, Serbia and Jasmina Ibrahimpašić, PhD - Biotechnical faculty, University of Bihać, Bosnia and Herzegovina

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FOLIAR DISEASES OF BARLEY AND WILD BARLEYS FROM THE FERTILE CRESCENT

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Key words: Hordeum vulgare, Hordeum spontaneum, Hordeum bulbosum, Pyrenophora teres f. teres, Pyrenophora teres f. maculata, Rhynchosporium commune, Pyrenophora graminea, Fertile Crescent, Turkey

ABSTRACT:

In April 2021, a survey was conducted in Sanliurfa province of Turkey located in the Fertile Crescent region, and foliar diseases occurring on barley (Hordeum vulgare) and wild barley species (Hordeum spontaneum and Hordeum bulbosum) were determined. A total of 77 barley fields, 31 Hordeum spontaneum populations, and 2 Hordeum bulbosum populations were investigated in Sanliurfa. Among the barley fields and Hordeum spontaneum populations, spot and net forms of net blotch caused by Pyrenophora teres f. maculata and Pvrenophora teres f. teres, scald caused by Rhynchosporium commune, and leaf stripe caused by Pyrenophora graminea were found. The net form of net blotch was found to be the most common disease in barley fields, followed by the spot form of net blotch, scald, and leaf stripe. Two barley fields were disease-free. The most common disease in Hordeum spontaneum populations was found to be the net form of net blotch, followed by scald, spot form of net blotch, and leaf stripe. No disease was observed in the 1 Hordeum spontaneum population. The net form of net blotch was found in 2 Hordeum bulbosum populations investigated.

1. INTRODUCTION

Barley (Hordeum vulgare) is the second most-produced cereal crop in Turkey after wheat. In Turkey, barley is produced in 3197042.7 hectares with a production of 6193553 tonnes in 2021. Şanlıurfa province of Turkey located in the Fertile Crescent region is an important barley growing area. In Şanlıurfa province, barley is produced in 101043.9 hectares with a production of 127239 tonnes in 2021 [1]. Wild barleys *Hordeum spontaneum* and *Hordeum bulbosum* naturally grow in Şanlıurfa. This area is part of the Fertile Crescent region where the domestication of barley and other cereals occurred [2, 3]. Barley and wild barley species (*Hordeum spontaneum* and *Hordeum bulbosum*) are common in that area [4, 5, 6]. Barley is affected by a number of diseases that limit its production [6]. Wild barleys are important disease-resistance sources [5, 6, 9, 10]. *Hordeum spontaneum* is the progenitor of modern-day barley [2, 3]. *Hordeum bulbosum* is placed in the secondary gene pool of barley [10]. In this study, diseases occurring on barley (*Hordeum vulgare*) and wild barley species (*Hordeum spontaneum* and *Hordeum bulbosum*) in Şanlıurfa province of Turkey located in the Fertile Crescent region were determined.

2. MATERIALS AND METHODS

A survey was conducted in Sanliurfa province Turkey between 11-15 April 2021 and diseases occurring on barley (Hordeum vulgare) and wild barley species (Hordeum spontaneum and Hordeum bulbosum were detected. Sanliurfa province is located in southeastern Turkey. A total of 77 barley fields were investigated in Sanliurfa. Six, 9, 9, 1, 8, 6, 4, 9, 4, 2, 3, 4 and 12 barley fields in Hilvan, Siverek, Harran, Ceylanpınar, Viranşehir, Eyyübiye, Akçakale, Suruç, Birecik, Halfeti, Bozova, Karaköprü and Haliliye districts were examined for the presence of diseases, respectively. In addition, 31 Hordeum spontaneum populations and 2 Hordeum bulbosum populations were examined for the presence of diseases. One, 3, 3, 3, 2, 3, 3, 1, 6, 4, 1 and 1 Hordeum spontaneum populations in Hilvan, Siverek, Harran, Ceylanpınar, Viranşehir, Akçakale, Suruç, Birecik, Halfeti, Bozova, Karaköprü, and Haliliye districts were investigated, respectively. Two Hordeum bulbosum populations were examined in the Harran district. For wild barley species, at least 10 plants were examined in each population. Plants were examined macroscopically and diseases were diagnosed according to their symptoms. For this purpose, Zillinsky (1983) [11], Mathre (1982) [7], and Zaffarano (2011) [12] sources were used. The scale developed by Saari and Prescott (1975) [13] was used to determine the severity levels of the diseases.

3. RESULTS AND DISCUSSION

Among the barley fields and *Hordeum spontaneum* populations, spot and net forms of net blotch caused by *Pyrenophora teres* f. *maculata* and *Pyrenophora teres* f. *teres*, scald caused by *Rhynchosporium commune*, and leaf stripe caused by *Pyrenophora graminea* were found. The net form of net blotch was found in 2 *Hordeum bulbosum* populations investigated (Table 1). The net form of net blotch was found to be the most common disease in barley fields, followed by the spot form of net blotch, scald, and leaf stripe. In these barley fields, the prevalence of the net form of net blotch disease varied between 1% and 60%, while the severity values ranged between 3-7. The prevalence of the spot form of net blotch disease was between 1% and 60%, and the severity values were between 3-9. The prevalence of barley

scald disease ranged between 1%-60% and the severity values ranged between 3-7. The prevalence values of the barley stripe disease ranged between 1%-70%. Two barley fields were disease-free in Hilvan and Haliliye districts.

The most common disease in Hordeum spontaneum populations was found to be the net form of net blotch, followed by scald, spot form of net blotch, and leaf stripe. In these populations, the prevalence of the net form of net blotch disease ranged from 1% to 70%, while the severity values were between 3-7. The prevalence values of the barley scald were between 1% and 40%, and the severity values were between 3-5. The prevalence of the spot form of net blotch ranged between 1% and 50%. The severity of leaf stripe disease was between 1% and 3% in the 2 populations observed. No disease was observed in the 1 Hordeum spontaneum population in the Haliliye district. In the barley survey, all 4 diseases were observed in Hilvan, Siverek, Harran, Eyyübiye, Akçakale, Suruç, Karaköprü, and Haliliye districts. Barley leaf stripe disease caused by Pyrenophora graminea was not observed in Viransehir, Birecik, and Bozova districts, while the spot form of barley net spot form of net blotch disease caused by Pyrenophora teres f. maculata was not observed in Halfeti district. Among the Hordeum spontaneum populations, Pyrenophora teres f. teres was observed in Hilvan, Siverek, Harran, Ceylanpınar, Viransehir, Akcakale, Suruc, Birecik, Halfeti, and Bozova districts. Pyrenophora teres f. maculata was observed in Hilvan, Suruç, Birecik, Halfeti, and Bozova districts. Rhynchosporium commune was observed in Siverek, Harran, Ceylanpınar, Viransehir, Halfeti, Bozova, and Karaköprü districts. Pyrenophora graminea is found in the Siverek district only.

The net form of net blotch disease was detected in 2 *Hordeum bulbosum* populations studied in the Harran district. The prevalence of this disease in these populations was found to be 70% and 40%, while the severity of the disease was found to be 3 and 5.

These diseases reported in this study are common diseases in Turkey. In other studies, in addition to these diseases, powdery mildew, leaf (brown) rust, spot blotch, and stem rust were also observed in barley fields as well as in wild barley populations [14, 15, 16, 8, 5, 17, 18, 19, 20, 6, 21, 22]. Scald and net blotch diseases are also common in the World and in Turkey [7]. Barley stripe is a problem in areas where no certified seed or chemically treated is used [7, 8].

Karakaya et al (2016b) [5] determined the diseases occurring in *Hordeum spontaneum* populations in 8 provinces located in the Fertile Crescent section of Turkey. In their study, scald, powdery mildew, both forms of net blotch, semi-loose smut, loose smut, leaf rust, and barley stripe diseases were detected among the *H. spontaneum* populations. The most common disease was scald followed by powdery mildew and net blotch. In their study, no disease was observed in nine of the 40 *H. spontaneum* populations investigated. Karakaya et al (2020) [6] determined the diseases occurring on *Hordeum bulbosum* plants in Bingöl, Turkey. Powdery mildew caused by *Blumeria graminis* f. sp. *hordei*, leaf rust (brown rust) caused by *Puccinia hordei*, spot form of net blotch, and scald were found in *H. bulbosum* populations. Powdery mildew was the most common pathogen followed by leaf rust, spot

form of net blotch, and scald. Five out of 27 *H. bulbosum* populations were disease-free. Wild barley populations with no disease may have the potential to be used in disease-resistance studies. Saraç Sivrikaya et al [22] examined the barley and wild barley (*Hordeum spontaneum*) diseases in Batman province and surrounding areas of Turkey. Scald was the most common disease in barley fields examined. Spot form of net blotch, barley stripe, the net form of net blotch, spot blotch caused by *Cochliobolus sativus*, powdery mildew, and brown rust followed scald disease. Scald was also the most common disease among the *H. spontaneum* populations. Spot form of net blotch, the net form of net blotch, powdery mildew, and brown rust followed the scald disease. In the investigation area, 2 *H. spontaneum* populations were disease-free. Wild barleys are important gene sources for obtaining disease-resistant plants. Disease-free wild barley populations could be used in obtaining barley plants resistant to diseases [5, 6, 9, 10, 23].

Table 1: Barley (*Hordeum vulgare*) and wild barley (*Hordeum spontaneum*, *H. bulbosum*) diseases observed in Şanlıurfa province of Turkey (*Ptt: Pyrenophora teres f. teres, Ptm: Pyrenophora teres f. maculata, Rc: Rhynchosporium commune, Pg: Pyrenophora graminea,* Pre: prevalence, Sev: severity)

No	Plant	Ptt	Pre	Sev	Pt	Pre	Sev.	Rc	Pre	Sev	Pg	Pre	Location
					т								
1	H. vulgare				х	3	5	х	10	5	x	3	Hilvan
2	H. vulgare										х	3	Hilvan
3	H. vulgare												Hilvan
4	H. vulgare							x	5	3	x	3	Hilvan
5	H. vulgare	х	5	3	х	3	3	х	10	3	х	1	Hilvan
6	H. vulgare	х	10	3	х	5	3	x	10	3	x	3	Hilvan
7	H. spontaneum	х	3	3	х	1	3						Hilvan
8	H. vulgare	х	3	3	х	2	3						Siverek
9	H. vulgare	х	3	5	х	1	5						Siverek
10	H. vulgare	х	5	5	х	5	5				х	3	Siverek
11	H. vulgare	х	5	3							x	5	Siverek
12	H. vulgare	х	3	3				х	15	3			Siverek
13	H. vulgare							X	30	3			Siverek
14	H. vulgare	х	5	5	х	5	5	x	30				Siverek
15	H. vulgare	х	1	5	х	1	5	X	20	5	x	10	Siverek

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16	H. vulgare	х	1	5	х	1	5	х	20	5			Siverek
17	H. spontaneum	х	3	5				х	15	5	X	1	Siverek
18	H. spontaneum	х	5	3				X	5	3			Siverek
19	H. spontaneum										X	3	Siverek
20	H. vulgare	х	1	5									Harran
21	H. vulgare	х	20	3	x	20	5	х	30	5	X	10	Harran
22	H. vulgare	х	1	5	x	1	5						Harran
23	H. vulgare				x	1	5						Harran
24	H. vulgare	х	1	5				X	20	5	X	10	Harran
25	H. vulgare	х	1	5				х	1	5			Harran
26	H. vulgare	х	1	5									Harran
27	H. vulgare	х	1	5		1	5				X	20	Harran
28	H. vulgare	х	1	5									Harran
29	H. spontaneum	х	1	5									Harran
30	H. spontaneum							х	30	3			Harran
31	H. spontaneum	х	1	5									Harran
32	H. bulbosum	х	70	3									Harran
33	H. bulbosum	х	40	5									Harran
34	H. vulgare	х	3	5				х	5	5			Ceylanpınar
35	H. spontaneum	х	1	5									Ceylanpınar
36	H. spontaneum	x	1	5				x	1	5			Ceylanpınar
37	H. spontaneum	X	1	3									Ceylanpınar
38	H. vulgare	x	3	5	x	3	5	x	20	5			Viranşehir
39	H. vulgare	х	3	5	х	3	5	х	60	7			Viranșehir

Table 1: Barley (*Hordeum vulgare*) and wild barley (*Hordeum spontaneum*, *H. bulbosum*) diseases observed in Şanlıurfa province of Turkey (*Ptt: Pyrenophora teres* f. *teres*, *Ptm: Pyrenophora teres* f. *maculata*, *Rc: Rhynchosporium commune*, *Pg: Pyrenophora graminea*, Pre: prevalence, Sev: severity) (continued)

ne.	<i>Chynchosporium</i> c	Jomm	<i>inc</i> , 1 g	5. <i>i yren</i>	opno	i u gi ui	nincu, i	rie. pre	valence	, 500. 5			Sintiliaea)
40	H. vulgare	х	10	5	x	10	5						Viranşehir
41	H. vulgare	х	30	7	X	30	7						Viranşehir
42	H. vulgare	X	40	7				X	5	5			Viranşehir
43	H. vulgare	x	1	3	х	1	3						Viranşehir
44	H. vulgare	х	1	3	х	1	3						Viranşehir
45	H. vulgare	x	10	5									Viranşehir
46	H. spontaneum	x	60	7				x	40	5			Viranşehir
47	H. spontaneum	х	1	3									Viranşehir
48	H. vulgare	x	25	5									Eyyübiye
49	H. vulgare	х	10	7	х	3	5						Eyyübiye
50	H. vulgare	х	1	5				х	10	5			Eyyübiye
51	H. vulgare	х	5	5	х	5	5						Eyyübiye
52	H. vulgare	Х	1	5									Eyyübiye
53	H. vulgare	х	1	5	х	1	5						Eyyübiye
54	H. vulgare	х	3	5	х	3	5	х	1	5	х	10	Akçakale
55	H. vulgare	х	2	5							х	1	Akçakale
56	H. vulgare	х	1	5	х	1	5						Akçakale
57	H. vulgare	х	1	3									Akçakale
58	H. spontaneum	х	1	5									Akçakale
59	H. spontaneum	х	1	3									Akçakale
60	H. spontaneum	х	1	5									Akçakale
61	H. vulgare	Х	1	3									Suruç
62	H. vulgare	X	20	5									Suruç
63	H. vulgare	X	1	3									Suruç
64	H. vulgare	X	1	5				х	5	5			Suruç

Table 1: Barley (*Hordeum vulgare*) and wild barley (*Hordeum spontaneum*, *H. bulbosum*) diseases observed in Şanlıurfa province of Turkey (*Ptt: Pyrenophora teres f. teres, Ptm: Pyrenophora teres f. maculata, Rc: Rhvnchosporium commune, Pg: Pyrenophora graminea,* Pre: prevalence, Sey: seyerity) (continued)

11011		0		<u>s. 1 jit</u>	mopile	n a gi a	mmea	, 110 . pr	evalence			(10)	(commaca)
65	H. vulgare	х	20	5									Suruç
66	H. vulgare	х	70	9	х	60	9	х	10	7	х	40	Suruç
67	H. vulgare	х	20	3	х	20	3						Suruç
68	H. vulgare	х	60	3	x	30	3						Suruç
69	H. vulgare	х	10	5	x	1	5						Suruç
70	H. spontaneum	х	1	5									Suruç
71	H. spontaneum	х	70	7	x	50	7						Suruç
72	H. spontaneum	х	5	5									Suruç
73	H. vulgare	х	1	3									Birecik
74	H. vulgare	х	1	3									Birecik
75	H. vulgare	х	3	5	x	3	5	х	1	5			Birecik
76	H. vulgare	х	1	3									Birecik
77	H. spontaneum	х	1	3	х	1	3						Birecik
78	H. vulgare	х	3	3				Х	1	3	х	70	Halfeti
79	H. vulgare	x	1	5							х	1	Halfeti
80	H. spontaneum	х	5	5									Halfeti
81	H. spontaneum	х	3	5									Halfeti
82	H. spontaneum	х	1	5									Halfeti
83	H. spontaneum	X	5	5				х	10	5			Halfeti
84	H. spontaneum	х	20	5									Halfeti
85	H. spontaneum	х	3	5	х	1	5						Halfeti
86	H. vulgare	х	20	5	Х	5	5	X	20	5			Bozova
87	H. vulgare	х	10	5	х	10	5	х	20	5			Bozova
88	H. vulgare	х	50	5	х	10	5	X	50	7			Bozova

Table 1: Barley (*Hordeum vulgare*) and wild barley (*Hordeum spontaneum*, *H. bulbosum*) diseases observed in Şanlıurfa province of Turkey (*Ptt: Pyrenophora teres f. teres, Ptm: Pyrenophora teres f. maculata, Rc: Rhvnchosporium commune, Pg: Pyrenophora graminea,* Pre: prevalence, Sev: severity) (continued)

89	H. spontaneum	x	1	3	X	1	3						Bozova
90	H. spontaneum	x	5	3	x	3	3						Bozova
91	H. spontaneum	x	3	3	х	1	3	х	5	5			Bozova
92	H. spontaneum	x	3	3				х	3	3			Bozova
93	H. vulgare	x	5	5	х	3	5	х	1	5			Karaköprü
94	H. vulgare	x	1	3	x	1	3						Karaköprü
95	H. vulgare										х	10	Karaköprü
96	H. vulgare	x	1	3									Karaköprü
97	H. spontaneum							х	10	5			Karaköprü
98	H. vulgare												Haliliye
99	H. vulgare	x	5	5									Haliliye
100	H. vulgare	x	10	5									Haliliye
101	H. vulgare	x	1	5	x	1	5				х	5	Haliliye
102	H. vulgare	x	60	9	х	10	9	х	1	3			Haliliye
103	H.vulgare	x	5	5									Haliliye
104	H. vulgare	x	10	5	х	5	5	х	1	5			Haliliye
105	H. vulgare	x	5	5	х	5	5	х	20	7	Х	15	Haliliye
106	H. vulgare	x	1	5	х	1	5	х	1	5			Haliliye
107	H. vulgare										Х	20	Haliliye
108	H. vulgare	х	3	5							х	10	Haliliye
109	H. vulgare	x	2	5									Haliliye
110	H. spontaneum												Haliliye

Table 1: Barley (*Hordeum vulgare*) and wild barley (*Hordeum spontaneum*, *H. bulbosum*) diseases observed in Şanlıurfa province of Turkey (*Ptt: Pyrenophora teres* f. *teres*, *Ptm: Pyrenophora teres* f. *maculata*, *Rc: Rhynchosporium commune*, *Pg: Pyrenophora graminea*, Pre: prevalence, Sev: severity) (continued)

4. CONCLUSION

For implementing successful barley disease control programs it is necessary to know the diseases and their prevalence and severity levels. In this study, barley and wild barley diseases

occurring in Şanlurfa province of Turkey located in the Fertile Crescent region were determined. Both forms of net blotch, scald, and barley stripe diseases were present in the region. Control methods should be developed for these diseases. The net form of net blotch was found to be the most common disease in barley fields, followed by the spot form of net blotch, scald, and leaf stripe. The most common disease in *Hordeum spontaneum* populations was found to be the net form of net blotch, followed by scald, spot form of net blotch, and leaf stripe. Prevalence values of these diseases ranged between 1%-70%. The net form of net blotch was detected in two *Hordeum bulbosum* populations studied in the Harran district. The prevalence of this disease in these populations was found to be 70% and 40%. Resistant barley cultivars should be planted in areas with high disease intensities. In one *Hordeum spontaneum* population, no disease was observed. Disease-free wild barley population could be utilized in developing disease-resistant barley cultivars.

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COMPARATIVE ANATOMY AND PHYSIOLOGY OF THE PANCREAS OF HUMAN AND CATTLE

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Key words: pancreas, anatomy, physiology, hormones

ABSTRACT:

It has been 100 years since insulin was isolated from the pancreatic tissue of a calf fetus (1921), while today insulin is obtained from the pancreas of adult mammals taken at the slaughterhouse, and insulin is used to treat diabetes. As animal insulin was first used, with this paper we want to compare the structure and function of the human and bovine pancreas. The pancreas is a gland located in the retroperitoneal space - the anatomical space outside the abdominal cavity. It consists of a head, body and tail. It extends from the duodenum all the way to the spleen. There are many important roles that can be divided into endocrine and exocrine. The exocrine part of the pancreas consists of glandular acinuses that secrete secretion-pancreatic juice with a large number of enzymes important for the processes of digestion. This secretion is transported into the duodenum by the pancreatic excretory duct system. The hormonal function of the pancreas is related to special glandular cells, grouped into small islets (Langerhans islets), whose diameter is 0.3 mm. These cells release their products directly into the blood. There are two types of cells in the islets of Langerhans: insulin-secreting β -cells and glucagon-secreting α -cells. In addition to these hormones, the pancreas also secretes vagotonin.

1. The pancreas of human

1.1. Anatomy of the pancreas

The pancreas develops from the endoderm of the duodenal gyrus in the area of the hepatopancreatic ring, from which the liver and bile ducts develop. The pancreas consists of the head-caput pancreatis, body-corpus pancreatis, and tail-cauda pancreatis (Picture 1). The pancreas is 13-18 cm long, 3-4 cm wide, 1-2 cm thick and weighs 70-90 g. The gland is grayish-red in color and soft in consistency, and has a lobed structure. It is S-shaped and secondarily retroperitoneally located in the posterior abdominal wall where it extends from the duodenum to the spleen.

The main outlet duct of the pancreas-ductus pancreaticus Wirsungi extends its entire length and is located closer to its posterior wall. It is 2 mm wide and during its length receives numerous small branches and ends together with the bile duct-ductus choledochus in the descending part of the duodenum. Auxiliary outlet channel-Ductus pancreaticus accesorius Santorini is an auxiliary outlet channel of different lengths. It can be connected to the main outlet channel or not and ends in the duodenum or flows directly into the main outlet channel. It can also replace the main outlet duct (Picture 1) [1].



Picture 1. Structure of the human pancreas [1]

1.2. Exocrine part of the pancreas

From the main excretory canal, short lateral branches enter the connective tissue between the lobes and divide into long flat parts that enter the end secretory parts and are called acini cells - acini pancreatics. The exocrine part of the pancreas secretes 1-2 liters of juice per day,

which contains enzymes for digestion of proteins, carbohydrates and fats. The most important pancreatic enzymes are lipase and diastase.

1.3. Endocrine part of the pancreas

In the exocrine glandular tissue, Langerhans islets-insulae pancreaticae are incorporated, most of which are in the tail, slightly less in the body and least in the head of the gland (Picture 2). There are several types of these cells:

A-cells (α -cells) which make up 10-20% of the cells that lie in the peripheral zone of the islets and produce the hormone **glucagon**.

B-cells (β -cells) make up 80% of the cells that lie in the center of the islets and produce insulin.

D-cells make up 5% of the cells that lie on the edge of the islet and produce somatostatin.

PP-cells *(F-cells)* are found in rare islet cells in the lower part of the head of the pancreas and a **pancreatic polypeptide** is formed in them.

E-cells (epsilon) - stimulate hunger, and secrete ghrelin.





Picture 2. Representation of the endocrine part of the human pancreas [2]

2. Cattle pancreas

The pancreas of cattle is located in the dorsal part of the abdominal cavity and is closely related to the anterior part of the duodenum. It has the shape of a stretched quadrangle and is quite flat. It can be divided into three parts: the body of the pancreas-corpus pancreaticus, the right lobe or duodenal arm of the pancreas-lobus pancreatis dexter and the left lobe or splenic arm of the pancreas-lobus pancreatis sinister. The left lobe is shorter, thinner and flatter than the right. The right lobe touches the ventral surface of the right kidney. The part that connects both lobes rests on the liver and that part is pierced by the portal vein. They regularly have one ductus pancreaticus that exits the right lobe and affects the duddenum. This channel is extremely small. (Picture 3). The exocrine part of the gland is far larger than the endocrine. The endocrine part consists of the pancreatic islets-insulae pancreatis, the so-called insular apparatus, which consists of clusters of cells scattered between the exocrine acinuses [3].



3. Pancreatic physiology

3.1. Insulin

Insulin is the most important anabolic signaling molecule in the control of glucose, amino acid and lipid metabolism. Its synthesis and concentration increases during meals, and decreases after the absorption of nutrients. Glucose is the most important source of energy and maintaining glycemia is one of the important parameters of homeostasis. Insulin enables the transport of glucose through the cell membrane of almost all cells in the body. Glucose molecules pass through the cell membrane of target cells through specific transport proteins. These transport molecules are called glucose transport molecules or GLUT molecules (GLUT). There are at least seven GLUT molecules that have the ability to transport glucose to different cell types. Insulin binds to receptors on target cell membranes. The insulin receptor belongs to a group of transmembrane receptors that possess tyrosine kinase activity and contain two alpha (2α) and two beta (2β) subunits joined by disulfide bonds [4].

Insulin binds to alpha subunits on the outside of the target cell membrane, and beta subunits enter the cytoplasm through the membrane. When insulin binds to the alpha subunit on the outside of the receptor, autophosphorylation of the beta subunit occurs and the enzyme tyrosine kinase is activated on the intracellular side. In this way, insulin increases the permeability of the cell membrane and activates glucose transport proteins.



Picture 4. Structure of pro-insulin showing-C-peptide and the A and B chains of insuline

3.1.1. Physiological action of insulin

Insulin has a hypoglycemic effect because it reduces the concentration of glucose in the blood. It affects the metabolism of carbohydrates by reducing the concentration of glucose in

the blood due to its rapid entry into the cells of the liver, muscle and adipose tissue. Insulin does not affect the uptake and utilization of glucose in the brain.

Brain cells are permeable to glucose and can use it without the mediation of insulin if the concentration of glucose is high enough. In the liver, insulin helps to quickly store glucose in the form of glycogen from which glucose can be mobilized as needed. In the liver, insulin inhibits gluconeogenesis, ie the synthesis of glucose from non-carbohydrate compounds. Insulin stimulates the conversion of excess glucose into fatty acids if the amount of glucose in liver cells is greater than can be used or deposited as glycogen. Fatty acids are transported through the blood to adipose tissue where they are stored in the form of triglycerides. In muscles, insulin stimulates the active transport of glucose and amino acids from the blood to the cells, increases protein synthesis and at the same time inhibits their breakdown.

Insulin acts on the rapid transfer of glucose to muscle cells that use glucose as an energy source when they are active. If the muscles are not active, most of the glucose is deposited in the form of glycogen, which can later serve as a source of glucose for energy needs. Insulin acts on protein metabolism indirectly, stimulates protein synthesis, translation of informational RNA on ribosomes and transcription of DNA in the nucleus.

It stimulates the active transport of amino acids across the cell membrane, cell growth and prevents the breakdown of proteins. Together with growth hormone, it acts synergistically and affects the growth of the whole organism. Under physiological conditions, the breakdown of insulin takes place primarily in the liver, kidneys and muscles (about 70-80%), where it is inactivated by the enzyme insulin protease. These enzymes reduce disulfide bonds, separate the A and B chains, and form two unconnected chains of amino acids that are physiologically inactive. In addition to specific insulin cells, some other target cells have insulin receptors and have the ability to break them down.

Disorders in glucose metabolism occur due to defic ency (hypoglycemia) and excess (hyperglycemia) of blood glucose. Hypoglycemia occurs as a result of reduced food intake and negative energy balance in the body. As a result of hypoglycemia, insulin secretion is reduced, and the body activates other hormones (epinephrine and norepinephrine) to increase glucose levels. Hyperglycemia, or elevated blood glucose levels, occurs under normal physiological conditions after a meal. Excess glucose in the blood is deposited in the liver in the form of glycogen under the action of insulin. However, the lack of insulin as a consequence of reduced function of the endocrine pancreas, leads to disorders in the metabolism of carbohydrates, proteins and lipids and the appearance of diabetes (diabetes mellitus). There are two types of diabetes: insulin dependent diabetes (type I) and insulin independent diabetes (type II) [5]. Insulin-dependent diabetes usually occurs as a result of chronic or acute inflammation of the pancreas, followed by loss of beta cells. In insulin-independent diabetes, despite the presence of a sufficient amount of insulin in the blood, glucose does not enter the cells. In this type of diabetes, insulin is resistant to glucose, due to reduced function of pancreatic beta cells or reduced sensitivity of glucose to insulin [6].



Scheme 1. Mechanism of insulin secretion control

The β -cells of the islets of Langerhans' endocrine pancreas synthesize and secrete insulin in response to an increase in blood glucose (a). Normal blood glucose (b). The autonomic nervous system participates in the regulation of insulin secretion. Increased parasympathetic activity stimulates insulin secretion sympathetic activity has an inhibitory effect on insulin secretion (c).

3.2. Glucagon

Glucagon is a catabolic hormone, the opposite effect of which in insulin increases the concentration of glucose in the blood. Stimulates the breakdown of body reserves and maintains a stable blood glucose concentration. Glycogen synthesis and breakdown takes place mainly in the liver and muscles. In muscle, glycogen reserves are used for muscle contraction, and in the liver as a source of glucose for extrahepatic tissues.

Glucagon is a peptide in composition, containing 29 amino acids. It is synthesized in the α cells of the endocrine pancreas, first producing preproglucagon, which is converted to proglucagon by proteolytic degradation, and then to the active form glucagon [7].

In the α -cells of the islets of Langerhans, preproglucagon is deposited in into granules, and if necessary, it separates and secretes the molecule of active glucagon by exocytosis. In plasma, glucagon circulates freely and the half-life in the circulation is 5-6 minutes, after which it is broken down in the liver and kidneys.

Glucagon acts on target cells through receptors on the cell membrane. After binding to the receptor, a cascade reaction and activation of the G-protein that activates the enzyme adenyl cyclase occurs. This enzyme stimulates the production of cyclic adenosine monophosphate (cAMP) which then activates the enzyme protein kinase. In the further process, protein kinase activates enzymes that stimulate the breakdown of glycogen to glucose, which is then released from the liver cells into the blood.

The physiological action of glucagon and insulin is crucial for maintaining blood glucose homeostasis. By influencing the concentration of glucose in the blood, glucagon stimulates the secretion of insulin. As the concentration of glucose in the blood increases, the concentration of insulin increases, and the concentration of glucagon decreases. This joint action of both hormones has a positive effect on the metabolism of the body's cells. Glucagon acts in the liver on increased production of glucose from amino acids, gluconeogenesis and increased breakdown of glycogen, glycogenolysis, which increases the concentration of glucose in the blood. In adipose tissue, glycogen stimulates fat catabolism and the release of free fatty acids, and reduces the synthesis of triglycerides and cholesterol in the liver.

Regulation of glucagon secretion takes place by negative feedback, which is triggered when the concentration of glucose in the blood decreases. Low blood glucose (hypoglycaemia) leads to increased glucagon secretion. Increased release of glucose from liver cells increases glucose concentration and inhibits glucagon secretion by a negative feedback mechanism. Glucagon secretion is also affected by the increased concentration of amino acids in the blood that are used to produce glucose in the process of gluconeogenesis.

3.3. Somatostatin

Somatostatin is a petid hormone that is synthesized by delta cells of the endocrine pancreas. Somatostatin is also secreted from the hypothalamus and inhibits the secretion of growth hormone from the adenohypophysis. It is partially excreted into the blood and has an inhibitory effect on the gastrointestinal tract by reducing gastric motility as well as secretion and absorption in the intestine. In the pancreas, it acts locally on α and β -cells, inhibits the secretion of insulin and glucagon in the case when the concentration of glucose and amino acids in the blood increases. The secretion of somatostatin is stimulated by an increase in the concentration of glucose, amino acids and free fatty acids in the blood, as well as the secretion of some gastrointestinal peptides [8].

3.4. Pancreatic polypeptide

This pancreatic hormone is a flat peptide of 36 amino acids. It is synthesized and resembles pancreatic cells. Its role has not been fully elucidated, although it is thought to have an inhibitory effect on food intake. It has been noticed that the amount of this hormone increases after eating meals, especially those rich in protein. In addition to protein, increased secretion of this hormone causes starvation, physical exertion and acute hypoglycemia. Excretion is reduced by somatostatin or by intravenous glucose administration.

3.5. Other pancreatic hormones

Other pancreatic hormones: lipocaine and vagotonin. Lipocaine is a hormone-like substance that prevents liver obesity, stimulates the oxidation of fats, fatty acids and their release from

the liver into the tissue, stimulates the biosynthesis of phospholipids. Vagotonin stimulates the activity of the parasympathetic nervous system, primarily the vagus, lowers blood pressure, enhances intestinal peristalsis and activates the processes of blood formation-hematopoiesis. Finally, let's take a brief history of insulin. On January 11, 1922, animal insulin was first used on a fourteen-year-old boy in Tornot. Animal (animal: bovine and porcine) insulin remained for a long time as the only insulin. In 1950, the sequence of amino acids in human insulin was discovered. The first synthetic insulin was produced in the early 1960s, the first genetically synthetic human insulin was produced in 1977, and the first analog insulin in 1996 [9, 10].




Scheme 2. The main events of the hemical aspects of history of insulin study [11]

4. CONCLUSION

After observing the anatomy of the pancreas of man and cattle, it can be concluded that there is a great analogy between these two species in the structure, with certain differences of individual parts. The pancreas of both humans and cattle, observed from a physiological aspect, secrete the same hormones of identical or very similar action.

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CURRENT SITUATION OF STEM RUST IN THE CWANA REGION

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Key words: Berberis spp., CWANA, Puccinia graminis f. sp. tritici, stem rust races, Ug99

ABSTRACT:

Wheat is one of the most cultivated cereal crops in the world, including the CWANA region which includes Central-West Asia and North Africa. Abiotic and biotic factors reduce wheat yields considerably. Due to the global climate changes and sharply increasing world population, food security is becoming a major problem. Therefore, the protection of wheat from diseases and pests has become an even more important issue. Stem rust, also known as wheat black rust, is one of the most important biotic factors with negative effects on wheat yield. Factors like weather conditions, mutation, production of large amounts of uredospores, and sexual recombinations on Berberis spp. have important roles in the occurrence of stem rust epidemics. Berberis species are present extensively in the CWANA region and Southern Europe, especially in Turkey and Georgia. This pathogen forms numerous races which render wheat plants susceptible to stem rust. In particular, the Ug99 stem rust race and its variants spread rapidly in the CWANA region and caused serious devastation to the wheat plants. This paper reviewed studies on stem rust caused by Puccinia graminis f. sp. tritici which has caused great damage to wheat fields in the CWANA region in recent years.

1. INTRODUCTION

Wheat (*Triticum aestivum* and *Triticum durum*) is the most cultivated plant along with corn and rice, among cereals [35]. Due to its high adaptability to environmental conditions, wheat has spread around the world. Wheat has rich nutritional elements, and it provides the basic ingredients of many foods in the nutrition of human beings and especially in rural areas. Wheat provides roughly 20% of the total calories provided by plant-based foods of the world population [14] and contributes essentially to the casual nutrition and food security of a great rate of the world's population [43].

Abiotic and biotic stress factors have serious negative effects on wheat yields. Food safety and supply have seen the fateful concern as a consequence of global climate changes in the future. Therefore, the protection of wheat from diseases and pests has become a more significant issue [25]. It is estimated that approximately 20 % of the yield losses are due to wheat diseases every year [59]. Wheat rust diseases are among the most threatening biotic factors on wheat yield. Stem rust can cause significant yield losses when the climate is suitable, and the farmer use susceptible cultivars in the growing season [39].

Ug99 is the most destructive stem rust race in wheat yields due to the breakdown of important stem rust resistance genes in bread and durum wheat cultivars. This race was detected in Uganda in 1999 and spread all over Uganda in a short period of time. Additionally, it is reported from Kenya, Ethiopia, Sudan, Yemen, Iran, and Egypt in the following years [31].

Stem rust extended all over the wheat-growing areas around the World and caused serious rust epidemics in different periods. Therefore, still it is one of the most feared diseases in wheat-growing areas. The rust populations have to be strictly monitored for two significant reasons. First of all, new races may move from a wheat-growing area to another area that can be considered long-distance, either through wind-assisted or accidental human activities. This makes global, regional, and national quarantines impossible. Secondly, rust pathogens have an excessive ability to change and develop new races through sexual recombination and mutation. For this reason, the effect of changing races on commercial varieties, genetic stocks, and advanced lines should be determined, and new sources of resistance against new and dominant races should be revealed [45]; [56].

2. HOST: WHEAT

Wheat has spread around the world from the Fertile Crescent, known as the genetic source and homeland of wheat. Wheat plays a significant role in human nutrition, especially in rural areas. It contains nutrients and has high adaptability to insufficient environmental conditions. In addition, wheat is the staple food of many countries due to its suitable nutrient value, storage, and ease of processing. Wheat also provides about 20% of the total calories provided by plant-based foods to the world population. Thus, it is one of the most cultivated crops with corn and rice [35].

3. THE PATHOGEN

Stem rust as known also black rust of wheat is caused by *Puccinia graminis* f. sp. *tritici*. The pathogen is a heteroecious rust species belonging to the Basidiomycota [26]. The pathogen moves by airborne uredospores from one field to another field and over long distances. *Puccinia graminis* f. sp. *tritici*, in addition to wheat, infects *Berberis*, *Mahonia*, and *Mahoberberis* species. The life cycle of the pathogen consists of five different periods. Sexual recombination on *Berberis* species causes stem rust pathogen to develop new race variants and these may be responsible for new epidemics. Planting systems and climate conditions

play a significant role in the occurrence of major epidemics [18]; [16]; [52]. Weather conditions also are essential for transmission of the pathogen to Berberis species and encourage the formation of infection, pathogen survival, growth, and sexual recombination. Additionally, environmental conditions help the pathogen to complete the life cycle and especially the survival of the teliospores, their germination, and the infection of Berberis leaves by basidiospores [61]; [57]; [15]. The occurrence of infection under natural conditions relies on several factors. Teliospores germinate and produce basidiospores. These basidiospores reaches Berberis plants and infection occur. After infection spermagonia forms on Berberis leaves. The dikaryotic stage is established after the fertilization between compatible spermatium and receptive hypha. On the lower side of the leaf, aecia and aeciospores form. Aeciospores infect wheat plants. In wheat plants, uredia, and uredospores form. Uredospores infect the wheat plants and cause damage during the growing season. New uredia and uredospores form within a short time period and new infections occur. When plants are maturing in the late season, telia and teliospores form. The Berberis species has been known as a factor in the occurrence of major epidemics for a long time. The Asian and Southern European countries are known as the regions where Berberis species are seen in abundance [3]; [24]; [61]; [12]; [22]; [52]. It is known that the risk levels of pathogens are between high and very high due to the high presence of Berberis species in Azerbaijan, Russia, Turkey, and Georgia (Table 1).

Country	Region	Risk Rate
Afghanistan	Central Asia	0.05
Azerbaijan	Central Asia	1.19
Iran	Central Asia	0.64
Kazakhistan	Central Asia	0.61
Kyrgyzstan	Central Asia	0.14
Russia	Central Asia	1.17
Tajikistan	Central Asia	0.23
Turkmenistan	Central Asia	0.30
Uzbekistan	Central Asia	0.62
Georgia	West Asia	1.00
Turkey	West Asia	0.88
Syria	West Asia	0.33

Table 1: Region and risk ratios of countries where Berberis species are present [52].

4. THE EMERGENCE, DEVELOPMENT, AND DISTRIBUTION OF THE UG99

Ug99 stem rust race (TTKSK) caused major epidemics after the breakdown of the Sr31 gene, which provides resistance to stem rust with unheard-of virulence in 1998 in Uganda [42]. The Ug99 stem rust race and its variants, which caused devastating damage by breaking the Sr31 resistance gene, spread throughout Uganda in a short time. After being detected in Kenya in 2001, it caused severe epidemics between 2002 and 2004 [58]. This race was detected in Ethiopia in 2003, Sudan in 2006, and the same year by crossing the Red Sea and detected in Yemen. This race, which spread to large areas in such a short time, further spread to East African countries by air and seriously affected the wheat production in the region. It has been reported in the Broujerd and Hamedan regions of Iran in 2007 [31]. The severe stem rust epidemics occurred in Egypt in 2014, and samples were collected and analyzed. The TTKST, which is in the Ug99 race group, and the original Ug99 race were determined [40].

Using the North American stem rust differential set [44], the Ug99 stem rust race was designated as TTKS [58]. TTKS was encoded as TTKSK, with the addition of the Sr24, Sr31, Sr38, and SrMcN resistance genes to the North American differential set. The geographical distributions, emergence, and changes of the Ug99 race group have been investigated (Singh et al. 2006, 2008 2011) and the most up-to-date consequences of this race group have been reported. The evaluations about the current status of the monitoring systems of the U99 race group, which seriously threatens wheat production areas globally have been made. Seven races in the Ug99 race group were determined phenotypically in 9 countries in Africa, the Middle East, and Asia until 2010 [50], and 8 different races were reported in 13 countries in 2014 [51]. These reports show that the pathogen's races will continue to develop and spread in this geographic region. Among the races in this group, TTKSF [13], which was detected for the first time in South Africa in 2000, is the most striking race. This race is significantly virulent on local wheat varieties possessing Sr8b and Sr38 resistance genes. Taking into consideration that the Ug99 race group is affected by mutations, the TTKSF race is considered an ancestor of the PTKSF race. The Sr24, Sr31, Sr36, and Sr38 are important sources of resistance against stem rust in commercial cultivars and breeding materials. Thus, the virulence of P. graminis f. sp. tritici on these genes is significant due to the sustainably combat against the stem rust pathogen.

	-	-		
Race	Common name	Virulent	Year identified	Country and
		+/Avirulent -		Year of
				Detection
TTKSK	Ug99	+Sr31	1999	Uganda
				(1998/9), Kenya
				(2001)

Table 2: Ug99 and its lineages detected in different countries [47].

Race	Common name	Virulent +/Avirulent -	Year identified	Country and Year of Detection
TTKSK	Ug99	+Sr31	1999	Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009), Eritre (2012), Rwanda (2014), Egypt (2014)
TTKSF		-Sr31	2000	SouthAfrica (2000), Zimbabwe (2009), Uganda (2012)
TTKST	Ug99 + Sr24	+Sr31, +Sr24	2006	Kenya (2006), Tanzania (2009), Eritrea (2010), Uganda (2012), Egypt (2014), Rwanda (2014)
TTTSK	Ug99 + Sr36	+Sr31, +Sr36	2007	Kenya (2007), Tanzaniaa (2009), Ethiopia (2010), Uganda (2012), Rwanda (2014)

Table 2. Ug99	and its lineages	detected in	different	countries	[47]	(continued)
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Table 2: Ug99 and its	lineages detected in d	ifferent countries [47	1 (continued).
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Race	Common name	Virulent	Year identified	Country and
		+/Avirulent -		Year of
				Detection
TTKSP		-Sr31, +Sr24	2007	South Africa
				(2007)
PTKSK		+Sr31, -Sr21	2007	[Uganda
				(1998/9)?],
				Kenya (2009),
				Ethiopia (2007),
				Yemen (2009),
				South Africa
				(2017)

5. RECENT SITUATION OF STEM RUST IN CENTRAL-WEST ASIA AND NORTH AFRICA (CWANA)

Stem rust disease is one of the most important wheat leaf diseases because it has serious negative effects on yield and quality by causing a number of damages to various wheat parts in all areas where wheat is grown.

When weather conditions are favorable for the development of the disease, stem rust causes epidemics and significant yield losses on susceptible wheat cultivars that do not contain resistance genes. Stem rust disease has caused severe economic damage by creating epidemics from time to time around the world. The Sr24 and Sr31 genes have a significant role in resistance against stem rust and are used in the varieties developed in breeding studies. However, the Ug99 stem rust race detected in Uganda is virulent on all cultivars that possess the Sr31 resistance gene [42].

In Turkey, wheat stem rust surveys were conducted in 2007 and 2008. In 2007 and 2008, 43% and 25% of the inspected wheat fields were found as infected with stem rust, respectively. These surveys revealed numerous stem rust races in Turkey [28]. The most common stem rust race was TKTTC. In addition, stem rust races TKSTC, TKTSC, PKSTC, PKNTC, PKJSC, TKTTC, RKGTC, RKRTC, RTKTF, TKKTC, TKKTC, TKTTC, TKCTC, TKJTC, TTJTC, TKSTC, TKSTC, TKSTC, RTKTC, RTTJ, RTTTF, PKTTC, LRHPF and MRKTF were found. Under greenhouse conditions, Mert [27] evaluated the seedling reactions of 138 wheat cultivars against wheat stem rust races RTKTF, TKTTC, and RTTTC. Durum wheat cultivars Fırat 93, Diyarbakır 81, Eyyubi, Artuklu, Turabi, Balcalı 2000, Amanos 97, Svevo and Zenit, and bread wheat cultivars Yıldırım, Alpu 2001, Karacabey 97, Ahmetağa, Canik 2003, Tahirova 2000, İzmir 85, Köksal 2000, Menemen, Seri 82, Carisma and Basri Bey 95 were found resistant to these 3 races [29] determined the reactions of some wheat genotypes to stem rust races RTTTC, TKTTC, and RTKTF. Two durum and 1 bread wheat genotypes were found as resistant to these stem rust races. Stem rust disease has been reported from Eskisehir, Turkey in barley plants, which is one of the hosts of stem rust [17]. The stem rust disease was detected in wheat and barley in the survey studies carried out by Özdemir et al. [38] in Kırıkkale province of Turkey in 2015. İlgen et al. [23] reported that P. graminis f. sp. tritici was present in the wheat and barley growing areas of the Cubuk, Ankara, Turkey in 2016. Wheat stem rust was found to be common in the Kastamonu and Sinop provinces of Turkey [4]; [5]. Berberis species with aecial infection was reported from Kastamonu, Turkey [5]. Akçi and Karakaya [8] reported stem rust races occurring on Berberis species and wheat plants from the Kastamonu province of Turkey. TTTTF was the most common race from both Berberis species and wheat plants. Additionally, from Berberis species, stem rust races RTTTF, RTTTC, TTTTC, and TTKTF, and from wheat plants, stem rust races TTKTF, TTTTC, TTKTC, RTTTF, and RTTTC were reported. From wheat plants in Sinop province of Turkey TTTTF, TTKTF, RTTTF, RTKTF, and TTKTC stem rust races have been identified. TTTTF was identified as the most common stem rust race [7]; [9]. In

another study carried out in Sinop province of Turkey by Akci and Karakaya [10]. stem rust races TTTTF and TTKTF from *Berberis crataegina* plants were reported. In the study conducted by [11]. TTTTF, RTTTC, and RTTTF stem rust races were inoculated on the seedling period of 46 bread and 14 durum wheat varieties under greenhouse conditions and bread wheat cultivars Tahirova 2000, Yıldırım, Alpu 2001, Canik 2003 and Basri Bey 95 cultivars were found to be resistant to three stem rust races. They also reported that the durum wheat cultivars Sarı Çanak 98 and Fırat 93 were resistant to TTTTF race, Eminbey, Altıntaş 95, Zühre and Sarı Çanak 98 were resistant to race RTTTC and Eminbey, Altıntaş 95, İmren, Yelken 2000 and Zühre cultivars were resistant to RTTTF stem rust race.

Understanding the sources and origins of genetic variation in rust pathogen populations globally would simplify the development of better strategies to combat the disease. In Georgia Olivera et al. [37] analyzed the stem rust situation. Stem rust races PTCTF, PRCQC, PRCTF, PRPTF, PRCTM, PKPTF, TTRTF, TKPTF, PKPTC, PRDSC, TKFTF, QFCSC, MMMTF, TKKTF, MMCTC, MRCQF, NTCSF, PCHTM, PHHTC, PMCQF, PRCQF, PRCQP, PTHTP and PRFTF were reported. Some unusual combinations were identified, including the Sr22+Sr 24 and Sr13b+Sr35+Sr 37 combinations. These new virulent combinations will pose serious challenges to breeding programs because many of these combinations are used in breeding programs against the Ug99 race group. *Berberis* species are common in Georgia. This study shows that these sexual recombinations in stem rust populations in Georgia and the Caucasus region create an important source for the new races.

The *Puccinia graminis* f. sp. *tritici* samples were collected from different regions of Kazakhstan and analyzed by Rsaliyev et al. [46]. They reported that QHHSF and THMTF stem rust races were common races in all regions. They also reported that the RFRTF, RHMRF, TKRPF, and MHCTC races were widely detected in Akmola and Kostanay regions, and LHCSF, QKCSF, and LKCSF races were detected only in East Kazakhstan.

With the emergence of the Ug99 race group, stem rust has become a severe problem in East African wheat-growing lands. The potential impacts and threats of the disease pose a particularly severe problem for Iraq. The Sr24 and Sr31 resistance genes are detected to be effective against the identified races. In 2019, 27 stem rust samples were collected from Iraq, and stem rust races TKTTF, TKKTF, and TTKTT were determined The stem rust race TKKTF exhibited additional virulence on Sr36 as compared to TKTTF. However, the TTKTT stem rust race was placed in the Ug99 stem rust race group and showed virulence to Sr24 and Sr31 [34]. Stem rust races TKTTP and TKKTP with virulence to Sr24 resistance gene were reported from Turkey and Tunisia [33]. The authors pointed out that an increasing trend of virulence to Sr24 in pathogen populations is of great concern given the spread of the Sr24 resistance gene in widely grown wheat varieties around the world.

Thirteen stem rust samples collected from surveys in the south west Iran were analyzed and the races RTRTC, HRCTC, RRTF, TTPTC, TTTQF, JTHTC, and TTKSK were identified

[32]. This study demonstrated that the TTKSK race detected in Iran [30] has passed from East Africa to Iran and its spread is continuing.

The highland and plain of Ethiopia are considered the most suitable areas for stem rust evolution. Therefore, a study was conducted by Abebe et al. [2] to determine the distribution, densities, and virulence of stem rust races in Southern Tigray (Northern Ethiopia) in 2010. Sixty-six wheat fields were examined and P. graminis f. sp. tritici infection was present in 33.3% of the examined areas. As a result of the analysis of the collected samples, BBBBC, HHSTF, JRGSC, BBBLC, BHJBC, CCGBC, GMHJC, HRJJC, JTGDB, RRTTF, SKQNH, SPSSF, TCQJH, TTKSK, TTSNK, TTSSK, DBHQC, DBHSC GKJSF races are reported. In the severe epidemics that appeared in Southern Ethiopia between November 2013 and January 2014, losses of up to 100% occurred in wheat production areas. The 60 stem rust samples collected from these areas were analyzed, and the TKTTF, TTKSK, RRTTF, and JRCQC races were identified. It has been reported that the race causing severe losses in the epidemic was TKTTF which is also called the Digalu race [36]. In another study conducted in Ethiopia, TTRTF stem rust race was reported as a result of race analysis of isolates obtained from 60 stem rust samples collected from different regions. This report also showed that the TTRTF stem rust race was detected for the first time in Ethiopia [54]. Hei et al [21] reported stem rust race TTKTT from Ethiopia. In addition, a study was conducted by Yesuf et al. [55] to determine the disease intensity, distribution of dynamics pattern, and the genetic variability of Puccinia graminis f. sp. tritici in the Awash River Basin of Ethiopia from 2014/15-2019/20. They reported that about 71.7% of the wheat fields were affected by stem rust during the 2018/19 main season and about 63.7% in 2019/20. They found TTTTF, TKTTF TKKTF, and TTKTF stem races in this period. They also reported that the races were highly virulent and affected most Sr genes except Sr31 and Sr24. Another striking study conducted using 147 isolates derived from 121 wheat stem rust samples collected during the 2015-2016 growing season in Ethiopia. The isolates were analyzed and 12 different stem rust races were identified. TTKTF, TTKSK, JRCQC, TRTTF, and TTTTF stem rust races were determined as the most common. TKTTF and TTKSK were determined as the two most common races. Additionally, the TTKTT stem rust race was detected in Ethiopia [1].

In the surveys conducted in Eritrea in 2010, intensive stem rust infections were observed in wheat-growing areas. *P. graminis* f. sp. *tritici* was recorded in 95% of the 92 wheat fields examined in 50 different locations, and the disease severity was reported as higher than 40% [60]. The collected samples were analyzed in Canada, and PTKST, and TTKST stem rust races, which have virulent on Sr24 and Sr31 and avirulent on Sr21, were reported for the first time in Eritrea [60]. In another study conducted in Eritrea, TKKTF and TTKSK stem rust races were reported as a result of race analysis of isolates obtained from 60 stem rust samples collected in different regions. This report also showed that the TTRTF stem rust race was detected for the first time in Eritrea [41].

TTRTF stem rust race, known as the Sicily race, was detected in Spain, Tunisia, and Iran during the surveys carried out between 2018 and 2019. TKTTF and TTTTF stem rust races were detected in Africa and Southern Europe [19]. TKKTF stem rust race was detected in 10 different European countries, including Egypt, Iran, and Tunisia during the survey program and race analysis studies between 2019 and 2020 [20].

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EXAMINATION OF THE DIVERSITY OF NATURAL SOIL CONDITIONS ON THE GROWTH OF WILD GARLIC (Allium ursinum)

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Key words: wild garlic, soil, natural conditions

ABSTRACT:

Allium ursinum, popularly known as wild garlic, forest or bear onion is a medicinal plant that is present as natural populations and is to some extent conditioned by soil properties. This plant species shows numerous positive impacts on human health, which indicates its potential and the need for its detailed research.

The aim of this study was to determine the diversity of natural conditions for wild garlic grows and soil samples was performed from three localities in the municipality of Srebrenik. Soil samples was performed in well-known localities where the presence of wild garlic (Allium ursinum) in its natural populations was determined. After proper sampling, processing and preparation of soil samples, analysis of basic physical and chemical parameters was performed. In the soil samples taken, the texture and structure, content of hygroscopic moisture, pH value, electrical conductivity, the soil salinity and the humus content were determined.

Soil analysis showed that the plant species Allium ursinum is most favored by lighter, moist soils, rich in humus and nutrients, i.e. soils in which the reaction of the soil in the area is weakly acidic to weakly basic. Analysis of pH values as well as electrical conductivity (EC) and total dissolved solids (TDS) showed that increasing the anthropogenic impact increases the pH value as well as the specific conductivity and the soil salinity. All the obtained results are in accordance with the literature data, where it can be concluded that the presence of wild garlic (Alllium ursinum) is mostly determined by combination of environmental factors (i.e. soil, climate, topography, hydrology, etc.).

1. INTRODUCTION

Wild garlic (*Allium ursinum*), also known as "bear onion" is a wild plant of the genus Allium. It is a perennial species that has a short vegetation period (April-June) and is used as the first spring salad when only leaves are harvested from its natural habitat. An important part of the plant are the leaves used in the food industry, while the flowers and bulbs are also

edible. Wild garlic (*Allium ursinum*) has antiseptic, bacteriostatic and antiparasitic properties and is used in the treatment of hypertension, hyperlipemia and hypercholesterolemia in alternative medicine. [1] [2] Wild garlic leaves are highly prized as spices, salads or soups, and raw, pickled or as vegetables in gastronomy. [3] Wild garlic often grow in dense form, forming large coverings in hornbeam, oak and beech forests. [4] [5] Under such a dense carpet, competition for light and space takes place. [6] Wild galic (*Allium ursinum*) is a powerful competitor that affects the growth of other herbaceous plants through the soil where it accumulates phenolic phytotoxins.

Despite its wide distribution across different continents, it is a plant species specializing in certain habitats. [7] It is particularly sensitive to the presence of water, and grows best on light to medium-heavy, nutrient-rich, moist but well-drained soils, in locations in shade or partial shade. Although it prefers areas with high relative humidity, it also thrives on shallow and fairly dry calcareous soils. [8] However, both drought and stagnant water are appropriate factors for the growth and development of this plant species. *Allium ursinum* is also sensitive to pH, and thrives on soils with a soil reaction of 5.5 to 7.9, with any deviation leading to morphological changes.

Due to all the above, the aim of this study was to determine the physico-chemical analysis of soil at three different locations where wild garlic (*Allium ursinum*) is present in natural populations to examine the importance of environmental factors on the growth and development of this plant species.

2. EXPERIMENTAL PART

2.1 Material

As material on this work, three different soil samples from the area of Srebrenik municipality were used, on which wild garlic (*Allium ursinum*) is present in natural populations.

	imples and their location
Sample	Location
Sample I	Ostružna
Sample II	Dolić
Sample III	Ahmedinovac

Table 1. Name of samples and their location

Samples were collected between the end of one growing season and the beginning of another, in order to reflect the actual soil conditions at the beginning of the growth and development of wild garlic, and thus provide insight into the requirements of this plant species for the substrate on which it thrives. A large number of individual samples were taken at each microlocation, after which one average sample was formed in order for the obtained results to show the soil characteristics that prevail over the entire surface of a given microlocation. Soil sampling was performed to a depth of 20 cm, which is determined by the depth to which the

root system of the wild garlic develops. After sampling, samples were identified, followed by drying of the soil for 10 days, in order to achieve the air-dry soil required to perform the desired analyzes.

2.2. Methods

Within the physical-chemical analysis of the soil, the following parameters were determined: moisture content, pH reaction of the soil, determination of specific conductivity, the soil salinity and content of organic matter.

The hygroscopic moisture content was determined by the gravimetric method of drying at 105° C, where the hygroscopic moisture content was calculated according to the following relation:

hygroscopic moisture content (%) =
$$\frac{m_2 - m_3}{m_2 - m_1} x 100$$

where is:

m₁- mass of the empty glass container;

m₂- mass of the glass container with soil sample before drying;

m₃- mass of the glass container with soil sample after drying.

The pH reaction of the soil was determined by electrochemical method, measured on a pH meter Mettler Toledo 220. The pH value of all samples was determined in distilled water (active acidity) and in 1 M KCl (potential acidity).

The content of organic matter was determined by the Tyurin method (bichromatic method), where in addition to the analysis of soil samples, a blank test was performed, and the content of organic matter was calculated according to the following relation:

$$\% = \frac{(a-b)x0,0005172 \cdot 100}{m}$$

where is:

a- volume of Mohr salt (ml) used for blank titration x 0.1x10;b- volume of Mohr salt (ml) used for soil sample titration x 0.1x10;m- mass of soil sample.

The specific conductivity and the soil salinity were determined by the method of direct measurement using a conductometric cell, and then calculated using the following relation:

$$TDS\left(\frac{mg}{L}\right) = EC\left(\frac{mS}{cm}\right)x 640$$

3. RESULTS AND DISCUSSION

After the analysis, the contents of each analyzed parameter were calculated and presented graphically.

3.1. Moisture content

The moisture content in the surface layers of the soil is an important parameter in agriculture [9]. Soil moisture is a crucial variable in governing the water and heat among land surface and atmosphere through plant transpiration and soil evaporation. [10] The analysis showed that the highest content of hygroscopic moisture has sample II from the locality Dolić 12.38%, while the lowest content of hygroscopic moisture has the sample III from the locality Ahmedinovac. Sample I had an approximate value of moisture content as well as sample III 7.97%.

3.2. pH value

Graph 1. shows the pH value of the active and potential acidity of the analyzed samples. The highest pH value was shown by sample III locality Ahmedinovac, where the active acidity was 7.24, and the potential or hidden acidity was 6.36, where this soil can be classified as weakly acidic soil. Sample I had the lowest soil pH value at the Ostružna site, where the active acidity of this sample was 6.16 and the potential acidity was 4.64. Thanks to such low values of potential acidity, the soil from the Ostružna site is classified as an acidic area. The tested sample II originating from the Dolić locality had an active pH value of 6.44, while the potential acidity was 5.72, which classifies it as a weakly acidic soil.

The obtained average pH value coincides with the claims of Oborny et al. that Allium ursinum thrives best on soils with a pH value of 5.5 to 7.9, or on soils from weakly acidic to weakly basic soil reaction. However, the pH results from the Ostružna site support the views of many authors, who claim that wild garlic (*Allium ursinum*) can grow on soils of different properties, given that the Ostružna site is classified as acidic soils. Also, the results of the research point to the conclusion that man's activity influences the reaction of the soil, whereby in this case areas with stronger anthropogenic influences have a higher pH value.



Graph 1. pH values of analyzed samples

3.3. Specific conductivity of the soil and soil salinity (TDS)

It is known that the root system of the plant, when receiving cations in the soil solution, in order to achieve electrical balance, secretes hydrogen ions and the soil becomes acidic. [11] [12] Guided by this, it is very important to know the same, since each plant prefers a certain value of electrical conductivity.

Determination of soil specific conductivity (EC), which in this case is presented in μ S/cm, made it possible to calculate the soil salinity (TDS), which is expressed in mg/ L. The values of specific conductivity as well as the degree of salinity of the analyzed samples are shown in Graph 2. The lowest specific soil conductivity of 19.7 μ S/cm, i.e. the soil salinity of 12.608 mg/L was in the tested sample I from locality Ostružna, while in sample III from the Ahmedinovac site, which is under human influence, found the highest values of specific conductivity in the amount of 73.9 μ S / cm, i.e. the soil salinity of the soil of 47.296 mg/L. The EC and TDS values in sample II from the Dolić locality are between the values of the previous two localities, where the specific conductivity is 48.0 μ S/cm, and the degree of salinity of the soil is 30.72 mg/L. It is very easy to see that the plant species *Allium ursinum* thrives in a wide range of values of electrical conductivity and the soil salinity. These results clearly indicate in this case the conclusion of a directly proportional relationship between EC and TDS and human activity, i.e. the anthropogenic impact increases the values of specific conductivity and the soil salinity.



Graph 2. EC and TDS of analyzed samples

3.4. Organic matter content (humus)

The importance of organic matter in the soil is manifold because organic matter is a source of plant nutrients and has an irreplaceable role for the stability of soil aggregates. In addition, the most important prerequisite is good tillage, supports the movement of water and air in the soil, increases water retention in the soil (retention) and biogenicity, prevents erosion, strongly increases the buffering effect of the soil, preventing leaching of nutrients (nutrient retention in the root zone, causes a darker color of the soil, which results in faster heating, has a strong effect on reducing soil density, etc. [13]

Of the three examined soil samples with three different microlocations, the highest humus content was found in sample II originating from the Dolić locality, and amounted to 3.46%. According to Gračanin, the humus content of 3.46% classifies this soil as quite humus soil (Table 2.). Sample III from the Ahmedinovac locality also belongs to the mentioned group, considering that the percentage of humus in the examined soil sample from the given locality was 3.16. The lowest humus content of 2.87% was in the soil sample I from the Ostružna locality, which classifies this soil as weakly humus. However, the analysis showed that even a lower % of humus in the soil (sample I) does not represent a limiting factor for the growth and development of wild garlic.

(Method by Gracanin)			
Soil supply	Humus (%)		
Very poor in humus soil	<1		
Pooe in humus soil	1-3		
Enough humus soil	3-5		
Rich in humus soil	5-10		
Extremely rich in humus soil	>10		

Table 2. Limit values for humus content in soil

4. CONCLUSION

Based on the physico-chemical analysis of samples, it can be concluded that the reaction of the soil on which wild garlic grows largely depends on other physico-chemical properties of the soil, anthropogenic impact and environmental conditions prevailing at the site.

The analysis also showed that the plant species *Allium ursinum* is most favored by lighter, moist soils, rich in humus and nutrients, which are covered with litter and vegetation, and with a reaction of soils from weakly acidic to weakly basic. A minor deviation from the stated soil properties does not represent a limiting factor for the growth and development of wild garlic, which makes it a plant species adaptable and tolerant to different soil properties and types. However, the presence of wild garlic (*Allium ursinum*) in a certain locality is primarily determined by a combination of ecological factors (climate, relief, topography, hydrology and soil), with anthropogenic factor having a great influence on its morphology and properties.

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INFLUENCE OF MULCH ON MORPHOLOGICAL, BIOCHEMICAL AND ANTIOXIDANT PROPERTIES OF ARONIA (*ARONIA MELANOCARPA* ELLIOT)

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Key words: Chokeberry, Mulch, Antioxidant properties

ABSTRACT:

Black chokeberry fruits are rich sources of bioactive substances, including polyphenols and anthocyanins, and suitable raw material for production of functional foods. The aim of this research was to determine how mulching and the types of mulch can affect the most important morphometric, chemical and antioxidant properties of the fruit of black aronia. Covering the soil with straw has influenced the double increase in iron content, and the coverage of black plastic foil has contributed to the multiple increase in sugar in the fruit, compared to the classic growing of the aronia without mulch.

1. INTRODUCTION

Lesser-known fruits are highly valued fruit crops for their unique flavors, textures, and colors. They have been reported that to include significant amount of human health contents including high antioxidant content, vitamins, minerals, fiber, folic acid, etc. In addition to fresh consumption, lesser known fruits are widely used in beverages, ice cream, yogurt, jams, jellies and many other food products (Serce et al., 2010; Ercisli et al., 2012; Cuce & Sokmen, 2017; Alibabić et al., 2018; Skender et al., 2019, Skender et al., 2022).

Black chokeberry (*Aronia melanocarpa* Elliot) is a deciduous shrub native to North America and botanically clasiffied as belonging to the family Rosaceae (Seidemann, 1993). In the beginning of the 20th century, chokeberry was brought to Russian Federation botanic gardens, where it spread in the European part of the country. Around 1946 the plant was started to use in cultivation in the former Soviet Union. More recently it is also cultivated both in East European countries and Germany (Strigl et al., 1995). In breeding studies, new cultivars that have larger edible fruits were released. The plants have been known pest free (Scott et al., 2007). Aronia schrubs can reach 2-3 m height, which produce 30 small white flowers in a

umbel in May to June. At the harvest the shrubs had bright red or purplish black berries (black chokeberry) with diameter 6-13 mm and weight of 0.5-2 g (Fan and Rechits, 1977; Seidemann, 1993; Strigl et al., 1995; Ara, 2002).

Black chokeberries are highly valued by the fruit processing industry, which uses them mainly in fruit juice production (Snebergova et al., 2014). The high polyphenol content and multiple health benefits of chokeberry fruits were increased its popularity not only in scientific world but also fruit industry (Denev et al., 2012).

Several papers revealed the biochemical composition (Denev et al., 2012; Kulling & Rawel, 2008) of aronia fruits. Besides, this berry is a very rich source of anthocyanins, proanthocyanidins, and hydroxycinnamic acids (Jakobek et al., 2007; Wangensteen et al., 2014). Several factors including habitat, cultivar, maturation stage, fertilization, harvest date, etc. could affect the biochemical composition of chokeberries (Kulling & Rawel, 2008). In Bosnia and Herzegovina, the first orchards were established in the last couple of years (Skender et al., 2017).

The aim of this research was to determine how mulching and the types of mulch can affect the most important morphometric, chemical and antioxidant properties of the fruit of black aronia.

2. MATERIAL AND METHODS

2.1. Plant material and experimental design

In this study, Nero cultivar was used. The bushes were planted at a distance of 1.5 x 2 meters at 2014 on experimental field of the Biotechnical Faculty, University of Bihać. Manure and 100 kg/ha nitrogen (15-15-15; N-P-K) were incorporated before planting. The moderate continental climate prevails here with a mean day temperature of 11 °C, long and cold winters, and warm and often dry summers. The rows were watered with drip irrigation, as required. Plants were fertilized with 20 kg/ha P and K (0-20-20) and 60 kg/ha N (applied as a triple split in March, April, and May). Research was carried out in 2017 and 2018. The experimental polygon is divided into three parts. In one part, the plot was covered with black plastic film (black polyethylene film, 1.2 m wide and 0.058 mm thick), the second part covered with a 15 cm thick straw, and the third part was without mulch material and was the controlled part of the experiment. With each of these three parts of the experimental polygon, samples were taken in a period of full maturity in the amount of 2 kg. Care was taken to avoid unripe, demaged, or over-ripe fruits. Samples were frozen and stored at -20 °C until use which was usually no longer than 2 months.

2.2. Morphological and biochemical analysis

Laboratory analysis of fruits were carried out in the chemical laboratory of the Biotechnical Faculty in Bihać. The weight of the fruit were determined by digital balance. With all three plots, a sample of 100 fruits (n = 100) was taken, the individual weights of the fruit were measured in order to calculate the average value. Determination of the number of fruits per issue was done by direct counting. After measuring the fruit weight, the analysis of the contents of the following parameters was carried out: water, dry matter, ash, acidity, total and reducing sugars, sucrose, anthocyanins, phenols, iron, phosphorus, C vitamins and pH, in order to determine the influence of mulch on the content of the mentioned components. All these laboratory analyzes were done in four replicates. The water content was analyzed by drying with the addition of quartz sand. By this method, water is determined indirectly by measuring the residue that is lagging behind after drying. The addition of sand increases the surface of the sample and accelerates evaporation. It dries at 105 °C, to constant mass and measure. Determination of the dry matter content was done using a refractometer (Carl Zeiss refractometer, Model II). The ash content was determined by incineration at 600 °C until white powder was obtained (Analog Selector). Five g of dried fruit was measured, lighted directly and burned at 600 °C until white ash is obtained. Total and reducing sugars were analyzed by volumetric method by Luff Schoorl. (Sucrose content was calculated according to the relationship: sucrose = (total sugar - reducing sugar) x 0.95. Titratable acidity was determined by titration of a water solution of chokeberry homogenate with 0.1 N NaOH to an end point of pH 8.1. Determination of vitamin C content was performed by the method of iodometric titration. For polyphenol and anthocyanin sample preparation, 20 mL of methanol/HCl 2% (95:5 v/v) were added to 20 g frozen berries. After 60 min, the berries were homogenized and centrifuged for 15 min at 3000 rpm. The supernatant solution was filtered under vacuum in a 50-mL volumetric flask. The residue was extracted again in the same way. The solution was diluted to volume with methanol/HCl 2%. The extraction procedure was repeated twice for each sample. The total anthocyanin amount, calculated as cyanidin-3glucoside, was determined by means of the pH differential method (Giusti & Wrolstad, 2001). The results were expressed as mg of cyanidin-3-glucoside per 100 g of fresh weight (FW). Quantification data were the mean of 4 results. Total phenolic compounds were determined by Folin-Ciocalteau's methods (Singleton & Rossi, 1965). Results were expressed as mg of gallic acid per 100 g of fresh berries. Quantification data were the mean of 4 results. The phosphorus content was determined by spectrophotometric use of the ammonium-vanadate molybdate method (Gericke & Kurmies, 1952). Measurement of the pH value was carried out with the pH meter by immersing the combined electrode in the sample and reading the values on the device. The determination of the iron content was done using the atomic absorption spectrophotometry, on the atomic absorption spectrophotometer "Perkin Elmer" Analyst-800 with Zeeman correction (flame technique-FAAS). The combination of gases we used for analysis on AAS is acetylene-compressed air, which has reached a temperature of 2400-2700 °C.

2.3. Statistical analysis

The obtained results were processed by statistical methods of descriptive analysis and application of the main component analysis (PCA) (Hotelling, 1936). The above mentioned analyzes of the obtained data set are processed in statistical programs PAST, XLSTAT 13 and SPSS 21 (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

Table 1. shows the results of morphological, biochemical and antioxidant properties of black aronia (Nero cultivar) on various types of mulch material, and on the controlled plot without mulch.

As indicated in Table 1, the highest fruit weight, number of fruit per shoot, water content, total sugar, sucrose, reducing sugar, ash and total acidity values were obtained from the plot covered with black foil.

Tuble in results of the blady of the native ability properties on anterent types of match				
Traits	Black sheet plastic	Straw	Control	
Fruit weight (g)	0.89 ± 0.50	0.80 ± 1.23	0.68 ± 0.33	
Number of fruit per shoot	208.71	163.38	151.73	
Water (%)	75.86 ± 0.60	74.27 ± 1.01	73.94 ± 0.24	
Dry matter (%)	24.13 ± 0.60	25.80 ± 0.97	26.05 ± 0.24	
Total sugars (%)	21.32 ± 8.20	12.56 ± 4.74	4.48 ± 0.45	
Reducing sugar (%)	13.32 ± 5.12	7.85 ± 2.96	2.80 ± 0.28	
Sucrose (%)	7.59 ± 2.92	4.47 ± 1.68	1.59 ± 0.16	
Ash (%)	0.47 ± 0.09	0.36 ± 0.05	0.65 ± 0.04	
Titratable acidity (%)	1.30 ± 0.01	1.25 ± 0.03	1.19 ± 0.08	
Antochyanins	707.51 ± 24.44	577.95 ± 22.85	724.35 ± 45.91	
(mg CGE/100 g)				
Total polyphenols	3887.25 ± 230.98	3703.50 ± 422.89	3982.25 ± 32.74	
(mg GAE/ 100g)				
pH	3.65 ± 0.01	3.70 ± 0.00	3.73 ± 0.00	
Vitamin C	60.77 ± 5.81	72.63 ± 4.36	79.71 ± 6.70	
Phosphorus (g/kg)	0.42 ± 0.12	0.35 ± 0.02	0.48 ± 0.09	
Iron (mg/kg)	9.13 ± 0.39	9.94 ± 1.61	4.65 ± 0.85	

Table 1. Results of the study of 15 fruit aronia properties on different types of mulch

In addition in general types of mulching surface (straw, black plastic foil) increased the values above parameters in comparison with soil without mulch. These results suggest that the use of mulch for the cultivation of aronia may result in a higher yield, which can also increase the overall yield. The results of the measured fruit weight are in accordance with the results of Kawecki et al. (2006), and Kulling et al. (2008), and somewhat lower than the results of the study Strik (2003). The number of fruits (208.71) is also the highest in shrubs growing on black foil 208.71, and in the straw (163.38) and control plot (151.73).

The amount of sugar in the fruits of aronia is a very important feature and significantly affects its consumption value, but also on the value of its products, especially juice. Fresh fruits of

aronia are normally bitter and rarely used as fresh fruit. Increasing the amount of sugar in the fruit can affect the increase in its consumable use value, but also on the increase in the quality of the juice. This study showed that using a black plastic film to cover the soil can affect the increase in the content of sugar in the fruit. As can be seen from Table 1, the fruits harvested from the black foil plots had a much higher amount of total sugar (21.32%). This value is also higher than the results of the Ochmian et al. (2012) and Denev et al. (2018) where this value was 10.25% and 10.56-19.70%, respectively. The fruits harvested from plants grown on straw, had the highest content of iron (9.94 mg/kg) compared without mulch (4.65 mg/kg).

The fruits harvested from plants grown on the controlled plot (without malch), had the highest content of dry matter, ash, total phenol, anthocyanins, C vitamins and phosphorus. The total polyphenolic compounds of the ivestigated samples varied between 3703.53-3982.25 mg/100 g FW. These findings are in agreement with the available information from other studies reporting the polyphenol content of aronia berries in the range 1022-7849 mg/100g FW (Kohkonen et al., 2003; Oszmianski et al., 2005; Hudec 2006; Ochmian et al., 2012; Kapci et al., 2013; Denev et al., 2018).

The results of an anthocyanin analysis (577.955-724.357 mg CGE/100g) were in accordance with the results of other studies, where this amount ranged from 278.2-870 mg CGE/100g (Ochmian et al., 2012; Wangensteen et al., 2014; Denev et al., 2018).

By comparing numerical values, it can be concluded that mulching has influenced most of the tested properties of aronia fruits, especially on chemical and morphological properties. Covering the soil with straw or foil has doubled the amount of iron in the fruits, and the amount of sugar is multiply higher than the fruits from the control plot.

The analysis of the main components (PCA) of 15 properties of the aronia fruit, depending on the type of malch, or the covering of the billet, formed two synthetic (PC) variables. The first (PC 1) component contains 72.97% variance, and in the second (PC 2) 27.03% (Table 2).

with 15 variables				
PC (Principal components)	Eigenvalue	Variance (%)		
1	10.9453	72.968		

4.05474

 Table 2: Eigenvalues and variance on first two principal components (PC) estimated from the correlation matrix with 15 variables

Based on the inherent values, it was determined that the following properties are the most important factors in the construction of the PC 1 component, ie for describing the total variability of the data: fruit weight (0.30), number of fruits (0.28), water content (0.27), dry matter (- 0.27), total sugars (0.30), reducing sugars (0.30), sucrose (0.30), titratable acidity (0.30), pH (- 0.29), vitamin C (0.29) (Table 3). Ash content (0.36), antochyanins (0.49), total phenols (0.45), phosphorus (0.42) and Fe (-0.27) were key factors in the formation of PC 2 components (Table 3).

2

27.032

Abbreviations	Traits	PC 1	PC 2		
FW	Fruit weight (g)	0.30226	0.0013418		
NF	Number of fruit per shoot N	0.27675	0.19971		
W	Water (%)	0.27254	0.21474		
DM	Dry matter(%)	-0.2669	-0.23309		
TS	Total sugars (%)	0.30049	0.05368		
RS	Reducing sugar (%)	0.3005	0.05355		
SA	Sucrose (%)	0.3005	0.053572		
AS	Ash (%)	-0.20538	0.36436		
TA	Titratable acidity (%)	0.3021	0.016165		
ANT	Antochyanins	-0.057174	0.48765		
	(mg CGE/100 g)				
TPH	Total polyphenols	-0.12513	0.45206		
	(mg GAE/ 100g)				
pН	pH	-0.29442	-0.11241		
Cvit	Vitamin C	-0.29433	-0.11306		
PHO	Phosphorus (g/kg)	-0.16164	0.41964		
Fe	Iron (mg/kg)	0.25263	-0.27267		

Table 3: Principal component (PC) coefficients of 15 variables

On the basis of the two main (PC) components obtained, a PCA biplot was constructed on which the interrelations of all 15 fruit aronia properties were presented (Figure 1).



Figure 1. Distribution of the manner of covering of aronia beds using the analysis of the main components (PCA) and the interrelationship of the fruit characteristics

Also, the graph shows the clear distribution of all three methods of cultivation of the analyzed aronia depending on the mulch. Aronia grown on the control bed, without covering, was

separated with the highest values of dry matter, ash, anthocyanins, total phenols, pH, vitamin C and phosphorus content, the smallest fruit weight, the number of fruit per shoot, the content of water in the fruit, total sugars, reducing sugars, sucrose, total acidity and Fe content in the fruit.

The positioning of the aronia grown by the plastic foil is conditioned by the high values of the fruit's weight, the number of fruit per shoot, the total water content of the fruit, the total sugar, the reducing sugar, the sucrose, the total acidity, the smallest values of the dry matter content, the pH of the reaction and vitamin C. Mulch straw and its distribution is conditioned by the high value of the content of Fe in the fruit, and the low values of the content of ash, anthocyanins, total phenols and phosphorus in the fruit (Figure 1).

4. CONCLUSION

Mulching (covering) of the surface of the soil can affect the characteristics of the fruits of black aronia. Mulching influenced the increase the weight of the fruit, the number of fruits per outlet, water, total sugars, reducing sugars, sucrose, acidity and iron. In this study, the results showed that mulching with black foil affected the increase in the morphological and chemical values of the fruits, and the fruits of the classical cultivation without mulching had higher values of antioxidant properties. Covering the soil with straw has influenced the double increase in iron content, and the coverage of black plastic foil has contributed to the multiple increase in sugar in the fruit, compared to the classic growing of the aronia without mulch. This research has a practical significance because it can serve farmers as a guideline in the implementation of agrotechnical measures in the cultivation of aronia.

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INVENTARISATION AND CHARACTERIZATION OF FIVE GENOTYPES OF WALNUT (*JUGLANS REGIA L.*) IN THE AREA OF BUGOJNO

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Key words: walnut, Juglans regia L., genotypes, morphological traits

ABSTRACT:

The common walnut (Juglans regia L.) originates from areas stretching from the Caucasus Mountains in Iran to the east, through Turkmenistan and the Himalayan Mountains to Central Asia. Common walnut or domestic walnut is also known by the names: Persian, Greek, Carpathian, Royal (Mrva, 1995). The aim of this study was to select superior walnut genotypes in the area of Bugojno based on agronomically significant traits. Five walnut genotypes marked as G1, G2, G3, G4 and G5 were found, marked and tracked in the field and their coordinates were determined. For morphological analysis of the fruit, a sample of thirty fruits for each genotype was used. Phenotypic studies included monitoring and measurement of agronomically significant traits based on the IPGRI descriptor of Juglans species. A total of seven phenotypic traits were monitored: fruit width (mm), fruit thickness (mm), fruit length (mm), fruit weight (g), core weight (g), shell weight (g) and bulkhead weight (g). According to its morphological characteristics, the G2 genotype was distinguished, which was characterized by the best characteristics and the following values were recorded: fruit width (31.6 mm), fruit thickness (29.13 mm), fruit length (41.26 mm), fruit weight (13.29 g), stone weight (5.35 g), shell weight (7.52 g), as well as bulkhead weight (0.354 g). The lowest values of these parameters were recorded for genotypes G4 and G5. Genotypes with high values of production-significant traits (fruit weight, core weight) confirmed the hypothesis that not enough effort has been made so far to identify those genotypes that can become a commercially significant resource in the area of Bosnia and Herzegovina. Those desirable traits can still be found on individual walnut trees throughout Bosnia and Herzegovina.

1. INTRODUCTION

Walnut (*Juglans regia* L.) which is also known as Persian, Carpathian, Greek and domestic. It belongs to the most important types of fruit trees. Walnut is a fruit of the northern hemisphere with a temperate and subtropical climate. It is believed to be native to Asia Minor. It is grown in eastern Europe up to 52 ° north latitude, and in western Europe up to 56 °. Individual trees can also be found further north (650), but such trees rarely bear fruit. Walnuts are also found in Canada, North and South America, Asia and Australia.

The man began to cultivate walnuts later than he did with other fruit species. Before that time, one can only speak of the results of spontaneous selection, by which the isolated forms of walnut were adapted to different environments. Today, one can speak with certainty about adaptable types of walnuts for certain areas, regions, countries and even continents. There are few standard varieties that can thrive in a wider ecological environment as is the case with many continental fruit trees. Therefore, the adaptability of walnuts to various environmental conditions should be understood only conditionally, because each variety has special needs in terms of growing conditions.

The ecological conditions, which mostly limit the intensive cultivation of noble varieties of walnuts, are certainly climatic factors, and of the climatic factors, the most harmful are low temperatures at the beginning of the vegetation (late spring frosts). It is this sensitivity of walnuts in the spring, when due to low temperatures freeze male inflorescences (tassels) and shoots with leaves and female inflorescences, limiting their lifting only to suitable positions, these suitable positions are usually positions where vines grow and give birth successfully. By knowing the requirements of walnuts for soil properties and climatic conditions, the most favorable positions for safe and economical walnut production can be selected. In recent times, late noble varieties (grafted walnuts) can be found on the domestic market, which can grow and bear fruit regularly outside the vineyards. These varieties "wake up" late, so they most often "miss" late frosts that appear in a certain calendar period.

So far, a large number of studies have been published that indicate the possibility and justification of the use of morphological markers in the evaluation of walnut germplasm and the selection of superior genotypes adapted to research conditions. One such study was published by Sharma and Sharma (2001) in which they examined the genetic diversity of Persian walnut (*Juglans regia* L.) trees propagated by seed in terms of 15 different metric traits of the fruit and core.

In the study of germplasm Juglans regia in the area of Dibra in Albania, Zeneli et al. (2005) evaluated walnut variability and identified promising conservation materials. Significant genetic variations in pomological and phenological traits have been found in indigenous trees. It is hypothesized that the phenotypic variations are due to natural and anthropogenic selection that were strong forces in shaping the studied walnut population in the Dibra region.

Aslantas (2006) identified superior walnut (*Juglans regia* L.) genotypes in northeastern Anatolia, Turkey. The research material consisted of individuals within natural populations of walnut trees. Out of a total of 39,000 trees grown in the region, 20 genotypes were selected for evaluation based on yield and fruit characteristics. The best genotypes were CVWS 13, 46, 138, 150 and 193. These genotypes had a thinner shell (0.58 mm, CVWS 13), higher kernel mass (7.37 g, CVWS 138), and a tendency to lateral fruiting (63 % CVWS 193 and 60% CVWS 46 and CVWS 150).

Many researchers from the territory of Bosnia and Herzegovina have been researching plant genetic resources of fruit crops, which has resulted in the publication of scientific papers using modern molecular methods of plant breeding that are widely used in chestnut (Skender., 2010, 2013), almonds (Hasanbegović et al., 2021) and figs (Hadžiabulić et al., 2005). Literature sources citing research on indigenous populations and cultivated varieties of fruit trees, their wild relatives and free populations of fruit species have recently been a great challenge to a large number of researchers working in this field. Therefore, a group of authors (Hadziabulić et al., 2011, 2014, 2017; Hasanbegović et al., 2017, 2020a, 2020b; Skender et al., 2019; Šupljeglav Jukić A et al., 2020), indicates the existence of a large gene pool of fruit trees in order to preserve fruit. in the territory of Bosnia and Herzegovina, which can serve as a good starting material in breeding programs. In recent years, more and more researchers in Bosnia and Herzegovina have become involved in researching autochthonous and wild varieties and fruit types, which points to an increasing importance of preservation and the possibility of exploiting these plant genetic resources in breeding (Skender et al., 2015, 2017a, 2017b, Becirspahic et al. 2017a, 2017b). The aim of this study was to select superior walnut genotypes in the Bugojno municipality based on agronomically significant traits.

2. MATERIAL AND METHOD OF WORK

Superior genotypes based on agronomically significant traits (fruit size, kernel reproduction, regular fruiting) were isolated. Coordinates are marked for each selection genotype. Phenotypic testing involves monitoring and measuring agronomically significant traits based on the IPGRI descriptor (IPGRI, 1994) for the description of *Juglans* species.

Five walnut genotypes marked G1, G2, G3, G4 and G5 were found, marked and tracked in the field and their GPS coordinates were determined. For morphological analysis of the fruit, a sample of thirty fruits for each genotype was used. Phenotypic examinations included monitoring and measurement of agronomically significant traits and a total of seven phenotypic traits were monitored: fruit width (mm), fruit thickness (mm), fruit length (mm), fruit weight (g), kernel weight (g), shell weight (d) and septum mass (g). For morphological analysis of the fruit, a sample of thirty fruits for each genotype was used.

All measured values were entered into Microsoft Office Excel in which statistical analyzes were performed. Descriptive statistics with basic data were performed: mean value, standard

deviation, one-factor analysis of variance and statistical significance test LSD test for significance levels 0.05 and 0.01.

Description of walnut genotypes

Genotype: G1 Location: 44 ° 01'33.1 "N 17 ° 29'42.2" E Age: about 30 years Height: 6 m Fruit shape: elliptical



Figures 1. and 2: Tree and fruit of genotype G1

Genotype: G2 Location: 44 ° 01'25.3 "N 17 ° 29'35.9" E Age: about 40 years Height: 8 m Fruit shape: oval



Figures 3. i 4: Tree and fruit of genotype G2

Genotype: G3 Location: 44°01'22.5"N 17°29'36.5"E Age: about 75 years Height: 16 m Fruit shape: eliptical



Figures 5. i 6: Tree and fruit of genotype G3
Genotype: G4 Location: 44°01'42.2"N 17°30'28.8"E Age: about 75 years Height: about 20m Fruit shape: oval



Figures 7. i 8: Tree and fruit of genotype G4

Genotype: G5 Location: 44°00'48.6"N 17°30'06.8"E Age: about 60 years Height: about 15m Fruit shape: round



Figures 9. i 10: Tree and fruit of genotype G5

2.1 RESULTS

Genotype	Fruit width (mm)	Fruit thickness (mm)	Fruit length (mm)	Fruit weight (g)	Kernel mass (g)	Shell mass (g)	Septum mass (g)
G1	27,13±0,88	26,53±0,96	38,33±1,58	10,53±1,03	4,55±0,79	5,55±0,28	0,258±0,06
G2	31,60±1,54	29,13±1,89	41,26±2,74	13,29±2,24	5,35±1,21	7,52±1,32	0,354±0,09
G3	26,73±0,93	26,93±1,34	30,33±1,07	10,25±1,06	4,20±0,46	5,65±0,69	0,196±0,03
G4	22,13±0,96	22,26±1,00	32,00±1,83	7,84±0,96	2,92±0,48	4,63±0,46	0,268±0,06
G5	23,66±2,47	24,53±1,31	30,26±1,39	7,13±0,92	3,39±0,65	3,59±0,30	0,164±0,04
LSD 0,05	0,55	0,49	0,67	0,49	0,28	0,27	0,02
LSD 0,01	0,72	0,65	0,88	0,65	0,37	0,35	0,03
F	175,15*	108,66*	224,96*	96,50*	44,85*	117,30*	44,59*
Fcrit	2,43	* Statistical significance, ± standard deviation					

Table 1: Morphological characteristics of the fruit of the examined walnut genotypes

Based on the results of morphological measurements of walnut genotype characteristics in Table 1, it can be concluded that the lowest average fruit width was recorded in genotype G4 and was 22.13 mm, while the highest average fruit width was recorded in genotype G2 31.6 mm. The average fruit thickness ranged from 22.27 mm in genotype G4, to 29.13 mm in genotype G2. The lowest average fruit length of the examined walnut genotypes was 30.27 mm in genotype G5, while the lowest average fruit length value of 41.26 mm was in genotype G2. The average fruit weight of the examined walnut genotypes ranged from 7.13 g in the G5 genotype to 13.29 g in the G2 genotype. The lowest average kernel weight was 5.35 g in genotype G2. The lowest average shell mass was recorded in genotype G5 3.59 g, while the highest average shell mass was recorded in genotype G2.

The results of the one-factor analysis of variance indicate the existence of statistical significance in all examined parameters (fruit weight, kernel weight, shell weight, septum weight, fruit length, fruit width, fruit width and fruit thickness). To determine the existence of the least statistical significance that may exist between the examined genotypes, an LSD test was performed for significance levels of 0.05 and 0.01. The results of statistical significance are presented in letters in the graph. Different letters indicate the existence of statistical significance between the examined genotypes of walnuts.









Graph 1. Overview of morphological characteristics of examined walnut genotypes

Different letters above the average values indicate the statistical significance of the examined walnut genotypes

Aslantas (2006) identified superior walnut (Juglans regia L.) genotypes in northeastern Anatolia, Turkey. The research material consisted of individuals within natural populations of walnut trees. Out of a total of 39,000 trees grown in the region, 20 genotypes were selected for evaluation based on yield and fruit characteristics. The best genotypes were CVWS 13, 46,

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GV

138, 150 and 193. These genotypes had a thinner shell (0.58 mm, CVWS 13), higher kernel mass (7.37 g, CVWS 138), and a tendency to lateral fruiting (63 % CVWS 193 and 60% CVWS 46 and CVWS 150).

In his study Balta et al. (2007) described indigenous, seed-propagated walnut genotypes (Juglans regia L.) in central Bilitis in Turkey. Taking into account the mass of the kernel as an important agronomic trait of the fruit, 17 found genotypes whose kernel mass was above 5 g were described.

McGranahan and Leslie (1990) preferably superior walnut genotypes have a fruit and a kernel weight of 12-18 g and 6-10 g, respectively, and that the walnut kernel makes up about 50% of the total fruit weight. It is also desirable that the kernel has a light color.

However, more than three decades later McGranahan and Leslie (2012) point out that it is desirable that the shell of the fruit be smooth, well closed and not make up more than 50% of the total weight of the fruit. The fruits should belong to the category of "large" or "very large". The kernel should be large and lightly colored, weighing about 8-9 g and easily removable from half the shell. The tree should be at least moderately resistant to diseases and pests. These facts indicate that the breeding of walnuts has exceeded the limits of classical selection, and that more and more efforts are being made for planned hybridization and the creation of superior commercial varieties.

Genotypes with high values of production-significant traits (fruit weight, kernel weight and kernel share in fruit) confirm the hypothesis that not enough effort has been made in Bosnia and Herzegovina to identify those genotypes that can become a commercially significant resource, ie., those desirable traits can still be found on individual walnut trees throughout Bosnia and Herzegovina.

Skender et al. (2020) report the results of a survey in the period 2014-2015 which included 75 walnut genotypes from the area of northwestern Bosnia and Herzegovina, the average thickness of walnut fruit ranged from 23.27-37.15 mm, fruit width ranged from 22.47- 31.18 mm, fruit height was 24.71–43.30 mm, fruit weight 5.86–16.25 g, kernel weight 1.66–5.07 g and kernel percentage 26.96% –48.25 %. The highest weight of walnuts weight was recorded in GT 25 (16.25 g; 5.07 g), and the highest percentage of grains in the total weight of walnuts was 48.25% recorded in GT-51. The highest value for the weight of nuts among our estimated genotypes (13.29 g) was lower than the corresponding data published by Sen and Tekintas (1992); Atefi (1997); Sharma and Sharma (1998); Yarilgac et al., (2001); Sharma and Sharma (2001); Zeneli et al. (2005); Cosmulescu and Botu (2012); Cosmulescu (2013); Khadivi-Khub and Ebrahimi (2015) and Rezaei (2018).

According to a study by McGranahan and Leslie (1990), the weight of nuts and kernels in superior walnut genotypes should be 12–18 g, which is consistent with the results obtained in this study, where the G2 genotype was singled out as the superior genotype.

3. CONCLUSION

According to its morphological characteristics, the G2 genotype was distinguished, which was characterized by the best characteristics and the following values were recorded: fruit width (31.6 mm), fruit thickness (29.13 mm), fruit length (41.26 mm), fruit weight (13.29 g), kernel weight (5,35 g), shell weight (7.52 g), and septum weight (0.354 g). The lowest values of these parameters were recorded for genotypes G4 and G5. Genotypes with high values of production-significant traits (fruit weight, kernel weight) confirm the hypothesis that in the territory of Bosnia and Herzegovina, not enough effort has been made so far to identify those genotypes that can become a commercially significant resource, i.e. those desirable traits they can still be found on individual walnut trees throughout Bosnia and Herzegovina.

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"June 5th - World Environment Day"









QUALITY OF MILK OF PRAMENKA SHEEP BREEDS FROM BIHAC AND TRAVNIK

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Key words: sheep, milk, chemical composition, hygienic correctness

ABSTRACT:

One of the main agricultural activities in Bosnia and Herzegovina, and especially in the area of Una-Sana Canton and Central Bosnia Canton, is the production of milk, both beef and sheep. According to the Agency for Statistics of Bosnia and Herzegovina for 2011, the number of sheep in Bosnia and Herzegovina ranged around 1,021,000 heads and milk production amounted to about 17,610,000 liters. Sheep and goat milk production in the world is estimated at 20.6 million tonnes, of which sheep's milk accounts for 8.3 million tonnes. The structure and function of the mammary gland is crucial for milk production. The development of the mammary gland is influenced by a number of factors. One of the most significant is the action of hormones. In this paper, we analyzed milk from two sheep farms, from two areas, with respect to the chemical composition and hygiene of the milk. Studies have shown that there are no significant differences in the quality of milk on both farms, both in terms of chemical composition and hygiene of milk.

1. INTRODUCTION

One of the main agricultural activities in Bosnia and Herzegovina, and especially in the Central Bosnia Canton, is the production of milk, both beef and sheep. According to the data of the Agency for Statistics of Bosnia and Herzegovina for 2011, the number of sheep in Bosnia and Herzegovina was around 1,021,000 head, and milk production amounted to about 17,610,000 liters. World production of sheep's and goat's milk is estimated at 20.6 million tons, of which 8.3 million tons are sheep's milk. The largest producers of sheep's milk in the world are: Asia, Europe and Africa. Sheep are raised primarily for meat but also for milk processed into cheeses, the most famous of which are Travnik, Pag, Istria, Lika, Roquefort (France), Fetta (Greece), Pekorino (Italy), Manchego, La Serena Spain), Kachkaval

(Romania, Bulgaria), etc. [1,2]. In Bosnia and Herzegovina, the number of sheep has decreased, but there has been no significant increase in production per head. Ecological and, conditionally speaking, economic opportunities Large areas of grassland (55.4% of the total agricultural area), which can be most adequately exploited by sheep, provide opportunities for significant production management of sheep meat and cheese, both for local use and for export [3].

Sheep have different uses and can be raised very extensively, but also very intensively [4]. In extensive conditions, a sheep produces on average about 100 liters (without the amount that the lambs suck) of milk, and in intensive conditions, the East Frisian sheep produces about 350 liters and more [5]. It is very adaptable, ie it has strong acclimatization abilities. It withstands low and high temperatures without harmful consequences, as well as various edaphic and climatic conditions, such as droughts, heat, food and water shortages and the like. The areas of Central Bosnia and Una-Sana Canton are mountainous areas, with numerous and very high quality pastures. The tradition in agricultural production is sheep breeding, for the development of which there are almost optimal conditions. However, the current level of development of sheep breeding is at a very low level. Namely, in the last 15 years, there has been a large reduction in the number of sheep and a deterioration in the breed composition. The structure and function of the mammary gland are crucial for milk production.



Sheme 1. General structure of the udder (A) and structure of the milk alveoli (B)

2. MATERIAL AND METHOD OF WORK

The research was conducted in 2020 and 2021 on two private farms in the municipalities of Travnik and Bihać. The assessment of milk quality was performed in the laboratory of the company Poljorad from Travnik and the Veterinary Institute Bihać.

The sheep were milked twice a day (morning and evening) to measure the amount of milk.

Samples of raw milk from 30 sheep from each dairy farm were taken to test the physicochemical characteristics, and the following parameters were tested:

- milk fat content (%),
- crude protein content (%),
- lactose content (%),
- dry matter content (%),
- fat-free dry matter (%),
- number of bacteria (number / ml),
- somatic cell count (number / ml) and freezing point (C)



Picture 1. Milk samples taken for laboratory analysis

3. RESULTS OF WORK

Maasuurad	Farm			
parameters	A X	B X		
Daily amount of milk (g)	1.434	1,368		
Milk fat (%)	9,41	8,65		
Crude protein (%)	7,48	7,39		
Laktose (%)	2,48	2,44		
Dray matter (%)	22,44	21,35		
Fat-free dray matter (%)	9,61	9,36		
Freezing point (⁰ C)	0,6532	0,6221		
Number of bacteria (No/ml) Somatic cell count (No/ml)	2.991.304 1.980.328	3.034.261 2.054.458		

Table 1: Values of measured chemical parameters of sheep from two farms in Travnik

Table 1. shows that all analyzed milk quality parameters were higher on farm A, however, the differences were not statistically significant. Also, the parameters of milk hygiene were better on farm A, but also, the differences were not statistically significant.

	Farm		
Measured	Α	В	
parameters	Х	Х	
Daily amount of milk (g)	1,535	1,436	
Milk fat (%)	8,25	8,22	
Crude protein (%)	7,62	7,44	
Laktose (%)	2,56	2,51	
Dray matter (%)	21,57	22,88	
Fat-free dray matter (%)	9,92	9,56	
Freezing point (⁰ C)	0,6344	0,6301	
Number of bacteria (No/ml)	2.664.382	2.723.261	
Somatic cell count (No/ml)	1.896.426	1.865.402	

Table 2: Values of measured chemical parameters of sheep from two farms in Bihać

The data from Table 2 show that the milk from farm A was of better quality in terms of chemical composition. Regarding the hygienic correctness in the milk of sheep from farm A, there were fewer bacteria but more somatic cells in 1 ml of tested milk. The obtained values were not statistically significant. In the area of the Una-Sana Canton, the presence of an increased number of somatic cells from the genus *Staphylococcus aureus*, as the most common cause of mastitis, was proven [6].

When comparing the milk of sheep from the area of Travnik and Bihać, it can be seen that sheep from the area of Bihać had a slightly higher daily amount of milk, as well as dry matter and protein, while the amount of lactose was the same in both sheep and milk fat was more in the milk of sheep from the area of Travnik. Only this difference was statistically significant (9.03: 8.23%). Regarding the hygienic correctness of milk, it was better in sheep's milk from the Travnik area, in both examined parameters and the obtained values were statistically significant in the number of bacteria and the number of somatic cells in 1 ml of milk (Table 3.). Other authors also obtained similar results by controlling milk [7, 8, 9, 10].

	Farm		
Measured	Α	В	
parameters	X	X	
Daily amount of milk (g)	1,401	1,485	
Milk fat (%)	9,03	8,23	
Crude protein (%)	7,43	7,53	
Laktose (%)	2,54	2,54	
Dray matter (%)	21,89	22,22	
Fat-free dray matter (%)	9,49	9,74	
Freezing point (⁰ C)	0,6377	0,6323	
Number of bacteria (No/ml)	3.012.782	2.698.321	
Somatic cell count (No/ml)	2.017.393	1.828.914	

Table 3: Mean values of measured chemical parameters of sheep from the area of Travnik and Bihać

4. CONCLUSION

Analyzing the quality of milk in terms of chemical composition and hygiene on two farms in two different localities, it can be concluded that there are no statistically significant differences, ie that sheep's milk in both localities is approximately the same quality, both in terms of chemical composition and hygienic correctness.

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EFFECT OF LIGHT ON GROWTH AND SPORULATION OF ISOLATES STUDIED

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Key words: A.dauci isolates, A.alternata isolates, light, growth, sporulation

ABSTRACT:

Six selected isolates of Alternaria spp. were used in these studies, as follows: A: 1) FM-15; 2) BM-2; 3) CLA-108 monitoring A. dauci isolates; B: 4) Mr-114; 5) IM-14, and 6) Aa-82 monitoring A. alternata isolate. Testing of the effect of light on the growth and sporulation of selected Alternaria spp isolates was performed by sowing on PDA and exposure to the following lighting conditions: constant light, constant darkness, natural light/dark shift, UV lighting. The growth rate was determined after sowing by measuring the diameter of the colony after 3, 5, 7 and 10 days in five replications, while the intensity of sporulation was expressed through the number of conidia per 1 cm² of colony. The largest radial growth of colonies of all three isolates from group A studied was achieved when exposed to constant light, while the isolates from group B studied achieved a slightly higher increase under UV lighting. The effect of nutrient media on the intensity of sporulation in the isolates from group B studied was abundant, while sporulation in isolates from group A was absent except in UV lighting where it was very weak.

1. INTRODUCTION

Fungi belonging to the genus *Alternaria* cause a complex of diseases on carrots and greater yield losses than any other single pathogen. Changes in cultivation methods and introduction of modern intensive carrot production have additionally influenced the more intensive appearance of existing pathogens, especially those with a wide range of hosts, such as species of the genus *Alternaria*. The changes that these fungi can cause on carrot plants are spotting and drying of the leaves, brown rot of the roots, scorching of the seedlings, rot of the root neck and drying of the petioles. The economic damages caused by *Alternaria spp.* on carrots are multiple, because these pathogens can affect the reduction of sprouting and decay of seedlings, and thus the reduction of crop density in the field, the reduction of yield and root quality, and the reduction of seed germination in carrot seed production. The analysis of infected samples was carried out using several methods of detection and identification in

order to reliably determine which species of the genus *Alternaria* appear as pathogens of carrots in the territory of Bosnia and Herzegovina and Serbia, and to perform their characterization based on the study of pathogenic, morphological, rearing and molecular characteristics, all of which together should serve as a basis for development and taking timely and adequate measures for their successful suppression. The influence of different light sources on the growth and sporulation of six selected isolates of *Alternaria* spp. was tested by growing them on potato dextrose agar and using four different lighting methods, namely: 1) constant darkness; 2) constant light; 3) natural light/dark shift, and 4) constant UV illumination, with all isolates analyzing radial increase, average daily increment and sporulation.

2. WORK METHODS AND MATERIAL

The effect of light on the growth and sporulation of the six studied isolates of *Alternaria* spp. was studied during incubation of cultures on potato dextrose agar at 25 °C in different lighting conditions, namely: constant light, constant darkness, natural light/dark shift and constant ultraviolet light (UV). Inoculation of selected isolates was performed by transferring 5 mm diameter mycelial fragments obtained from the rim of seven-day-old colonies grown on potato dextrose agar to the center of a Petri dish using a spear needle. The growth of mycelium under different lighting was monitored daily by measuring the diameter of the colony for a period of 10 days. The effect of different lighting conditions on the growth of colonies of selected isolates of *Alternaria* spp. was calculated as the average daily increase, while the intensity of sporulation was expressed through the number of conidia per 1 cm² of the colony. Preparing preparations and determining sporulation were performed according to the procedure described above. The experiment was conducted with five replicates.

3. RESULTS



Figure 1. *Alternaria* spp. effect of four different types of lighting: a) constant darkness; b) constant lighting; c) natural light/dark change, and d) constant UV illumination, on macroscopic properties and growth of colonies of studied fungal isolates on potato dextrose agar.

Radial colony growth: out of the six isolates of *Alternaria* spp. tested, different light sources had a statistically significant effect on radial colony growth only in isolates from group A (CLA-108, BM-2 and FM-15) identified as *A. dauci*, while in isolates from group B (Aa-82, Mr-114 and IM-14) identified as *A. alternata*, different light sources did not have a statistically significant effect on their growth (Table 1 and Graph 1), i.e. the best colony growth isolates studied achieved when exposed to constant light, but there was a steady and good increase when exposed to other lighting variants. In contrast, the best increase in colonies of the studied isolates from Group A was when exposed to constant UV light, then when exposed to constant darkness, while these isolates had approximately the same increase when exposed to natural light/darkness shift and constant darkness (Table 1). Average daily increment: light did not have a statistically significant effect on the average daily growth of colonies in two isolates (Mr-114 and IM-14), while in other isolates of *Alternaria* spp. statistically significant effect on the observed property was found (Table 1 and Graph 1).

	Isolates						
Light conditions		Group A		Group B			
	CLA-108	BM-2	FM-15	Aa-82	IM-14	Mr-114	
Colony diameter (mm)							
Light/darkness	$42,83 \pm 5,49^{b}$ *	$59,67 \pm 1,86^{b}$	$68,00 \pm 7,75^{\mathrm{b}}$	$85,00 \pm 0,00^{a}$	$85,00 \pm 0,00^{a}$	$85,00 \pm 0,00^{a}$	
Constant light	$42,83 \pm 5,49^{b}$ *	$59,67 \pm 1,86^{b}$	$68,00 \pm 7,75^{\mathrm{b}}$	$85,00 \pm 0,00^{a}$	$85,00 \pm 0,00^{a}$	$85,00 \pm 0,00^{a}$	
Constant darkness	$46{,}33\pm5{,}65^{\mathrm{b}}$	$61,83 \pm 6,08^{b}$	$72,\!67\pm9,\!52^{\mathrm{b}}$	$85,00 \pm 0,00^{a}$	$85,00 \pm 0,00^{a}$	$85,00 \pm 0,00^{a}$	
Constant UV	$58{,}00\pm4{,}34^{\mathrm{a}}$	$74,67 \pm 3,67^{a}$	$85,00 \pm 0,00^{a}$	$\textbf{85,00} \pm \textbf{0,00}^{a}$	$85{,}00\pm0{,}00^{\mathrm{a}}$	$85,\!00\pm0,\!00^{\mathrm{a}}$	
Average daily increment (mm)**							
Light/darkness	$4,\!38\pm0,\!98^{\text{b}}$	$6,40 \pm 2,54^{b}$	$6{,}99 \pm 1{,}57^{\text{b}}$	$9{,}07\pm3{,}10^{\mathrm{b}}$	$9,01 \pm 2,99$ ^a	$9,14 \pm 4,24^{a}$	
Constant light	$4,\!48\pm0,\!98^{\text{b}}$	$6{,}39\pm2{,}54^{\text{b}}$	$7{,}19\pm1{,}57^{\text{b}}$	$9{,}07\pm3{,}10^{\mathrm{b}}$	9,01 ± 2,99 ^a	$9,14 \pm 4,24^{a}$	
Constant darkness	$4,71 \pm 1,64^{b}$	$6,36 \pm 2,49^{b}$	$7,\!45\pm2,\!02^{\mathrm{b}}$	$9,11 \pm 4,84^{b}$	$9,18 \pm 4,50^{a}$	$9,16 \pm 4,22^{a}$	
Constant UV	$5{,}90\pm1{,}13^{\mathrm{a}}$	$7,84 \pm 2,17a$	$9,05 \pm 3,19^{a}$	$12,41 \pm 1,81^{a}$	$11,\!27 \pm 4,\!15^{a}$	$9,10 \pm 4,81^{a}$	
Sporulation***							
Light/darkness	—	_	+	+	++++	+++	
Constant light	_	_	_	+	++++	+++	
Constant darkness	_	_	_	++	++	++	
Constant UV	+	+	+	++++	++++	++++	

 Table 1. Effect of different light sources on colony growth, average daily increment and sporulation of studied isolates of *Alternaria* spp.

* Values marked with the same letter do not differ statistically (Duncan test; $p \le 0.05$);

* Average daily increment calculated by formula: (D2 - D1) / (T2 - T1), where D2 and D1 is the diameter of the culture of the last and first measurement, and T2 and T1 time (in days) of the last and first measurement;

*** Sporulation: (-) no sporulation, (+) very weak, (++) weak, (+++) medium, (++++) abundant, and (+++++) very abundant sporulation.

Sporulation: the effect of light on the intensity of sporulation of the studied isolates from group A (CLA-108, BM-2, FM-15) and group B (Mr-114, IM-14, Aa-82) varied. Thus, for example, the sporulation of all three isolates from group B was very abundant when exposed to constant UV light, while in isolates from group A it was very weak. In all other three light sources, the sporulation of individual isolates was very variable (Table 1).



Graph 1 Effect of different light on radial growth of colonies (above) and average daily increment (below) in three selected isolates from group A (*A. dauci*) (left column) and group B (*A. alternata*) (right column).

It is seen from the data listed in Table 1 and Graph 1 that the tested isolates from group B had a uniform radial increase under different lighting conditions, while the isolates from group A had the largest diameter of the colony under constant UV lighting, then when exposed to constant darkness and constant light, and the smallest in light/dark shift.

4. DISCUSSION

Different light sources had a statistically significant effect on the radial growth of colonies only in isolates from group A (CLA-108, BM-2 and FM-15), while in isolates from group B (Aa-82, Mr-114 and IM-14) different light sources did not have a statistically significant effect on their growth. Thus, the isolates from group B studied achieved the best growth of colonies when exposed to constant light, but there was a uniform and good growth when exposed to other variants of lighting. In contrast, the best increase in colonies from the Group A isolates studied was when exposed to constant UV light, then when exposed to constant darkness, while these isolates had approximately the same increase when exposed to natural light/dark shift and constant darkness. The obtained results differ from the results [1] which found that the tested isolates of *Alternaria* spp. showed the fastest increase in light/dark shift,

slower growth when grown in constant light conditions, and the slowest growth when grown in constant darkness.

The effect of light on the intensity of sporulation of the studied isolates from group A (CLA-108, BM-2, FM-15) and group B (Mr-114, IM-14, Aa-82) varied, with the sporulation of all three isolates from the group B when exposed to constant UV light was very abundant, and in isolates from group A was very weak, while in all three other light sources the sporulation of individual isolates from both groups was variable. The obtained results differ from the literature on the effect of light on the sporulation of *Alternaria* spp., especially *A. dauci*, for the sporulation of which light/dark shift is necessary [2]; [3]; [1].

5. CONCLUSION

Isolates from group B showed a uniform growth of colonies under all different lighting conditions, while isolates from group A achieved the largest diameter of the colony under conditions of constant exposure to UV light.

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Seventh International Scientific Conference

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RESISTANCE BREEDING THROUGH GENETIC RESERVOIRS OF *AEGILOPS* SPELTOIDES

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Key words: Wheat, Triticum spp. Aegilops speltoides, disease resistance

ABSTRACT:

Tetraploid durum wheat (2n = 28, AABB) and hexaploid common wheat (2n = 42, AABBDD)are major crops used as food and forage. Diseases cause significant yield losses in wheat. Developing disease-resistant varieties by using resistance genes is a more economic and environmentally friendly solution than applying fungicides against disease-causing agents. Wild relatives of wheat are important genetic reservoirs for use in wheat (Triticum spp.) breeding strategies. Aegilops speltoides Tausch is one of the most exploited ancestral relatives of wheat. However, the recipient chromosome region cannot always be fully substituted by alien chromatin, or the alien loci of the donor may carry genetic material with adverse effects besides the introgressed target. Complicated immunity mechanisms of plants have provided a better comprehension following the recent advances in molecular biology. Recently, mapping technologies have been improved due to the invention of next-generation sequencing (NGS). Moreover, advances in molecular marker technology have elucidated key regulators of resistance in big-sized and complex genomes. Various QTLs/genes responsible for resistance to stem rust, leaf rust, and powdery mildew diseases have previously been identified in Aegilops speltoides. Improvements in genomic technologies, and gene editing technologies have much to offer in terms of targeting resistance loci more precisely, which will undoubtedly facilitate the introgression of alien genetic material. This review comprises the resistance breeding through wheat–Aegilops speltoides hybridization and gene transfer and gives perspectives about future implications of state of art technologies.

1. INTRODUCTION

Common wheat, which is an allopolyploid (6x = AABBDD = 42), is consisted of three genomes; A genome derived from T. urartu, B genome from Aegilops speltoides (Ae. speltoides) or a closely related species, D genome from Aegilops tauschii [1]. Wheat breeders have focused on obtaining high-yielding crops for a long time, resulting in the loss of valuable and rare alleles from the gene pool that provides high-quality grain (such as milling and baking quality), as well as resistance to abiotic/biotic factors. The significant yield decreases in wheat are caused by fungal agents. The world population is expected to reach more than 9 billion by 2050 [2]. Until then, boosting wheat yield by at least 50% is critical to meet the increasing needs of the growing world population [3]. Strengthening the fight against biotic stress factors is essential to achieving this goal. Complex agronomical traits including disease resistance can be improved using the rich presence of alien genes of the Aegilops genus. Aegilops species can naturally cross with wheat since the two are closely related [4]. Aegilops speltoides Tausch (Ae. speltoides) comes from the area of Fertile Crescent [5]. Introgressions of genes from Ae. speltoides (2n=14, SS) into the genetic background of wheat are an important source of genetic diversity. The B genome of wheat is closely related to the Sgenome of Ae. speltoides. Therefore, homoeologous pairing can occur between Ae. speltoides and wheat [6]. To assess the lines, cytogenetic (meiosis analysis, C-banding), genetic, and phytopathological techniques should be used following introgression [7].

The development of tightly linked markers to resistance genes has vital importance to confirm introgression during resistance breeding. Marker-assisted selection (MAS) provides the selection of individuals based on markers (DNA-based markers) linked to agronomically important traits. Selection with no molecular markers is challenging when multiple genes segregate. Selection using molecular markers is based on developing markers specific to the allele typical of *Ae. speltoides*. Advances in sequencing technologies have accelerated the discovery of molecular markers, increased accessibility of diverse and low-cost genotyping platforms, and obtained better QTL maps. Thus, they have allowed a more comprehensive understanding of functional genomics underpinning relationships between homoeologs [8]. Furthermore, possessing a large and complicated genome hindered the precise detection of introgression in wheat until recent advancements in sequencing. In addition, the availability of annotated reference sequence of wheat (The International Wheat Genome Sequencing Consortium (IWGSC), 2018) has helped detect target introgressions into wheat in terms of size and context. Moreover, developing SNP markers across the genome has smoothed the path of validating introgression [9].

On the other hand, there are issues about homoeologous recombination using alien genetic materials. Homoeologous recombination might be challenging due to colinearity between *Aegilops* genus and wheat and low recombination rates at centromeric or other chromosomal loci. The meiotic pairing between homoeologous chromosomes of wheat is hampered by the *Ph1* locus found on chromosome 5 of the B genome [10]. To induce meiotic pairing, wheat

deletion mutant, designated as ph1b, that has a deletion at Ph1 locus can be generated via radiation [11], or *Ae. speltoides* x wheat hybrid lines can be produced as nullisomic (a chromosomal type missing) for the 5B chromosome of wheat [12]. To increase the rate of meiotic pairing between homoeologous chromosomes, the generation of *Ph1* mutants is the most preferred method [10].

Another method for inducing homoeologous recombination is based on the genes that existed in *Ae. speltoides* [10]. Suppression of *Ph1* locus in wheat is an attractive characteristic of hybridization with *Ae. speltoides* since it has genes on 3S and 7S chromosomes for suppressing *Ph1*, unsimilar many other wild relatives [13]. Polymorphic suppressor loci of *Ph1* locus, *Su1-Ph1*, and *Su2-Ph1*, were mapped in *Ae. speltoides*. A molecular marker named 'Xpsr1205-3S' tightly linked to *Su1-Ph1* makes it easy to target *Su1-Ph1* for introgression into wheat. In a recent study, researchers introgressed *Su1-Ph1* into wheat to induce recombination between homoeologous chromosomes in wheat [14].

Linkage drag is also an issue during hybridization with alien species since long segments of alien chromosomes are transferred with undesirable genes. It hinders the usage of developed resistant recombinants due to undesired traits [10]. The addition of alien chromosomes to wheat genetic background is made via selfish Gc genes. Developing addition lines is troublesome due to the 2S and 6S chromosomes of Ae. speltoides that have gametocidal genes (Gc factors, Gc1a, and Gc1b) [1], [15]. Because Gc factors which are segregation distorters kill cells lacking them by breaking chromosomes in the monosomic addition states [16]. Transmission to the next generation is only possible when gametes stay alive with the Gc genes [17]. These genes might be advantageous when Gc genes are linked with genes controlling useful traits. Because they eliminate gametes carrying an undesirable allele which may be useful for selection [15].

Although there are issues using *Ae. speltoides* as mentioned above, there has been an acceleration in uptaking novel alleles, due to the availability of high-density marker arrays. High throughput screening of *Aegilops species* was first applied using the SNP genotyping platform (The Axiom Wheat-Relative Genotyping Array) designed for wheat relative species [18]. In another study, King et al. (2018) [1] hybridized *T. aestivum* 'Paragon' (male parent) and *Ae. speltoides* followed by backcrossing to 'Paragon' to generate introgression lines. Introgressions were distinguished using Affymetrix Axiom Array (35K SNP array in which 22258 SNPs were polymorphic between *Ae. speltoides* and wheat) and genomic *in situ* hybridization (GISH). High-throughput and high-resolution genotyping was performed; however, the researchers could not confirm which chromosome of wheat was recombined with *Ae. speltoides*. They reported that they were at the stage of developing chromosome-specific KASP markers from the SNP markers on the array. The researchers also added that they were working on developing lines lacking 2S *Gc* genes for mirroring research done by Friebe et al. (2003) [19] who reported successful deletion of preferential transmission genes in *Ae. sharonensis*.

Work to date has been focused on breeding through the use of *Ae. speltoides* as a resistance reservoir against leaf rust, stem rust, tan spot, *Septoria nodorum* blotch, spot blotch, powdery mildew, and stripe rust diseases. To our best knowledge, there is no recent review covering disease resistance breeding for all these diseases using *Ae. speltoides*, which we discussed further in the following section of this review.

2. DISEASE RESISTANCE INTROGRESSION INTO WHEAT

2.1. Leaf rust resistance

This disease is one of the most important foliar diseases, caused by *Puccinia triticina* Erikss. (Pt). Although over 80 Lr genes have been identified for leaf rust resistance, only a limited number of alien-originated genes were transferred into recipient wheat [20]. Up to now, Lr28 [21], Lr35 [22], Lr36 [23], Lr37 [24], Lr47 [25], Lr51 [26] and Lr66 [17] resistance genes were identified or transferred into wheat using Ae. speltoides. To enhance leaf rust resistance of spring and winter wheat varieties, Leonova et al. (2017) [27] used STS primers that cosegregate with the LrAsp5 resistance gene for screening plants in terms of genetic material transfer from Ae. speltoides. In a recent study, Lr28, LrSp, and Lr47 genes introgressed from Ae. speltoides were mentioned as promising for use to achieve durable resistance since they are efficient against leaf rust in Western Siberia and the Southern Urals [28]. The researchers also advised the use of Lr47 and Lr35 as a gene combination in Russia. In terms of resistance breeding, creating coordination between neighboring countries is vital to combat P. triticina due to the migration possibility of the pathogen. In another study [29], the Lr47 gene introgressed from the 7S chromosome of Ae. speltoides has been reported as highly efficient to most Pt races. But this gene has not been cloned yet and the regulatory network of this gene was still a question mark. In this study [29], RNA samples of lines carrying this gene were analyzed three days after inoculation using a Pt race, THTT. Differentially expressed genes (DEGs) encoding receptor-like kinases (RLKs) were identified, and transcriptional changes associated with the Lr47-mediated resistance were profiled via transcriptomic strategies. Identified transcription factor genes are important resources for breeding and the transcriptome data will hopefully make it easier the cloning process of this gene.

2.2. Stem rust resistance

The stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is one of the most devastating biotic stress factors. Although it was under control in the last 50 years thanks to efforts in crop resistance, the appearance of a highly virulent pathotype designated as Ug99 (formally named TTKS, then TTKSK) and its global migration have been a globally major threat. This race is virulent to the most widely-deployed resistance genes and 90% of the cultivated wheat issusceptible in the world [30]. Fortunately, screening *Ae. speltoides* showed the presence of a resistance gene against *Puccinia graminis* f. sp. *tritici* (*Pgt*) including Ug99. Although several genes including *Sr32* [31], *Sr39* [22], and *Sr47* [32] were transferred to wheat by developing

wheat-alien translocations, there are only a few successfully introgressed commercial cultivars due to the negative effects linked to these genes or lack in wheat background [33]. In 2009, Mago et al reported that *Sr39* holds resistance to all known pathotypes of U99 [34]. The researchers developed and validated PCR markers associated with the *Sr39* gene (Sr39#22r and Sr39#50s) for discriminating resistant and susceptible genotypes. Thus, researchers obtained shortened *Ae. speltoides*-wheat translocation that carries linked *Sr39* and *Lr35* genes. In another study, Mago et al. (2013) [35] reported developing PCR markers associated with the *Sr32* gene and *Ae. speltoides*-wheat recombinants. Furthermore, they identified a novel resistance gene on the 2S#1 chromosome, designated as '*SrAes1t*'. Recombinants that have resistance genes were found efficiently resistant against U99.

Resistance against multiple races of Pgt can be provided by transferring resistance genes through chromosome engineering. However, until recently, chromosome engineering studies were limited in number due to the low efficiency of conventional cytogenetic methods. In a study [36] combining DNA marker technology with chromosome engineering strategy, ph1bmutation was employed to reduce alien chromatin, and using molecular markers specific for alien translocations, 4 recombinants carrying Sr39 were verified out of 40 recombinants that have the most reduced alien chromatin.

On the other hand, the Sr47 gene is a strong candidate for resistance since its resistance is high-level and broad-spectrum against Pgt including Ug99. Klindworth et al. (2012) [32] mapped five SSR markers associated with Sr47 to 2BL chromosome. In another study [37], a distinguishing STS molecular marker associated with Sr47 was developed and validated for further use. However, markers developed in these two studies had their own limitations. In a recent study, Klindworth et al. (2017) [38] physically mapped 28 molecular markers associated with the Sr47 gene to facilitate gene pyramiding into a single linkage block with other resistance genes on chromosome 2B.

2.3. Tan spot and Septoria nodorum blotch (SNB) resistances

Tan spot disease is caused by the necrotrophic fungal agent designated as *Pyrenophora triticirepentis* which produces necrotrophic effectors. Resistance is often maintained through deficiency of sensitivity to necrotrophic effectors (NE) encoded by the virulence genes of the pathogen, showing a recessive inheritance pattern. By using high-throughput wheat 90 K SNP assay, Zhang et al., (2019) [39] identified a novel tan spot resistance gene designated as *TsrAes1* on the short arm of the 2S chromosome that exhibits dominant inheritance and NEindependent resistance mechanism. The 2S chromosome of *Ae. speltoides* was engineered through the chromosome engineering method to induce meiotic homoeologous recombination. The researchers introduced the gene to wheat following physical mapping. To produce recombinants with minimum alien chromatin, SNP-derived PCR markers associated with *TsrAes1* were developed for marker-assisted selection (MAS). Septoria nodorum blotch disease is caused by the necrotrophic fungal agent designated as *Parastagonospora nodorum*. NE-independent SNB resistance gene called *SnbAes1* originated from *Ae. speltoides* was identified by Zhang et al., 2019 [39] using a 90 K SNP assay. SNP-derived PCR marker associated with *SnbAes1* was used for MAS for introgression of this gene into wheat.

2.4. Spot blotch (foliar blight/leaf blight) resistance

Spot blotch disease is caused by *Cochliobolus sativus* (anamorph: *Bipolaris sorokiniana*). Climate change has a dramatic influence on weather causing high temperatures and atypical raining patterns, which affected the emergence of this pathogen. Kaur et al (2021) [40] used 89 backcross introgression lines (DSBILs) developed from crosses of *Ae. speltoides* and *T. durum* cultivar to develop a marker for SB resistance. QTLs associated with SB resistance were mapped using SNPs obtained by genotyping by sequencing (GBS) which is a third-generation marker development technology.

2.5. Powdery mildew and stripe rust resistances

Powdery mildew is a serious disease in many areas of the world, caused by Blumeria graminis f. sp. tritici (Bgt). Miller et al. (1988) [41] transferred the Pm12 resistance gene by crossing Ae. speltoides with wheat. In another study, the transfer of the Pm32 resistance gene was achieved by Lapochkina et al. (1996) [42]. Later on, this gene was described as novel by Hsam et al., 2003 [43]. Moreover, molecular markers (such as SSR, SNP, and KASP) have been used for identifying resistance genes that originated from Ae. speltoides. In a recent study [44], Pm53 was mapped onto chromosome 5BL and introgressed into soft red winter wheat with the help of closely linked SSR and SNP markers. KASP markers are known as cost-effective and flexible in the adjustment. Recently, KASP markers were developed from distinctive SNPs defined among the exon sequences of Pm21, PmV, and Pm12 resistance genes (only Pm12 originated from Ae. speltoides, two others from Dasypyrum villosum) and their homoeologous sequences for gene pyramiding efficiently [45]. In a recent study, Dhillon et al. (2020) [46] worked on QTL mapping for powdery mildew and stripe rust (caused by Puccinia striiformis f. sp. tritici) resistance originated from Ae. speltoides. The researchers developed Triticum durum-Ae. speltoides backcross introgression lines and performed QTL mapping based on SNPs identified using GBS. Eleven novel QTLs were identified as associated with resistance, which can be further used as a source of resistance in breeding programs.

3. FUTURE PERSPECTIVES

Reverse genetics approaches, developing molecular marker technologies, gene editing technologies and radiation technologies have much to offer for mining novel alien resistance alleles. Reverse genetics is modifying the target gene's activity for understanding gene function by examining phenotypic consequences. Targeting Induced Local Lesions in

Genomes (TILLING) is a reverse genetic strategy for supporting functional genomics by generation and screening of chemical-based mutations in populations. TILLING can be used for Ae. speltoides to expand genetic diversity, which was used in the discovery of useful genes in Ae. tauschii [47]. Durable resistance can be maintained by the deployment of multiple resistance genes (gene stacking or gene pyramiding) into cultivated wheat crops. Advances in sequencing technology and newly emerged genotyping platforms that are fast, flexible, and cost-effective have facilitated precise pyramiding of target locus. In addition, using genome editing technologies that maintain targeting loci precisely (such as CRISPR/Cas9) has a great potential in terms of gene pyramiding with less or no linkage drag. CRISPR/Cas9 technology offers simultaneously targeting multiple genes which can be a subject of gene pyramiding of resistance genes originating from Ae. speltoides. Until recently irradiation for producing translocation was only used by Sears (1956) [48] who transferred the Lr9 resistance gene via irradiation from Ae. umbellulata. On the other hand, Singh et al. (2016) [49] and Verma et al. (2016) [50] used irradiation to produce translocation by radiation of hybrids of Ae. kotschyi. To the best of our knowledge, there is no study for gene transfer from Ae. speltoides to wheat through irradiation, which can be the subject of research. Overall, even though there are crossability and incompatibility issues while using Ae. speltoides as a source, Ae. speltoides can significantly contribute to wheat breeding with the emergence of new tools and the identification of novel genes with broad-spectrum resistance for breeding purposes.

4. CONCLUSION

Wheat has been through a genetic bottleneck due to continuous selection pressure and cultivation of domesticated crops, which induced narrowing in the gene pool. Ae. speltoides is in the secondary gene pool of wheat, namely, its one genome is homoeologous to wheat. To combat with fast pathogen evolution and the effects of global warming, interspecific hybridization is an untapped source to transfer useful traits into wheat. Until recently, there was not a quick and accurate way to identification and characterization of interspecific transfer. It is also important to manage gene transfer with no or few deleterious genes. The emergence of last technologies in genomics in addition to specific hybridization techniques has resulted in the systematic exploitation of alien genetic materials' variation. As an alien genetic resource, Ae. speltoides is known as the most exploitable species among the genus Aegilops. S genome of Ae. speltoides is genetically close B genome of wheat, and it carries diverse genes of resistance to several diseases. Moreover, most lines of Ae. speltoides carry a gene promoting the induction of homoeologous recombination [10]. Up to date, there are only a few reviews [4], [10], [13] about resistance breeding in wheat through the usage of Ae. speltoides. In this review, some cases of resistance gene transfers from Ae. speltoides to wheat have been described and future potential implications have been mentioned. We believe that considering this literature gap, covering the general approaches and latest advancements in this topic will be a useful source for the readers.

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OCCURRENCE *PSEUDOPERONOSPORA CUBENSIS* (BERK. & M.A. CURTIS) ROSTOVZEV (CUCUMBER DOWNY MILDEW) IN THE MUNICIPALITY OF CAZIN ON GHERKIN CUCUMBER HYBRIDS

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Key words: cucumber gherkin, Pseudoperonospora cubensis, hybrids

ABSTRACT:

Berkeley and Curtis first reported cucumber downy mildew in 1968 from Cuba. The gherkin cucumber downy mildew, caused by pseudo fungus Pseudoperonospora cubensis (Berk & M. A. Curtis) Rostovzev is one of the economically most significant diseases. Symptoms on cucumber are angular lesions that are limited by the leaf veins. During periods of wet foliage from dew, irrigation or rainfall, initial lesions may be conspicuously waterlogged. In our area, the disease appeared in the early eighties of the last century and has been occurring regularly every year since then. During 2021, the occurrence of P. cubensis in the area of the municipality of Cazin was monitored at 4 locations with 4 hybrids–Regal F1, Passamonte F, Harmonie F1 and Kybria F1. The disease was determined by the microscopic method. A scale from 0–5 was used for determination of infection intensity on leaves of the examined hybrids. The intensity of infection (Index of illness) of the pathogen was analyzed using Townsend Heuberger method. High index of illness were determined for hybrid Passamonte F1 (I=55), medium height Index of illness was for a hybrid Regal F1 (I=32). Good resistance was registered on the hybrids Harmonie F1 (I=10) and Kybria F1 (I=5). Of the protection measures of gherkin cucumber, in addition to the application of fungicides, good results are achieved by growing resistant hybrids. The results of this work show that it is best to grow hybrids for this area Kybria F1 and Harmonie F1, because they are the most resistant to P. cubensis.

1. INTRODUCTION

Cucumber (*Cucumis sativus* L.) belongs to the genus *Cucumis* in the family *Cucurbitaceae* and is an economically important vegetable. There are three wild or semi – wild cucumber varieties: *C. sativus* L. var. hardwickii, *C. sativus* L. var. sikkimensis, *C. sativus* L. var.

xishuangbannanesis. Cucumber is indigenous to India and probably originates from the foothills of the Himalayas [1, 2].

At present, cucumber is the fourth most widely cultivated vegetable after tomato, cabbage and onion [3]. Cucumber has versatile uses in culinary, therapeutic and cosmetic purposes [4, 5]. Nutritional and epidemiological researches have shown various benefits of cucumber. For example, cucumber contains abundant nutrients and has crunchy texture and unique flavor, so it is a quintessential vegetable used for a variety of dishes, and it is also indispensable for salad, soup and smoothie. Cucumber is rich in superior hydration and phytochemicals, which have diverse health benefits including weight loss, anti – inflammation, remedy for multiple diseases of eczema, constipation, hypertension, atherosclerosis, cancer, etc. [6]. Recent studies found that the presence of kaempferol in cucumber is an important antidiabetic agent [7]. Furthermore, cucumber is popularly used for natural beautification and for skin treatments [8].

Cucumber gherkin is the main vegetable crop in Una – Sana Canton. The largest areas are in the municipality of Cazin. During growth process, gherkin cucumber hybrids might be affected by multiple diseases and pestsresulting in decrease of yield and quality. Downy mildew caused by *Pseudoperonospora cubensis*, is the major disease followed by powdery mildew (*Sphaerotheca fuliginea*) during spring and rainy season [9].

Some pathogenic fungi including Alternaria tenuis, Fusarium equisett, Phytophthora capsici, Botrytis cinerea and Cladosporium tenuissimum cause rotting and high post – harvest losses of cucumber [10]. The major insect pests in cucumber including Diabrotica undecimpunctata, Acalymma vitatum, Bactrocera cucurbitae, Raphidopalpa foveicollis, Epilachna implicate, Myzus persicae, Aphis gossypii, Anasa tristis, Trialeurodes vaparariorus, Bemisia tabaci and B. Argentifolii [11, 12].

Many diseases caused by viral, bacterial, fungal and nematode pathogens severely affect the cultivation and production of cucumber. Viruses infecting cucumber belong to three genera: *Potyvirus, Cucumovirus* and *Crinivirus* [13]. *Pseudoperonospora cubensis* was first described by Berkeley in Cuba in 1868 [14]. *Pseudoperonospora cubensis* can be found worldwide, causing significant yield losses in the USA, Europe, and Asia [15]. It has been found in over 70 countries, across diverse environments (semi – arid to tropical) [16]. It can infect over 50 different species in 20 genera [17]. *P. cubensis* and its cucurbit hosts cannot survive freezing temperatures; consequently it overwinters in warmer climates.

Current taxonomic classification indicates that *P. cubensis* belongs to *Stramenopiles* (kingdom), *Oomycota* (phylum), *Oomycetes* (Class), *Peronosporales* (order), *Peronosporaceae* (family), and *Pseudoperonospora* (genus) [18].

When the cucumber plant is infected by P. *cubensis*, the upper side of the leaf surface exhibits yellowish – brown lesions with irregular form, and gray sporangium layers appear on the

lower side of the leaf surface under high humidity. Multiple lesions merge after serious infection, which causes the leaves to turn yellow and wither [19]. The reduction in cucumber yield caused by cucumber downy mildew is detrimental if no control countermeasures are applied in the early infection stage of *P. cubensis*. The damage of cucumber downy mildew to cucumber yield and quality increases over time, and cucumber downy mildew has become an important factor limiting the yield and quality of cucumbers [20].

In the municipality of Cazin *Pseudoperonospora cubensis* occurs every year and causes great economic damage to gherkins. The first research on the resistance of gherkins hybrids to diseases and their yields in the area of Cazin and Una – Sana Canton was done by Delalić [21].

The aim of this work is during 2021 to monitor the occurrence of *P.cubensis* in the municipality of Cazin in 4 locations with 4 hybrids – Regal F1, Passamonte F1, Harmonie F1 and Kybria F1. The research will be used by agricultural producers in the municipality of Cazin and beyond to select the most resistant gherkin in cultivation.

2. MATERIAL AND METHODS OF WORK

During 2021, the occurrence of *P.cubensis* in the area of the municipality of Cazin was monitored at 4 locations with 4 hybrids – Regal F1, Passamonte F1, Harmonie F1 and Kybria F1. Diagnosing the cause of the disease was determined based on typical symptoms on plants and microscopic method. During the vegetation, samples of diseased cucumber leaves were collected and the fungus was determined by microscopic method in the microbiological laboratory. The semi – permanent microscopic preparations of fungus were made [22], and microscopic observations and painted conidia and conidiofora were observed. The assessment of the infection was carried out of the scale 0 - 5. Intensity of infection is calculated according to Townsend – Heuberger. For each hybrid, 200 sheets were selected by random selection. The infected leaves are ranked according to the scale: 0 = no symptoms; 1 = 1 - 10%; 2 = 10 - 25%; 3 = 25 - 50%; 4 = 50 - 75%; and 5 = 75 - 100% of infected leaves. The index of the disease was calculated according to the Townsend Heuberger formula:



Where is I% = index of the disease; n = degree of infection by scale; v = number of sheets per category; N = highest degree of infection; V = total number of sheets examined. Based on the disease index, the resistance of the hybrids was determined in the following way: 0-very resistant hybrid (all plants are completely healthy, index of disease 0); I – resistant (index of disease 10); II = medium – resistant (disease index 11 – 25); III = medium sensitive (index of disease 26 – 50) and IV = very sensitive (index of disease above 50).

3. RESULTS AND DISCUSSION

3.1.Symptoms and properties of fungi

The fungus was detected in 2021. on 60% of the examined plants. During the vegetation, symptoms of the fungus were observed on cucumber plants. On cucumber, initial symptoms appear as water – soaked lesions on the lower leaf surface during periods of leaf wetness, and irregularly shaped chlorotic lesions that are vein bound appear on the upper leaf surface (Picture 1). As the disease progresses, the lesions may coalesce with large area of the leaf tissue turning necrotic and eventual death of the leaves (Picture 2). One of the key diagnostic features of downy mildew infection is the presence of purple – gray colored fuzzy growth with sporangia and sporangiophores observed on the underside of the leaves after early morning dews or rainfall. An additional diagnostic feature is the lemon shaped sporangia and dichotomously branched sporangiophores observed under the microscope. The fruits are not affected by infection, but they are stunted and of poorer quality, and therefore lose their market value.



Picture 1. Symptoms of downy mildew caused by *P. cubensis* on the upper surface of cucumber leaf. Initial chlorotic irregularly shaped vein – bound (Photo by: Delalić Z.)



Picture 2. Cucumber leaf showing large area of necrotic lesion at the later stage of downy mildew infection (Photo by: Delalić Z.)

Symptoms vary depending on the host plant, but *P. cubensis* has some consistent identifying characteristics. It is a foliar pathogen that causes chlorotic lesions on the adaxial leaf surface. Primary lesions are between 3 - 10 mm, as the disease progresses the lesions combine to form larger lesions that can eventually cover the entire leaf [23]. The disease is characterized by a "downy" or "felt" appearance which is due to the sporangia found on the abaxial side of the leaf. The lesions can be angular and restricted by the veins of the leaf, particularly on *Cucumis sativus* (cucumber) [24].

3.2. Indexes of illness and resistance hybrides

The indexes of illness ranged from 0 - 51%. In 2021 as the most sensitive was hybrid Passamonte F1 (I=51). In the group of medium resistant is hybrid Regal F1 (I=32), very resistant was Harmonie F1 (I=10). The highest resistance *to P.cubensis* was registered on the Kibria hybrid F1 (I=5) (Table 1.) Hybrids less sensitive to *P.cubensis* should be selected for cucumber cultivation [25].

Table 1. Index of finitess and resistance hybrids in 2021. Of the multicipanty Cazin				
Hybrid	Index of illness (%)	Resistance /sensitivity of hybrids		
	2021			
Passamonte F1	51	very sensitive		
Regal F1	32	medium resistant		
Harmonie F1	10	very resistant		
Kybria F1	5	very resistant		

Table 1. Index of illness and resistance hybrids in 2021. of the municipality Cazin

The climatic conditions of the municipality of Cazin and beyond in the Una - Sana Canton favor the development of this pathogen. The ecological and biological characteristics of P. cubensis are dependent on climatic factors. Sporangia fungi can retain their infectivity in a wide range of 1 - 16 days, depending on temperature, relative humidity and solar radiation. For this type of infection, it is necessary for the plants to be wet for some time at a certain temperature, but the number of hours of wetting with the corresponding temperature (°C) must be constant and at least 50-60 hours [26]. Therefore, rain, fog, dew, irrigation of plants by sprinkling or rain, continuous humidification of plants for at least six hours, high humidity (90 - 100%), with optimal temperatures of 15 - 25 °C, are almost ideal ecological conditions for strong epiphytoses [27]. The reasons for the high infestation of gherkins with these pathogens are: poor draining soil, less distance in a row and between rows, because the crop is less well ventilated. The application of fungicides often does not provide sufficiently effective and safe protection of cucumber. The main problem is the impossibility of applying fungicides during the harvest due to the length of the waiting period for most fungicides. There are a large number of fungicides to control this pathogen, and these are agents based on: azoxystrobin, bacaroxychloride, metalaxyl, copper hydroxide, cineba, etc. [28].

4. CONCLUSION

In the area of the municipality of Cazin and Una – Sana Canton, there is a long tradition of growing gherkins, which is top quality. The market in EU countries has provided exports, which is why the area of gherkins is increasing. During the cultivation of cucumbers from sowing to harvest, agricultural producers must also perform prophylactic and therapeutic measures to protect gherkins from diseases and pests. Of the fungal diseases it is still the most dangerous for gherkin plants *Pseudoperanospora cubensis*.

The pathogenic fungus *P. cubensis* (cucumber downy mildew) regularly occurs on cucumber plants in the municipality of Cazin and beyond in the northwestern part of Bosnia and Herzegovina and causes significant damage to the leaves, which is manifested in the total yield. The fungus was detected in 2021. on 60% of the examined plants. During the vegetation, samples of diseased cucumber leaves were collected and the fungus was determined by microscopic method in the microbiological laboratory. The indexes of illness ranged from 0 - 51%. In 2021 as the most sensitive was hybrid Passamonte F1 (I=51). In the group of medium resistant is hybrid Regal F (I=32), very resistant was Harmonie F1(I=10). The highest resistance to *P.cubensis* was registered on the Kybria hybrid F1 (I=5). Hybrids less sensitive to *P.cubensis* should be selected for cucumber cultivation. Based on research, the recommendation to agricultural producers is to grow Kybria F1 hybrids. Other measures are certainly important: agro – technical (crop rotation, balanced fertilization, irrigation), application of fungicides and other protection measures.
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ENERGY EFFICIENCY AND RENEWABLE ENERGY SOURCES

"June 5th - World Environment Day"



ENERGY EFFICIENCY IN PUBLIC BUILDINGS

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Key words: Energy efficiency, public buildings

ABSTRACT:

Considering the critical amount of power consumption in public buildings, sustainable energy use can present an opportunity to tackle the climate change and air pollution problems in B&H. This paper provides a brief overview of the existing studies, the legal framework and the major donor programs for EE in B&H. Moreover, it presents the financing of EE projects and provides an estimation of potential energy savings and benefits. Finally, the paper describes the implementation process of the EE projects and summarizes the major barriers to public sector EE implementation.

1. INTRODUCTION

Baring in mind that buildings are globally responsible for 60% of electricity use, 12% of water use, 40% of waste, and 40% of material resource use, investing in energy efficiency in public buildings measures can yield substantial energy savings, while supporting economic growth, sustainable development and creating jobs. Energy efficiency is one of the easiest and most cost-effective ways to both combat climate change and reduce energy costs for consumers. [2] For example, each 1€ invested in EE avoids more than 2€ on energy supply spending. Energy efficiency can also increase the resilience and reliability of the electric grid and provide various community and health benefits. [10]

Some of the usual EE measures to reduce energy spendings are: thermal insulation of facades and roofs, increasing insulation thickness, replacement of doors and windoors, installation of a radiator thermostat heads etc. In addition to this, raising awareness and educating building occupants and users on their energy behaviors can encourage lower energy consumption.

Review paper

According to the Typology of Public buildings in B&H, the total number of public buildings in B&H is 7.600, of which 4.419 in the Federation of B&H and 2.908 in the Republic of Srpska. Public administration bodies often own or occupy a considerable number of old buildings intended for various uses (offices, schools, hospitals, warehouses etc.). More than 3.400 public buildings in FB&H have been built before 1987 and only 157 after 2010. Depending on each municipality's size and competencies, public buildings in the Federation of B&H cover cca. 5.1 million m² of the total heated surface in BiH, which is nearly 57% of the overall heated surface of the public buildings in B&H. As to International Energy Agency data, the energy intensity of BiH is 0.5 toe/1000\$ of GDP. For comparison purposes, it is 4 times higher than the average in the European Union, which means that an average European country earns 4 times more national income than B&H for the same amount of consumed energy. [7]

2. REVIEW OF EXISTING STUDIES AND DOCUMENTS RELATED TO ENERGY EFFICIENCY IN B&H

In the past 10 years the following relevant studies and reports related to Energy Efficiency are conducted:

- In-Depth Review of Energy Efficiency Policies and Programmes, prepared by the Energy Charter Secretariat in 2012,
- Energy Strategy of the Republic of Srpska up to 2030, developed by the Government of Republic of Srpska in 2012,
- EE in Buildings in Bosnia and Herzegovina, conducted under Western Balkan Energy Efficiency Study, prepared by The World Bank in 2013,
- Scaling-up Energy Efficiency in the Western Balkans, prepared by The World Bank in 2014.
- The Action Plan for Energy Efficiency in the Federation of B&H for the period 2016-2018 (in accordance with the Law on Energy Efficiency in the Federation of B&H),
- National Environmental Action Plan (NEAP-BiH), prepared by the Federal Ministry of Physical Planning and Environment, FB&H and Ministry of Physical Planning, Civil Engineering and Ecology, RS,
- Energy Efficiency in Buildings in the Contracting Parties of the Energy Community, conducted by the company Energy Saving International (ENSI) for the Energy Community.
- Action Plan for the Use of Renewable Energy Sources of FB&H, prepared by the Federal Ministry of Energy, Mining and Industry,
- Framework Energy Strategy of Federation of B&H by 2035, prepared by the Government of FB&H,
- Typology of Residential Buildings in B&H, prepared by UNDP in 2017,

- Case Study "Energy efficiency and renewable energy sources in Bosnia and Herzegovina" developed by Sustainable Development Goals Fund in 2017,
- Cantonal Environmental Protection Plan (KEAP) developed by the Government of Canton of Sarajevo in 2017 and amended in 2021,
- Guidelines for Conducting Energy Audits and Energy Certification, prepared by the Ministry of Physical Planning,
- Strategic Plan and Program of the Energy Sector Development in FB&H,
- The Study "Support to meet the requirements of the Energy Comunity Treaty for SEE focusing on energy efficiency & renewable energy" was prepared by the EU,
- Energy Efficiency Finance Study was prepared by the Development Bank of Austria in 2015,
- Study of Energy Characteristics of 252 Public Buildings conducted by CETEOR,
- Development Strategy of the Federation of Bosnia 2021-2027 prepared by the FB&H Government,
- Green Cantonal Action Plan for Sarajevo (GCAP) prepared by the Government of Canton of Sarajevo in 2021,
- Study on energy efficiency in buildings used by people in social need in the Sarajevo Canton, developed by the Ministry of Spatial Planning, Construction and Environmental Protection of the Sarajevo Canton and the Government of Sweden,
- Energy Efficiency Study for 300 Public Buildings in Sarajevo Canton,
- Study of Energy Consumption State in Main Health Centres in Canton Sarajevo With Calculation of Possible Energy Savings by Energy Efficiency Measures Implementation, and
- Energy Efficiency Action Plan of the Federation of Bosnia and Herzegovina for the period 2019-2021 (EEAPF).

3. LEGAL FRAMEWORK RELATED TO ENERGY EFFICIENCY

The laws, regulations and decrees relevant to EE are:

- Law on Energy Efficiency in the Federation of Bosnia and Herzegovina (2017),
- Law on Electricity and Amendments to this Law (2015),
- Law on Use of Renewable Energy Sources and Effective Cogeneration in Federation B&H and Amendment to this Law (2014),
- Law on Public Enterprises of FB&H (2005),
- Law on Physical Planning and Utilization of Land at the Level of the Federation of Bosnia and Herzegovina and various Amendments to this Law (2010),
- Law on Environmental Protection and Amendment (2009),
- Law on Fund for the Environment Protection of the Federation of Bosnia and Herzegovina and proposed Amendment to this Law (2017),
- Legislation on District Heating,

- Ordinance on conditions for persons conducting energy certification of facilities and amendments (2012),
- Guidelines for conducting energy audits and energy certification (2018),
- Ordinance on technical requirements for thermal protection of buildings and rational use of energy (2009),
- Ordinance on conditions for giving and subtracting authorizations for performing energy audit and energy certification of buildings (2018),
- Decision on the reference price of electricity (2015).

The Energy Efficiency Information System of FB&H (https://iseefbih.ba) provides a more detailed overview of the legal and strategic framework and action plans and programs related to energy efficiency.

4. ENERGY EFFICIENCY FINANCING

Investment in the upgrade of public infrastructures such as public buildings provides high energy-saving potential. Although it is a cost-effective solution, budgetary constraints of its owners, who are often municipalities or other city bodies, lead to challenges in providing financing for these projects. For example, the legislation related to public agency budgeting and financing in FB&H includes *Law on Budget in FB&H, Law on Financial Management and Control in the Public Sector, Regulation on Allocation of Funds from Revolving fund for EE projects, Regulations on Accounting of Budget in FB&H and Law on Accounting and Audit in FB&H.*

Both Federation of B&H and Republic of Srpska have established an Energy efficiency and environmental Fund, covering monitoring of implementation and reporting on achieved savings. The Revolving Fund provides sustainable financing for the energy efficiency sector in Bosnia and Herzegovina because the budgets do not have enough funds for EE projects. The users of the EE Revolving fund can be legal entities, municipalities, cities, bodies and organs of the federal and cantonal administrations and other public institutions, non-profit organizations and non-governmental bodies (individual craftsmen, companies, facilities) which want to implement EE measures through a revolving fund loan. [4]

The energy efficiency laws of both entities and the draft law of Brčko District recognize ESCOs (Energy service companies) and energy performance contracting. However, the ESCO market is not functioning due to the big implementation gaps which exist in public procurement, multi-year budgeting and adoption of model ESCO contracts.

5. EXISTING DONOR PROGRAMS RELATED TO EE IN B&H

Many multinational and bilateral financial institutions have been active in B&H and have launched several projects related to energy efficiency.

The World Bank conducted a comprehensive assessment of the options for scaling up EE projects in buildings in the Western Balkan countries in 2013/2014 and started implementing the *Bosnian Energy Efficiency Project* (BEEP). Since 2016, within BEEP energy efficiency measures have been implemented in the buildings of public institutions in the Federation of BiH. To date, more than 25 million BAM has been invested in the reconstruction of buildings, which resulted in reducing CO₂ emissions by 4,564 tones per year. In addition to increasing comfort for more than 457,000 users, the Project also results in creating of more than 1 300 green jobs. Buildings of high social importance, such as educational and health institutions, are the focus of the Project, along with other public buildings. Since the start of the project, a total of 56 facilities have been renovated, 40 of which are educational, 10 are health, and 5 are other public institutions. The extension of the BEEP project is secured through the preparation of the Additional Funding (BEEP AF) project in 2021. So far, under AF BEEP, 40 public buildings and 2 roof replacements in FB&H are confirmed to be retrofitted. The selected buildings include 29 cantonal and 11 federal-level public buildings. [1]

Since 2013, United Nations Development Program (UNDP) is implementing the Green Economic Development Project (GED), financed by the Government of Sweden, the Environmental Protection Fund of FBiH and the Environmental Protection and Energy Efficiency Fund of Republic Srpska. The Project partners are the Ministry of Foreign Trade and Economic Relations of B&H, the entity's ministries for spatial planning, cantonal ministries and other institutions. So far, within this project, Energy management institutionalization has been introduced in 8 cantons, the Decision on obligatory EMIS data input is adopted in 6 cantons, 2 Energy Efficiency Action Plans have been developed, over 4,900 users have been trained to use the EMIS system, over 270 detailed energy audits on public sector buildings were conducted to identify the most effective measures to improve energy efficiency, the Revolving fund within the Environmental Fund of Federation of B&H which enables investments into energy-efficiency projects on public, business and industry buildings was established, energy efficiency retrofits on 229 public buildings across the country are conducted, modernization of public lighting system is implemented in nine municipalities and over 67,700 citizens have participated in educational events and workshops.[8]

European Bank for Reconstruction and Development (EBRD) established the Western Balkans Sustainable Energy Financing Facility (WeBSEFF), to provide debt financing for renewable energy and industrial energy efficiency projects to small and medium-sized enterprises (SMEs) in the Western Balkans. WeBSEFF is working closely with local partner banks which conduct a full credit assessment of each borrower and their project proposals. Partner banks under WeBSEFF in B&H are Raiffeisen Bank and UniCredit Bank. Overall, the Facility's investments in projects have contributed to saving more than 103 million kWh of energy and more than 32 million kg of CO_2 emissions per year. Furthermore, EBRD established a legal framework to support Energy service companies (ESCOs) that can successfully implement EE projects in the public sector. In February 2019, Sarajevo Canton joined <u>EBRD Green Cities</u>. This framework is designed to help cities to articulate a sustainable development vision and their strategic objectives as well as define actions and investments necessary to address priority environmental issues.

EBRD and the European Union (EU) provided a financial package for improvement in EE of public buildings in Canton Sarajevo under the Green City Action Plan. The investments cover 29 schools and related facilities, 6 kindergartens, 3 student dormitories and 2 health centers. The energy savings are estimated to be around 13.7 GWh and would result in 4,774 tonnes of CO_2 savings annually. Energy efficiency measures within this project include the introduction of cleaner and more efficient heating, better insulation, better lighting and overall improvements. [3]

U.S. Agency for International Development (USAID) launched the Energy Policy Activity to help B&H coordinate, manage and improve transparency in the gas and electricity sectors while providing targeted technical assistance to improve and simplify the energy policy environment and legal framework. Throughout this project, USAID develops and/or recommends legislative and other measures at all levels of government to ensure that the B&H energy sector is compliant with EU requirements. USAID also assists the BiH Ministry of Foreign Trade and Economic Relations and relevant entity ministries in improving energy strategies and action plans, including the development of the National Energy and Climate Plans. Additionally, USAID is helping B&H develop a cyber security roadmap for the energy sector. [9] The project also supports a strong public outreach and awareness program to promote a market-based energy sector and educate the general public about the benefits of the changes taking place in the energy sector.

6. POTENTIAL ENERGY SAVINGS AND BENEFITS

The potential of the energy savings from the typical EE measures is mainly achieved by:

- replacing the old joinery with the new more energy-efficient. With this measure, the average amount of cca. 35% energy savings can be achieved in comparison to the baseline depending on the condition and surface of the building,
- installing the thermal insulation. With this measure, it is possible to save the average amount of cca. 26% of energy,
- replacing old heat supply systems with modern boiler(s) for central heating systems is a measure that can save up to 20% of energy, and
- insulating of the ceiling/roof can save up to 21% of the energy.

Since the return period for the investment can depend on many parameters (heating fuel is one of the most important ones), the time required to recover the cost of the EE investments is relatively long: *approx. 15 years for the replacement of windows and doors, 16 years for the building insulation, 28 years for the roof insulation and 4 years for the investments to replace old heat supply systems.* The payback periods for EE investments can be long, but they

provide several other benefits such as improved thermal comfort, extended lifetime of the building and overall visibility and image of the facility. The main environmental benefit achieved with very high energy performance public buildings is the reduction of the primary/useful/final energy demand, e.g. for space heating/cooling, water heating, air conditioning as well as a reduction in the consumption of electricity. Using energy more efficiently means burning less fossil fuels, which reduces the emission of pollutants into the atmosphere, soil, and water while also reducing the need to procure additional fuels, allowing us to conserve the environment by leaving those resources in the ground.

7. IMPLEMENTATION PROCESS FOR PUBLIC BUILDINGS EE PROJECTS

The first step in the implementation process for EE projects for public buildings is to perform detailed energy audits and conduct all on-site measurements of the selected facility(ies) to determine energy losses and the current energy efficiency of the heating and lighting system and building user pattern. These audits gathered in the Detailed Energy Audit Report should include, inter alia: *Site visit and data collection, Analysis of energy characteristics of the buildings and characteristics of energy consumption and cost management, Identification of the presence of potential asbestos-containing materials (ACMs), Analysis of energy costs and modeling of energy consumption, Analysis of possible measures for increasing energy efficiency of the buildings, Defining measures for increasing the energy efficiency of the buildings in comparison of energy, economic and environmental-related effects of proposed measures, Comparative analysis of similar purpose, Defining recommendations for energy management within the building, Measures for the safe management of ACMs, Preparation of Draft Final Report and the Preparation and delivery of a final report on a detailed energy audit.*

After the established and agreed steps for the implementation of the energy audit, the terms for

detailed measurement of the public building should be agreed upon. This is one of the first actions for the preparation of project documentation for obtaining of urban planning permit and construction permit. If the client has existing project documentation, urban permit and construction permit for the building, he shall present all documents and projects to the engineering team during the visit and if the documentation is valid, then it is temporarily given to the project company for reverses and serves as the basis for the design of the conceptual and detailed design.

The team of architects shall take the dimensions of the interior and exterior spaces with the characteristic elements. When measuring windows and doors, both their dimensions and their characteristics (frame material, number of windows, mode and opening scheme, door filler) shall be determined. The engineering team shall inspect the condition of the complete

envelope/facade and its water tightness, the roof structure with the layout of the attic spaces and the condition of the support structure and its ability to carry loads. Moreover, the engineering team shall inspect heating, cooling and electrical installations in detail. If the building has centralized hot water preparation for individual local boilers, the appointed engineer shall inspect its condition, take all the necessary dimensions, measures and capacities to prepare the analysis and make a proposal for improving or changing the hot water preparation system. The engineers who examine the position of the luminaires and the installations are obliged to inspect all security systems in order to identify any security problems. The electrical engineer shall propose the project documentation to reduce the peak load (reactive power) and thus the consumption of electricity. If the roof is changed or works on the installation of the thermal façade are performed, it is necessary to dismantle and reinstall the lightning protection installation on the part treated with energy measures in order for these to be operational.

When all the necessary and relevant data are collected, they shall be analyzed and used to create the project documentation - the Conceptual and Detailed design.

The conceptual design contains the technical description with the proof of the surfaces, the ground layout of the building in the plot, the appropriate graphic documentation for the developed technical documentation, and, if necessary, a rough example of the works, materials and equipment. Depending on the complexity and technical structure of the building, it may contain other designs and appendices if they are significant for determining the urban-technical conditions for the construction areas not covered by the relevant detailed planning document, as well as other conditions for the preparation of the detailed design (technological description), processes, technological drawings, description of the application of particular construction technology, cost estimation and preliminary environmental impact assessment. The Conceptual design will be used for obtaining the urban permit.

Preparation of the Detailed Design for obtaining a construction permit and performing of works begins after obtaining the urban permit. The detailed design is a set of mutually harmonized phases of projects that provide a technical solution of the building, display the placement of the building in the space, and prove the fulfillment of essential requirements for the building, other requirements stipulated by law, special regulations, norms and technical standards. The detailed design is made based on the conditions given in the location information, i.e. urban permit. The detailed design, depending on the technical structure of the building, can be made of one or more parts – phases and must include the name and registration of the legal entity that performs the design, signatures of responsible designers for all parts of the project, project assignment certified by the investor, general and special conditions, standards, norms and regulations for an object or operation, technical description for all works with surface proof, object and estimate of works, materials and equipment, the architectural project, construction project, hydro installation project, electrical installation project, the mechanical project, technological project, project external design, measures defined by the environmental permit, if the purpose of the building is defined as an activity

that can endanger the environment, study on geotechnical studies of the bearing capacity of the underlying soil, study of fire protection, the study of safety at work, and other contents in accordance with special regulations. Designers may also be obliged to make an Elaborate on the Occupational Safety of both participants in the work and users of the public institution and an Elaborate on the management of construction waste.

Special emphasis should be placed on the preparation of technical description, bill of quantities and drawings details. This will be the basis for the preparation of tender/bidding documentation for the execution of works. When all project phases are completed and the estimated cost is calculated, the design documentation is submitted to the client for comments and revision, after which it should be submitted to the competent municipal authorities for obtaining a construction permit or in accordance with the Law on Spatial Planning.

The next step in the project implementation is the procurement phase. Depending on the needs of the project, the market situation and the needs of the contracting authority, it is necessary to decide on the type of public procurement framework. 4 procurement options are possible: open tendering, restricted tendering, negotiation with or without prior publication of a contract notice and direct method. The choice which procurement procedures will be apply depends on whether the procurement is being done in line with the local legislative framework or the procurement process in conducted in line with the international financial institutions procedures. After choosing the procurement type, it is necessary to create tender documentation which contains the minimum relevant information in relation to the selected contract award procedure: procurement notice, invitation to submit requests for participation/offer (initial and final), technical specifications, qualification criteria for selection of the most favorable bid, draft or basic elements of the contract and other relevant documents and explanations.

The procedure for review and evaluation of bids is to be performed by the procurement commission with the support of the external experts who are authorized by the contracting authority, on the basis of the conditions and requirements from the tender documentation. To ensure the integrity of the competitive process, the evaluation of proposals must be undertaken objectively, consistently & without bias toward particular suppliers. The bids submitted by qualified bidders should be evaluated by applying the award criteria set out in the tender documentation, choosing the most economically advantageous tender or the lowest price.

An evaluation team will examine each tender received and make recommendations as to which bidder is the most qualified one. Once the final decision has been made on the tender award to the particular bidder, the tender administrator creates the tender results notification which is in letter form and then sent to all participating contractors.

The next steps are Contract engrossment and execution. Contract engrossment is the process of preparing the final agreed form of contract and its schedules and appendices so that it can be executed. Contract execution is the process of signing an agreed contract, after which its terms become binding on the parties to the contract.

Along with the procurement of a Contractor for the work execution, it is necessary to do the procurement for the Supervision of works, in order to ensure the works are performed in accordance with applicable laws and best practices. The Supervision of works includes inter alia: conducting regular site visits, coordinating and regular monitoring of the status of project activities through preparation and updating of implementation plans and schedule-liaising on a regular basis with the Contractor, preparation of monthly and periodic progress reports, review of design documents comprehensiveness, project closure and preparation of Final Project report.

8. BARRIERS AND CHALLENGES FOR EE INVESTMENTS IN THE PUBLIC SECTOR

The main challenges for EE implementation in the public sector in BiH can be classified as: *legal and regulatory barriers, equipment and service provider barriers, public end-user barriers, financing barriers, and the need for capacity building.*

The legal and regulatory barriers refer to complex government structure, limited implementation of the Law on Energy Efficiency, lack of institutional focus on EE, restrictions on borrowing by public agencies, restrictive procedures that may prevent retention of energy cost savings, public procurement rules and procedures, inability to sign long-term contracts, no provisions for performance contracting, lack of comprehensive Public-Private Partnership law and the low energy tariffs for electricity and heat.

Barriers related to the equipment and service providers are limited demand for energy services in the public sector, weak and fragmented energy services marked, high project development costs, limited knowledge and understanding, lack of experience with performance-based contracting and the limited availability of equity funds and poor access to commercial financing.

Public building owners or users often lack the technical background and expertise to understand EE methods and technologies for reducing energy consumption and/or replacing the consumption of fossil fuels with renewable energy sources. This is the first barrier related to the public building end-users and other barriers refer to no discretionary budgets for capital investments in EE projects, varying responsibilities for energy procurement and bill payment, limited experience with energy service providers, low existing comfort levels and poor structural condition of buildings and limited data on building characteristics.

The financial barriers are related to the unwillingness of the lenders to provide debt financing to public agencies, the lenders' perception of high risk and the lack of customized financial products. Moreover, the project financing requires collateral and many public agencies may

find it difficult to provide it. In addition to this, EE projects are generally smaller in the terms of the amount of financing, which leads to high transaction costs.

Finally, there is a need to extend the capacity of the public sector decision-makers, energy managers, service providers and lenders in order to understand the importance of EE projects and to obtain information and skills to implement such projects.

9. CONCLUSION

Since most of the public buildings in B&H were built before the demands for reduction of energy use and have been poorly maintained throughout the years, they can be considered a significant contributor to the elevated energy consumption in B&H. Due to inefficient insulation, obsolete heating systems and inadequate lighting, buildings in BiH consume more energy per square meter compared to countries in similar climates, which makes the B&H economy one of the most energy and carbon-intensive in the region.

A number of strategic documents at the level of entities, region and local communities in Bosnia and Herzegovina have dealt with energy consumption and possibilities for its reduction in both public and residential buildings, but due to the complex legal and regulatory barriers and other challenges, not many projects have been fully implemented. Bearing in mind that increasing the energy efficiency leads to energy savings, better comfort and a cleaner environment, it is necessary to have a systematic approach to implement the projects in the field of EE.

The implementation process starts with the building analysis, and it aims to primarily reduce energy consumption. In addition to this, it aims to improve buildings' structural stability, integrity, and aesthetics, as well as to prolong buildings' life cycle, enhance indoor thermal and air comfort, and provide a healthier environment for the occupants.

Given the sector's massive potential for comprehensive and sustainable socio-economic growth in the country, the matter of its development must be approached strategically, taking into account all B&H capacities and resources but also the areas with room for improvement and the shortcomings to overcome.

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"June 5th - World Environment Day"







REDUCTION OF LOSSES IN WATER DISTRIBUTION NETWORKS USING ZONAL WATER METERS

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Key words: Water Losses, Water Distribution Networks, Zonal Water Meters, Losses Reduction, Water Supply Enterprise

ABSTRACT:

Water losses are inevitable and are conditionally acceptable up to 15% in water supply systems, 5-8% in the case of newly build water supply networks, and 1-3% for main water supply pipeline. However, most public water companies have problems with uneconomical and irrational water losses. One of the biggest challenges in public water systems is to reduce water losses to an economically acceptable minimum. Zonal measurement instruments of water flow in combination with a memory-communication system have proven to be a very successful method of detecting water losses and initial water leaks from the system. This paper aims to discuss whether the use of such measuring instruments represents an economically optimal and technically flexible solution in the maintenance of public water companies. Qualitative research method, based on comparative case study analysis, is used to achieve the goals of this paper. Results proved that monitoring water losses by the method of night measurement results in the detection of faults, which are manifested by water losses. After detecting the increased flow on the diagram, which is formed by the data sent from the zonal flow meters, the places of water leakage in the field are detected and the fault is repaired. This paper concludes that using the zonal water meters in Water Distribution *Networks is an efficient and economically acceptable method for detecting the unaccountable* waters and water losses due leakage in the water supply systems.

1. INTRODUCTION

Due to the constant increasement in the costs of water supply, the suppliers are pressured to improve effectiveness of water supply providing enterprises preduction of water losses [1]. Bosnia and Herzegovina is ranked as regional leader and seventh in Europe from the aspect of the amounts of drinking water per capita, which is estimated to 9.46 cubic meters [2]. To satisfy the demand for drinking water, considering its limitied and decreasing character, and water supply of the population, which is in a constant growing, effective use of the water resources most be achieved [3]. For example, capital city of Bosnia and Herzegovina is facing a water crisis for a long time, as the water losses are estimated to be more than 70%, which caused severe water supply reductions [4].

Several studies, such as the one conducted by A. Nasirian, M.F. Maghrebi, S. Yazdani in 2013 [5] and G. Venkateshin in 2012 [6] researched about this relationship and developed methods to enhance energy efficiency of water supply systems. Perrone and a group of researchers in 2011 concluded that if the water demand is reduced by 20%, that will result in the reduction of required energy consumption by 20% as well [7].

Many of the mentioned factors that affect the amount of losses can be controlled by the companies providing the service, such as: the time of detection and the reaction fastness [8]. Numerous researchers indicated that the quick leakage detection issue of and the intervention on the leakage source are a crucial one [9]. When the lowest water losses are maintained with the lowest operating costs, it can be said that the goal is achieved [10].

However, despite the encouraging stories about success, water supply systems worldwide still have high levels of water losses [11]. The guidelines that the International Water Association (IWA) developed established the relation to the determination, analysis, and assessment of the losses of water distribution networks, which enabled the comparison of losses in various water supply networks and the assessment of their amounts [12]. Numerous studies showed that the recommended methods by the IWA should be applied worldwide [13].

Several questions need to be asked in the diagnostic approach are to be asked during the diagnoses phase and set of solutions/recommendations are given depending on the answer. For the question regarding to the amount of water that is being lost, it is required to do the Water Balance calculations. To answer the question regarding the location of the water leakage or lost, it is required to do the network audit. To answer the question related to reasons for the loss, it is recommended to review the network practices. After that, to prevent such reasons, it is recommended to develop appropriate strategies. Finally, to sustain and maintain the system, it is recommended to implement procedures for training and operation [14].



Picture 1: A typical district metered area [14].

2. RESEARCH METHODOLOGY

Qualitative Comparative Research method is used in this paper and will be based on comparing the results achieved in the selected case study of Komunalno LTD Bosnanski Petrovac by using the Zonal/District Metering Approach, with recommendations and results of other case studies were the same approach is used.

This paper aims to answer the question about whether using the Minimum Night Flow Analysis in determining the leaks of water networks can be beneficial in Bosnia and Herzegovina.

Total of 3 case studies are compared in this paper. Beside the Enterprise Komunalno, which is a local company, 2 other cases are taken in the comparison.

Evaluation criteria will focus on the amount of water usage measured on zonal/district meters for a period of the last 5 years. The trend of increasement or decrement will be commented and noted. Measures used will be included in the analysis process.

2.1 District/Zonal Metered Areas (DMA)

The Zonal/District Metered Area (DMA) is one of methods of Active Leakage Control (ALC) Methodology, which is a vital cost-effective and efficient management of leakage in water supply systems. The DMA is defined as an area of the water supply system or network, that is having (in ideal case) about 2000 properties, which are supplied from a signle input measured point and pressure controlled. The objective of this method is the reduction of the real losses to the level that is acceptable for economical aspects, and to keep up this level during the application of the proactive actions and strategies. Numerous advantages are achieved using this method, such as: easy management of the netowrk as the area is divided into smaller areas; easier application of ALC; faster deterction of leaks based on the monitoring system; improved optimisation of pressure; less water losses; bigger financial savings. However, this

method is having some disadvantages as well, such as the complaint of users about the pressure reductions and the dead-ends in the system causing water quality issues [15].

2.2 The Minimum Night Flow Method (MNF)

This methodology was initially developed and applied in the UK, but nowadays is internationally accepted[16] and has proven its efficacy in many other countries [17].

Determining the sectors in which higher levels of leakage occur, special attention was paid to night flow measurement. With this method, priority locations for repairs can be determined on a daily basis, and those are those with a higher flow during the night hours. Even in systems where universal metering applies, uncertainties in the determination of leakage rates arise for different factors such as inherent meteringerrors, the fact that meters register volumes instead of flow rates, or just because all meters are notbeing simultaneously read [18].

The night flow method is basically based on two types of budget. The first budget is based on calculations of water losses per hour of minimum night flow obtained by subtracting the night consumption from flow users. The second part of the budget consists of determining water losses in the minimum night flow hour and they must be extrapolated over a period of 24 hours. With the equation that connects the flow and pressure together with the development of pressure during the day at a significant point in the network, the extrapolation process is performed. The behavior of the whole network is determined on the basis of one characteristic point [19].

The simplified method would not be used if they had a reliable network model. The simplified method is a good starting point for observing the system, and its accuracy depends on the ability of the node to represent the pressure transmission of the entire network, because the flow rate depends on the pressure in the node [19].

3. RESULTS AND DISCUSSION

Total of four case studies are included in this section among which one is local, and it is the Case of Komunalno Ltd. Bosanski Petrovac, while other case studies are published in scientific papers, and they include:

- The Zarqa water network in Jordan case study [20] / Analysis done by AL-Washali, T., Sharma, S., AL-Nozaily, F., Haidera, M., and Kennedy, M. in 2018.

- Zomba City water networkin Malawi case study [21] / Analysis done by Zvikomborero Hoko and Jessy Alida Chipwaila in 2017

3.1 Case Study 1: Bosanski Petrovac water network / Bosnia and Herzegovina

Detection of one major fault using data obtained from zone/district flow meters, such as flowrates and pressure for the case study of Komunalno Ltd., is shown in picture 3. A

significant jump in the flowrate can be observed compared to the steady state in that zone. To the team of workers who constantly follow the diagrams, it was a signal for a breakdown in the distribution network, where they move immediately to discover the exact location of the leak. Finding the exact location of the leak was easier because it was already known in advance which district meter gave the signal and in which zone it was located. After detecting the approximate locating of the uncontrolled water leak, an electro-acoustic device is used, the so-called geophones for precising the location, and then wearing and repairing the fault. After repairing the fault, it was seen that the flow and pressure are within the established limits. It is more difficult to detect minor faults, with leaks of smaller amounts of water, but they can also be observed based on several days of monitoring using the Minimum Night Flow method, when the diagrams show a small but constant increase in flow during the night. Such leakages, in case that there are no zonal flow meters installed and combined with a memory-communication system, could not be detected in a short period of time, and water losses and leaks would last for weeks, especially in rocky areas where ground infiltration ratio is high.



Picture 2: Review of the pressure and flow-rate diagram of the flow meter -Komunalno Ltd. Bosanski Petrovac

Picture 3 shows the monthly consumption for the selected zonal water meter, where it can be seen how the introduction of this fault detection system has reduced water consumption compared to previous years.



Picture 3: Overview of multi-years water consumption diagrams registered on the selected zonal flow meter -Komunalno Ltd. Bosanski Petrovac

The information received from the enterprise Komulano Ltd. Bosanski Petrovac, was processed and presented five-year period in table 1.

5-years water consumption registered on the zonal flowmeter in m ³													
	2017	2018.	2019.	2020	2021								
Januar	19456,2	9658,1	5917,5	4004,1	4701,6								
Februar	16911,48	11847,6	4001,4	3636,0	4499,1								
March	16472,7	14972,4	3737,7	3866,4	6632,1								
April	15098,4	1424,6	3362,4	4537,8	5007,6								
May	13069,5	9242,1	4016,7	3773,7	4524,3								
Jun	12589,4	7345,8	6628,5	4519,8	5522,4								
July	10435,5	7588,8	5688,5	4970,7	6264,0								
August	9915,3	5497,2	4188,6	6621,3	7173,0								
September	8037,9	3843,0	3175,2	5836,5	4566,6								
October	78808,4	4629,6	3942,0	3746,7	5564,7								
November	10470,6	3722,8	1107,9	4392,0	4808,7								
December	13854,6	3972,0	702,9	5843,7	5805,0								

Table 1: Monthly review of the amount of water in m³ that has passed through the zone flow meter for a period of 5 years

This reduction, along with the timely detection of uncontrolled water leaks, was accompanied by the reconstruction of existing and construction of new parts of the water supply network.

3.2 Case Study 2: The Zarqa water network / Jordan

The main source of the Zarqa water supply network is imported water from the Disi project, which consists of 99 groundwater wells. According to GIS records, the length of the water supply network is 2447 km. The pipes that make up this network are composed of the following materials: polyethylene, galvanized iron, ductile iron, steel and cast iron. The network is almost fully pumped with average pressures from 10 to 30 m, except for small parts in the network where it is supplied by gravity or a combination of both. The water is supplied to customers through interlinked distribution areas located within five administrative zones:Rusaifah, Al-Azraq, Beerian, AL-Hashimia, and Dhulail [20].

Picture 4 shows the results of pressure and flow measurements during the experiment. The range of pressures in DMA can be seen in Figure 4a where we have three characteristic points, namely the entry point of pressure, the point of medium pressure rise and the point of high pressure rise. The measured pressures at the other two points of the identical range are shown in Figure 4b where we see the drop at midpoint 2 on Monday, January 4, 2016 from 9:00 a.m. to 6:00 p.m. In Figure 4c, due to the possibility of changing the valve position, the pressure drops coincided with the flow drop, from 43 to 5 m³ / h [20].



Picture 4: Flow and pressure measurements in the DMA: (a) range of pressure in the DMA; (b)measurement in further two points in the DMA with pressure collapse in one point; (c) flow and average pressure relationship in the DMA [20].

Based on the MNF analysis, the leakage level of the network was estimated at 16.1 million m^3 /year. Accordingly, the leakage from reported and unreported failures was estimated at 2.4 million m^3 /year. There is also a background leak estimated at 1.8 million m^3 /year. Based on the sum of the volumes of reported and unreported failures and background leaks and the difference in the estimated volume obtained on the basis of the MNF method, hidden losses in the amount of 11.8 million m^3 /year [20].

3.3 Case Study 3: Zomba City water network / Malawi

The Zomba water supply network is located in the southern part of Malawi. The water supply system originally consisted of a conventional treatment plant on the Mulunguzi River. Its capacity was 6,000 m³/day consisting of 17 tanks with a capacity of 25 to 455 m3 and a 90 km long pipeline with a pipe diameter ranging from 25 to 200 mm. During the period 1994–2001, the water supply system was expanded through the construction and commissioning of a 3.4 Mm3Mulunguzi Dam, an additional 12,200 m³/d capacity treatment plant, storage tanks with a total storage capacity of 9,750 m3; and a total of 41.3 km of pipeline of PVC, GI and ductile iron with diameters ranging from 80 to350 mm. The Zomba water supply system produces about 400,000 m³ per month. This network covers 89% of the population, which is an average of 6,000 connections [21].

Water losses of the city water supply system amount to 30 to 40%, and the reason for this is the high pressures due to the geography of the terrain, which lead to the rupture of the pipe in the pipeline. Study trials in the specific areas of Airwing, Malonje and Sadzi were carried out due to reported problems, as these areas are supplied from individual independent reservoirs.

UFW showed the percentage of total water delivered and based on that Airwing had the smallest distribution losses of 13%, and Malonje had the largest in the value of 62%. The reason Airwing has the least distribution losses lies in the fact that the Airwing Army serves a bulk meter equal to 50 connections [21].

High pressures in the system of 40 to 114 m lead to bursting of pipes in the water supply network, which is a big problem in Malonja and caused sudden increases in water leakage from the tank during the minimum flow [21].



Picture 5: Malonje tank outflow pattern for the period 31 March 2009-6 April 2009[21].



Picture 6: Sadzi tank outflow pattern for the period 6 April 2009–10 April 2009[21].

In this study, the actual losses were caused by high pressure along with the age of the Airwing pipe. As for Malonje and Sadzi, the losses shown were lower than reported, and the losses themselves were caused by meter errors [21].

3.4 Discussion

The three case studies analysed in this paper proved that the water loss is an expected and a common issue in all water supply networks. However, the variation ratio of the amount of the water lost depends on several factors.

In the case of Bosanski Petrovac, it was clear that the investment in the network and materials pipes was crucial for improving the performance of the water supply network and in reducing the water losses. Considering the role of the Zonal meters, it helped in detecting the location of the leakage, and using additional instruments, an effective solution was found and successful implemented.

This can directly bring an answer to the research question set by authors, and it can be said that using the Minimum Night Flow Analysis in determining the leaks of water networks can be beneficial in Bosnia and Herzegovina. Also, this paper proved that using the Zonal/District Metering Area approach is useful in detecting the leakage and apply active measures. The MNF analysis helped in detecting small-sized leakages, as they were difficult to detect using only the DMA approach.

As for the case of Zarqa water network, it might be concluded that the testing time was too short to set some concrete conclusions, and therefore, huge gab was noticed between the estimated leakage level based on the MNF analysis and the detected amount. Also, it has to be said that the detection time is quite satisfying in general, as well as the reaction/recovery time. However, it could be recommended further to run a long study that will follow the network analysis and the water meters for longer period to establish a satisfying correlation.

The high unaccounted-for water (UFW), as well as pipe bursts, proved to a major issue in the third case study, as the water losses in the study areas exceed the acceptable values by far. In such cases, there is a need of considering the solution that will satisfy the Economic Leakage Level, where the planned interventions on changing/replacing pipes, installing new measurement equipment and other solutions are done in accordance to a feasibility study. However, doing nothing in such cases is not an option, as with the time progressing, it is expected for losses to increase, and that will cause potential water supply crisis.

4. CONCLUSION

The water losses is a common problem that is faced by all water suppliers and providers. It is not expected, neither economically acceptable to eliminate this problem entirely, as it would not be cost-effective. Several methods are used for detecting the water leakage, and several actions are introduced for reacting to such problems. This paper reviewed the literature regarding the main theme, which is the water loss in water supply networks, as well as methods used for reducing the amount of the lost water. Using the comparative qualitative analysis of different case studies and literature findings, authors of this paper concluded that the Zonal/District Metering Area approach is helpful in developing countries, such as Bosnia and Herzegovina, as it helped in detecting major water losses that are due to a fault/failure in

the system. Also, this paper concluded that, using the Miminum Night Flow methods could be of a big assest in detecting small water leakages, if the network of the zonal meters is well established and dense. This paper concluded that, at some point when the water losses are higher that the acceptable ranges, intervention that are cost-effective must take place, but without exceeding the ELL. Finally, this paper concluded that the analysis time should be long enough to establish a precise, or at least an acceptable, relationship between the estimated values of water leakage and the measured once on field.

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ENVIRONMENT, NUTRITION AND HEALTH







PHYSICO-CHEMICAL AND MICROBIOLOGICAL CONTROL OF DRINKING WATER OF KLJUČ MUNICIPALITY

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Key words: physico - chemical indicators, microbiological indicators, water supply

ABSTRACT:

This paper presents the results of testing the hygienic correctness of the municipal water supply in Ključ in 2017 and 2018. Samples were taken from the city water supply network. The following physico – chemical indicators were examined: humidity, pH value, consumption of KMnO₄, ammonia, nitrates, nitrites, chlorides, iron and microbiological indicators: Escherichia coli, Colifom bacteria, Enterococci and Clostridium prefrigens. The results of the study showed that water from the city watercourse mainly meets the conditions prescribed by the Regulation.

1. INTRODUCTION

Water is one of the most important substances in nature, and it is an irreplaceable natural asset that is necessary for the life of living beings. About 70% of the Earth's surface is covered with water. Water builds 50-90% of the weight of every living organism, and maintains plant and animal life, as well as human life on Earth [1]. Apart from drinking and washing in households, it is also used for irrigation in agriculture, recreation and sports, as one of the most important industrial raw materials, and is irreplaceable in most technological processes. In many parts of the world, even in our country, there is a gradual destruction and pollution of water sources. Many of the products of human activities end up in water, while others, emitted into the air or on the ground, again end up in watercourses [2]. Uncontrolled pollution of surface and groundwater, which is rapidly increasing, reduces the amount of quality water sources. The main problems related to water are: organic pollution, eutrophication, acidification and heavy metals. A special problem is the content of organic matter, and especially the increasing presence of nitrogen compounds [3].

2. WATER SUPPLY OF KLJUČ MUNICIPALITY

There are no installed energy capacities in the municipality of Ključ and in the past period, mostly studies of hydropower capacities on watercourses in this region have been conducted. Possibilities for energy use of the rivers Sanica and Korčanica may be of interest only to individual, small users, because it is possible to build a plant with a capacity of up to 0.1 - 1.0MW. With the spring of Sanica or the canyon part of Sanica, the needs of the settlement of Sanica and its industrial and economic potentials could be met. This interest can be only in the case that today's energy supply can be difficult and unreliable or is a limiting factor in the development of the economy of this region. The project for the protection of drinking water sources in the Okašnica water supply system was prepared in 2004, and, accordingly, the A protection zone was fenced off and warning signs were installed. The following year, the Sanica drinking water protection project was built - the municipalities of Bosanski Petrovac and Ključ.[6] The length of flood risk rivers in the municipality is 4.5 km according to data from 2011. In the period from 2005 to 2011, the length of flood risk streams was reduced by 2 km. The municipality is continuously, and in accordance with the possibilities of the Budget and other available sources, working on the arrangement of watercourses, rehabilitation and cleaning of the riverbed. The population of Ključ municipality is supplied with water from the Ključ city water supply system and local / local water supply systems [10]. Public company "Ukus" d.o.o. Ključ manages the Ključ city water supply system, as well as the local water supply systems Gornji Dubočani, Donji Dubočani, Lubica - Šehići, Prhovo - Plamenice, Crljeni, Krasulje - Bušotina, Bajrami - Dedići - Husići, Kalabe - Kazići, and since August 2015 the water supply systems in the area of the local community Velagići. The city water supply system Ključ, the water supply system Gornji Dubočani, Donji Dubočani, Lubica -Šehići and Prhovo - Plamenice use water from the Okašnica spring, whose water intake is at least 25 L/s, to supply the population with water. The Crljeni water supply system has its own springs "Vreoci i Kadinca", with a water intake of 2 L/s, while the Krasulje water supply system uses water from its own source of the drilled well "Bušotina" whose water intake is 4 L/s. Water supply systems Bajrami - Dedići - Husići, Kalabe - Kazići, and water supply systems in the area of the local community Velagići use water from their own sources. The control of water from the mentioned water supply systems is performed regularly by taking samples once a month. About 85% of households in the municipality of Ključ have a wellregulated drinking water supply. All springs belong to the Una basin. About 10 kilometers of asbestos-cement pipeline are still in operation on the city water supply system, namely the Okašnica-Palež supply pipeline Ø 250, the Zgon-Humići settlement pipeline Ø 150, and the city pipeline on the Faik curve stretch - petrol station Ø 150. During the summer dry period, water is delivered to the Zmajevac - Velagići water supply system by means of a pressure system which pumps water from the Šehići reservoir to the Zmajevac pumping station. The second part of the water supply system of the municipality of Ključ is managed by "Mrkva montaža" d.o.o. The key is the Sanica water supply system - the Trebunj water supply system. Other water supply systems in the local communities of Biljana, part of Krasulja, Donja

Sanica, Kamičak, part of Zgon - Crljeni and Gornja Sanica are privately owned - the owners manage the water supply system. The current total length of the primary water supply network (without connections) is: 176 km. The current number of consumers of water from public water supply is 8,076 (13,087 inhabitants according to the 2013 census in settlements supplied with water from water supply systems managed by JP "Ukus" d.o.o. Ključ), and according to estimates for 2014, 150 households do not have water supply network. In the zone of water supply systems managed by JP "Ukus" d.o.o Ključ. Only the settlements of Egrlići, Mali Rejzovići and part of Gornji Dubočane are not connected to water supply systems. Water losses on the city water supply system are very high and range around 66% (affected amount of water - invoiced amount of water). The company successfully eliminates visible faults, while it does not have the appropriate equipment for more efficient fault detection.[6]

2.1 Drinking water control

Regular monthly quality control of drinking water is performed in the health institution "Institute of Public Health of Una-Sana Canton Bihać", on the prescribed number of samples taken and transported to Bihać by an authorized employee of JP "UKUS" d.o.o. Ključ. Occasionally there are six-month and annual water quality controls [4]. Due to heavy rainfall, water turbidity occurs at springs several times a year. Through local media, citizens are informed about water quality, when due to increased turbidity, they are urged to boil water before use. In this paper, physical-chemical and microbiological analyzes of drinking water of the municipality of Ključ for the sampling period 2017-2018 are presented.[7]

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3. RESULTS OF DRINKING WATER TESTING IN KLJUČ MUNICIPALITY

Test parameter	Unit of	Reference		January	Februar	March	April	May	June	July	August	Septemb	October	Novemb	Decemb
	measure	value	Method	2017	y 2017	2017	2017	2017	2017	2017	2017	er 2017	2017	er 2017	er 2017
Colour	-	acceptable	BAS EN ISO 7887	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits
Smell	-	acceptable	BAS EN 1420-1	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits
Taste	-	acceptable	BAS EN 1420-2	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits
Residual chlorine	mg/l	0,5	Spectrofotometri c	0,43	-	0,31	0,27	0,36	0,17	0,43	0,56	0,47	0,34	0,36	-
Turbidity (for water surface treatment)	NTU	max. 1,0	DAG EN 190	0,45	-	0,60	2,3	0,70	0,55	0,35	0,40	0,76	0,45	5,3	-
Turbidity (for other types of water)	NTU	acceptable	BAS EN ISO 7027*	-	1,1	-	-	-	-	-	-	-	-	-	0,56
рН	pH unit	6,5 - 9,5	BAS EN ISO 10523*	7,6 (17 C)	7,3 (21,3 C)	7,3 (17,4 C)	7,3 (18,7 Q	7,3 (19,5 C)	7,4 (23,4 C)	7,4 (20,1 C)	7,8 (20,5 C)	7,4 (17,7 Q	7,5 (22,5 Q)	7,2 (22,1 Q	7,4 (18,5 C)
Electrical conductivity	µS/cm	2.500	BAS EN 27888*	438 (17,1 C)	404 (21,2 Q	376 (17,4C)	441 (19 C)	382 (20 C)	396 (23,6 C)	394 (19,7 C)	397 (20,5 C)	400 (17,8 C)	466(22, 6 Q	414 (21,5 C)	416 (18,5 C)
Chlorides	mg/l	250	BAS ISO 9297*	1,06	1,06	1,42	2,12	3,54	2,12	0,71	2,13	1,42	1,77	1,42	1,06
Consumption of KMnO4	O ₂ mg/l	5,0	Calculating from BAS EN ISO 8467	1,53	1,40	1,27	1,02	0,76	0,80	0,19	0,06	0,45	2,02	1,52	1,26
Permanganate index	O ₂ mg/l	-	BAS EN ISO 8467	-	-	-	-	-	-	-	-	-	0,51	0,38	0,32
Amonium	mg NH4 ⁺ /l	0,50	Spectrofotometri c	0,028	0,09	0,07	0.05	0,06	0,00	0,059	0,00	0,00	0,00	0,095	0,00
Nitrates	mg NO ₃ 7/l	50,0	Spectrofotometri c	1,65	3,59	2,28	3,19	2,64	2,57	2,37	2,32	4,00	4,28	3,51	2,59
Nitrites	mg NO ₂ ^{-/1}	0,5	Spectrofotometri c	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Iron	mg/l	0,2	Spectrofotometri c	0,003	0,01	0,02	0,01	0,01	0,018	0,013	0,02	0,002	0,00	0,0007	0,00

Table 1: Results of physical - chemical analysis of central water system water for 2017

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Table 2: Results of physical - chemical analysis of central water system water for 2018

Test parameter	Unit of measure	Reference value	Method	January 2018	Februar y 2018	March 2018	April 2018	May 2018	June 2018	July 2018	August 2018	Septemb er 2018	October 2018	Novemb er 2018	Decemb er 2018
Colour	-	acceptable	BAS EN ISO 7887	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	-
Smell	-	acceptable	BAS EN 1420-1	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	-
Taste	-	acceptable	BAS EN 1420-2	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	fits	-
Residual chlorine	mg/l	0,5	Spectrofotometri c	-	0,38	0,43	0,3	0,38	0,26	0,34	0,19	0,30	0,34	0,30	-
Turbidity (for water surface treatment)	NTU	max. 1,0		-	0,80	1,7	1,1	0,83	2,7	0,98	0,44	0,35	0,50	0,35	-
Turbidity (for other types of water)	NTU	acceptable	BAS EN ISO 7027*	0,54	-	-	-	-	-	-	-	-	-	-	-
рН	pH unit	6,5 - 9,5	BAS EN ISO 10523*	7,1 (22,5 C)	7,2 (23 C)	7,5 (20 C)	7,1 (20 C)	7,4 (20,5 C)	7,3(19,9 C)	7,3 (19,8 C)	7,0 (24 C)	7,3 (22,5 C)	7,3(18,8 C)	7,3 (18,5 C)	-
Electrical conductivity	µS/cm	2.500	BAS EN 27888*	400(22, 4 C)	394 (23 C)	352 (20 C)	344 (21 C)	368(20, 3 C)	386 (20 C)	397 (19,4 C)	395 (24 C)	392(22, 2 Q	398(18, 9 C)	400(18, 5 C)	-
Chlorides	mg/l	250	BAS ISO 9297*	1,77	2,13	0,71	2,48	0,71	1,06	0,71	0,35	3,55	1,42	2,13	-
Consumption of KMnO4	O ₂ mg/l	5,0	Calculating from BAS EN ISO 8467	3,03	2,53	3,29	3,79	3,54	4,55	4,55	2,02	3,04	3,29	3,28	-
Permanganate index	O ₂ mg/l	-	BAS EN ISO 8467	0,77	0,64	0,83	0,96	0,90	1,15	1,15	0,51	0,77	0,83	0,83	-
Amonium	mg NH4 ⁺ /l	0,50	Spectrofotometri c	0,00	0,109	0,007	0,028	0,00	0,043	0,00	0,002	0,094	0,096	0,001	-
Nitrates	mg NO ₃ ⁻ /l	50,0	Spectrofotometri c	3,00	1,46	2,25	2,40	2,33	2,27	0,91	2,04	2,68	2,66	2,64	-
Nitrites	mg NO ₂ ^{-/l}	0,5	Spectrofotometri c	0,00	0,00	0,00	0,00	0,001	0,00	0,00	0,007	0,001	0,00	0,00	-
Iron	mg/l	0,2	Spectrofotometri c	0,00	0,005	0,010	0,002	0,00	0,021	0,006	0,005	0,006	0,002	0,002	-

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Types of microorganisms	Unit of measure	Reference value	Method	January 2017	February 2017	March 2017	April 2017	May 2017	June 2017	July 2017	August 2017	September 2017	October 2017	November 2017	December 2017
Escherichia coli (E.coli)	cfu/100 ml	0/100 ml	BAS EN ISO 9308-1*	0	3	0	0	0	0	0	0	0	0	0	3
Coliform bacteria	cfu/100 ml	0/100 ml	BAS EN ISO 9308-1*	0	25	0	7	0	0	0	0	0	0	0	20
Enterococci	cfu/100 ml	0/100 ml	BAS EN ISO 7899-2*	0	3	0	0	0	0	0	0	0	0	0	3
Clostridium prefringens ¹	cfu/100 ml	0/100 ml	Sulfitni agar	-	-	-	-	-	-	-	-	-	-	-	-
Number of colonies on 22 C	cfu/1 ml	100/ml	BAS EN ISO 6222:2003	-	-	-	-	-	-	-	-	-	-	-	-
Number of colonies on 37 C	cfu/1 ml	20/ml	BAS EN ISO 6222:2003	-	-	-	-	-	-	-	-	-	-	-	-

Table 3: Results of microbiological analysis of water in the central water system in the municipality of Ključ in 2017

¹Needed only if the water originates from or is influenced by surface waters

* Indicates only accredited methods

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Types of microorganisms	Unit of measure	Reference value	Method	January 2018	February 2018	March 2018	April 2018	May 2018	June 2018	July 2018	August 2018	September 2018	October 2018	November 2018	December 2018
Escherichia coli (E.coli)	cfu/100 ml	0/100 ml	BAS EN ISO 9308-1*	1	0	0	0	0	0	0	0	0	0	0	-
Coliform bacteria	cfu/100 ml	0/100 ml	BAS EN ISO 9308-1*	15	0	0	0	0	0	0	0	0	0	0	-
Enterococci	cfu/100 ml	0/100 ml	BAS EN ISO 7899-2*	0	0	0	0	0	0	0	0	0	0	0	-
Clostridium prefringens ¹	cfu/100 ml	0/100 ml	Sulfitni agar	0	-	-	-	-	-	-	-	-	-	-	-
Number of colonies on 22 C	cfu/1 ml	100/ml	BAS EN ISO 6222:2003	-	-	-	-	-	-	-	-	-	-	-	-
Number of colonies on 37 C	cfu/1 ml	20/ml	BAS EN ISO 6222:2003	-	-	-	-	-	-	-	-	-	-	-	-

Table 4: Results of microbiological analysis of water in the central water system in the municipality of Ključ in 2018

¹Needed only if the water originates from or is influenced by surface waters

* Indicates only accredited methods

4. CONCLUSION

Based on the research conducted in this paper, we can conclude that drinking water from the central water system of the municipality of Ključ for most of the year meets the requirements of the Ordinance on the health of drinking water "Official Gazette of BiH, No. 40/10.[5]

The results of the physical-chemical analysis for 2017 showed that one of the samples in April, May, August and November did not meet the requirements of the Ordinance on the health safety of drinking water. In August, the residual chlorine was elevated, did not correspond to the reference values, and in all other months it was water turbidity. The results of the microbiological analysis for 2017 showed that one of the samples in February, April and December did not meet the requirements of the Ordinance.

The results of the physical-chemical analysis for 2018 showed that one of the samples in March, April and June did not meet the requirements of the Ordinance on the health safety of drinking water. In all three samples, the turbidity was that which did not correspond to the reference values. The results of the microbiological analysis for 2018 showed that one of the samples in January did not meet the requirements of the Ordinance on the health safety of drinking water, while in all other months the samples were in order.

Increased turbidity in some samples is caused mainly by heavy rainfall, but taking into account all these facts we can conclude that the physical - chemical and microbiological properties of drinking water from the central water system of Ključ municipality in 2017 and 2018 meet the Ordinance on water safety drink.

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ASSESSMENT OF IRON INTAKE AND RISK OF IRON PRESENT IN MINT HERBAL TEA INFUSIONS CONSUMED BY PRESCHOOL CHILDREN

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Key words: risk assessment, hazard coefficient, herbal tea, heavy metals

ABSTRACT:

Iron is an essential element for the human body and plays a key role in the functioning of all cells. Its main role is to carry oxygen through the blood from the lungs to all cells. Iron deficiency causes anemia, which is a major public health problem. One of the most vulnerable groups in the population are children in development. On the other hand, excess iron taken up in the body accumulates in vital organs, mostly in the liver, so excessive accumulation can cause serious health consequences. Providing the population with a sufficient amount of nutritious and hygienically healthy food is the foundation of public health protection. According to the current Law on Food of BiH, the main goal is to ensure a high level of human health protection, and the measures applied should be based on risk assessment, which is based on the assessment of the intake of harmful substances. An anonymous survey on 200 preschool children from the municipality of Travnik collected data on body weight, age, frequency and amount of consumption of mint herbal tea infusions. Analysis of infusions of 20 samples of mint herbal tea for Fe content by atomic absorption spectrophotometry (AAS) showed that the average iron concentration was 0.01775 mg / kg. The values of average daily intake of Fe (I_{Fe}) decreased with the age of the surveyed children and were lower than the values of the oral reference dose of iron, therefore the values of hazard coefficients of iron (HQ_{Fe}) in relation to age decreased and were <1. By consuming the analyzed infusions of herbal mint tea, children consume iron necessary for the functioning of their body in quantities that do not have a harmful effect on their health.
1. INTRODUCTION

Herbal infusions, after water, are the most commonly consumed beverage in the world, and it is estimated that 18 to 20 billion cups of tea are consumed daily in the world [1]. This can be attributed to their pleasant taste and smell, but also to their health effects [2]. Thanks to the high content of menthol, which gives a refreshing taste and aroma, mint herbal tea is one of the most commonly consumed herbal teas. In addition to having a refreshing effect, it also has antioxidant, antitumor, anti-inflammatory, antimicrobial and anti-allergic effects [3,4]. It contains a large amount of essential oil characterized by a high percentage of menthol, and alkaloids, carotenoids, flavonoids, polyacetylenes and terpenoids [5], but also trace elements that include essential metals (Fe, Mn, Zn, Cu) that play an important role in the human body, but their excessive and prolonged intake can cause harmful effects on the human body [6]. The presence of non-biogenic trace elements such as Pb and Cd in traces can pose a major health risk [7]. Iron is a heavy metal that is one of the essential elements for the human body, so it plays an important role in traces in maintaining normal functions [8,9]. It is a very important element for the normal development and functioning of the neurological system in children in early preschool age, and as a cofactor of enzymes for the synthesis of serotonin and dopamine, plays an important role in emotional and psychomotor development of preschool children [10]. There are no clear data on how many children of this age have iron deficiency, but according to WHO estimates it is present in most preschool children in developing countries, and 30-40% in developed countries. WHO data from 2001 showed that 30% of children under the age of 4 and 48% of children aged 5-14 are anemic in developing countries [11]. A study conducted on the population of FBiH showed that 18.4% of children under 5 years of age have iron deficiency, and 11.3% of children aged 5-15 years [12].

As early as the 16th century, Paracelsus (1493-1541), considered the founder of modern toxicology, stated that "a dose of a substance makes it poisonous" [13], so that excessive intake of nutrients into the human body can have negative health consequences. Although iron is an essential metal, excessive intake can have various toxic effects on the functioning of the liver, heart, pancreas, and the possible development of diabetes mellitus, hormonal disorders, but also fatalities in children [14,15]. Excessive iron intake in the child's body acts as a catalyst for the reaction of free radicals, which cause damage to cells, tissues and organs [10]. Excess Fe is deposited in the human body, mostly in liver cells, where more than 60% is stored. For children aged 4 to 8 years, the recommended daily intake of iron is 10 mg [16].

2. MATERIALS AND METHODS

For the analysis of iron content, 20 samples in the original packaging of mint herbal tea from different manufacturers were sampled on the free market. Infusions were prepared from them by holding filter bags in 200 ml of deionized water heated to a temperature of 85°C for 5 minutes. After this time, the filter bag was removed and the infusion was allowed to cool to room temperature, after which the iron concentration in each of the 20 prepared infusions was measured by atomic absorption spectrophotometer (AAS) as well as in a blank. Standard solutions of iron concentrations of 0.01, 0.05 and 1 mg/l (standard Fe solution for AAS, Fluka Analytical, 1000 mg/l \pm 4 mg/l) were used for AAS calibration.

An anonymous survey of randomly selected 200 preschool children from the municipality of Travnik, collected data on body weight, age, frequency and amount of consumption of these infusions of herbal mint tea.

The hazard coefficient of iron, as an instrument of quantitative health risk assessment of anonymously surveyed children consuming these infusions of herbal mint tea, was calculated as the ratio of average daily iron intake (I_{Fe}) by consuming these infusions of herbal mint tea and the corresponding oral reference dose for iron (R_fD):

$$HQ_{Fe} = I_{Fe}/R_{f}D$$
 (1)

To calculate the average daily iron intake by consuming infusions of this herbal tea, data obtained from anonymous surveys and analysis of infusions of sampled mint herbal teas for iron content by AAS method are needed, according to the formula:

$$I_{Fe} = \frac{CxC_{r}xE_{f}xE_{d}}{B_{w}xA_{t}}$$
 (mg/kg BW/day) (2)

Where: I_{Fe} -average daily iron intake (mg / kg BW / day); C – average iron concentration in the analyzed samples of mint herbal tea infusions expressed in (mg / kg); C_r – average amount of mint herbal tea infusion consumed (ml / day); E_f - average frequency of consumption of mint herbal tea infusions (days / year); E_d – duration of exposure expressed in years; B_w – average body weight of the surveyed preschool children (kg); A_t – average period of exposure of the organism expressed in days.

3. RESULTS AND DISCUSSION

The results of the analysis of samples of mint herbal tea infusions on iron content, shown by labels from IM1 to IM20, are given in Table 1.

Sample	Concertation Fe (mg/kg)	Sample	Concentration Fe (mg/kg)	
IM1	0,020	IM11	0,022	
IM2	0,001	IM12	0,018	
IM3	0,020	IM13	0,018	
IM4	0,021	IM14	0,018	
IM5	0,025	IM15	0,020	
IM6	0,001	IM16	0,022	
IM7	0,021	IM17	0,019	
IM8	0,001	IM18	0,018	
IM9	0,023	IM19	0,020	
IM10	0,026	IM20	0,021	
Mean=0,01775 mg/kg, SD=0,0075385				

Table 1. Concentration of Fe in analysed samples of herbal mint tea infusions

*IM- infusion of herbal mint tea

Based on the following parameters collected by surveying preschool children and analysis of samples: C = 0.01775 mg/kg, $C_r = 0.2 \text{ ml}$, $E_f = 288 \text{ days/year}$, $E_d = 2$, $B_w = 21.395 \text{ kg}$, $A_t = 730$ (2 years) days, the value of average daily iron intake (I_{Fe}) was calculated by consuming the analyzed samples of mint herbal tea infusions (Table 2).

Table 2. Average daily intake of Fe by consuming herbal min tea infusion with respect to age of surveyed children

Age of surveyed	Average daily intake Fe		
children(years)	(mg/kgBW/day)		
4	2,140x10 ⁻⁴ ±0,0000154		
5	$1,831 x 10^{-4} \pm 0,0000211$		
6	1,587x10 ⁻⁴ ±0,0000221		

Based on the value of the average daily intake and tabular data for the oral reference dose (R_fD) for iron, prescribed by the European Food Safety Authority (EFSA), the value of non-carcinogenic iron hazard coefficient (HQ_{Fe}) was calculated in relation to the age of the surveyed children. (Table 3).

Table 3. Values of oral reference dose for iron and hazard coefficients in relation to the age of the surveyed children

Age of surveyed	RfD	Hazard coefficient Fe	
children (years)	(mg/kg BW/dan)	HQ _{Fe}	
4	5,0	4,281x10 ⁻⁵ ±0,0000031	
5	5,0	$3,662 \times 10^{-5} \pm 0,0000042$	
6	5,0	$3,174 x 10^{-5} \pm 0,0000044$	

Diagram 1 graphically shows the values of iron hazard coefficients by consuming infusions of herbal mint tea, prepared by holding filter bags in water for 5 minutes, compared to the age of the surveyed children.



Diagram 1. Values of iron hazard coefficient with respect to age of surveyed children

4. CONCLUSION

The results of this study showed that the average daily intake of iron (I_{Fe}) by consuming infusions of herbal mint tea decreases with age, because with age body weight and body surface area of children increases. By reducing the average daily intake of iron, the hazard coefficients of iron (HQ_{Fe}) taken by consuming infusions of herbal mint tea also decrease with age. Since the

values of iron hazard coefficients is $(HQ_{Fe})<1$, the values of the average daily intake were less than the oral reference dose of iron (R_fD) , children consume iron necessary for their health from analyzed herbal mint tea infusions and are not in health risk.

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CHEMICAL COMPOSITION OF TRADITIONAL TRAHANA FROM THE UNA -SANA CANTON

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Key words: Trahana, Chemical compotion, Una-Sana canton

ABSTRACT:

Trahana is a traditional fermented product made from wheat flour. After fermentation, the dough is cut into smaller pieces and rubbed by hand through a sieve until a specific granular shape is obtained which is then dried in the sun and then at room temperature. It is usually consumed as a soup. In this paper, chemical analysis of nine trahana samples from the Una-Sana Canton was performed. Next values were obtained: water content ranging from 8.32% (sample A1) to 12.08% (sample A2); pH value of 4.24 (with A4); degree of acidity °SH from 0.68 (sample A8) to 3.43 (sample A2); ash expressed on dry matter from 0.52% (sample A8) to 0.92% (sample A1). The following values were determined by colorimetric color measurement: the lowest L* value was recorded for sample A2 (66.82), and the highest for A5 (81.44); the lowest a* value was determined for sample A8 (1.25), and the highest for sample A2 (3.59). The lowest b^* value was recorded for sample A9 (13.61), and the highest for A2 (24.32).

1. INTRODUCTION

The ethnographic resources of Bosnia and Herzegovina include gastronomy and traditional food production. The richness of gastronomy and gastronomic diversity in Bosnia and Herzegovina is a consequence of its natural geographical features, cultural and historical heritage, customs, habits and traditions of its ethnic groups. Bosnia and Herzegovina has various gastronomic resources of regional and national character and its gastronomy is developing under the influence of East and West. Thanks to the geographical location of the cuisine and history, Bosnian-Herzegovinian cuisine contains elements of Turkish, Greek and Mediterranean, as well as other European cuisines. When talking about traditional Bosnian specialties, tarhana soup should be

singled out in the category of savory dishes [1]. Mehmed Pargan in the book "Old and artistic crafts in Bosnia and Herzegovina" (2011) states that the artisans of Ascia took care of the wealth of different dishes that were created on the basis of the crossing of East and West cultures. Today, throughout Bosnia and Herzegovina, you can find aščinice (public kitchens where oriental dishes are prepared) that inherit the old Bosnian cuisine, and the menu includes dishes such as bey's soup, tarhana, Bosnian pot.

Tarhana has been consumed in Bosnia and Herzegovina since the reign of the Ottoman Empire, and one of the general characteristics of Turkish cuisine is the consumption of soup (broth) at the table. Tarhana was brought by the Turks from Central Asia, and it is also consumed in the Middle East, in Anatolia, Hungary, and Finland [2]. After the establishment of the Ottoman state in the 15th and 16th centuries, tarhana was widely consumed [3]. The first Turkish cookbook, Melceü't-Tabbâhin (The Cook's Refuge), was published in Istanbul in 1844, the fact that the author of the book was a doctor of medicine, Mehmet Kamil, shows that the relationship between nutrition and health was known at that time. In the first part of this book, tarhana is mentioned among Turkish dishes. One of the oldest written recipes for tarhana can be found in Ali Eşref Dede's —Food Bookletl, known to have been written before 1859 [4].

In the Una-Sana Canton, the traditional home-made tarhana soup is called trahana, which is related to the word trati, because it is one of the procedures for making the pasta needed to make this dish. The basic characteristic of making the dough for trahana is that after fermentation, the dough is cut into smaller pieces and manually rubbed through a special solution until a specific granulated form is obtained, which is then dried in the sun, and after drying it is packed in canvas bags.

2. MATERIALS AND METHODS

The paper analyzes samples from a total of 9 trahane producers from the The paper analyzes samples from a total of 9 trahane producers from the Una-Sana Canton. Manufacturers marked with codes A1 and A2 differ from others in that they have industrial equipment for the production of trahana, other manufacturers perform the entire production process manually. The resulting mixture was allowed to ferment for five to seven days at room temperature. In manufacturers A1 and A2, trahana is ground by hand with the addition of flour, dried in electric drying chambers for about 10 hours with the circulation of heated air in the chamber. Other producers perform the trahana production process in households. During the fermentation of the dough, which takes three to four days, depending on the manufacturer, the dough is periodically kneaded every day, then spread on a sieve with the addition of flour. After spreading, the trahana is dried in the sun for two to three days, and then the dried trahana is sifted and stored. Chemical analysis of trahana samples: water content was determined by AOAC method A926.07, pH using pH-meter according to AOAC method 940.23 [5], degree of acidity expressed in ° SH by

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Shulerd method [6], total ash expressed in relation to dry substance by incineration of the sample. Instrumental color measurement was performed with an LCC-A11 colorimeter (LABTRON, United Kigda).



Picture 1: Production of trahana (Foto: Jahic 2022)

3. RESULTS AND DISCUSSION

Table 1 shows the results of chemical analyzes of trachana samples.

Producers	pН		Acidity	°SH	Water cont	ent %	Total As	sh %
	$X \pm SD$	CV %	$X \pm SD$	CV %	$X \pm SD$	CV%	$X\pm SD$	CV%
A1	4.45 ± 0.03	0.697	$2.90\pm0,44$	22.85	8.32 ± 0.72	8.641	$0.92{\pm}0.02$	1.883
A2	$4.30{\pm}0.03$	0.714	3.43±0.66	19.207	12.08 ± 0.58	4.825	$0.60{\pm}0.01$	1,667
A3	4.35 ± 0.02	0.563	2,59±0.23	8.695	10.22 ± 0.35	3.401	0.58 ± 0.04	7.097
A4	4.24 ± 0.02	0.535	2.62 ± 0.65	24.637	10.53 ± 0.37	3.540	0.61±0.02	2.518
A5	4.40 ± 0.05	1.200	2.44 ± 0.43	17.774	10.52 ± 0.38	3.606	0.67 ± 0.01	0.866
A6	4.36 ± 0.05	1.090	2.47 ± 0.30	12.341	10.59 ± 1.76	12.514	0.55 ± 0.01	1.056
A7	$4.36{\pm}0.05$	1.085	2.01±0.43	21.189	9.04 ± 0.46	5.107	0.69±0.01	0.841
A8	$6.4l\pm0.07$	1.100	0.68±0.15	22.341	$8.77{\pm}0.52$	5.894	$0.52{\pm}0.02$	3.846
A9	$4.91{\pm}0.04$	0.850	2.14±0.43	20.177	9.18 ± 0.89	9.656	0.57±0.10	16.736
Average	4.64±0.69	14.877	2.36 ± 0.76	31.961	9.92±1.18	11.900	0.63±0.12	18.899
Average grade interval (α=0,05)	4.19≤µ≤	5.09	1.86≤µ <u>≤</u>	≤2.86	9.15≤µ≤1	0.69	0.55≤μ≤	0.71

Table1. Results	of chemical	analysis of trahana	samples $(n = 18)$
		2	

 $\overline{A1} - A9$ - manufacturer codes, X - mean value, SD - standard deviation, CV - coefficient of variation, μ - mean value in the set, α - risk of error

According to the results shown in Table 1, the evaluation interval of individual parameters of chemical analysis was determined with the risk of error α =0.05. The pH value was determined to be min. pH value 4.19, and max. value 5.09. The lowest value for the degree of acidity (°SH) is 1.86, and the maximum is 2.86. According to the results for water content, min. value is 9.15%, and max. value 10.69%. The lowest value for ash expressed as dry matter is 0.55%, and the max. value 0.71%.

According to Tanguler [7], after the drying process, tarhana contains an average of 6-10% moisture, and the pH value ranges from 3.3 to 5.0. Drying is a critical operation in the production of tarhana. As a result of an improperly performed drying process, product discoloration and poor rehydration may occur. Therefore, if the product dries faster, the better the quality of the rehydration and the shorter the drying operation time [8,9].

Table 2 shows the results of the colorimetric measurement of the color of trachana samples.

					1 ()	
Producers						
	L		a*		b*	
	$X \pm SD$	CV %	$X \pm SD$	CV %	$X \pm SD$	CV%
A1	74.08±3.50	4.725	2.30 ± 0.43	18.645	22.34±0.73	3.247
A2	66.82±82	5.041	3.59±1.11	31.049	24.32±0.94	3.884
A3	80.67±1.49	1.850	1.95 ± 0.61	31.467	15.41±1.17	7.577
A4	80.55±1.59	1.980	1.76 ± 0.50	28.445	15.16±0.54	3.549
A5	81.44±0.99	1,221	1.90 ± 0.39	20.764	14.60±0.53	3.643
A6	79.61±2.34	2.937	1.67 ± 0.46	27.298	15.30±0.73	4.784
A7	72.08±3.19	4.419	2.51±0.33	13.030	16.38±0.53	3.213
A8	78.06±4.28	5.483	1.25 ± 0.27	21.312	13.66±0.90	6.597
A9	79.21±3.17	4.003	2.13±0.53	24.857	19.61±1.27	6.491
Avarage	76.95±4.94	6.415	2.12±0.66	31.285	17.42±3.77	21.615
Avarage grade interval (g=0.05)	73.72µ≤80.18		1.69≤µ≤	<u>-</u> 2.55	14.96≤µ≤	19.88

Table 2. Results of color measurement of trahana samples (n = 18)

A1 - A9 - manufacturer codes, X - mean value, SD - standard deviation, CV - coefficient of variation, μ - mean value in the set, α - risk of error

According to the results shown in Table 2, the rating interval of the color measurement results with the risk of error α =0.05 was determined. For the L parameter, it was determined that the min. value 73.72, and max. value 80.18; for the measured parameter a*, min. is a value of 1.69, and the max. 2.55. For the measured parameter b*, min. is a value of 14.96, and the max. the value is 19.88.

There are different types of tarhana, according to the Turkish Institute for Standardization (TS-2282) the following are defined: flour tarhana, goce tarhana (broken wheat), semolina tarhana and mixed tarhana [10]. The difference between them is in the use of wheat flour, shredded wheat and semolina separately or as a combination in the recipe [11,12]. In addition to wheat flour, wheat flour can be used in the production of tarhana as well as the flour of other types of cereals, as well as the flour of legumes [13].

The shelf life of tarhana is variable, but due to its low moisture content and pH, it can generally be stored for 2-3 years without any signs of spoilage [9,13,14,15].

According to the results shown in Tables 1 and 2, the interval for evaluating the results of color measurements with the risk of error $\alpha = 0.05$ was determined. Nowadays, consumer demands and expectations regarding the specifics of product quality, its properties and characteristics of products bearing the label of a certain quality are increasing, and special importance is given to the branding of agri-food products. Promoting products that have certain characteristics could make a significant contribution to the rural economy. The direct connection of an agricultural product with an area brings added value to that area because it makes it more recognizable and desirable for tourists to visit.

Based on the provisions of the Law on Food, in 2018-2021 the Rulebook on Quality Systems for Food Products [16] was adopted in Bosnia and Herzegovina, which prescribes the procedure for protection of labels guaranteed traditional specialty in Bosnia and Herzegovina and for submitting applications for registration and submitting objections for labels guaranteed to be a traditional specialty of food products at the level of the European Union. The Rulebook on the Appearance and Manner of Using the Trademark of Designation of Origin, Geographical Indication and Guaranteed Traditional Specialty of Food Products [17] prescribes the appearance and manner of using the trademark, geographical indication and guaranteed traditional specialty food products, and the manner of issuing the mark.

4. CONCLUSION

In the international market, there is strong competition in the placement of food products. In this struggle for competitiveness, traditional products are increasingly valued, ie products whose special characteristics derive from the value of their ingredients, methods of production and processing, and the climate from which they come. Greater education on the nutritional and protective properties of food, and increased purchasing power of consumers in the European Union have led to increased demand for food products that are an indispensable part of the culture and traditions of a region. We expect that the results of this work will be useful in creating opportunities for branding traditional products of Una-Sana Canton, including trahana

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CHEMICAL MIGRATIONS FROM POLYETHYLENE TEREPHTHALATE BOTTLES INTO DRINKING WATER

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ABSTRACT:

Packaged drinking water is mostly sold on the market in polyethylene terephthalate (PET) bottles. PET is a very inert polymer. However, under certain conditions, chemicals such as monomers, antimony, 2-aminobenzamide and non-intentionally added substances (NIAS) can migrate from PET bottles to water. The amount of migrants in the water depends on the quality of the PET bottle (raw materials and technology of PET bottle production), the bottling process and the conditions of storage and distribution. Studies, depending on the factor and the intensity of the influence of factors, show variability in the results, and in the case of the influence of the color of PET bottles contradictions. Variability can be attributed to a variety of analytical methods and exposure conditions. Although studies of migration of proven migrants under standard conditions do not show exceeding the prescribed limits, the safety of PET bottles for drinking water should still be under question, because in some cases, the origin of some chemicals found in water packaged in PET bottles is not clearly determined. The paper presents an overview of typical migrating chemicals from virgin PET bottles, influencing factors and toxic effects on the consumer.

1. INTRODUCTION

Bottled water is the fastest growing group of products in the beverage industry, mainly due to the world population growth and the strong marketing of bottled water [1]. Transparency Market Research estimated that the global bottled water market will grow by 6.44% per year between 2017 and 2024 [2]. Share of packaging materials in the European bottled water industry is: PET

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bottles (87.4%), glass (12.4%), aluminum (0.1%) and cardboard (0.1%) [3]. Article 3 of the Regulation (EU) No 1935/2004 on materials and articles intended to come into contact with food requires that materials and articles do not release chemicals into food, react with food, or affect food sensory properties. Annex I of the Regulation (EU) No 10/2011 on plastics food contact articles (FCA) and food contact materials (FCM) is a positive list of substances used in the production of plastic FCA and FCM. The list contains: a) monomers or other starting substances; b) additives, excluding colors; c) polymer production aids excluding solvents; d) macromolecules obtained by microbial fermentation. Plastic FCM and FCA must not release these substances into food in quantities exceeding the specific migration limits (SML, mg/kg) specified in this Annex. For substances for which SML is not listed in Annex I is applicable overall migration limit (OML) of 60 mg/kg or 10 mg/dm² of material contact surface [4,5]. Nonintentionally added substances (NIAS) can also migrate from PET. NIAS are impurities in raw materials, reaction intermediates formed during production, products of decomposition or reactions [6]. The challenge is to identify and evaluate NIAS very harmful properties. Some NIAS remain undetected by any of the analytical methods [7] (Figure 1).



Figure 1. Non-intentionally added substances categories [7].

2. TYPICAL MIGRANTS FROM POLYETHYLENE TEREPHTHALATE BOTTLES

PET is a very inert and most common plastic in the production of water bottles: PET 78.8%; PC (polycarbonate) 12% and HDPE (high density polyethylene) 9.2%[8]. PET bottles are produced in sizes: 0.25; 0.33; 0.5; 1; 1.5; 2; 5; 6 and 25 L. They are trasparent, unbreakable, low weight and recyclable and they retain CO_2 in carbonated beverages. They are 33% easier today than they were 30 years ago [9]. Typical migrants from PET bottles into water are: monomers, antimony, 2-aminobenzamide and NIAS (acetaldehyde, formaldehyde, oligomers ...) [10]. SMLs for PET migrants are given in the table <u>1</u>.

Trastics Regulation (adapted from [11]).				
Substances	Function	Specific migration limit (SML)		
Mono- and diethylene glycol ¹	Monomer	30 mg/kg		
Terephthalic acid	Monomer	7.5 mg/kg		
Isophthalic acid	Monomer	5 mg/kg		
Antimony trioxide	Catalyst	0.04 mg/kg (calculated as Sb)		
Acetaldehyde	NIAS	6 mg/kg		
2-aminobenzamide (anthranilamide)	Additive	0.05 mg/kg		

Table 1. Specific migration limits for typical polyethylene terephthalate migrants in accordance with the Plastics Regulation (adapted from [11]).

2.1 Monomers

According to data available in the scientific literature migration of monomers from PET cannot be exceed SML. Störmer et al. [12] proved it for the migration of monoethylene glycol (MEG) and terephthalic acid (TPA). Diethylene glycol (DEG) migrates negligibly little due to significantly higher molecular weight than MEG. Calculated by arithmetic progression from the determined residual concentration of TPA (30 mg/kg) in PET, the migration of TPA from PET for the worst case was 30 μ g/L (5 μ g/dm) after storage for 10 days at 60 °C. And then it was below the SML of 7.5 mg/L [11,3]. According to Kim and Lee [13] the migration of TPA was below 0.94 μ g/dm after storage for 10 days at 40 °C. Park et al. [14] analyzed the migration of TPA, IPA and terephthalic acid dimethyl ester from 56 PET bottles into water (also into 4% acetic acid, 20% ethanol and n-heptane) for 30 days of storage at 60 °C. Specific migration was in all cases under 0.1 g/L. Adverse effects of IPA and TPA have been demonstrated on experimental animals [15]. Ingestion of MEG causes intoxication and mild gastritis. MEG itself has low toxicity. However, it is metabolised to several toxic metabolites such as glycol aldehyde, glycolic acid, glyoxylic acid and oxalic acid, which can cause acidosis, renal failure and death after a delay of 4 to 12 hours [16].

2.2 Antimony

Antimony trioxide (Sb₂O₃) is a polymerization catalyst that lags behind in PET. Keresztes et al. [17] found residual concentrations of Sb in the walls of PET bottles of Hungarian mineral waters in the range of 210-290 mg/kg. The concentration of Sb released from PET into water after storage of one year was in the range of 0.7-0.8 μ g/L and did not exceed 1 μ g/L even after three years of storage. Even under extreme light and temperature storage conditions, the concentrations of Sb were below 2 μ g/L. Welle and Franz [18] tested 67 PET samples from the European market and concluded that the migration of Sb from PET bottles cannot exceed the

¹Including stearic acid ester with ethylene glycol.

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SML of 40 μ g/L. Maximum migration levels even after three years of storage at room temperature did not exceed 2.5 μ g/L and were, in any case, below the European limit of 5 μ g/L for drinking water. Dogan and Cebi [20] monitored the growth of Sb content in Turkish bottled waters every two months during the year. It was expected to be higher in smaller bottles (0.5 L) than in larger bottles (1.5 and 5 L), but always below European limit. Sunlight affects the migration of Sb less than storage times and temperature [20]. Reimann et al. [21] concluded that the migration of Sb is higher in water in dark than in transparent bottles, and Westerhoff et al. [22] observed 4 times higher migration of Sb in ultrapure water in transparent bottles than in blue colored bottles of the same dimensions at 60 ° C and 10 days. Study of Shotyka et al. [23] on the contrary, did not find the impact color PET bottles on migration of Sb. The concentration of Sb in carbonated water was twice higher than in non-carbonated water, which suggests the influence of CO₂ on the release of Sb [24]. The form in which Sb is present in PET can also affect migration into water. Even when Sb is added as Sb₂O₃, Sb is present as a glycolate complex in the polymerization step because Sb₂O₃ is soluble in ethylene glycol medium [25]. Sb₂O₃ is classified as a possible human carcinogen (Group 2B). Exposure to antimony can lead

 Sb_2O_3 is classified as a possible human carcinogen (Group 2B). Exposure to antimony can lead to nausea, vomiting and diarrhea when exposure to concentrations exceeds the level of maximum concentration in a short period [26].

The tolerable daily intake (TDI) for Sb is 6 g/kg body weight, resulting in a 360 g daily intake for a 60 kg adult person. Consumption of 3 L mineral water from PET bottles with a concentration of 2 μ g/L will result in only 1.7% TDI for an adult. A baby with 5 kg body weight drinking 0.75 L of mineral water from a PET bottle results in only 5% TDI. The migration of Sb from PET bottles to beverages is negligible from the point of view of consumer protection [27].

2.3 Additives

2.3.1 2-Aminobenzamide

The compound 2-aminobenzamide (anthranilamide) is a chemical scavenger of acetaldehyde (AA). A huge excess of 2-aminobenzamide added to PET can itself migrate, due to its small molecules, high diffusion rate and relatively low SML (50 μ g/L). The exceed in mineral water was found in the range of results (26.6 to 55.3 μ g/L) obtained by Franz et al. [28] after 60 days of storage at 40 °C, depending on the size of the bottle and the concentration of 2-aminobenzamide in the wall of the bottle. On the other hand, storage at room temperature at the end of the shelf life results in significantly lower concentrations between 14.1 μ g/L and 25.7 μ g/L. Therefore, in real storage conditions, the migration of 2-aminobenzamide was below SML [27]. This compound irritates the eyes [29]. Poly (m-xylene adipamide) (MXD6) (1% w), oxygen scavenger, can also be used as an AA scavenger even in the presence of anthranilamide [30].

2.3.2 Other additives

Phthalates (plasticizers) are not used in PET material [3]. However, some studies have found phthalates in water bottled in PET bottles. The source of contamination by phthalates can be: atmosphere, aquatic environments, food and beverages, background pollution that may occur during sample preparation. The filling line can contaminate mineral water with di-n-octyl phthalate (DOP) and cup resins with DEHP [31]. Some phthalates are found in water from recycled PET bottles with 20-30% recycled content [32]. Phthalates are known endocrine disruptors that affect lower semen quality, neurodevelopment, childhood asthma, low birth weight, endometriosis, low testosterone, type 2 diabetes and breast/uterine cancer [33].

2.4 Non-intentionally added substances

2.4.1 Acetaldehyde and formaldehyde

Acetaldehyde (AA) and formaldehyde (FA) are formed at high temperatures during the production of PET bottles [34]. Usually, the concentrations of AA in PET bottles are in range from 1 mg/kg (bottles of mineral water) to 10 mg/kg (bottles of soft drinks). The lower concentration of AA in mineral water bottles is due to the use of scavengers. Depaolini et al. [35] find that concentrations of AA in PET bottled water samples on the Italian market are a negligible health risk for consumers. The migration of AA from PET bottles can never exceed SML due to low concentrations of AA in the wall of the PET bottle even assuming total mass transfer of AA. However, the migration of AA into the mineral water can easily exceed the organoleptic threshold because it is low and lies between 10 g/L (retro-nasal) and 25 g/L (orthonasal). In this case, changes in the taste of water, PET bottles do not comply with the sensory requirement of Article 3 of the Regulation 1935/2004. The diffusion of AA and FA is affected by temperature, storage time and carbonization. Dogan and Cebi [19] monitored the migration of AA every two months of the year, which grew with prolonged storage time at 20 ° C. The largest quantities were observed in the smallest PET bottles (0.5 L). All migration outcomes were found to be below the migration limit at the end of the storage period. Studies have confirmed the impact of CO₂ on promoting diffusion of AA into water. Nawrocki et al. [36] and Wegelin et al. [37] gave opposite conclusions about the influence of sunlight on the migration of AA and FA.

The NIAS reaction product of AA and ethylene glycol is 2-methyl-1,3-dioxolane. This product makes AA in PET "invisible" for scavengers. Anthranilamide does not react with 2-methyl-1,3-dioxolane. After migration into mineral water, 2-methyl-1,3-dioxolane regenerates AA. This could be the reason why scavengers of AA like anthranilamide do not reduce the AA concentration in natural mineral water to zero, even if a large amount of scavenger is added in the production of PET preform [11].

FA, as well as AA due to high volatility has a high chance to migrate from PET bottles to water and lead to changes in taste and smell of bottled drinking water, which then makes PET incompatible with Article 3 of the Regulation 1935/2004. According to study of Redžepović et al. [34] in all cases the concentrations of AA and FA were significantly lower than the allowed level of migration from PET packaging. AA is the most dominant among volatile migrants. It has been rated as probably carcinogenic to humans [38]. The level of AA produced during the production of PET bottles can be reduced by controlling the thermal exposure of the polymer [39].

The concentration of FA in PET bottles is usually lower than that of AA, it is usually 1:10. Mutsuga et al. [40] detected levels of AA and FA in PET bottles from Japan, Europe and North America in the ranges: AA of $8.4 - 25.7 \ \mu g/g$; $5.0 - 13.1 \ \mu g/g$; $9.1 - 18.7 \ \mu g/g$, and FA of $0.8 - 3.0 \ \mu g/g$; $0.5 \ \mu g/g - 1.2 \ \mu g/g$. The highest levels of FA and AA in Japanese bottles are attributed to differences in formulations and packaging production. FA is also a chemical of concern. Some studies suggest that long-term occupational exposure to FA is associated with certain types of cancer such as leukemia and is classified as carcinogenic to humans. Some people are very sensitive to FA, while others are not [26].

2.4.2 Oligomers

PET oligomers are cyclic oligomers (dimer to nonamer) and linear [41]. Oligomers are NIAS with a relatively low migration limit of 50 g/L and high concentrations in the wall of the PET bottle, especially PET cyclic trimmer [27]. The highest concentrations of PET oligomers were found for the PET cyclic trimer with 2922 mg/kg, followed by the cyclic tetramer and pentamer with 749 mg/kg and 303 mg/kg, respectively. The cyclic trimer migrates and represents 98% of all surface oligomers in PET [26]. Cyclic dimers are sensitive to hydrolysis, and it is generally assumed that cyclic oligomers (classified as Cramer III in the toxicity class) are transformed into linear ones, which are less toxic (Cramer I toxicity class). A recent study showed that linear oligomers dominate the human body after digestion. PET oligomers were evaluated for their associated In silico toxicity of Cramer III and showed no warning of genotoxicity [42]. The migration limit of 50 g/L has been exceeded for high temperature applications [28]. When PET was heated to a temperature above 260°C, the increase in alicyclic oligomers was between 5 and 20 times higher than the cyclic oligomers [13].

Due to the high molecular weight of PET oligomers, specific migration at room temperature conditions is negligible, but may increase at high temperatures, e.g. cooking or baking in the microwave, which is not the case with consuming bottled water [43].

2.4.3 Other non-intentionally added substances

Dattilo et al. [30] found seven NIAS migrants in low concentrations of which three benzene, styrene and furan were classified as CMR² or potentially CMR, and 2-methyl-1,3-dioxolane, acetone, butanone, and limonene are not classified as CMR substances.

Bisphenol A (BPA) is not used in the production of PET bottles, but several studies have confirmed the presence of BPA, a proven endocrine disruptor [38], in water packaged in PET. Low concentrations (up to 4 ng/L) of BPA were observed in PET bottled water before and after sun exposure for 15 and 30 days [44]. Toyo'oka and Oshige [45] found in bottles from Japan the concentrations of BPA from 3 ng/ L to 10 ng/L, which remained the same before and after heating PET bottles at 50° C for 8 h, which means that PET material cannot be incriminated as source of BPA. It has been suggested that the water itself may be contaminated before filling, the source of BPA in PET water bottles may be cups.

Analysis of 23 metals in PET bottles was performed by Westerhoff et al. [22]. The highest concentrations were found of Co (27 mg/kg), Cr (0.11 mg/kg), Fe (1.3 mg/kg) and Mn (0.34 mg/kg), respectively. The relatively low levels of concentrations of metals observed in the PET polymer material relative to Sb, explain why researchers have not found significant traces of these metals in bottled water in studies on the migration of these metals into bottled drinking water. According to Reimann et al. [21] more metal is leached from glass (Ce, Pb, Al and Zr) than from PET bottles.

3. CONCLUSION

Migration studies of typical migrants from non-recycled bottles under normal storage conditions until the end of the shelf life show very low diffusivity of migrants and minimized risk of migration from packaging to water. The exceed of the organoleptic threshold is possible during the migration of acetaldehyde into mineral water.

Manufacturers of PET bottles, producers of bottled water, distributors, as well as consumers themselves should know all the factors that initiate and/or intensify migration in order to control these processes for greater safety for consumers.

The safety of drinking water bottled in PET bottles is questionable by some research that has identified some NIAS substances found in bottled drinking water (phthalates, BPA) whose origin in water has not been clearly established.

All this suggests that further researches on migration due to NIAS substances, controversy in the results of some studies on the impact of some factors on migration, accumulation of migrants in the body (cumulative effect) and their synergistic effect in a mixture with chemicals from other sources are needed to reduce uncertainty.

²CMR - carcinogenic, mutagenic and reprotoxic.

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COMPARATIVE CADMIUM CORRELATED GENE EXPRESSION IN DOMESTIC VS. HYBRID KALE VARIETIES FROM BOSNIA AND HERZEGOVINA

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Key words: Gene expression, heavy metals, kales, phytoremediation, RT-PCR

ABSTRACT:

Kales (Brassica oleracea L. var. acephala D.C), are ancient Mediterranean vegetables, known for their nutritional values, and very popular cultivars in the Herzegovina region, the southern part of Bosnia and Herzegovina. Kales are known as heavy metal accumulators, but some are very tolerant to heavy metals as well. To evaluate and confirm the expression levels of several cadmium heavy metal correlated genes (HMA2/4, YSL3, FP3/6 and ATX1), the Real-Time PCR was used. The results showed linear gene expression with increasing cadmium concentration. The lowest gene expression was observed in domestics kales, whereas in hybrid kales, the gene expression was significantly higher.

These results indicate a better ability of domestic kale varieties to tolerate CdCl2 than hybrid kales. Based on the obtained data, we can conclude that domestic Herzegovina kales are better candidates for human consumption, whereas the hybrids show exceptional cadmium absorption abilities, indicating their possible role in phytoremediation processes.

1. INTRODUCTION

Cadmium is a common heavy metal, as a pollutant extremely toxic due to its solubility in water [1]. Found in the industry chain production of fertilizers, alloys, detergents, batteries, petroleum, and pigments [2]. For humans, by the International Agency for Research on Cancer and the US National Toxicology Program, cadmium compounds are considered as cancerogenic elements [3]. In plants, it disturbs photosynthesis, several enzyme activities, sugar metabolism, decreases water uptake and nutrients and therefore inhibits plant growth rate [4].

In order to remove cadmium from the soil, *Brassica oleracea* can be used. Brassicae species are known as metal accumulators and have been evaluated as potential phytoextraction plants [5]. Several Brassicae species can accumulate relatively high amounts of toxic metals without visible symptoms. In addition to the fact that they are used in the human diet very often as food crops, and have been proven to accumulate heavy metals, that leads to potential contamination of the food chain and endangering of human health. On the contrary, these facts are desirable plant characteristics in process of any phytoremediation [6]. Brassica species are well-known phytoextractors, mainly due to their intrinsic tolerance to heavy metals and considerable above-ground biomass production [7].

According to the study conducted by Šutković et al., 2016, HMA₂ and HMA₄ genes are essential heavy metal accumulators, involved in Zn/Cd homeostasis, and among others, showing very strong interaction with Farnesylated protein 3 (FP₃), Farnesylated protein 6 (FP₆)/HIPP₂₆, Mitogen-activated protein kinase 2 (MAPK₂), Metal-nicotianamine transporter (YSL₃) and Anti-Oxidant 1 (ATX₁) gene [8].

During the evolution process, most of the plants have developed various cellular and molecular adaptation mechanisms that are required to tolerate and survive various heavy metal stresses. A set of different genes contributes to heavy metal hyper-accumulation which are mostly expressed in the hyper-accumulator plants, compared with non-hyper accumulators [9]. Heavy metal accumulator (HMA) protein family, such as HMA₂, HMA₃, and HMA₄ that ensure heavy metal tolerance to plants and show overexpression during heavy metal stress [10], [11]. The Yellow Stripe-like (YSL) family protein (different name: metal-nicotianamine transporter) belongs to the oligopeptide transporter family, initially characterized in Arabidopsis thaliana. Multiple YSL genes are found in diverse plant species, including monocots, dicots, gymnosperms, ferns, and mosses, having in a total of 8 members [12]. In general, the metal-nicotianamine transporter (YSL₃) gene has been involved in the root-to-shoot transport of heavy metals. The Histone-lysine N-methyltransferase (ATX₁) protein, belongs to the family of metallochaperone molecules that escort copper to distinct intracellular targets [13]. ATX proteins control root growth by regulating cell production, cell cycle duration, and the transition from cell proliferation in the root apical meristem (RAM) to cell elongation [14]. ATX₁ protein is involved in defence against oxidative stress by relating to copper metallochaperones which helps copper to reach its destination [15]. FP₃ (farnesylated protein 3) and FP₆ (farnesylated protein 6) proteins in Arabidopsis thaliana are known to function as ubiquitous proteins, as in other diverse plants, both having a heavy metal-binding domain (HMA) and an additional isoprenylation motif on Cterminus [16]. Furthermore, in Brassica oleracea FP3 and FP6 proteins interact with acyl-CoAbinding protein 2 (ACBP₂), a mediate cadmium Cd (II) tolerance protein, suggesting its role in Cadmium homeostasis [8]. The Arabidopsis thaliana HIPP family (HIPP₂₂, HIPP₂₆, and HIPP₂₇) are shown to be expressed during Cd treatment [17], [18].

2. MATERIAL AND METHODS

2.1. Plant growth and root assay analysis under cadmium treatment

In the study, three domestic kale varieties (marked as kale 1, 6 and 9) and one hybrid kale (BB) was used, as seen in table 1.

No	Regions (Country, city, village)	Geographical coordinates
1	BiH, Mostar, Blagaj	43°25 North, 17°88 East
6	BiH, Stolac, Dubrave	44°82 North, 18°57 East
9	BiH, Stolac, Rivine	43°53 North, 17°53 East
В	Netherland sort (Brassica oleracea L. Italica group - Bonanza F1)	Netherland

Table 1: Kale varieties used in this study

Brassica oleracea var. acephala seedlings were grown using Tap-of-paper method [19]. Seeds were treated with different concentrations of Cd (control – distillated water, 50, 100, 200, and 500 μ M of CdCl₂) and incubated for 5 days at 27 °C in growth chamber where they were exposed to light for 16 hours a day. When plants reached their sufficient growth, root length was measured. After the root length measurement, the values were entered for statistical analysis. Descriptive statistical analyses of root length results were performed using GraphPad Prism 18.3.0. All samples were stored at -80 °C prior to RNA isolation.

2.2. Primer design

Primer design for the annotated genes in *Brassica oleracea*, as shown in table 2, were done with an incorporated NCBI server tool, called PRIMER-BLAST [20]. The selected primers were chosen upon the following parameters: min annealing temperature of 57 °C, maximum of 62 °C and optimum of 60 °C, 50 to 60 % of GC, and the primers should be 18-22 bp in length.

Genes	Sequences
Ubiquitin 2 (UBQ2)	F - ATATTCGTGAAGACGCTG
	R - CTCAACTGGTTGCTGTG
Metal-nicotianamine transporter 3 (YSL3)	F - TTCCTGGGGAATCATGTGGC
	R - CCCGTCTCCGAGGATCAATG
Heavy Metal Associated protein 2 (HMA2)	F - AAGGTCCAACGGCTATGGTG
	R - CTCGTCTTGGTCTGGTTGCT
Farnesylated protein 3 (FP3)	F - CGGACTGGAAAACATGCTGC
	R - TGCGGGTAAGCATAAGACGG
Farnesylated protein 6 (FP6/HIPP26)	F - TACGGGACACCACCTTGTTG
	R - GAAGATGGACTGCGAAGGCT
Anti-Oxidant 1 (ATX1)	F - TTAGGCCTTGGCGGTTTCAG
	R - CGCTGTTTTGCAGACCGTAT

Table 2: Oligonucleotides used in the present research

2.3. RNA isolation and cDNA Synthesis

The total RNA isolation was performed based on a standard CTAB protocol [21], but slightly optimized. New optimized procedure was based on homogenization tissue in CTAB extraction buffer and selective precipitation of RNA with LiCl. RNA concentration and purity were determined using Thermo Scientific µDrop Plate and RNA integrity was confirmed by 1 % agarose gel. Isolated RNA was stored at -80 °C. The presence of contaminating genomic DNA can give false positives in qPCR, therefore the isolated RNA was treated with DNAse prior to cDNA synthesis. DNAse I (RNAse-free) kit from Thermo Fisher Scientific was used according to manufacturer's instructions. Complementary DNA (cDNA) is generated from an RNA template by reverse transcription using SCRIPT cDNA Synthesis Kit from Jena Bioscience according to manufacturer's instructions. qPCR amplification was performed on the Step One Plus system by Applied Biosystems®.

2.4. Gene expression analysis

The qPCR reaction was prepared using the Maxima SYBR Green/ROX qPCR Master Mix by Thermo ScientificTM according to manufacturer's instructions. qPCR thermal cycle was adjusted under the following conditions: PCR initial activation step 95 °C for 0 min, followed by 35 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. Ubiquitin 2 (UBQ2) is used as a house keeping gene. To validate the specificity of the reaction by checking for primer-dimers or nonspecific amplification, a melt curve analysis was performed. Data are presented graphically where Y-axis in graphs represents $2^{-\Delta\Delta Ct}$, which analyzes the relative changes in gene expression.

2.5. Statistical analysis

Root length data were presented graphically as mean + standard deviation. Significant difference between each treatment was accepted if p < 0.05, calculated and presented with GraphPad Prism 18.3.0 statistical software. Relative fold expression levels were determined using the $2^{-\Delta\Delta Ct}$ method. Relative gene expression levels were obtained by dividing the extrapolated transcript levels of the target genes by the levels of the internal control.

3. RESULTS

In Picture 1 we present the average root length in domestic and hybrid sorts with standard deviations. As it can be observed in Picture. 1, the longest roots are in Kale 1, followed by 6, 9, and B. For both Kale 1 and 9, the root length drops at 50 μ M, rise to 100 μ M, and then drops until 500 μ M. For kale 6, however, it rises at 50, and then again at 200 μ M. In hybrid sort, by increasing Cd concentrations, the root length drops.

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Picture 1: Average root length of domestic and hybrid sorts

Real-time PCR was used to determine the relative expression of genes involved in Cadmium accumulation, with a minimum of 2 to 4 replications. However, with some genes, we had 7 to 8 replicates. Additionally, results whose melt curve generated a single peak were used, which means that in the reaction only one product (gene of interest) was produced.

To validate the specificity of the reaction by checking for primer-dimers or nonspecific amplification, a melt curve analysis was performed. Data are presented graphically where Y-axis in graphs represents $2^{-\Delta\Delta Ct}$, which analyzes the relative changes in gene expression.

In the following figures, we represent the expression levels of the genes analysed.



Picture 2: HMA2 and YSL3 expression levels for kales 1, 6, 9, and B

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Picture 3: ATX1 and FP3 expression levels for kales 1, 6, 9 and B



Picture 4: HIPP₂₆ expression levels for kales 1, 6, 9 and B

As shown in pictures 2-4, we observe different gene expression patterns with increasing Cd concentration. In HMA₂, YSL₃, and HIPP₂₆, in general, we observe a linear expression profile with increasing Cd concentration in comparison to control. Cadmium stress-induced approximately 4 to 8-fold changes in HMA₂ expression, but a lower change in expression is observed in YSL₃, from 1.24 to 7.5-fold up-regulation. However, in ATX₁ and FP₃ the fold expression does not show linear expression. Only ATX₁ in kale 9 shows a linear increase of expression in comparison to control, from 0.5 in 50 μ M to 7.7 in 500 μ M Cd concentration fold change, where the expression of kale 1 and kale B is irregular and in kale 6 it shows down-regulation. In general, in kale 6 we observe the lowest gene expression in comparison to other kales, where even HMA₂ expression is observed to be lowest. In picture 3, the FP₃ expression profile is nonlinear as well. Kale 1 and kale 9 show over-expression of the gene, by increasing

the Cd concentration (from 0.98 to 2.9-fold). In kale 6 the FP_3 is down expressed in all Cd concentrations.

4. **DISCUSSION**

One of the initial goals of this study was to analyze the domestic kale varieties on their ability to sustain cadmium stress and to find the most tolerant and most accumulative kale varieties.

Cadmium, as one of the most toxic heavy metals, raised in the recent year's many concerns as it is found in natural soil, limiting crop yield and threatening food safety worldwide [22]–[24]. The genome of *Brassica Oleracea* was published first in 2016 [25] and its metabolism pathways regulating heavy metal stress remain still unknown. Although, its genomic sequence is very similar to *A. thaliana* and *Brassica Napus*, where especially the HMA genes show more than 90% similarity with *Brassica Oleracea* [8], the major genes regulating metabolism activity during heavy metal stress are not yet understood. Therefore, in comparison to *Arabidopsis thaliana* and *Brassica Rapa*, a homology-based approach was implemented to annotate similar genes in *B. Oleracea*. Through this approach, we selected and annotated the major genes, currently known to be involved in Cd accumulation (Table 2). Using an *in-silico* approach, besides the most common genes, two potential new genes (ATX₁ and YSL₃) are proposed to be involved in cadmium stress regulation, as shown and previously supported by Šutković *et al.*, 2016. A key mechanism in the protection and survival of plants under heavy metal stress is regulation of its gene expression [26].

The domestic kale varieties were collected from the Herzegovina region for a good reason. This region is known to have a high concentration of heavy metals, especially contaminated with cadmium, a very common heavy metal acoriding to Sefo et al., 2010 [27]. Sefo showed that Cd in the soil of Herzegovina exceeds limited values and therefore, being without visible symptoms, these plants could have high accumulating capabilities or are simply tolerant to cadmium and other heavy metal impacts. Kales are the most popular vegetables in Herzegovina, used as a major food supplement in human nutrition but also as livestock food (older leaves of the plant). It is grown in small production areas, in gardens, and mostly from its seed production. Given its nutritional and dietary value, kales are rich in free sugars, organic acids, lipids, and minerals [28].

Based on the results of root assay analysis we can conclude that the domestic varieties show much higher tolerance, expressed in root length, during $CdCl_2$ stress. In Picture 1, we can see that in kale 1, root length for control, 50 and 100 was about 5x higher than in 200 and 500. In kale 6 and 9 the values were similar; futhermore, in kale the values rised at 50 and 200, whereas in kale 9 values rised at 100 and then drop. But in kale B, values keep decreasing with the increase of Cd concentration. Because the root is in direct contact with the heavy metal, they are

affected more than other parts [29]. Other studies also showed that the root length was higher at smaller concentrations [30]. This phenomenon is known as hormesis which is a "overcompensation" response to a disruption in homeostasis and it is considered to be a general toxic effect of various chemical substances [31].

To see the expression levels of the target genes, those already know to be involved in Cd homeostasis, and those predicted to be correlated to cadmium stress, we performed the RT-PCR. HMA (heavy metal accumulator) 2 and 4, FP₃ (farnesylated protein 3) and FP₆/HIPP₂₆ (farnesylated protein 6), Yellow Stripe-like 3 (YSL₃) and histone-lysine N-methyltransferase (ATX₁) were checked for their expression levels in comparison to control (non-treated plants, only grown with distilled H₂O under the same light and temperature conditions).

As shown in the introductory part, heavy metal accumulator (HMA) protein family members, HMA₂ and HMA₄ ensure the heavy metal tolerance to plants, showing overexpression during heavy metal stress [10], [11]. Translocation of cadmium, along with Zn is regulated by HMA₂, as shown in A. thaliana, barley, rice, and wheat, and other plant organisms. HMA2 expression is closely correlated to the expression of HMA4, which controls the process of hyper-accumulation of metals, both being up-regulated during heavy metal stress [32]. HMA₂ in our study was used as control gene for additional verification of heavy metal effects on plant metabolism. According to our results, as shown in Picture. 2. HMA₂ is increasing its expression with increasing Cd concentration linearly. Similar results were shown in *A.thaliana*, where HMA₂ regulates the homeostasis of heavy metal by transporting Zn and Cd metal ions to the plant stem [33]. Further, a genome-wide study showed that HMA₂ from Brassica napus is strongly correlated to cadmium expression [34]. Similar findings were confirmed beforehand in 2016, based on a homology search, HMA₂ gene from *Brassica rapa* shared more than 95% similarity with *B. oleracea* [8]. In Brassica rapa L. ssp. Chinensis the HMA₂ expression was 16-fold more in comparison to their control, showing significantly higher expression in comparison to Herzegovina domestic species with 9.4-fold up-regulation in 500 µM concentration and 3.6-fold increase in hybrid kale.

A significantly smaller fold increase is seen hybrid, being highest at 200 μ M CdCl₂ concentration, with a 1.7-fold increase. These results represent the first expression analysis of HMA₂ gene for *Brassica oleracea L. var. acephala D.C.* and are in line with previous research data done in other plants [10], [34]–[36].

The Yellow Stripe-like (YSL) family protein (different name: metal-nicotianamine transporter) belongs to the oligopeptide transporter (OPT) family, initially characterized in *A. thaliana* [12]. OPT proteins belong to a small gene family in plants, which includes 17 in Arabidopsis [57], 27 in rice [37], 20 in Populus, and 18 in Vitis [38] and 9 predicted OPT proteins in *Brassica oleracea*, found in NCBI main page, accessed at date 7th February 2020 [39]. Oligopeptide

transporters (OPTs) are membrane-localized proteins, contribute to several biological processes, mainly in different transport mechanisms [40].

OPT protein family is known to be involved in different processes: nitrogen mobilization, glutathione transport heavy metal sequestration and long-distance metal distribution [37], [41]–[45]. The YSL proteins are involved in direct root-to-shoot transport of heavy metals [13], controlling the remobilization of Fe, Zn, and Cu in the form of metal–NA chelates in *Arabidopsis thaliana* [46].

In Picture 2, based on the gene expression results we obtained, it's observable that all kales show linear expression growth in folds by increasing the cadmium concentration. The domestic kale 9 shows a significant 7.5-fold increase in comparison to control, in 500 μ M concentration; having 4.3-fold higher expressions than domestic kale 6; and 2.4-fold higher expression than hybrid kale B in 500 μ M concentrations. Domestic varieties have significantly higher expression fold in 100 μ M and 500 μ M concentrations, when compared with hybrid ones, whereas in 50 μ M and 200 μ M concentration there is no significant fold change in expression. These results indicate and confirm a strong involvement of YSL₃ proteins in Cd homeostasis, as shown in similar studies [13], [29], [47], [46].

In 1997, Lin *et al.* showed that ATX_1 protein belongs to the family of metallochaperone molecules, escorting copper to distinct intracellular targets [48]. ATX_1 protein is involved in defense against oxidative stress by relating to copper metallochaperones, which helps copper to reach its destination [15]. Later on, in addition to copper ions, ATX_1 is confirmed to transport iron ions [49], [50].

ATX1 also acts as a chromatin modifier so regulating the expression of several genes (Ding, 2009). ATX1 protein controls root growth by regulating cell production, cell cycle duration, and the transition from cell proliferation in the root apical meristem to cell elongation [14].

In Picture 3, kale 9 has the highest gene expression levels of ATX₁ with increasing amounts of Cd, showing a 7.7-fold increase in comparison to control at the 500 μ M Cd concentration. This is the first study the analyses the ATX₁ expression level under cadmium stress in *Brassica Oleracea*. Currently, only in *Solanum torvum* plant, the ATX₁-Like protein expression was analyzed in correlation to Cd stress, where the ATX₁ expression after 1 day of treatment under 100 μ M Cd concentrations was down-regulated [51]. However, in other organisms, the ATX₁ homolog from *Saccharomyces cerevisiae* is directly up-regulated due to reactive oxygen toxicity caused by Cd stress [52]. Furthermore, there is a significant linear increase of ATX₁ expression with the increase of Cd concentration within hybrid at 50 μ M when compared to other concentrations. The only significant change of fold expression of ATX₁ in domestic kales is seen in 100 μ M Cd concentrations, with a 3.98-fold change in comparison to control. However, the expression levels ATX₁ in kale 1 and B were irregular, but in kale 6 it shows significant down-regulation, as seen in *Solanum torvum* [51].

A. thaliana FP_3 (farnesylated protein 3) and $FP_6/HIPP_{26}$ (farnesylated protein 6) with a heavy metal-binding domain (HMA) and an additional isoprenylation motif on C-terminus function as ubiquitous proteins [16].

FP₃ and FP₆ are shown to be up-regulated in *Arabidopsis thaliana* during Cd and Zn stress [57] and as well in *Brassica Juncea* [53].

Besides, through an *in-silico* study done on *Brassica oleracea*, FP₃ and FP₆ proteins interact with acyl-CoA-binding protein 2 (ACBP₂), a mediate cadmium Cd (II) tolerance protein, suggesting and confirming its role in cadmium homeostasis in this plant [8].

In our study, as seen in picture 3, the FP₃ expression profile shows a nonlinear pattern. Kale 1 and 9 shows linear up-regulation FP₃ by increasing the Cd concentration (from 0.98 to 2.9-fold), whereas kale B show down regulation of the FP₃ gene and kale 6 slightly downregulation. The FP3 expression in the domestic varieties and hybrid kale variety show a slightly different pattern, where domestic kales show a linear growth of gene expression, with a small decrease of 0.4-fold in 500 μ M Cd in kale 6. These results are in line with data published in 2010, where a 100 μ M CdCl₂ treatment was undertaken, lasting for 3 days, in the plant *Solanum torvum Sw. cv. Torubamubiga* [51]. The hybrid, on the contrary, shows a statistically significant 4.4-fold gene expression increase at 50 μ M Cd stress, and then a huge decrease of gene expression to 1.5 fold at 500 μ M concentration. Similar results were not reported before.

In Picture 4, in domestic kale 6, the HIPP₂₆ showed a significant 1.7-fold increase at the concentration of 100, but with the increase of Cd concentration, the gene expression dropped to 0.7-fold. In general, the HIPP₂₆ is down-regulated in domestic kales with a 0.86-fold drop in gene expression and significantly up-regulated in hybrid kales with a 2.6-fold increase in expression. It already reported that the transcription of many HIPPs is altered in response to heavy metal stresses [54]. In a study done on yeast, HIPP₂₆ and HIPP₂₇ proteins conferred Cd-resistant to a Cd-sensitive strain [17]. In addition, [55] showed that HIPP₂₆ can bind Cd²⁺, Cu^{2+,} and Pb²⁺ and over-expressing HIPP₂₆ in *A. thaliana*, enhancing tolerance to Cd²⁺. However, in *A.* thalliana and Noccaeacae rulescens a down-regulation is reported for HIPP26 under Cd stress [56]. This phenomenon might be explained due to the fact that the cadmium ions were combined with Zn, Ni, and C, or with Cupper and Palladium, where HIPP26 reacted differently. However, in our study we use a different concentration of CdCl₂ only combined with distilled water, so excluding the possibility that other heavy metals could affect the HIPP₂₆ expression under different Cd concentrations.

5. CONCLUSION

Analyzing the effects of cadmium chloride on the root growth in all kale varieties, we concluded a significant cadmium impact on reducing root growth in all varieties, with the least impact on the domestic variety kale 6. For the first time, an analysis of the expression of cadmium-related genes in *Brassica* oleracea L. var. acephala DC was performed. Based only on the root assay results, we can conclude that the domestic kale varieties show tolerant behavior to cadmium stress, as seen in kale 6, verifying the adaptive mechanisms of Brassica to cadmium stress, whereas the hybrid kale varieties showed significantly lower root length compared to the domestic ones.

In essence, the expression level of HMA₂, YSL₃, HIPP₂₆ genes were in a linear expression pattern with increasing the Cd concentration, with exceptions of ATX_1 and FP₃, whereas a clear down-regulation of expression is an observed in domestic varieties.

It's seen that in kale 6 all genes showed lower expression rate in compression to other domestic and hybrid kales, and even a down-regulation of FP₃, ATX₂, and HIPP₂₆ genes, demonstrating the ability of this particular kale to be the most tolerant to cadmium stress. This is clearly in line with the root assay analysis, where kale 6 shows significantly the longest roots under all concentrations of cadmium treatment. This data could suggest that kale 6 is the best candidate in the food diet for the people in the Herzegovina region. Through the gene expression analysis, kale 9 is shown to have on average a 4.8-fold increase in gene expression (in 500 μ M CdCl₂ concentrations) in comparison to control, whereas kale 6 shows the lowest increase with a 1.6fold increase of expression. Therefore, it is recommended that kale 9 should be avoided as a vegetable in the human diet, as it is shown to be one of the major accumulators of cadmium in this study, indicating its potential use in phytoremediation.

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FIVE-YEAR EVALUATION OF PATIENTS WHO UNDERWENT THYROID SURGERY BASED ON PHD FINDINGS

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Key words: hyroid gland, hyroid cancer, PHD finding, perative tretman

ABSTRACT:

Diagnosis of nodal changes in thyroid gland is based on hormonal evaluation, ultrasonic diagnostics, thyroid scintigraphy and cytologic puncture. Based on volume or localization, relation with surrounding organs and how nodes are scintigraphically displayed, cytological punctuation is indicated. It gives important information about further therapy and type of surgical approach.

Aim: Evaluate all operated patients with changes in thyroid gland during the period from 2013 to 2018 at the Cantonal Hospital "Dr. Irfan Ljubijankić "Bihać and get information on the number and type of tumors operated based on pathohistological findings (PHD).

Materials and methods: The conducted study was a retrospective, descriptive clinical study which included 208 patients who underwent surgery of the thyroid gland from 2013 to 2018. Patients are divided according to PHD diagnosis, age, gender and geographical affiliation. For calculating significance of frequency differences and procedural representation we used Chi square test and for samples where the number of expected values is lower then 5, we used Fisher test.

Results: Our research included 208 patients, 84% women and 16% men. Prevalence of women in each Una-Sana Canton municipality and in each age category is predominant. 15.5% of patients had thyroid cancer and 176 patients or 84.5% had benign thyroid changes. No significant difference was found in the representation of individual forms of thyroid cancer based on gender distribution. An exception is colloidal struma, which has been shown to be significantly more common in women than in men.

Women in our population are significantly more likely to suffer from all thyroid diseases. There is no difference in the prevalence of individual forms of thyroid disease in relation to the gender of patients. An exception is colloidal struma, which is significantly more common in women than in men. This article is also a sort of evaluation of the current state of health care in the field of thyroid diseases, since it is the first work of this kind in the Una-Sana Canton and can be used in the future as a starting point for monitoring and improving the treatment of these diseases.

1. INTRODUCTION

The thyroid gland is located at the front of the neck, just below the cricoid cartilage, and consists of two lobes connected to the bridge (isthmus). Its follicular cells produce two major hormones, tetrajodtironin (thyroxin T4) and triiodothyronine (T3). After binding to receptors in the nucleus, these hormones act on cells of almost all tissues, altering the expression of a range of protein and enzyme gene products. They are necessary for the normal development of the brain and somatic tissues of the fetus and newborn, and at each age monitor the metabolism of proteins, carbohydrates and fats. In addition, parafollicular cells (C cells) secrete calcitonin, which is released upon hypercalcemia and lowers calcium levels. All the reactions necessary for the synthesis and release of thyroid hormones are controlled by TSH, which is secreted by thyrotropic pituitary cells. TSH secretion is controlled by negative feedback.

Thyroid gland pathology includes a large number of diseases, congenital anomalies, inflammatory diseases, which include, more and more often, autoimmune diseases, malignant tumors like anaplastic thyroid cancer, as one of the most malignant and invasive tumors in human pathology [1].

The incidence of thyroid nodules is 50% of the total population [2]. The most common benign changes in the thyroid gland are multinode strums and thyroid adenomas. Only 4% of all thyroid nodes are malignant [3]. The most common malignant thyroid tumor is papillary cancer (85%) [4]. It's followed by follicular carcinoma, anaplastic carcinoma and medullary carcinoma. For further diagnosis of thyroid nodules, we also use FNA - fine needle aspiration [5]. The thyroid scintigram is obtained with a gamma camera after giving a radioisotopes (123I, 131I, or 99mTc pertechnetat) with the creation of a graphical representation of isotope accumulation [6]. Places of increased (warm) or decreased (cold) accumulations make the detection of cancer possible. Thyroid carcinomas are found in <1% of hot nodes as opposed to 10–20% of cold nodes [7].

2. RESEARCH OBJECTIVES

Evaluation of all operated patients with changes on thyroid who were treated at the Cantonal Hospital "Dr. Irfan Ljubijankić" Bihać in the period from 2013 to 2018 and get the informations about number and type of operated tumors based on PHD result which was obtained after

conducted operative therapy. In addition, it's necessary to analyze representation of thyroid tumors in particular municipalities of Una-Sana Canton and their representation according to gender and age structure of patients.

3. METHODS AND MATERIALS

Conducted study is retrospective, descriptive clinical study with one group of respondents. The research included 206 patients of both genders (175 females and 33 males) and age between 37 and 88 years who were exposed to surgical procedure of thyroid in Cantonal Hospital "Dr. Irfan Ljubijankić" Bihać in period from 2013 to 2018. Examined patients were hospitalized under diagnosis od thyroid tumor confirmed based on clinical picture, laboratory findings, thyroid ultrasound and puncture of suspicious changes.

Besides laboratory biochemical tests, one of the primary diagnostic tests in pathology of thyroid is ultrasound. After we did ultrasound of the thyriod, we detected, nodule changes and marked them based on BTA U-classification, we then followed recommendations for further processing [8]. According to ATA (American Thyroid Association) recommendations for further processing of suspicious thyroid nodels include their size, ultrasound characteristics and clinical findings [2].

The results were processed using Microsoft Office Excel 2007 and SPSS version 24 (IBM). For calculating significance of frequency differences and procedural representation we used Chi square test and for samples where the number of expected values is lower then 5, we used Fisher test.

4. **RESULTS**

Our study included 208 patients who were exposed to surgical procedure, of which 33 (16%) were males and 175 (84%) were females. Their were included in research based on pathohistological report. Of the total 206 operated patients, 32 of them or 15,4% had diagnosis of thyroid cancer and 176 patients or 84,6% had benign changes of thyroid diagnosed.

4.1. Follicular thyroid adenoma

From the total number of patients and with the pathohistological analysis of operative material, 27 patiens or 13% had thyroid adenoma. Of which were diagnosed 8 thyroid adenoma in the municipality of Sanski Most (30%), 3 patients (11%) in Bosanska Krupa, 4 cases in Cazin (15%), 6 in Bihać (22%), 1 in Bužim (4%) and 5 in Velika Kladuša (18%).

Based on gender distribution it has been found that 14,28% of the total patients had follicular thyroid adenoma of which 6,1% were males. No significant difference was found in the incidence of follicular adenoma based on gender distribution, p = 0.317.



Chart 1: Age structure of patients with thyroid follicular adenoma

4.2. Lymphomatous thyroid struma

Of the 208 patients enrolled in the study, lymphomatous strums were proven in 16 patients or 8%. Based on the gender distribution, 8.57% of the total number of female patients had lymphomatous thyroid strum, or 3.03% of male patients. No significant difference was found in the incidence of lymphomatous struma based on gender distribution, p = 0.459.



Chart 2: Number of patients with lymphomatous struma in the Una-Sana Canton by municipalities

4.3. Colloid thyroid struma

From the total number of patients, 133 patients or 64% had a pathohistological diagnosis of colloidal thyroid struma. From that number, 25% of them were in municipality of Cazin, 32% in Bihać, 22% in Velika Kladuša, 5% in Sanski Most, 8% in Bosanska Krupa, 5% in Bužim, 2% in Ključ and 1% in municipality of Bosanski Petrovac.



Chart 3: Age structure of patients with colloid struma in the Una-Sana Canton

From 133 patients with colloid thyroid struma, 123 of them were females with 10 male patients. Colloid thyroid strum had 70,28% female and 30,3% male patients of total observed patients. A significant difference in frequency was found based on gender distribution at p < 0.001.

4.4. Follicular carcinoma of the thyroid gland

Of the total number of patients included in the study, 8 or 4% had follicular thyroid cancer. Of these, 1 patient in Bosanska Krupa (or 12%), 2 in Cazin (25%), 1 in Bihac (12%), 1 in Ključ (13%) and 3 (38%) patients in Velika Kladuša. 50% of patients with thyroid follicular cancer belonged to the age group of 61-70 years.

4.5. Papillary thyroid cancer

Of the total number of proven thyroid tumor changes, 21 patients or 10% of patients hadpapillary carcinoma of the thyroid gland. Of these, 1 patient in Bosanska Krupa (5%), 4 patients in Cazin (19%), 9 in Bihać (43%), 1 in Bužim (5%) and 6 in Velika Kladuša (28%). Graph 4. and 5. show the territorial and gender structure of patients with papillary thyroid cancer.



Chart 4: Number of patients with papillary thyroid cancer in the Una-Sana Canton area by municipalities



Chart 5: One third of the subjects with papillary thyroid cancer were between the ages of 50 and 60, with another 33% over the age of 60.

Of the 21 patients with proven papillary cancer of thyroid gland, 19 (90%) were female patients, with another 2 (10%) male patients. Overall, of the total observed patients, papillary carcinoma was proven in 10.8% of female patients and 6.06% male patients. No significant difference was found based on gender distribution, p = 0.6065.

4.6. Medullary thyroid carcinoma

In the area of Una-Sana Canton, of the total number of patients (208), two medullary thyroid carcinomas were proven in the study period, one patient in municipality of Cazin and one in Bužim. Regarding the age structure, one patient belonged to the age group 21-30 and other one to the 41-50.

4.7. Anaplastic thyroid carcinoma

Anaplastic thyroid cancer was found in one patient in municipality of Cazin. The estimated incidence of this tumor in Una-Sana Canton is less than 1% of all thyroid tumors found. The patient was over 71 years old.

5. DISCUSSION

The incidence of thyroid cancer has increased in the last five years in the United States. Siegel and el al. conducted a study which showed that there was a 1 percent increase in the 5 years they conducted the study [9].

With regard to specific thyroid tumors, the best prognosis is for papillary and follicular thyroid cancer if they are treated on time, the prognosis is worse as the rate of dedifferentiation increases so that the total rate of survival for anaplastic thyroid cancer is only about 2 percent [10].

Fortunately, the most common thyroid gland tumor diagnosed in the world is well-differentiated papillary thyroid cancer, which is easily treated. This is confirmed by our study in which we proved that papillary thyroid cancer is the most common thyroid tumor in the Una-Sana Canton.

In the last twenty years, 80% of patients with newly diagnosed thyroid cancer smaller than 2 cm have been successfully treated. This indicates the importance of early recognition and treatment of thyroid tumors [11].

In our study, the average age of the patients was more than 60 years and the prognosis of the disease outcome is worse in a given period of life, which is discussed in the research conducted by Gilliand et alwhere they proved that best age for treatment of thyroid gland is 45. Total survival rate in such cases was 96-98%. This finding indicates the need to introduce screening procedures for the early diagnosis of thyroid gland tumors to increase the survival rate in the Una-Sana Canton area as well [12].

In a study conducted by Rahbari et al. thyroid gland diseases has generally been shown to be almost three times more common in women than men [13]. As the degree of tumor differentiation increases, or as opportunity of successful treatment decreases, so does the gender representation of the tumor equals. The results of our study are in line with the earlier research. Of the total number of patients included in the study, 84% were women and 16% were men.

We did not find a similar study that looked at representation of each form of thyroid tumors in the Una-Sana Canton area or any region similar to Canton in population.

In our study, we did not prove that there was a significant difference in representation of individual thyroid diseases based on gender distribution. Only, a significant difference in incidence was found based on gender distribution in patients with colloid struma.

In this study, we evaluated the representation of indvidual thyroid tumors in the Una-Sana Canton and their prevalence in individual Canton municipalities. In this way, we have created a sort of registry that can be used as a reference research in the future, by which each next research can be compared.

6. CONCLUSION

Our research confirms the results of studies conducted in Europe and the US that show that thyroid disease is much more common in women than in men. We have proven that our patients are, on average, older than patients in the world who are surgically treated for thyroid cancer. This is explained by the still poorly developed health care sistem and indicates the need to introduce screening and other methods for early identification and treatment, since long-term prognosis is directly dependent on the degree of disease progression at the time of diagnosis.

This article is also a kind of evaluation of the current state of health care sistem in the field of thyroid pathology, since it is the first such study in the Una-Sana Canton and can be used as a starting point for monitoring and improving the treatment of these diseases in the future.

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EFFECTS OF MELATONIN ON MORPHOLOGICAL CHARACTERISTICS IN MELISSA OFFICINALIS L. AND VALERIANA OFFICINALIS L. UNDER HEAVY METAL INDUCED STRESS

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ABSTRACT:

Heavy metals, due to the impossibility of their decomposition, represent a serious environmental and nutritional problem. The accumulation of essential and non-essential heavy metals in living organisms reduces normal growth and development, causing acute poisoning, disease and even death of organisms. Melatonin, a hormone, has recently been shown to be an important multifunctional molecule in protecting plants from oxidative stress because of its ability to directly neutralize reactive oxygen species.

The response of lemon balm and valerian (germinated in water or 0,1 mM melatonin) to increased concentrations of Zn and Cd, as well as the changes in the chloroplast pigments of plants during abiotic stress were determined.

In contrast to lemon balm, significantly higher concentrations of chlorophyll a, b and carotenoids were obtained in valerian leaves treated with exogenous melatonin (1.73, 0.59 and 1.12 mg/g FW) compared to control leaves (1.14, 0.35 and 0.72 mg/g FW). Cadmium treatment had a negative effect on all morphometric characteristics of both plant species. This negative effect is reduced in plants treated with melatonin, which confirms a positive effect of melatonin in the growth and development of plant species, especially under the influence of chemical stressors.

1. INTRODUCTION

To date, numerous publications have been published on the presence of melatonin in plants and plant products with a wide range of concentrations, from a few picograms to a few micrograms per gram of tissue [1, 2].

Melatonin concentrations vary not only among plant species but also among varieties of the same species, and depend on growth rate, location, specific plant organ, as well as harvest time and season [3, 4]. Melatonin has so far been isolated from algae and from more than 20 mono- and dicotyledon flowering families.

Heavy metals are transition metals with a density over 5 g/mL, and show marked environmental toxicity [5]. They are found in air, water, food, and soil. Certain heavy metals such as iron, cobalt, copper, zinc, and manganese are necessary for many functions, normal growth and development of living organisms and are labeled as essential. The recent results supported the theory that melatonin protects plants from stress conditions induced by oxidative stress at the cellular level, especially in valerian, by elevating the activity of antioxidative enzymes in first developmental phases of the plant [6]. Melatonin might be a receptor-independent free-radical scavenger and a broad-spectrum antioxidant; or it can stimulate antioxidant enzymes or augment the activities of other antioxidants to protect plant tissues from oxidative damage [7].

2. MATERIAL AND METHODS

The experiment was set up in field conditions by the split-plot method in three replications. The selected plants were treated with heavy metals, cadmium and zinc, and melatonin as a pre-treatment. The concentrations were determined according to the Rulebook on Maximum Permitted Quantities for Certain Contaminants in Food [8]. The soil treatments shown in Table 1 were used.

Treatment label	Treatment	Concentration (mg/L)
1	Control	_
2	Cd (CdSO ₄ solution)	15
3	Zn (ZnSO ₄ solution)	3000
4	Melatonin 0,1 mM	23,23
5	Melatonin and Cd	23,23 i 15
6	Melatonin and Zn	23,23 i 3000

Table 1. Soil treatments and labels

Lemon balm seeds are sown in germination pots, covered with only a thin layer of fine soil, and watered regularly with water and aqueous solution of melatonin, concentration 100 μ M. Unlike lemon balm, valerian seedlings were obtained in seedlings from a local herbal collector. One part

of seedlings was kept in water and the other in an aqueous solution of melatonin (100 μ M), 48h, in the dark, after which the plants were planted in a permanent place. Sampling of lemon balm plants was done in nice and dry weather, before flowering. Leaf samples were taken from the last fully developed leaf. The root samples were first well washed with water, dried, and chopped for further analysis. The following parameters were measured:

- 1. Plant height,
- 2. Diameter of plants,
- 3. Biomass of fresh plants, roots and stems,
- 4. Biomass of dried plants, and
- 5. Root development (Fig. 1).



Figure1. Sampling and morphological parameters measuring

2.1. Determination of chloroplast pigments

The process of extraction and determination of pigments was performed quickly, in the dark due to the photosensitivity of the pigments. Leaf sample (0,1 g) was weighed and transferred to a mortar. About half a teaspoon of quartz sand, MgCO₃ powder (to neutralize the acidity), and 10 mL of acetone were added to the sample. After maceration, the mixture was quantitatively transferred with acetone to a test tube in a vacuum flask. The filtrate was quantitatively transferred to a 25 mL volumetric flask. The absorbance was measured at 662, 644 and 440 nm with acetone as a blank. The pigments concentration was presented as mg/L [9].

3. RESULTS AND DISCUSSION

Numerous studies prove that toxic concentrations of heavy metals contribute to changes in the morphological characteristics of plant species, leading to slow growth, reduction of plant diameter and height, and reduction of plant organ biomass [10]. The influence of exogenous melatonin and accumulation of heavy metals on morphometric and some physiological parameters of examined plant species are shown in Figures 2, 3, and 4.

Under the influence of zinc and cadmium, there was a statistically significant decrease in the height of valerian, while in lemon balm a significant reduction is observed only in plants treated

with cadmium ions. Exogenous melatonin in valerian did not improve the growth of this plant, but in lemon balm an increase in height is observed. Also, in plants treated with heavy metals and pre-treated with melatonin, there is no negative effect of metals on plant growth, where the same height was measured in both plant species in the case of cadmium treatment or even more in the case of zinc treatment. This phenomenon can be explained by the fact that clear signs of this element excess occur when its concentration in the dry matter exceeds 300 to 5000 mg/kg. In such cases, the plants have lower growth, reduction of the root system, the formation of small leaves and necrosis. Same results were obtained measuring the plants diameter.



Figure2. Average values of lemon balm (A) and valerian (B) height, diameter, and root development (cm)

Changes in lemon balm biomass indicate that both heavy metals have an inhibitory effect, although there are statistically significant differences only in Cd-treated plants compared to the control. Exogenous melatonin alone had no effect on the mass of fresh balm plants. However, treatment with Cd in plants pre-treated with melatonin had a smaller effect on the reduction of biomass, which confirms the effect of melatonin in protecting plant cells from stress. Lemon balm root biomass is reduced most after treatment with heavy metals compared to stem biomass.

Exposure of valerian plants to Cd ions did not cause a significant reduction in biomass compared to the control. In contrast, Zn had a stimulating effect on biomass and caused a statistically significant increase in fresh mass compared to control. Exogenous melatonin alone had no effect on the mass of fresh valerian plants, and it does not seem to influence in improving the resistance of plants to the action of heavy metal ions. The obtained results of valerian root and stem biomass are similar.

Root development was also measured from the morphological characteristics in this experiment. Increasing the length of the main and lateral roots improves the availability of water and nutrients. Lemon balm did not show statistically significant changes in root development during treatment with Cd or Zn compared to the control. Similarly, no statistically significant differences occur in plants treated with increased concentrations of Cd and Zn with melatonin pre-treatment. However, treatment with exogenous melatonin has a mild stimulating effect on root development in relation to control.



Figure3. Average values of fresh and dry lemon balm mass, fresh root, and stem mass (g)

Valerian shows slightly different results. Namely, Cd has an inhibitory effect on the root development of this plant, while Zn again did not cause statistically significant differences compared to the control, but the differences occur in relation to plants treated with Cd ions. Exogenous melatonin had a mild stimulating effect on this morphological characteristic compared to the control, but also increased the resistance of Cd-treated plants in terms of increasing root development.



Figure4. Average values of fresh and dry valerian mass, fresh root, and stem mass (g)

Bihać, 09 - 10 June 2022.

One of the first experiments on the effect of melatonin on some morphological characteristics of plants was in 2000, where it was shown that in tissues melatonin interacts with auxin in the control and production of roots and shoots of St. John's wort, *Hypericum perforatum* L. [11].

Recently, papers have emerged that reveal that lower concentrations of melatonin have a stimulating effect on the root as observed in St. John's wort, wild mustard leaves, cherry root and wolfberry. In all studies, the ability of melatonin to improve lateral root growth multiplies the action of IAA. Lower amounts of melatonin are more effective inducers of root growth than any higher dose tested [12, 13].

According to the conducted research, transgenic rice plants (*Oryza sativa* CV. Dongjin) rich in melatonin showed higher biomass, but also delayed flowering and yield [14].

The positive effects of melatonin are often enhanced under stressful conditions, when the plant adapts to changes in the environment, which further suggests the importance of melatonin as a mediator of behavior. The allegations are confirmed by recent research in which it was found that in tomatoes (*S. Lycopesicum*), the promotion of lateral roots by melatonin is mediated by nitric oxide signaling, although auxin action mediated by melatonin has also been shown [15]. Therefore, auxin-dependent, and auxin-independent melatonin is likely to be involved in modulating root growth [16].

The parameters of photosynthesis are significantly related to the content of chloroplast pigments, primarily chlorophyll a, b and carotenoids. Heavy metals are known to interfere with photosynthesis by inhibiting chlorophyll biosynthesis and disrupting the structure of thylakoid membranes.

Decreases in chlorophyll content may be due to decreased chlorophyll synthesis due to enzyme activity, replacement of Mg with heavy metals in the chlorophyll structure, reduction of essential metal sources involved in chlorophyll synthesis (eg Fe²⁺ and Zn²⁺), chloroplast membrane structure impairment, size, and synthesis of chlorophyll [17]. The average concentration of chlorophyll a and the standard error in lemon balm is 1.60 ± 0.052 , chlorophyll b 0.54 ± 0.022 , and carotenoids 1.05 ± 0.044 mg / g FW (Fig5).

Elevated cadmium concentrations affected the reduction of chlorophyll a from 1.74 to 1.61 mg/g FW, as well as carotenoids from 1.21 to 0.96 mg/g FW in lemon balm leaves. The content of chlorophyll b cadmium had no effect. Treatment of plants with zinc did not affect significant changes in the content of chloroplast pigments compared to the control. Interestingly, plants treated with melatonin in contaminated soil with cadmium and zinc show similar values of plant hormones with control, which indicates the possibility of melatonin in the defense of plants against the harmful effects of heavy metals.

The average concentration and standard error of chlorophyll a in valerian is 1.36 ± 0.083 , chlorophyll b 0.47 ± 0.035 , and carotenoid 0.90 ± 0.054 mg/g FW (Fig6).

In contrast to lemon balm, significantly higher concentrations of chlorophyll a, b and carotenoids were obtained in valerian leaves treated with exogenous melatonin (1.73, 0.59 and 1.12 mg/g FW) compared to control leaves (1.14, 0.35 and 0.72 mg/g FW).



Figure5. Average values of Chlorophyl A, Chlorophyl B, and Carotenoid concentration in lemon balm (mg/g)

Also, heavy metal treatment had a similar stimulant effect. It is assumed that the applied concentration of heavy metals was lower than the concentration that could affect the reduction of plant pigments. On the other hand, this plant species is considered a weed and as such more easily withstands the imposed conditions of oxidative stress.



Figure6. Average values of Chlorophyl A, Chlorophyl B, and Carotenoid concentration in valerian (mg/g)

Melatonin can act as an antioxidant against free radicals produced during plant photosynthesis. Greater melatonin degradation can therefore occur in older leaves, which contain higher amounts of chlorophyll and a higher degree of photosynthesis [18].

Examining the effect of exogenous melatonin on the aging of perennial rye leaves (*Lolium perenne* L.) in the dark, Zhang and coworkers found that melatonin-treated leaves retain their green color longer compared to controls at eight days of treatment. It has increased the concentration of endogenous melatonin, which can directly suppress dark-induced leaf aging [19].

4. CONCLUSION

Exogenous application of melatonin had a positive effect on the morphological characteristics of lemon balm, increasing the height, diameter of the plant, and root development, while in valerian, the positive effect of melatonin is reflected in increasing plant weight. As expected, cadmium treatment had an extremely negative impact on all morphometric characteristics of both plant species. The entry of Cd through the Ca channel into plant leaves reduces water utilization, causing stoma closure in many plant species and leading to decreased transpiration rate and inhibition of photosynthesis by adverse effects on chlorophyll metabolism, resulting in growth inhibition and nutrient imbalances. The negative effect of cadmium is reduced in plants treated with melatonin, which confirms the hypothesis of a positive effect of melatonin in the growth and development of plant species, especially under the influence of chemical stressors. Zinc, on the other hand, did not cause statistically significant changes in the parameters mentioned in relation to control plants. The observed reduced values of most morphometric parameters can

also be attributed to the lack of bioavailable zinc in the soil medium. Exogenous melatonin is a potential plant biostimulator, in prolonging the life of seeds after exposure to stress and slowing down the aging of leaves after flowering plants. Therefore, its application in agronomy could result in a good, feasible and inexpensive method of preventing stress and improving plant growth and development under different conditions.

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GERMAN WORDS IN THE CONCEPTUAL FIELD: FOOD AND HEALTH -LINGUISTIC ANALYSIS

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ABSTRACT:

Ecolinguistics, or the ecology of language, emerged as a new paradigm of linguistic research in the 1990s within sociolinguistics.

Just as ecology studies the interaction of living organisms with each other, so the ecology of language studies the influence and interaction between languages and between languages and their environment and the society in which they are located.

In this paper, the influence of the German language on the Bosnian language and the resulting semantic adaptation of words of German origin are discussed, with special focus on the conceptual field of "food and health". The aim of this work is to analyze the adaptation processes and to describe what changes a foreign German word has undergone from the moment of "borrowing" to the formation of the basic form - which in linguistics is called a replica - and what changes occur in a replica from the moment of integration onwards in the system of the recipient's language, the Bosnian language.

Additionally, this paper points out the representation of German words in the field of "food and health" and their frequency in the Bosnian language.

1. INTRODUCTION

In order to analyse the frequency of Germanisms in the Bosnian language, a corpus of over a thousand Germanisms was created. The corpus consists of collected Germanisms within the work *Germanisms in the press of the Bosnian Krajina*, where Germanisms were excerpted from

the most widely read newspapers in the Bosnian-speaking area of the Bosnian Krajina: *Dnevni* Avaz, Krajina, Moja Sana and Reprezent.

In this paper, the focus will be set on the analysis of the semantic adaptation of Germanisms from the conceptual field of "food and health".

The theoretical basis of this paper is primarily the study of Rudolf Filipović *Languages in Contact*, ie. his terminology and model of language borrowing-

According to Uriel Weinreich, Filipović points out three basic elements of language borrowing, from which every language research is based: the place of contact, the bilingual person and the consequences of using more than one language. Filipović points out that *the languages in contact* are those that are alternately used by the same speaker. He calls this person *bilingual*, this phenomenon *bilingualism* and the consequence of this phenomenon *interference*.

When two language systems come into contact with each other, words are taken from one language to another. Languages involved in this process are specified as the *giver language* and the *recipient language*.

The element ronounced by the speakers of the giver language is called the *model*, and the borrowed element that is pronounced by the speakers of the recipient language is a *replic*.

When taking lexemes from the language of the giver to the language of the recipient, in our case from German to Bosnian, certain changes occur at different levels.

2. TAKING AND ADAPTING FOREIGN WORDS TO ANOTHER LANGUAGE SYSTEM

When taking foreign words into another language, a process of adjustment or adaptation occurs. This process is necessary since words are often taken in a phonological, orthographic or morphological form that does not correspond to the recipient's language system.

The word changes as much as it takes to fit better in the new system. According to R. Filipović, the categories at which changes may or may not occur are: phonological, morphological, semantic and lexical (Filipović 1986).

Adaptation is a long-term process, and its final form undergoes primary and secondary changes according to which it is divided into primary and secondary adaptation.

The task of the analysis of adaptation process is to describe what changes the foreign word has undergone in the primary phase, occuring from the moment of borrowing to the formation of the final form - which in contact linguistics is called a replica, while secondary changes occur on the replica from the moment of integration into the recipient's language system.

Different language levels also require different approaches to the problem. Theorists of contact linguistics agree, that the loanword must first go through the process of forming a phonological form. Since, the phonological systems of the two languages never completely coincide, the phonological elements of the giver language o the native ones that are most similar in

articulatory-acoustic properties. The problem of a phonological mismatch is solved by omitting or inserting domestic phonological elements.

Phonological adaptation is always followed by morphological adaptation. At the time of transition to a new language system, each foreign word brings morphological characteristics, of the type of word to which it belongs, with it. As morphological systems are different, the giver must harmonize and adapt its morphological features to the rules of the recipient's language. This may, but does not have to, reshape the basic shape of the loanword.

The adaptation of Germanisms at the semantic level is specific in that it belongs to the realm of the human mind, as opposed to the phonological and morphological adaptation that can be characterized as mechanical adaptation (Talanga 2002).

3. PROCESSING THE ANALYSIS OF THE SEMANTIC ADAPTATION PROCESS OF GERMANISMS

In this paper, the emphasis is placed on the analysis of the process of semantic adaptation of Germanisms in the Bosnian language, both in the primary and secondary phase. Germanisms that are frequent from the conceptual field of "food and health" have been singled out from the corpus. These are: *beštek, birtija, brezle ili brizle, buhtle, bruh, celer, cinkova mast, dinstati, ementaler, escajg, fasung, faširati, fil, filovati, flaster, flaša, flašica, flaširanje, flek (fleka), fras, frišak, frištik, gemišt, geršla, giht, griz, haringa, kifla, knedla, košpica, krigla, krofna, krompir, kuga, kuglof, mileram, pakung, pleh, princes krofne, puter, rendgen, rerna, ribati, roštilj, saft, sala, senf, supa, šampita, šarlah, šerpa, šlag, šmekati, šnicla, šnita, šolja, špajz, špica, šprica, šporet, štanglica, čokolade, štapić, štruca, šunka, vaga, vekna.*

In this analysis, we will adhere to the principles and methods of Rudolf Filipović, ie. Hope's system of division, which Filipović accepted in a modified form. In his methodology, Filipović puts emphasis on the separation of the adaptation process into two phases and states "

"By applying primary and secondary adaptation at the semantic level, we have obtained a new division that meets the needs of our analysis of semantic changes and changes in the meaning of loanwords" (Filipović: 1986: 161).

As at other levels, Filipović uses a three-part classification.

Comparing the meaning of the model word and the replica, Filipović classifies changes into three degrees of adaptation: zero semantic extension, narrowing of meaning and expansion of meaning. This division is based on the distinction between primary and secondary adaptation.

Primary adaptation includes: zero semantic extension (no difference in meaning between model and replica), narrowing of meaning in number (change from multiple meanings to one meaning) and narrowing of meaning in meaning field (change from general meaning to special meaning).

Secondary adaptation includes expanding the number of meanings and expanding the field of meaning. In the secondary adaptation, changes related to metaphor, metonymy, pejoration and ellipse occur (Sočanac 1992).

3.1. Zero semantic extension

Zero semantic extension occurs when the meaning of the German loanword (replica) remains unchanged and fully corresponds to the original meaning of the German model after entering the Bosnian language system. Filipović marks those changes *transfer of meaning*. This is especially true of terms whose meanings are precisely defined. These are, for example, professional titles from various fields

Usually, these terms belong to specialized areas suach as health, food and drink, technology, construction, sports, agriculture and others, as well as names of members of various movements, teachings and directions or nouns that denote the bearer of a trait or composition. By taking over these terms and their expressions, their meaning is retained. Analyzing selected Germanisms from our corpus in the field "food and health", it is evident that in most cases these arem models belong to the word type with one or few meanings and only one specific meaning is transmitted. Rarely, multiple meanings of a single word are transferred to the recipient's language. Therefore, the *zero semantic extension* group can be further divided into two groups.

> The first group consists of models with one assumed meaning, such as in our corpus:

 $birtija < Wirtshaus^3$ (inn), brizle < Briesel (roast lamb or veal breasts), bruh < Bruch (hernia), buhtla < Buchtel (homemade and baked puff pastry), cinkova mast < Zinksalbe (ointment containing zinc oxide), ementaler < Emmentaler (hard cheese), flaša < Flasche (bottle), geršl < Gerste (barley dish), giht < Gicht (a disease that affects the tissue around small joints), $gris < Grie\beta$ (coarsely ground wheat), haringa < Hering (fish species), kifla < Kipfel (crescent-shaped pastries), knedla < Knödel (round shaped and possibly stuffed potato dough), krigla < Krügel (a glass from which to drink beer), kuga < Koge (plague), kuglof < Gugelhupf (kind of cake), pakung < Packung (type of hair treatment), rendgen < Röntgen(gerät) (X-ray body imaging device), šarlah < Scharlach (scarlet fever, contagious childhood disease) šprica < Spritze(accessories in the form of a glass cylinder with a piston and a hollow needle for injecting drugs). It should be noted in particular that the models of our corpus, which also belong to this group, are often compound words composed of two words. Compound models in our corpus belong to

³ The dictionaries used in translating Germanisms into Bosnian are: *Dictionary of Bosnian* by Ibrahim Čedić et al., *Dictionary of Bosnian* by a group of authors Senahid Halilović, Ibrahim Palić and Amela Šehović and the *Great Dictionary of Foreign Words* by Klaić Bratoljub.

the *Determinativkompositum type*, where each word within a compound has its own meaning and function in the formation of the compound. The second part of the compound defines the morphosyntax and semantics of the whole word, while the first part has the function of modifying the semantics of the second part of the compound.

In the models of our corpus it is evident, that this was not recognized when taking German compounds into Bosnian and the whole compounds were transferred and functioned as a whole into the basic form. Therefore, they adapted as such in phonetic and morphological level aswell and fully integrated into the bosnian language. In the conceptual field of food, one example of a compound model with one assumed meaning is *escajg* < *Esszeug* (pribor za jelo).

> The second group consists of models with two or more assumed meanings:

pleh < *Blech* (lim; baking tray),

flaster < *Pflaster* (medically self-adhesive to protect the injured area; concrete pavers;

transferred meaning: one who clings to whom)

flek < *Fleck* (stain and darkened spot; shadow on the lungs; patch, eg on shoes),

cug < Zug (train; chess move combined with iz -iz cuga means at once),

špic < *Spitze* (top; the peak of the season; the core of the stone fruit; type of dog)

3.2. Narrowing of meaning

Narrowing of meaning is the most common occurrence in the semantic adaptation of foreign words. Comparing Germanisms in Bosnian and German, we can conclude that words have more than one meaning in German. In the process of semantic adaptation to the Bosnian language, most of the meaning was retained, which was necessary due to the naming of a specific term or object. It represents the specialization of many meanings of the model into one specific meaning. This general tendency is called *narrowing of meaning*.

This concept can refer to the narrowing of the number of meanings, which is a very common occurrence. Additionally, some loanwords, show a-further narrowing in the field of meaning. It is a double narrowing: in number of meanings and in the semantic field.

The corpus of this research confirms the tendency to narrow the model in number and convey one specific meaning of the model in the following examples:

beštek \leq Besteck (cutlery, Duden 1/3)⁴

⁴ The first number indicates which meaning is recorded in the *Duden Universalwörterbuch dictionary*, and the second number indicates how many meanings the dictionary lists under the entry of that model. This data is relevant in order to notice a narrowing in the number. All meanings in German are taken from the above dictionary and the translations are authorial.

The meaning of the word *das Besteck* in German: 1. cutlery (*Essbesteck*) 2. A set of instruments composed for a specific medical purpose (*für einen bestimmten medizinischen Zweck zusammengestellter Satz von Instrumenten*) 3. position of a ship at sea (*Ortsbestimmung eines Schiffes auf See*)

fasung < Fassung (food tracking, Duden 6b/6)

The meaning of the word *die Fassung* in German: 1a. edge - often artistically made - for the purpose of attaching an object (*der Befestigung eines Gegenstands in etwas dienende, oft kunstvoll ausgearbeitete Umrandung, Einfassung*) 1b. a hoop used to capture and collect water, eg on a well (*dem Auffangen, Sammeln von Wasser* (*besonders eines Brunnens*) *dienende [ausgemauerte] Umrandung*) 1c. Screw or clamp holder (*Haltevorrichtung zum Festschrauben oder Festklemmen*) 2. linguistic form; formulation (*sprachliche Form, Ausformung; Formulierung*) 3. color painting or gilding of wood or stone sculpture 4. self-control (*Selbstbeherrschung*) 5. the grasping (das Ergreifen) 6. large quantity (*Ladung - größere Menge*) fil < Füllung (stuffing, mixture Duden 2a/3)

The meaning of the word *die Füllung* in German: 1. filling (*das* Füllen) 2a. mixture, which is added to a particular dish for enrichment (*Masse, die zur Anreicherung in bestimmte Speisen hineingefüllt wird*) 2b. dental filling (*Masse, die den Hohlraum in einem Zahn nach dem Ausbohren ausfüllt*) 2c. material in mattresses, quilts, pillows

(Material in Matratzen, Federbetten, Kissen) 3. door panel (Türfüllung)

rerna < Röhre (oven, the part of the stove where groceries are baked, Duden 3/6)

The meaning of the word *die Röhre* in German: 1. long cylindrical hollow body [with smaller diameter], mainly used for transporting gases or liquids (*langer zylindrischer Hohlkörper [mit geringerem Durchmesser], der vor allem dazu dient, Gase oder Flüssigkeiten weiterzuleiten*) 2. a small tubular vessel (*kleiner röhrenförmiger Behälter*) 3. baking and frying tube (*Back-, Bratröhre*) 4a. Electronic tubes, in particular radio or television tubes (*Elektronenröhre, besonders Radio- oder Fernsehröhre*) 4b. Fluorescent tube, neon tube (*Leucht[stoff]röhre, Neonröhre*) 5. Screen, TV (*Bildschirm, Fernsehgerät*) 6. underground passage of a tubular building (*röhrenförmiger unterirdischer Gang eines* Baus)

Narrowing of meaning in the field of meaning occurs very rarely. In fact, it is difficult to determine exactly how much the field of meaning decreases or narrows. These are examples that have already undergone a narrowing in number and show further narrowing in the field of meaning. Examples of narrowing in the field of meaning in our corpus are the models *šolja*, *brile*, *gemišt*.

For example, the replica *šolja* < *Schale* in German has, according to Duden, eleven meanings. Among other things, it denotes *a hemispherical bowl (zdjelu oblika polukugle)*. In the Bosnian language, the word is not used in that sense. The field of meaning has been narrowed and the word is used in the sense of "a small vessel with a handle on the side from which coffee or tea is drunk". In order to confirm this emphasize this diminution, the diminutive of the word cup *(šoljica)* is often used. In German, a word with this meaning is not used.

3.3. Extension of meaning

In the secondary phase, the integrated foreign word may retain its meaning in the language of the recipient, or acquire additional meanings that do not exist in the language of the giver, therefore undergoing an extension of meaning. In this phase, the loanword loses precision, and gains in breadth of meaning (Filipović 1986: 169-170). Two basic conditions are required for the extension of meaning: complete phonological, morphological and semantic integration of the loanword into the system of the recipient's language and its free use within that language. Therefore, the expansion of meaning appears exclusive in the- secondary adaptation. Secondary semantic adaptation includes expanding the number of meanings and expanding the field of meaning. Starting from Hope's definition, Filipović lists four main types of semantic changes a) metaphor (similarity according to the meaning), b) metonymy (interconnection according to the meaning), c) folk etymology (similarity according to the form), e) ellipsis (interconnection according to the form). (Filipović 1985: 179)

3.3.1. Metaphor

To illustrate the influence of metaphor, we can use the example of germanism šlauf < Schlauch. In the primary phase of the adaptation, two meanings from the German language were transferred: "hose" and "swimming belt". The second meaning served as a basis for expanding the meaning, ie. creating a new metaphorical meaning of the word šlauf in the sense of "layers of fat around the waist". In the Bosnian language, the loanword šlauf is used with all three different meanings. The first two meanings were transferred in the primary adaptation and became frequently used in the Bosnian language, and with their productivity in the Bosnian language system a new metaphorical meaning was created.

In addition to the possibility of gaining a metaphorical meaning, the word can remain of neutral meaning or acquire a pejorative nuance. Mostly with the use of metaphors, pejorization follows. In our corpus, the germanism *šlauf* in the sense of "layers of fat around the waist" is used in a pejorative sense in most cases.

3.3.2. Metonymy

The A loanword whose meaning has expanded, among other things, and under the influence of the factor of has undergone metonymy is, for example, the Germanism *štapići*, with the meaning *sticks* (salty snacks oblonged shape).

The German loanword *štap* < *Stab* was taken into Bosnian with the meaning "piston" or "aid for work or walking". In this form and with these meanings, the word *štap* becomes active and subjected to change at different levels as any domestic word. By adding the suffix -ić, which forms a diminutive in the Bosnian language, a new word *štapić* (noun in the singular) was formed. By adding the suffix -i which forms the plural of sticks - *štapići* in the sense of "elongated snacks of small sticks". The term *štapići* (small sticks) is used in various compounds with the meaning of "small stick shape", such as *riblji štapići, čarobni štapić, dirigentski štapić...*

3.3.3. Ellipse

In the case of the ellipse, elements are omitted. When it comes to Germanisms in our corpus, this is mostly the case with compounds in which the whole meaning is taken over, but already in the primary phase one part is lost during the formation of the replica in the primary phase. In the Germanism *rerna* < *Backröhre* (oven), the first part of the compound is omitted. The example of šlag < Schlagsahne (whipped cream) or *rendgen* <*Röntgengerät* (X-ray) shows the omission of the second part of the compound word.

4. CONCLUSION

By classifying Germanisms according to the respective thematic areas, we came to the conclusion that Germanisms are quite common and frequent in the conceptual field of "food and health", and a corpus of 68 Germanisms are formed to analyze adaptation processes at the semantic level.

The results show how complex the adaptation of Germanisms in this field are in relation to phonological and morphological adaptation, Especially, considering that this is not a mechanical adaptation, since this area belongs to human linguistic creativity.

Based on the analysis of adaptive changes in the primary phase, we can conclude that the analyzed Germanisms are mostly taken into the Bosnian language with their specific meanings such as. *birtija, brezla, celer, gris*, etc. In the entire analyzed corpus, there was a narrowing of the number of meanings in four cases, and in three examples a further narrowing in the field of meaning was observed.

Unlike the primary adaptation, in which the beginning and the end are clearly defined, the secondary adaptation is clearly determined in terms of the beginning, ie. forming a primary replica based on the primary model. The ending cannot be determined, as the replica may change during the development of the Bosnian language. These changes are no longer associated with the German language because they are always related only within the Bosnian language system and according to its rules. This is evident in the examples of Germanisms in which, the meaning

was expanded by using metaphor, metonymy, pejorization and ellipse in the phase of secondary adaptation.

In conclusion, we observed that every Germanism in the secondary adaptation becomes a model that can serve to create both new lexemes and new meanings. Therefore, the process of secondary adaptation remains open as long as Germanism is used in the Bosnian language.

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MEDICINAL PROPERTIES AND PRESENCE OF WILD CHESTNUT IN ALTERNATIVE MEDICINE OF THE POPULATION OF UNA-SANA CANTON

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Key words: wild chestnut, escin, saponin, healing

ABSTRACT:

Unlike the sweet chestnut, the wild chestnut fruit is not edible, but is harvested during the fall season for its medicinal properties. Due to its medicinal properties, the most commonly used in alternative medicine is the seed; however, the plant itself is still medicinal, including its buds, flowers, leaves, bark, and even the root. Wild chestnut saponin prevents the accumulation of fluid in the body, and has a positive effect on veins. The primary active ingredient found in wild chestnut extract is escin, which is used as an aid in the therapy of veins and capillaries because it reduces capillary damage, strengthens its walls and protects against edema. Wild chestnut is represented in the alternative medicine of the population of Una-Sana Canton with over 65.5% of the total number of respondents, in the form of various preparations and for the treatment of various health problems, varicose veins, blood clots, cuts, wounds, skin and wrinkles.

1. INTRODUCTION

Traditional and folk medicine mark the medical practice which is determined by tradition, that which the people came up with, used and perfected to transfer it to younger generations [1]. They represent a very dynamic area, and throughout history they have been transmitted mainly through oral lore, as literacy was at a low level. Since antibiotics were not invented at the time and hygiene care was not common and was at a low level, the fight to improve health was focused mainly on eradicating unhygienic habits. The limitations and inefficiencies of conventional medicine in the treatment of certain diseases enable the development of commercial alternative medicine, which is becoming a growing industry and a very lucrative business [2]. The wild

Bihać, 09 - 10 June 2022.

chestnut tree (*Aesculus hippocastanum*) is mostly grown as a decorative tree in parks and gardens in Europe, although it is, in fact, native to Asia Minor. Seeds and seed bark have been widely used in European herbal medicine since the 16th century.

Unlike real chestnuts, wild chestnut seeds are not edible, although they are used as animal feed as a specially prepared seed meal [3]. Its positive effects and help in treating veins and capillaries of the lower extremities have been proven. Namely, saponin escin lowers the damage to capillaries, strengthens their walls and protects against edema. Studies have also shown that it reduces the formation and influence of enzymes that break down the inner walls of capillaries as early as ten days after the start of treatment.

For problems with veins, it helps with chronic venous insufficiency, for example, fatigue especially in legs, itchiness in the varicose veins, tensed legs, pain and inflammation of the varicose veins. It contains vitamin P, which is responsible for the high resistance of capillaries and reduces their permeability.

In all parts of the stem, as well as in the fruit, there is saponin which dilutes the blood and makes it less sticky, cures skin diseases. The extract is the most suitable as a medicine, however tea from the buds and flowers or tinctures of flowers is often drunk as well, against gout, neuralgia and rheumatism. Tea from brown bark and young bark cures hemorrhoids, inflammation and varicose veins, enlarged prostate, especially varicocele and varicose veins of the legs and relieves pain caused by them [4]. Numerous studies such as [5], [6], have led to the approval of the use of wild chestnut for the treatment of chronic venous insufficiency in the legs [7].

2. GOAL AND TASK OF THE RESEARCH

The main motive for conducting experimental research is to investigate the use and impact of herbal medicine which is used in traditional medicine, and is made from the plant material of wild chestnut. The set hypothesis is that the correct use of herbal medicine made according to the traditional recipe, which is mainly transmitted from "knee to knee" in the examined area, can alleviate health problems and improve a person's health.

3. METHODS AND MATERIALS

Interviewing specialists in the field, cultivation, collection and use of medicinal plants was conducted in the city of Cazin in the period from May to October 2021. The survey collects 20 respondents ranging from 18 to over 45 years of age who have some knowledge and experience in traditional use of wild herbs, whether for medicinal, nutritional or other purposes.

	QUESTION		
1.	Is there a wild chestnut tree near your home?		
2.	Do you collect parts of the tree (fruit, leaf, bud or bark) of young branches?		
3.	you experienced any of the illnesses or conditions, such as:		
	a)	Diseases of the blood system	
	b)	Skin diseases	
	c)	Arthritis	
	d)	Mucus cough	
	e)	Skin aging (wrinkles)	
	f)	Hemorrhoids	
4.	Have you ever used any of the chestnut-based products?		
5.	If so, evaluate the effectiveness of the product?		

Some respondents also mentioned traditional recipes of wild chestnut, then showed their own preparations (teas, juices, oils, fats, tinctures ...)

4. RESEARCH RESULTS AND DISCUSSIONS

The interview was conducted within 20 people ranging from the ages of 18 to 45, who traditionally collected, grew, used, consumed and prepared certain preparations of wild chestnut. The survey consisted of questions which relate to the popular name of the medicinal plant, place of collection and time of collection, parts used to make various preparations for the treatment of certain illnesses and recipes for making certain preparations.





DO YOU KNOW THE WILD CHESTNUT PLANT

20 re-roomine



DO YOU COLLECT A PLANT




Graph 1. Results of the survey

Through the analysis of the collected data, we established that 13 out of 20 respondents know the examined plant species, and have easy access to wild chestnut trees, as well as the fact that all 13 respondents use or have used the plant species in treating certain health problems.

Participants confirmed during the interview that wild chestnuts are mainly used to alleviate certain illnesses, so coming to a conclusion, 12,5% or 2 respondents use the plant in the treatment or alleviation of varicose veins and thrombus in the legs, 43,8% or 6 respondents use herbal species for the treatment of various injuries, burns, wounds and cuts, 25% or 3 respondents drink tea to solve respiratory problems (productive cough) while 12,5% or 2 respondents use various tools to improve skin elasticity and prevent wrinkles. Most of the respondents use the mentioned medicinal plants in the form of tea, tincture, ointments and creams, while 9 out of 13 respondents confirmed the effective action of the preparation.Wild chestnut fruit contains 30 to 40% water, 38 to 42% starch, 5,.2 to 10,8% protein, 3,0 to 7,2% fat, 1,6 to 2,8% cellulose and 0,98% minerals [8].

It contains saponin escin, tannins, flavonoids and coumarin derivatives as well as vitamins B1, C and P [4]. After the test for saponins and the presence of the same, the escin content of 2,68% was isolated by extraction, which is in line with the research [7]. According to research [9], the mass fraction of saponin components can be affected by the use of various solvents, and the solvent used in our research is ethanol.

5. CONCLUSION

A survey conducted with a total of 20 participants found that 65% of respondents know the studied plant species, and apply it in everyday life to treat various health problems. The ratio of female to male respondents, in percentage, was 75:25, ranging from ages between 18 to 50 years old.Percentages of dealing with the mentioned diseases/conditions were: 43,8% - skin diseases (injuries, burns, swellings, wounds ...); 25% - mucus cough; 12,5% skin aging; 12,5% vascular

disease (varicose veins, blood clots) and 6.3% hemorrhoids or chronic diarrhea. The share of the most active part of the saponin escin plant was 2,68%. Keeping in mind, that wild chestnut supplements are not tested sufficiently for security, and their use is not recommended for pregnant women, nursing mothers, children and those with medical conditions who are taking medication.

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POTENTIAL IMPACT OF TRAFFIC NOISE ON THE ENVIRONMENT AND HUMAN HEALTH

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Key words: traffic noise, noise impact, human health

ABSTRACT:

It is known that traffic noise is a significant environmental problem of all urban ecosystems, and which is also one of the most widespread sources of such ecosystems. In addition to the potential impact on the environment, traffic noise also has a significant impact on humans and their health.

The paper presents individual measurements of noise intensity in the narrower area of the city of Bihać at two measuring locations with the highest frequency of traffic flow. Control measurements were performed in the inner part of the city at two traffic intersections on Bedem – intersection 1 (mark: R1) and at the intersection of Irfana Ljubijankica Street and city bypass – intersection 2 (mark: R2). For individual measurements, a mobile dB meter was used – Sound Level Meter – Datalogger PRO 318 type II IEC 651. At both locations, a total of 60 individual measurements were performed that did not exceed values greater than 90 dB or ranged from 60 to 90 dB.

The measured noise values were statistically processed (t-test) and indicate that there is a statistically significant difference in noise intensity between these two locations.

1. INTRODUCTION ABOUT NOISE AND TRAFFIC NOISE

The fact of the matter is that we are surrounded by various noises. Noise perception is subjective – some sounds can offer a pleasant experience while for others an unpleasant one, i.e. noise. The definition of environmental noise in the legislation is identical. According to the Law on Noise [1], "noise is any sound whose value exceeds the permitted level prescribed by law, given the place, time and environment in which people reside." The same law defines the concepts of noise

sources and noise emissions. Noise sources can be various objects and actions from which the sound spreads, while noise emission represents the noise level that the source transmits to the surrounding space, ie. surroundings.

2. THE IMPACT OF TRAFFIC NOISE

The term noise is often associated with the term traffic noise in the environment, which nowadays has an increasing impact on the environment. Generally speaking, in urban ecosystems, traffic noise, among other environmental pollutants, is dominant. Due to the spatial branching of city streets and the intensity of road traffic, noise is the most dominant factor that contributes to the overall noise levels in all urban areas [2]. However, in addition to road traffic as a mobile source of environmental pollution, there are other sources such as stationary, point and nonpoint pollution, etc. [3]. In practice, the road is a linear source of noise that spreads cylindrically.

Some previous research in the EU has shown that about 40% of the EU population is exposed to road traffic noise higher than 55 dB. The seriousness of this environmental problem is evident by the fact that about 170 million EU people live in so-called "gray areas" where noise levels cause severe feelings of discomfort during the day (noise levels ranging between 55 and 65 dB) [4]. The World Health Organization (WHO) defines noise above 65 dB as noise pollution. Strictly speaking, noise becomes harmful above 75 dB, and painful above 120 dB. To prevent psychosocial effects, it is recommended that external noise be below 55 dB during the day and 40 dB at night. The WHO has documented seven categories of adverse effects on human health through noise pollution. Hearing impairment, speech intelligibility, sleep disorders, cardiovascular disorders, mental health disorders, reduced ability to perform tasks, negative social behavior and anxiety are seven harmful categories that WHO directly correlates with noise from the individual's immediate environment [4,5].

2.1 The state of traffic vehicles in Bosnia and Herzegovina with special reference to the Una-Sana Canton

The number of registered vehicles in Bosnia and Herzegovina (BiH) in the last two years, 2019 and 2020, was reduced by 67.020 vehicles, or 5.7% [6, 7]. In the previous year 2021, a total of 1.152.743 road vehicles were registered in BiH [8], which compared to 2020 (1.108.711 d/cv) represents an increase of 44.032 vehicles, ie of 3.97 %. Table 1 provides comparative data on the total number of registered road vehicles in BiH for 2019, 2020 and 2021.

• Data Entities	• Number of registered motor vehicles in 2019	• Number of registered motor vehicles in 2020	Number of registered motor vehicles in 2021	Difference in the number of registered vehicles (%)
F BiH	703.038	671.108	690.600	2.9
RS	432.925	401.412	423.965	5.6
BD	39.768	36.191	38.178	5.5
Σ	1.175.731	1.108.711	1.152.743	3.97

Table 1. Tatal number of registered mater subjets in 2010, 2020 and 2021	Γ <i>(</i> /	7 0	٦.
Table 1. Total number of registered motor venicles in 2019, 2020 and 2021	10,	1,0	1

Note: FBiH: Federation of Bosnia and Herzegovina; RS: Republic of Srpska; BD: Brčko District

Road traffic in the Una-Sana Canton (US Canton) plays a dominant role in the work of environmental pollutants. In the US Canton in the past 2019 and 2020, a total of 118.553 motor vehicles were registered [2]. According to the data of the Ministry of Internal Affairs in 2021 there were a total of 70.946 motor vehicles in the US Canton with the following condition (Table 2).

Table 2: Number of motor vehicles in the US Canton for 2021.⁵

Procedure	• 1	• 2	• 3	• 4	• 5	• 6	• 7
Σ	3.551	9.258	4.316	40	920	52.861	10.620

Note: 1:Vehicle registration for the first time in BiH; 2:Re-registrations; 3:Change of ownership of the vehicle; 4:Temporary vehicle registration; 5:Issuance of test boards; 6:Extension of vehicle registration; 7:Check out the vehicle

In recent years, the growth rate of registered motor vehicles in the department of Bihać is much more pronounced than in other Canton Departments. In 2021, the highest number of registered motor vehicles was in the municipality of Bihać 18.413, while in the municipality of Bosanski Petrovac their number is the lowest and amounts to only 1.855 (Picture 1).

⁵ Source: Cantonal Ministry of Internal Affairs of the US Canton for 2021



Picture 1: Number of registered vehicles in the US Canton for 2021

3. NOISE LEVEL CALCULATION METHODS

In European countries, there are several different methods used to calculate noise levels. Approaches to individual methods are different. For example: Germany uses DIN 18005 standards and RSL guidelines [9]; The United Kingdom applies the CoRTN method [10]; Switzerland uses the StL-86 method [11]; Austria according to ÖAL guidelines 23 [12]; France according to the national method [13] and Croatia uses the RLS-90 method [14].

In Bosnia and Herzegovina, a unique methodology for measuring noise intensity and evaluating noise levels has not been established at the state level, although it is possible to apply the German national method. In practice, noise standardization is often done in accordance with legal acts at the entity level – F BiH, RS and BD.

For example, in the FBiH, measurements are performed according to the group of standards BAS ISO 140 for field measurements, laboratory and other measurements. Noise measurement is performed with equipment – phonometer that meets the recommendations of IEC 651 and 804 Type 1. and EN60804 with integration and averaging [1].

Noise protection is carried out in the day and night. However, the same time periods are not observed for the mentioned methods of calculating noise levels. For example, two time periods are used by: Bosnia and Herzegovina, Germany, Austria, Switzerland and France. For example:

- "day" period from 06^{00} to 22^{00} and
- ,,night" period from 22^{00} to 06^{00} .

The countries that use the three time periods are: the Netherlands, Denmark, Sweden, Belgium and Croatia. Specifically, the three periods are day-evening-night:

- ,,day" period from 07^{00} to 19^{00} ;

- , evening" period from 19^{00} to 23^{00} and
- "night" period from 23^{00} to 07^{00} .

According to EU regulations, all member states need to converge their legislation on noise in accordance with the guidelines of the EU Directive (2002/49/EC), which provides the basis for the covergence of methods of calculating noise levels.

4. MATERIAL AND METHODS

At each measuring point, there were several individual measurements during the day $(06^{00} \text{ to } 22^{00})$. At each location, 30 individual measurements were performed that lasted 15 minutes. For individual measurements, a mobile dB meter Sound Level Meter – Datalogger PRO 318 type II IEC 651 was used (Picture 2). A total of 60 individual measurements were performed at both locations. Control measurements were performed in the inner part of the city at two traffic intersections on Bedem – intersection 1 (mark: R1) and at the intersection of Irfana Ljubijankica Street and city bypass – intersection 2 (mark: R2).



Picture 2: Mobile sound level meter - Datalogger PRO 318 type II IEC 651

5. RESEARCH RESULTS WITH DISCUSSION

The measurement results showed that there is a statistically significant difference in noise intensity between the control locations – R1 (The inner part of the city at two traffic intersections on Bedem) and R2 (The intersection of Irfana Ljubijankica Street and city bypass). The measured values did not exceed 90 dB and ranged from 60 to 90 dB. Control sites R1 and R2 have a similar impact on the environment and human health. The measurement results represent the measured values of noise intensity given in the Picture 3.



Picture 3: Measured values of noise intensity

The presented measurement results (Table 3) were processed by Student's test (t-test) with a significance level of 0.1% (t_{0.001}).

Location Parameters	• R ₁	• R ₂
Min	63.2	63.5
Max	79.2	87.6
Average	71.203	77.045
St. error	0.811	1.138
Variance	19.085	37.537
St. Deviation	4.369	6.127
Coeff. of variation	6.135	7.952
t value	-4.1804	0.00010317

Table 3. Statistical processing of measurements from the location R1 and R2

6. CONCLUSIONS

The measured minimum and maximum values of noise intensity at the localities R_1 (65.40 - 76.0 dB) and R_2 (67.80 - 85.6 dB) were, according to the WHO and other literature sources, in intervals that have an impact on human health (insomnia, stress, speech, etc.).

The fact that the number of registered vehicles in the US Canton, ie. specifically in Bihać (18.413), the increase indicates the potential for harmful effects of traffic on human health.

Based on the obtained results, it is possible to qualitatively assess the state of endangerment by traffic noise in the observed area, and in addition, to plan the implementation of appropriate protective measures (noise barriers, speed reduction, etc.).

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PREVENTION AND INTERVENTION MEASURES OF OBESITY IN ADOLESCENTS

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Key words: obesity, body weight, prevention and intervention measures

ABSTRACT:

Obesity means increase in total fat mass, not total body weight as a result of an imbalance between energy intake and energy consumption. Weight gain does occur at the expense of body fat mass, but with the application of modern procedures for determining body composition, certain deviations have been observed. Weight gain does not have to be due to fat, but muscle mass, and people with increased muscle mass can not be classified as obese, and it is similar in people who have increased body weight at the expense of body water in the presence of generalized edema and ascites. People with optimal body weight can have a higher normal weight than the reference one, and this form of obesity is called sarcopenic obesity. Obesity in adolescents can lead to various health problems, the most common of which are depression, asthma, gene varum, and non-alcoholic steatohepatitis. If preventive measures and interventions are not taken in time, obesity will eventually lead to much more serious health problems such as diseases of the cardiovascular and endocrine systems. Factors contributing to the development of obesity are low socioeconomic status, sedentary lifestyle and reduced physical activity.

1. INTRODUCTION

Caloric intake is due to higher energy intake than for a long period of time and due to inadequate energy consumption. In the form of energy reserves, excess calories are stored in the body as, for example: (glycogen, fat). If there is a balance between food intake and energy consumption, we will prevent obesity. The health consequences that obesity can cause are really wide, and it is combined and occurs in pediatric age, and is an important factor for many diseases in adulthood, and in certain situations due to untimely prevention results in death. Obesity in adolescents can lead to various health problems, the most common of which are depression,

asthma, gene varum, and non-alcoholic steatohepatitis. If preventive measures and interventions are not taken in time, obesity will eventually lead to much more serious health problems such as diseases of the cardiovascular and endocrine systems. Factors contributing to the development of obesity are low socioeconomic status, sedentary lifestyle and reduced physical activity.

2. METHODS OF WORK AND MATERIALS

For the purposes of this paper, we used body mass index (BMI) as one of the more relevant methods for obtaining data when it comes to obesity in adolescents. The age range of the respondents ranged from 2 to 20 years. The oldest respondent was 20 while the youngest respondent was 2 years old. The study was conducted in a prospective-descriptive study, and was conducted over a 12-month period among adolescents. Subjects included in the study were adolescents with obesity, both sexes. Data were collected continuously for 12 months. For the purposes of this research, two surveys were formed. Survey I is intended for respondents with obesity and has several sections. The first part included the socio-demographic characteristics of the respondents, while the second part of the survey included the presence of risk factors for obesity. The third part of the survey is related to the current disease. The fourth part of the survey contained data on the treatment of obesity and prevention and control in order to reduce risk factors for complications in the cardiovascular and endocrine systems. Survey II contained respondents' knowledge of obesity and the consequences it can have on their health.

3. RESULTS

The results are presented in a table, and the table shows the number of respondents, the gender of the respondents, the age of the respondents, the number of obese, the risk of obesity and the normal range. The results showed the following:



Age of the	BMI	Normal	Number of obese	The risk of obesity
respondents		range		
2	10	50	95	85
3	12	14 – 18	20 - 32	18 – 19
4	14			
5	16			
6	18			
7	20			
8	22			
9	24			
10	26			
11	28			
12	30			
13	32			
14				
15				
16				
17				
18				
19				
20				

Table 2: Age of the respondents, BMI, normal range, number of obese, the risk of obesity

The presented results showed that the normal age limit is between 14 and 18 years, and that the risk of obesity is between 18 and 19 years of age. The number of obese is in the high range the ages of 20. Taking into account the presented results, and the corrected diet and increased physical activity for the purpose of reducing body weight in boys and girls will significantly contribute to our health, both physical and mental. By properly controlling the intake of nutrients in our body, we will prolong the life of our organic system.

4. **DISCUSSION**

The basic principles of obesity therapy are reflected in the methodology, gradual and long-term changes in diet. Repeating a short-term diet does not improve, but in order to improve it is necessary to balance a low-calorie diet. Physical activity and psychological support greatly

improve therapy and give positive results. One of the important factors in the goal of prevention is actually the recognition of genetic, environmental factors or combinations risk factors for obesity code children. Determination of BMI once a year for all children and adolescents, will allow us to recognize the phenomenon early obesity based on assessment BMI. By identifying families at risk based on family history, birth weight child or socioeconomic factors we will contribute to the prevention and control of obesity in adolescents. Support and promotion of breastfeeding, and education parents to promote a healthy way diet will greatly contribute to the reduced incidence of obesity.

5. CONCLUSION

We can conclude that by educating the family to recognize the importance physical activity and proper nutrition for health their children, restricting television and video game viewing and recognizing the significance of changes in quantity ingested foods greatly contribute to the control and prevention of obesity. The prevalence of obesity is increased and is clearly associated with certain diseases and is rapidly increasing. The goal of health professionals is prevention and early detection obesity in children and adolescents and promoting a healthy lifestyle.

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QUALITY AND ACCEPTABILITY OF TRADITIONAL COOKED CHEESES FROM THE AREA OF UNA-SANA CANTON

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Key words: cooked cheese, chemical composition, sensory analysis, color

ABSTRACT:

Cheese is an extremely valued food all around the world, including in our areas, Bosnia and Herzegovina has a long tradition in a family production of dairy products. With the development of technology, various types of cheese have also developed. So cheese smoking has become widespread in cheese production. It is known that this method of canning also improves sensory properties such as taste, smell, texture, consistency. Traditionally, boiled chees is made from cow's milk, and recently producers have also produced it from goat's milk and a mixture of goat's and cow's milk. The aim of this work was to examine the quality and properties of cooked cheeses made from cow's milk based on the chemical compositions and sensory properties of cheese samples from the USK area. Sensory and chemical quality was tested on 8 samples of cheese (4 non-smoked and 4 smoked). The proportion of dry matter, water, fat, protein, salt, acidity (pH value), sensory analysis, and cheese color were analyzed in the samples. The results of the analysis show that according to the share of water, cheeses are classified in the category of semi-hard cheeses. Based on the obtained data, it can be concluded that there is no large deviation in the chemical composition of cheese produced in different rural households from the Una-Sana Canton area. During the sensory analysis, all domestic traditional cooked cheeses made from cow's milk belong to the good quality category, while the sample from the market belongs to the middle-quality category. Based on the results of acceptability, we can conclude that consumers are more inclined to consume domestic cheese.

1. INTRODUCTION

Bosnia and Herzegovina, compared to many larger countries, can boast of quality cheeses. In addition to the natural resources of this country, the successful development of livestock is due to

the unique climate and specific region, as well as huge grazing areas, which gives us the conditions for the production of premium cheeses. It is very important to accept and respect the domestic tradition of making cheese, as well as the climate is a very important feature for making cheese, because in the cheese it must be felt regional vegetation. By definition, cheeses are fresh products or products with different degrees of ripeness produced by separating whey after coagulation of milk (cow's, sheep's, goat's, buffalo's milk, and/or mixtures thereof), skimmed or partially skimmed milk, cream, whey, or a combination of these raw materials. [1]. In the local markets, one of the most common cheeses is homemade fresh cheese produced in the traditional way. Then the second most important is the cooked cheese that is produced in rural households of Una-Sana Canton and beyond in BiH. Homemade cooked cheese has the shape of a roll, of different dimensions. When we talk about types of cooked cheese, there are two types: smoked and unsmoked. Smoking food not only prolongs its shelf life but also improves its taste [2]. Therefore, it is possible that the use of smoking can positively affect the sensory quality of cheeses produced from different types of milk, whose taste and smell are not acceptable to individual consumers. In addition, these cheeses are valuable food products due to their high nutritional value. Cow's milk is used as a raw material for the production of boiled cheese, and recently some producers also produce it from goat's milk, ie a mixture of goat's and cow's milk [3]. In addition to the technological process of production, the quality of cheese depends on the chemical and microbiological quality of milk [4]. Cooked cheese from rural households is produced according to a traditional recipe. The basis of the production process is the creation of coagulation using lactic acid or rennet. In this paper, domestic cooked cheeses made from cow's milk from the area of Una-Sana Canton, collected from different rural households, were researched. This research aims to examine the physicochemical composition and sensory properties and acceptability of cheese by consumers. The content of dry matter, water, protein, salt content, pH value, and color of cheese during storage days were analyzed. The sensory properties of cheese samples were determined by the scoring method and based on the obtained results, homecooked cheeses were categorized.

2. MATERIAL AND METHODS

Eight cheese samples were used in the study. Samples of cooked cheese made from cow's milk (4 unsmoked and 4 smoked) were collected from the Una-Sana Canton. The method of production of traditional boiled cheese from cow's milk is described below. Cheese analyzes were performed in the laboratory of the Biotechnical Faculty, University of Bihać. A more detailed overview of the cheese samples used is shown in Table 1.

Sample label	Manufacturer	Place	A type of cooked cheese	Cheese price (KM/kg)	Weight (g)	Packing
S-1	OPG	Ključ	Unsmoked	10.00	993.8	-
S-2	OPG	Sanski Most	Unsmoked	5.00	861.85	-
S-3	OPG	Bosanski Petrovac	Unsmoked	5.00	1044.99	-
S-4	Pađeni d.o.o.	Bileća	Unsmoked	8.50	367.28	+
S-5	Poljorad	Travnik	Smoked	5.50	408.36	+
S-6	OPG	Ključ	Smoked	10.00	1000.02	-
S-7	OPG	Bosanska Krupa	Smoked	4.00	397.07	-
S-8	OPG	Velika Kladuša	Smoked	5.00	809.77	-

Table 1. Data on cheese samples

The method of production of traditional cooked cheese

The production of traditional boiled cheese from cow's milk on this area is based on heating raw milk to 90-95°C and its direct acidification with sour whey, buttermilk or acid. The obtained curd can be mixed with certain additives, or most often it is salted, shaped in molds, pressed, which gives the consistency of cheese for cutting. Cooked milk cheese can be consumed immediately after production, but also after a longer storage period. In addition to casein, whey proteins also coagulate, which contributes to higher nutritional value and yield of cheese.

2.1. Physicochemical analysis of cheese

The chemical composition (dry matter, water, fats, proteins, salt) of cheese samples were analyzed by standard analytical methods. The pH value of the cheese was tested with a pH meter (Lutron PH - 220S Electronic Soil pH meter).

2.2. Sensory analysis of cheese

The sensory properties of the collected cheese samples (appearance, consistency, color, smell, and taste) were assessed by a panel group of 14 evaluators. The evaluation was performed in the laboratory of the Biotechnical Faculty, using the scoring method with a system of 20 weighted points [5]. All sensory properties were previously clearly defined and described. Each property was rated on a scale of 1 to 5, and then the score was multiplied by a significance factor to obtain weighted points. Based on the total number of weighted points (maximum 20 points), samples of smoked and unsmoked cheeses were classified into classes.

2.3. Instrumental determination of cheese color

Instrumental measurement of cheese color was performed with a colorimeter (A22112040131, Model No LCC-A11, Labtron Equipment LTD, UK). Three color parameters were determined: L (brightness), a (green), and b (yellow). Before each measurement, the instrument was standardized with white and black ceramic tile. The measurement was performed by placing a cheese sample measuring approximately 1×1 cm. The sample is matched and the reading is started on a computer program. The measurement was performed on the days of storage, the first, the third, sixth, and ninth days of storage.

2.4. Statistical data processing

The results of the analyzed samples are presented as mean values of repetition \pm standard deviation. All results were processed in Microsoft Excel, Office 2019.

3. RESULTS AND DISCUSSION

3.1. Physicochemical characteristics of homemade cooked cheese

The results of physicochemical characteristics of cheese samples made from cow's milk (smoked and unsmoked) are shown in Table 2. Physicochemical analyzes of cheese samples showed good and uniform quality, even though there are no written instructions, but the production process is passed from generation to generation. The results show that of the analyzed cheese samples, the highest dry matter content has the sample S4 (62.57%), and the smallest sample S3 (40.27%). The highest percentage of protein has the sample S3 (23.66%), and the lowest the sample S1 (18.25%). The salt content of the samples varies from 1.20% in sample S7 to 3.55% in sample S2. Such oscillations in salinity indicate different taste preferences in cheese production. Maretić (2015) [6] in his paper states similar results, where the salinity of cheese is 2.84%. Among the analyzed cheeses, 6 of them are characterized by a similar content of milk fat (homemade cheeses without declaration). The acidity of the analyzed samples was typical for rennet (pH ranging from 5.52 to 5.77), except for sample S6 which was characterized by a relatively high pH value (6.18). An increase in pH may occur during the storage of cheeses due to proteolysis of casein. According to Van Nieuwenhove et al. [7], the pH of cow's and goat's milk cheeses is similar. Fangmeier et al. [8] came to the same conclusions about pH in relation to cream cheeses made from cow's and goat's milk. Based on the chemical composition of the cheese, except for the main factor of cheese quality, ie. raw materials and types of milk, the quality of the cheese is affected by the season of production and the length of smoking. In addition, the quality of the cheese depends on the use of heat treatment of pasteurization during production [9].

Parameter	S1	<i>S2</i>	<i>S3</i>	<i>S4</i>	<i>S5</i>	<i>S6</i>	<i>S</i> 7	<i>S8</i>
Dry matter	52.52±	51.75±	$40.27\pm$	$62.57\pm$	41.55±	$50.05\pm$	46.98±	52.08±
Dry muller	2.19	0.35	0.61	0.32	0.76	0.18	0.32	1.68
Watow	47.47±	$48.24\pm$	$59.72\pm$	$37.43\pm$	$58.44\pm$	$49.94\pm$	$53.014\pm$	$47.91\pm$
water	0.13	1.07	1.55	0.97	0.2	0.52	0.14	0.32
Eat	27.55±	$22.91\pm$	$33.33\pm$	$15.88 \pm$	$17.02\pm$	$26.18\pm$	$23.83\pm$	$29.02\pm$
ГШ	1.55	1.88	2.01	2.03	0.11	1.2	0.41	0.36
Duotoing	18.25	$21.78\pm$	$23.66 \pm$	$19.48 \pm$	$23.29\pm$	$21.93\pm$	21.95±	$21.05\pm$
Froteins	±0.1	0.22	0.01	0.41	0.0002	0.0004	0.0001	0.0005
Salt	1.67	$3.55\pm$	$2.72 \pm$	$2.06 \pm$	$1.24\pm$	$1.94\pm$	$1.20\pm$	$2.01\pm$
Sall	±0.03	0.37	0.12	0.15	0.15	0.15	0.06	0.07
$_{n}U$	5.63±	$5.70\pm$	$5.55 \pm$	$5.52\pm$	$5.77\pm$	$6.18\pm$	6.01±	$6.06\pm$
рп	0.19	0.19	0.12	0.39	0.04	0.03	0.07	0.03

Table 2. Physicochemical composition and properties of cheese

Based on the categorization of cheeses according to water/dry matter content, sample S4 belongs to hard cheeses, semi-hard cheeses include S1, S2, S6, S7, and S8, while samples S3 and S5 belong to the category of semi-soft/soft cheeses (Table 3). In his research, Roštan (2014) [10] obtained similar results, where his samples according to the dry matter content belong to the group of semi-hard cheeses.

Table 3. Categorization of cheeses according to water/dry matter content

Water content	Dry matter content	Cheese group	Samples
<34	≥66	Very hard	
34-45	55-66	Hard	S4
45-55	45-55	Semi-hard	\$1,\$2, \$6,\$7,\$8
55-80	20-45	Semi-soft/soft	S3, S5

3.2. Results of instrumental analysis of cheese color through storage days

Table 4 shows the results of cheese color through storage days. It can be seen that the L value (brightness) of fresh cheeses slightly increases during the storage period in all analyzed cheese samples. Evert - Arriagada et al. (2014) [11] noted an increase in L value in fresh cheeses during 7 days of storage. According to a study by Sant'ane et al., (2013) [12], a decrease in L value can be observed during the storage of fresh cheeses made from cow's and goat's milk. The a * parameter corresponds to the color range green (-a *) or red (+ a *). The values of the parameter a *, move in the negative color range and decrease during the storage period. In this way, the

color of all cheeses leans slightly towards green. The b * parameter corresponds to the color range yellow (+ b *) or blue (-b *). The values of the b * parameters of the tested cheeses are in the positive color spectrum, so the tested cheeses are in the yellow color spectrum. The average a * value of (green) domestic cooked cheeses during the storage period ranged from -3.73 to -5.22 with a tendency to constantly decrease the specified parameter during the storage period. The B * value of the analyzed cheeses in this study ranged from 13.08 to 21.07, which also decreased during storage in all analyzed cheese samples. Comparing the b * values measured in domestic cooked cheeses during the 1st and last day of storage, it can be concluded that in all samples this parameter decreased during storage.

	Storage days	<i>S1</i>	<i>S2</i>	<i>S3</i>	<i>S4</i>	<i>S5</i>	<i>S6</i>	S 7	S 8
	1. day	78.73± 2.83	82.58 ± 3.40	79.65 ± 0.78	78.1± 3.90	75.36± 1.58	78.82± 5.06	76.53± 4.57	82.83± 4.79
	3. day	$\begin{array}{c} 87.67 \pm \\ 0.75 \end{array}$	$\begin{array}{c} 88.46 \pm \\ 0.11 \end{array}$	$\begin{array}{c} 89.96 \pm \\ 0.60 \end{array}$	89.54± 1.32	$\begin{array}{c} 84.22 \pm \\ 0.74 \end{array}$	86.48± 2.13	$\begin{array}{c} 89.05 \pm \\ 0.84 \end{array}$	86.83± 1.16
L value	6. day	$\begin{array}{c} 88.01 \pm \\ 1.81 \end{array}$	$\begin{array}{c} 88.25 \pm \\ 0.06 \end{array}$	$\begin{array}{c} 86.62 \pm \\ 0.88 \end{array}$	$\begin{array}{c} 88.35 \pm \\ 0.98 \end{array}$	$\begin{array}{c} 83.96 \pm \\ 0.98 \end{array}$	$\begin{array}{c} 87.73 \pm \\ 0.83 \end{array}$	$\begin{array}{c} 87.44 \pm \\ 0.97 \end{array}$	$\begin{array}{c} 89.6 \pm \\ 0.91 \end{array}$
	9. day	90.12± 0.71	$\begin{array}{c} 88.45 \pm \\ 0.45 \end{array}$	$\begin{array}{c} 86.54 \pm \\ 0.82 \end{array}$	91.96± 1.25	84.51± 1.17	$\begin{array}{c} 88.08 \pm \\ 0.83 \end{array}$	88.43± 1.62	90.39 ± 0.49
	1. day	-4.8± 0.22	-3.75± 0.86	-4.40± 0.27	-5.22± 0.69	-5.18± 0.52	-4.73± 0.54	-4.95± 0.76	-3.73± 0.85
	3. day	-2.56± 0.33	-1.99± 0.07	-2.5± 0.15	-1.7± 0.09	-3.48± 0.29	-3.13± 0.64	-1.81± 0.23	-2.70± 0.37
a valua	6. day	$\begin{array}{c} -2.43 \pm \\ 0.48 \end{array}$	-2.21± 0.06	-2.81± 0.35	-2.80 ± 0.50	-3.54± 0.34	-2.85± 0.41	-2.40± 0.32	-1.44± 0.16
<i>u vuiu</i> c	9. day	-1.59± 0.15	-1.98± 0.29	-2.84± 0.30	-0.81± 0.38	-3.43 ± 0.36	-2.77± 0.17	-2.01± 0.21	-1.27± 0.25
	1. day	$\begin{array}{c} 18.01 \pm \\ 0.15 \end{array}$	14.91± 1.45	21.07± 1.78	13.08± 0.64	$\begin{array}{c} 18.10 \pm \\ 0.69 \end{array}$	13.52± 1.04	$\begin{array}{c} 18.64 \pm \\ 0.49 \end{array}$	13.16± 1.11
	3. day	16.74± 0.74	$\begin{array}{c} 14.18 \pm \\ 0.34 \end{array}$	$\begin{array}{c} 18.68 \pm \\ 0.75 \end{array}$	11.61 ± 0.09	17.03 ± 0.32	13.41 ± 0.29	$\begin{array}{c} 14.31 \pm \\ 0.13 \end{array}$	13.43 ± 0.15
b value	6. day	16.67± 1.00	14.5± 0.39	$\begin{array}{c} 19.02 \pm \\ 0.68 \end{array}$	11.22± 0.56	17.37± 0.52	$\begin{array}{c} 13.51 \pm \\ 0.27 \end{array}$	$\begin{array}{c} 15.45 \pm \\ 0.22 \end{array}$	$\begin{array}{c} 13.55 \pm \\ 0.40 \end{array}$
	9. day	$\begin{array}{c} 15.33 \pm \\ 0.34 \end{array}$	$\begin{array}{c} 14.25 \pm \\ 0.22 \end{array}$	$\begin{array}{c} 18.52 \pm \\ 0.63 \end{array}$	11.18± 0.33	16.42± 0.13	13.19± 0.62	15.13 ± 0.29	$\begin{array}{c} 13.22 \pm \\ 0.38 \end{array}$

Table 4. Color indicators of tested samples of smoked and unsmoked cheeses

Data represent mean values (\pm SD) of three replicates.

3.3. Sensory characteristics of homemade cooked cheese samples

Sensory analysis was conducted by a panel group of 11 evaluators who evaluated the aroma, consistency, color, appearance, and taste of samples of homemade cooked cheeses made from

cow's milk (smoked and unsmoked). The results of the evaluation are presented in a graph (Figure 1) where a comparison of the average scores of different samples of cooked cheeses can be clearly seen.



Figure 1. Distribution of weighted points of sensory properties (appearance of crust. smell/odor. taste. color and consistency) and total weighted points of cheese samples

Conducted sensory analysis of all evaluated properties of samples of cooked cheeses showed the high sensory quality of the product. The sensory quality of cheeses produced in rural households was as good as that of cheeses produced in industrial conditions. The sensory characteristics of cheese depend on several factors, including the type of milk, the quality, and composition of the raw material, as well as the production technology itself. However, the use of raw materials of different quality and the identified differences in the chemical composition of the tested types of cheese did not affect the results of sensory evaluation. Such results of sensory evaluations can be caused by smoking. The smoking process affects sensory characteristics. Natural smoke contains many fragrant active substances, ie phenolic compounds, which play an important role in smoking. According to Spiel et al. [13]. when it comes to goat cheeses only, young consumers most often prefer smoked cheese, followed by soft cheese, cottage cheese, processed cheese, and ripe cheese. Moreover, Fangmeier et al. [8], analyzed 6 cream kinds of cheese made from cow's, goat's, and buffalo's milk and their mixture. Respondents most often claimed that they would like to buy cheeses exclusively from cow's milk. Cheeses made from cow's milk usually have a more desirable taste, smell, and texture than goat's milk cheeses, especially after storage. The obtained results show that the tested cheeses (smoked and unsmoked) from cow's milk had a similar

sensory quality. Therefore, it can be concluded that natural smoking can be used to increase the sensory quality of cheeses. According to the results of the mean values of the total weighted points, the highest grade was given to sample S6 (homemade smoked cheese), while samples S1, S2, and S3 received a slightly lower grade. In summary, based on the overall results of an evaluation of cheese samples by a system of 20 weighted points, based on the achieved scores, a range of 12.83 to 18.90 out of a possible 20 was obtained, and it can be concluded that most analyzed cheese samples belong to good quality products. Table 5 shows the categories of cheeses according to the obtained weighted points. Sample S6 belongs to the category of excellent quality, while samples S1, S2, S3 and S5 are in the category of good quality.

Quality category	Weighted points	Cheese samples
Excellent	17.6 - 20.0	S6
Good	15.2 - 17.5	S1, S2, S3, S5
Medium	13.2 - 15.1	S4, S8
Acceptable	11.2 - 13.1	S7
Unacceptable	<11.2	

Table 5. Cheese quality categories according to obtained weighted points

3.3.1. Acceptability of cheese samples (smoked and unsmoked) according to the hedonic scale

The results of the acceptability test of homemade cooked cheeses are shown in Table 5. Based on the data determined by the hedonic scale, the basic statistical parameters (x, s, Cv), as well as the desirability percentage, were calculated (Table 6). Students of the Biotechnical Faculty participated in the evaluation of the acceptability of samples of cooked cheeses.

Table 6. Results of evaluation of smoked and unsmoked cheese samples using the hedonic scale

C		C	heese sample.	s (smoked a	nd unsmoke	ed)		
Scores	S1	S2	S3	S4	S5	S6	S7	S8
9	4	1	2	1	2	8	1	0
8	3	5	2	1	3	2	0	4
7	0	2	4	1	3	2	1	3
6	2	2	2	2	3	2	2	2
5	1	1	0	1	3	1	1	2
4	1	0	1	3	0	1	3	3
3	0	0	0	0	2	0	3	1
2	0	0	0	2	0	0	3	0
1	0	0	0	0	0	0	2	1
Total	11	11	11	11	16	16	16	16

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X	7.36	7.27	7.09	5.18	6.37	7.68	3.87	5.68
S	1.80	1.19	1.44	2.27	1.86	1.66	2.24	2.08
Desirability (%)	90.90	100.00	90.90	54.54	87.50	93.75	18.75	68.75
Cv	24.45	16.37	20.31	43.82	29.20	21.61	57.88	36.61

Legend: Data represent mean values (± SD) of three replicates. x = average / mean; s = standard deviation; Cv = variability coefficient.

According to the hedonic scale, the mean desirability of cheese samples ranged from 3.87 to 7.68, while the desirability percentage ranged from 18.75 to 100%. The highest percentage of desirability (above 90.90%) and the best marks (above 7.00) were given to samples S1, S2, S3, and S6, home-made cooked cheeses, without declaration. A slightly lower score (6.37), but still with a high desirability percentage (87.50%) was achieved by the S5 sample, a control sample from the market. Of all the analyzed samples, the least desirable samples were S4 (54.54%) and S7 (18.75%). Based on the results of the research, it can be concluded that consumers are more inclined to consume homemade cooked cheeses. Therefore, further research should focus on researching the acceptability of larger groups of consumers.

4. CONCLUSION

The nutritional value of cheeses depends on the type of milk used in their production. The chemical composition of the analyzed cheese samples made from cow's milk was very similar. According to the water/dry matter content, cheeses S1, S2, S6, S7, and S8 belong to semi-hard, and samples S3 and S5 to semi-soft/soft cheeses. The salt content of the samples varies from 1.20% to 3.55%. Such oscillations in salinity indicate different taste preferences in cheese production. The color of all the cheese samples was lighter. The sensory evaluation did not show significant differences between cheeses, regardless of the type of cheese (smoked or unsmoked). Moreover, the sensory quality of cheeses produced in small rural households was as good as that of cheeses produced in industrial conditions. Therefore, the application of smoking has a positive effect on the sensory quality of cheeses. The analysis of the acceptability of different cheese samples, among consumers, using a hedonic scale with 9 possible ratings, for the most desirable samples of cooked cheese made from cow's milk (unsmoked or smoked) has the characteristics of a guaranteed traditional specialty precisely because of its growing prevalence in BiH, and it differs in the method of production or traditional composition from other similar cheeses.

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Tea and its ingredients are one of the important components for maintaining health and reducing the risk of various diseases. Next to water, it is the most commonly consumed drink. It has significant antioxidant, anti-inflammatory, antimicrobial, anticancer, antihypertensive properties. The method of preparation of tea drinks affects the content of biologically active substances and their stability, so it is important to investigate the influence of the parameters of the preparation of tea solutions on the content of biologically active substances. In this paper, the influence of tea brewing time at constant temperature on the content of total phenols will be determined and examined, anthocyanins, flavonoids. Two types of tea (nettle and mint) were taken from two domestic producers. The teas were treated in time intervals of 5, 10, 15, 20, 25 and 30 minutes at a temperature of 800C. The obtained results indicate that there is an increase in the content of phenols and flavonoids until a certain time, after which there is a drop in concentration. Anthocyanin content was found only in some treatments. It was also determined, based on the results obtained by the Mann-Whitney U test, that for most treatments there is a statistically significant difference in terms of phenolic and flavonoid content between two producers for the same type of tea.

1. INTRODUCTION

Utica dioica is the most common species of the *Urticaceae* family known as nettles and one of the most studied medicinal plants in the world. It is a herbaceous perennial plant and has a long history of use for various types of health problems, and is used in nutrition. Extracts of *Utica dioica* are important areas in the development of drugs with numerous pharmacological activities.[1] It contains a large number of phytochemicals, the most important of which are phenols, flavonoids, tannins, scopoletins, sterols, fatty acids, polysaccharides, isolectins and sterols, which *Utica dioica* is associated with its antibacterial, antioxidant, analgesic, anti-inflammatory, antiviral, anti-colitic, anti-cancer and anti-Alzheimer effects.[2]

Total phenol content ranges from 0.9298 to 2.3846 mg GAE/L in water extracts, and from 0,9649 to 19924 mg GAE/L in ethanol extracts.[3]

Mentha piperita L., Lamiaceae has a long history of safe use both in medicinal preparations and as a flavoring in food and sweets. The biological activity of Menta piperita is primarily related to the phytochemical composition, the main ingredients of which are monoterpenes (menthol and menthylacetate), and phenolic compounds such as flavonoids, phenolic acids. They show antioxidant, antiallergic and other types of pharmacological activity.[4] M. piperita contains the following phenolic compounds which include isorhoifolin, mentoside, piperitoside, nevadensin, mentocubanone, dimethoxyand dimethylsudachitin, 5-hydroxy-6,7,3,4hymenoxin, tetramethoxyflavone, eriocitrin, luteolin-7-rutinoside, xanthomicrol, gardenin B and D, 5-Odemethylnobiletin, 5,3,4-trihydroxy-6,7,8-tetramethoxyflavone, eupatorin, salvigenin, luteolin, sorbifolin, thymusin, timonin, apigenin, 5,6-dihydroxy-7,3,4-trimethoxyflavone, sideritoflavone, 5,6-dihydroxy-7,8-3,4-tetramethoxyflavone, ladanein, pebrelin, acaceti, luteolin-7-glucoside, eriodictyol-7-glucoside, narirutin, hesperetin, diosmin, rosmarinic acid, rhoifolin, 5,7dihydroxychromone-7-rutinoside, luteolin-7-glucuronide, and caffeic and lithospermic acids.[5]

Menta piperita is used as an aid in gastrointestinal infections, digestive disorders (intestinal colic, enterocolitis and flatulence), respiratory disorders (bronchitis, laryngitis, tracheobronchitis and convulsive cough), pharyngitis (analgesic), nausea, nervous or mental fatigue and mou hygiene.[6] Tea is the most popular beverage that can be served hot or iced all over the world. Tea infusion has a distinct taste, aroma and contains essential components important for nutrition and human health.[7] The health effects of tea on health depend on the availability of biologically active substances in tea infusions. Therefore, most authors indicate that it is important to consider optimal or better conditions for extraction in order to achieve the greatest possible number of biologically active vats in tea infusions.[8] This will first of all depend on the conditions of preparation of tea infusions. Studying the effect of brewing time on antioxidant activity and polyphenol content in tea can lead to more information on how to prepare the product more efficiently. Today, tea for consumption as a drink is prepared using loose tea and

tea preparations using tea bags. Preparing tea using filter bags is more practical and they are used more and more today. The conditions for preparing tea infusions are important for the extraction of as many antioxidants and other biologically significant compounds as possible. Therefore, the biological activity of tea infusions is correlated with preparation conditions, infusion time and temperature, and the size of tea leaf particles.[9]

In this context, in these observations we focused on the influence of preparation time, i.e. the length of time of brewing tea infusions on the content of phenols, flavonoids and anthocyanins. This work is primarily carried out to determine the most optimal conditions for the preparation of tea infusions.

2. MATERIAL AND METHODS

2.1. Samples

Two types of bags were selected: mint and nettle, from two different producers, taken from the market shelves. Both producers are from Bosnia and Herzegovina.

2.2. Preparation of tea infusions

The tea bags were boiled in 240 ml of water at 80°C for 5, 10, 15, 20, 25 and 30 minutes. The solutions are then allowed to cool to room temperature before analysis.

2.3. Analysis of biologically active components with tea solutions

2.3.1. Determination of total phenols

In the prepared samples, total phenols were determined by the Folin-Ciocalteu method[10], and the results were recalculated from the gallic acid calibration curve. The color intensity is proportional to the concentration of phenol in the sample, and was measured at a wavelength of 765 nm. After the sample was allowed to stand for 2 hours at room temperature, a photoLlab 6600 UV-VIS WTW spectrophotometer was used to measure absorbance.

$$y = 0,002x + 0,0678$$

 $R^2 = 0,9987$
where are:

y-absorbance at 765 nm,

x – concentration of gallic acid (mg/L),

R2 – coefficient of determination.

2.3.2. Determination of flavonoids

A modified colorimetric method with AlCl₃ was used to determine the total flavonoids[11], and a standard quercetin solution was used to create the base line.

$$y = 0.0193x + 0.3734$$

 $R^2 = 0.9914$ where are:

y – absorbance at 415 nm,

x – concentration of quercetin (mg/L),

R2 – coefficient of determination.

2.3.3. Determination of anthocyanins

Total anthocyanin concentration was determined by the pH differential method, which is based on the anthocyanin structural transformation that occurs with a change in pH[12] Anthocyanin concentration is calculated thus:

Anthocyanin = $(A \times M \times FR \times 1000)/\varepsilon \times 1 \pmod{kg}$ A - Absorbance of a sample, which is calculated thus A = (A513 - A700) pH 1 - (A513 - A700) pH 4,5M - 449,2 FR - dilution factor (DF) ε - Molar absorptivity, 26 900 1 - the length of the cuvette, 1 cm

(M and ε were taken as a dominant type of anthocyanin, namely for cyanidin-3-glucoside)

2.4. Statistical processing of measurement data

To compare the content of individual biologically active components in tea infusions from two manufacturers at the same temperature, the Mann-Whitney U test was used. This test is used to compare two sample mean values that come from the same population, that is, to check whether two sample mean values are equal or not.

3. WORK RESULTS AND DISCUSSIONS

The content of total phenols and flavonoids is higher in mint tea infusions compared to nettle in all extraction treatments. Anthocyanin concentration determined as cyanidin-3-3glucoside mg/l ranged from 0.413 mg/l to 1.865 mg/l, and was not detected in most treatments.



Figure 1. Average value of total phenol content in *Menta piperita* tea infusion as a function of time from two manufacturers (mg GAE/(2g/240ml))



Figure 2. Average value of total phenol content in *Utica dioica* tea infusion as a function of time from two manufacturers (mg GAE/(2g/240ml))



Figure 3. Average value of flavonoid content in *Menta piperita* tea infusion as a function of time from two manufacturers (mg Q/(2g/240ml))



Figure 4. Average value of flavonoid content in *Utica dioica* tea infusion as a function of time from two manufacturers (mg Q/(2g/240ml))

According to research, the increase in the level of phenol and antioxidants in tea infusions is influenced by the increase in exposure time (extraction), the intensity of mixing, the size of the leaves and the porosity of the tea bags.[13] Despite the fact that there are differences in the quality of tea, which often depends on the method of packaging, drying, place and method of harvesting tea, in this paper we focused on two producers and two types of tea. According to the

obtained results shown in Figures 1, 2, 3 and 4, it is clear that for all producers and types of tea, extending the extraction time increases the content of total phenols and flavonoids at a temperature of 80 Cup to a certain extraction time, which depends on the type of tea, but from the manufacturer. The maximum value of total phenols and flavonoids in tea infusion for mint tea from both manufacturers is found at 20 minutes. Further extension of the extraction time does not achieve any effect, and even the results are slightly lower if the extraction is extended to 30 minutes at the same temperature. For nettle tea, the maximum extraction values of total phenols and flavonoids are achieved in a period of 15 to 20 minutes, which depends on the tea manufacturer.

Approximately up to 5 minutes of infusion time for lightly packed tea bags is sufficient to extract almost more than half and up to 80% of the water-soluble total phenols and anthocyanins.

In 2012, Armoskaite et al. also confirmed in their research that as the extraction time increases, the amount of extractable compounds increases. They found that the highest rate of extraction of extractive compounds as well as phenolic compounds was observed in the first 10 minutes of extraction, while after that the rate of extraction decreased. They found that the amount of extractable compounds increased by only 25 to 40% within 20 minutes. The same author states on the example of black tea that different methods of tea production also cause a marked difference in the chemical composition of tea.[14]

Yao et al., 2006 confirmed that in the case of using tea bags, the quality of the paper used to pack the tea bags is also important and affects the extraction conditions.[15]

Due to the statement by most authors that there are differences in the chemical composition for the same types of tea and the same treatments, the Mann-Whitney U test was performed. In addition, Armoskaite et al. 2012 stated that both cultivation and processing of tea affect the chemical composition of tea. Usually, the Mann-Whitney U test is used when the data are ordinal or when the assumptions of the t-test are not met. Mann-Whitney U is sometimes difficult to interpret because results are presented in group rank differences rather than group mean differences.

The test showed that for most measurements there is a statistically significant difference, except for the phenol content in *Utica dioica* tea after 5 minutes of brewing and in the flavonoid content after 30 minutes in *Menta piperita* tea.

4. CONCLUSION

With an increase in the extraction time, that is, the exposure time, the content of total phenols and flavonoids in the tea extract increases at a temperature of 80 Cuntil a certain extraction time, which depends on the type of tea, but also on the manufacturer. The maximum concentration of total phenols and flavonoids in mint tea is achieved after a treatment of 20 minutes, while for nettle tea it ranges from 15 to 20 minutes.

Anthocyanin content was found only in some treatments and ranged from 0.413 mg/l to 1.865 mg/l. Also in all treatments in which it was detected, the highest values were observed in the 15-minute treatment.

In all treatments, 50 to 80% of total water-soluble phenols and flavonoids are isolated in a 10minute treatment, and even in a smaller treatment, which means that it is enough to extract them for up to 10 minutes or less.

There are statistically significant differences in the content of phenols and flavonoids for most measurements, as shown by the Mann-Whitney U test.

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RISK FACTORS THAT INCREASE SYSTOLIC BLOOD PRESSURE

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Key words: systolic blood pressure, risk factors, consequences

ABSTRACT:

Systolic blood pressure values greater than 140 mmHg are considered to be elevated blood pressure, which can result in multiple damage to various parts of the human body. The aim of this paper is to show how risk factors such as increased blood fats, elevated blood sugar, cigarette smoking, myocardial infarction and stroke lead to increased systolic blood pressure and the consequences of high systolic blood pressure. The research will determine the relationship between the age of patients, gender, body weight of patients with the occurrence of increased systolic pressure, and seek to determine which risk factors contribute most to the occurrence of elevated systolic blood pressure. Respondents are patients of the family medicine clinic, Banja Luka Health Center (100 patients) who have elevated systolic blood pressure. The research was done in a period of 6 months during 2021. An equal number of male and female patients were included, and the average age of the patients was 65 years. Based on the results of the research, we came to the conclusion that patients older than 35 have high systolic blood pressure, and that there is a significant difference in systolic blood pressure, gender and age.

1. INTRODUCTION

According to the definition of the World Health Organization (WHO), normal systolic blood pressure values do not exceed 140 mmHg. All systolic blood pressure values above this value are considered high blood pressure. High blood pressure is a systemic disease that can result in multiple injuries to different parts of the body. The blood that the heart pumps through the body

encounters resistance from the walls of arterial blood vessels. The greater the amount of blood that the heart pumps and the narrower or stiffer the arteries, the higher the blood pressure. Systolic (upper) blood pressure is the result of left ventricular contraction. As systolic blood pressure rises, so does the incidence of cardiovascular disease. If there are additional risk factors (high cholesterol, high blood sugar, smoking, thickening of the left ventricular heart muscle), the frequency of heart attack, but also stroke is even more prevalent. High blood pressure is a condition that must be treated for life with a change in lifestyle [2].

2. METHODS OF WORK AND MATERIALS

The aim of this study was to determine the relationship between age, body weight, sex and blood pressure (systolic mmHg). The research is aimed at examining whether age, body weight and sex affect elevated systolic blood pressure.

100 respondents participated in the research. The respondents are patients of the family medicine clinic of the Banja Luka Health Center, with elevated blood pressure values (systolic mmHg). The research was conducted in the period from April 10, 2021 to October 10, 2021.

Elevated blood pressure values were measured with a mercury sphygmomanometer in all 1 00 subjects under the same conditions.

Data were statistically processed using Microsoft Office Excel 2007. For statistical processing of research data were used: Descriptive analysis; Pearson's correlation coefficient; T-test and X²-test [1,3].

3. RESULTS

The study included 100 respondents, of which 50 (50%) were men and 50 (50%) were women, median age 65, aged 35 to 88 years. The sexual structure of subjects with elevated systolic blood pressure values is shown in Graph 1.



Graph 1. Gender structure of respondents

The graph clearly shows that an equal number of male and female patients with elevated systolic blood pressure participated in the study.

Table 1 shows the mean value of elevated systolic blood pressure (CP) in the subjects.

Table 1. Mean KP value	
Average value of KP	162.10
Maximum value of KP	210
The most common value of KP	150
Interval of variation	70
Mean absolute deviation	13.04
Standard deviation	15.38
Coefficient of variation	9%

Table 1. Mean blood pressure values

A descriptive analysis of the study shows that the range of systolic blood pressure of the subjects is 70 mmHg. The percentage of variability is 9%, which means that the variability of systolic blood pressure in the subjects is very weak. The maximum value of systolic blood pressure is 210 mmHg, and compared to the normal defined value of systolic blood pressure, it can be concluded that this is a very high value of systolic blood pressure, while the most common systolic blood pressure is moderately elevated and 150 mmHg.

The first correlation analysis compared the values of systolic blood pressure and age. A Pearson correlation coefficient of 0.097 was obtained, indicating that no correlation was expressed. This can also be seen in the scatter diagram (Graph 2).


Graph 2. Scattering diagram

The study examined whether there is a relationship between age and elevated systolic blood pressure, ie whether age affects elevated systolic blood pressure, assuming it does not. A T-test was used. The calculated value of T-test statistics is 0.97, and the tabular value with significance level $\alpha = 0.05$ is 1.66. Since the tabular value is higher than the calculated value, we conclude that there is no statistically significant difference in systolic pressure values compared to age.

The second correlation analysis compared systolic blood pressure and body weight (kg). A Pearson correlation coefficient of 0.257 was obtained, indicating that no correlation was expressed. This can also be seen in the scatter diagram (Graph 3).



Graph 3. Scattering diagram

The study examined whether there is a relationship between body weight (kg) and elevated systolic blood pressure, ie whether body weight (kg) affects elevated blood pressure values, assuming no effect. A T-test was used. The calculated value of T-test statistics is 2.63, and the tabular value with significance level α = 0.05 is 1.66. Since the tabular value is less than the calculated value, we conclude that there is a statistically significant difference, ie there is an effect of body weight (kg) on the level of systolic blood pressure.

The third correlation analysis compared the values of systolic blood pressure and the age of the female subjects. A Pearson correlation coefficient of 0.056 was obtained, indicating that no correlation was expressed. This can also be seen in the scatter diagram (Graph 4).



Graph 4. Scatter diagram

The study examined whether there was a link between female subjects and elevated systolic blood pressure, ie whether the age of the female affected elevated blood pressure values, assuming no effect. A T-test was used. The calculated value of T-test statistics is 0.56, and the tabular value with significance level α = 0.05 is 1.66. Since the tabular value is higher than the calculated value, we conclude that there is no influence of female gender and age of the subjects on the level of systolic blood pressure.

The fourth correlation analysis compared the values of systolic blood pressure and the age of the male subjects. A Pearson correlation coefficient of 0.138 was obtained, indicating that no correlation was expressed. This can also be seen in the scatter diagram (Graph 5).



Graph 5. Scattering diagram

The study examined whether there was a link between male subjects and elevated systolic blood pressure, ie whether age of male influence on elevated blood pressure values, assuming no effect. A T-test was used. The calculated value of T-test statistics is 1.38, and the tabular value with significance level α = 0.05 is 1.66. Since the tabular value is higher than the calculated value, we conclude that there is no influence of male gender and age of the subjects on the level of systolic blood pressure.

The study examined whether there is a significant difference between men and women when it comes to age and systolic blood pressure, assuming no difference. The X test -test was used . The calculated value of X² statistics is 239.45, and the tabular value with significance level $\alpha = 0.05$ is 124.34. Since the tabular value is less than the calculated value, we conclude that there is a statistically significant difference between men and women when it comes to age and systolic blood pressure.

4. DISCUSSION

Our research has shown that elevated systolic blood pressure values are found in middle-aged and elderly subjects. A T-test was used to test the hypothesis that body weight had a lesser effect on systolic blood pressure. The calculated value of T-test statistics is higher than the tabular value (p <0.05). We reject the set zero hypothesis and accept the alternative hypothesis - there is a statistically significant difference in systolic blood pressure in relation to body weight. An X² test was used to test the null hypothesis that there was no difference in systolic blood pressure between men and women. The calculated value of the X²-test is higher than the tabular value (p <0.05). We reject the null hypothesis and accept the alternative hypothesis - there is a statistically significant difference in the level of systolic blood pressure between male and female subjects.

5. CONCLUSION

Based on the results of the research, the following can be concluded:

- people over the age of 35 have a problem with elevated systolic blood pressure,
- equal number of men and women with elevated systolic blood pressure,
- there is a significant difference in the values of systolic blood pressure in relation to body weight, ie there is an influence of body weight on the level of systolic blood pressure,
- there is a significant difference in systolic blood pressure values relative to gender and age.

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THE EFFECT OF THE BIOSTIMULATING FERTILIZER VERAMIN ON THE RASP QUALITY OF THE RASPBERRY PLANT (RUBUS IDAEUS)

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Key words: Polka, fertilizers, biostimulators, Veramin, control

ABSTRACT

This paper analyzes the influence of foliar application of the biostimulative fertilizer Veramin on some of the quality elements of the raspberry variety Polka. The research was conducted on the site of Ribić Island in the area of the city of Bihać, and according to the system of control and treatment, a total of 12 quantitative and qualitative properties were analyzed depending on the influencing factors, namely: the total of sugar content, reducing sugars, invert sugars, sucrose, water content, dry matter, total acidity, vitamin C, total phenols, total flavonoids and antioxidant capacity and fruit mass.

After the analysis, it can be concluded that raspberry plants treated with Veramin are characterized by lower content of total acidity (1.39%), dry matter (12.71%) and vitamin C content (20.60 mg / 100 g fresh weight). Based on the obtained results, it was shown that Veramin had a positive effect on the weight of the raspberry fruit and certain chemical and antioxidant properties of the fruit compared to the control treatment.

1. INTRODUCTION

According to the pomological classification, raspberries belong to the group of berries, and according to importance, to the order *Rosales*, family *Rosaceae*, subfamily *Rosoidae*, genus *Rubus*, species *Rubus ideus L*. Although having a short tradition of intensive cultivation in Bosnia and Herzegovina, it has been the leading variety in Bosnia and Herzegovina since 2013. Raspberries have long been known for their medicinal properties, as confirmed by [1], which states, that the *Rubus* plants and their fruits were collected in nature by the ancient Greeks and Romans and used for medicinal purposes fresh or processed.

The aim of this research is to understand the influence of the fertilizer *Veramine* with biostimulative effect and the effect on the quality elements of raspberry fruits. Test fertilizer *Veramine* is a liquid biostimulative foliar fertilizer based on Aloe Vera extract. Aloe Vera plant extract contains nutrients, polysaccharides, amino acids and enzymes. Amino acids that are an integral part of these fertilizers and stimulate protein synthesis in plants and any fertilizer enhanced with amino acids, is recommended for use in stressful situations.

Raspberries in the genus mostly use potassium, then nitrogen, and phosphorus [1].

The basic task of any applied feeding model is to achieve the highest possible efficiency of fertilizer, as well as the rationality and economic justification of its use. This research also showed certain differences in the quality of raspberry fruits that were treated, and within them, after conducting a statistical analysis of the results, certain conclusions were made in terms of fertilizer application.

2. MATERIAL AND WORK METHODS

The research was conducted on a raspberry orchard of the *Polka* variety, located at the site of "Ribića Otoka", in the settlement of Bakšaiš, located in the area surrounding the town Bihać, with a total area of approx. 9.5 ha. Raspberries are grown in a trellis system of free individuals with a type of backrest commonly called the T-system. According to [2] this area was mapped as alluvial-carbonate sandy soil on sands. It is located in the lower parts of the valley along the Una River and often alternates alluvially with alluvial, carbonate, loamy and clayey poorly gluey soils. The characteristics of the land in this locality is high natural drainage, water is lost very quickly, and the soil is thus very drained.



Picture 1: Experimental plantation - plot "Ribića Otoka" [3]

Fruit sampling was performed at full harvest, in late July and early August. Harvesting was performed for each marked field, with samples from all three fields treated with *Veramine* being combined into one average sample, as well as for the control field. Raspberry fruits were taken

from all parts of the plant, to avoid possible mistakes that may occur due to various microecological factors.

3. RESEARCH RESULTS AND DISCUSSIONS

Results of chemical analysis of raspberry fruit.

Polka raspberry fruit was examined, and the obtained results of the average values based on the examined chemical properties were presented. Thereby a standard deviation in the results of statistical data processing (One-Way ANOVA and Tukey - test) occurred, due to the volume of the results and the large number of individual tables. The obtained results for all the mentioned parameters are shown in three tables (chemical, antioxidant properties and fruit mass), according to the statistical processing of the data.

	0		1 1		1 7		
Treatment	Total sugars	Reduced	Inverted	Sucrose	Water	Dry matter	Total
		sugars	sugars		content	content	acidity
Veramine	$6,29 \pm 0,46^{a}$	$3,32 \pm 0,11^{\circ}$	$2,96\pm0,35_a$	$2,81 \pm 0,34^{a}$	$87,29 \pm 0,39^{a}$	12,71±0,39 ^c	1,39±0,16 ^b
Control	$5,46 \pm 0,28^{b}$	${\begin{array}{*{20}c} 4,04 & \pm \\ 0,24^{b} & \end{array}}$	$1,42 \pm 0,21^{b}$	$1,35 \pm 0,20^{b}$	$\begin{array}{c} 86,\!20\pm \\ 0,\!27^{b} \end{array}$	13,80±0,27 ^b	1,60±0,09 ^b
ANOVA test	p ≤ 0,05	p ≤ 0,05	p ≤ 0,05	p ≤ 0,05	p ≤ 0,05	$p \le 0,05$	$p \le 0.05$

Table 1. Average values of chemical properties of Polka raspberry fruit

Based on the data shown in table, depending on the treatment, a statistically significant difference was found in the observed chemical properties of *Polka* fruit (total sugars, reducing sugars, invert sugars, water content, dry matter content and total acidity). Foliar fertilizer factor with biostimulative effects was shown to affect some of the observed chemical properties of *Polka* raspberry fruit ($p \le 0.05$). From previous research, [4]; [5]; [6], it can be seen that the dry matter content in the fruit of raspberries, grown in the conventional way, ranged from 10.04% to 14.49%.

When it comes to the content of reducing sugars, based on the Tukey test, no statistically significant differences between the fruits analyzed from the fields, treated with *Veramine*, and control fields, was found (p > 0.05). The studies of total sugars in the fruits of the *Polka* variety, grown organically and conventionally in different locations in western Pomerania (Poland), conducted by [7], showed of content of 5.82% in organic cultivation.

[4] conducted a research as well on the *Polka* variety, in the from 2006 to 2007 (published 2008) and registered significantly higher values of the total sugar content in the fruit (7.10% and 8.42%), compared to the conventional method of cultivation. The average higher content of

inverted sugars (6.82%) in conventional cultivation of raspberry cultivar *Polka* over two experimental years compared to this study was published by [4].

[8] examined the influence of three types of biostimulative fertilizers (Atonik SL, Biochikol 020 PC and Tytanit) on the fruit yield and quality of three Polish raspberry varieties: *Pokusa, Polka* and *Poranna rosa* between the years 2006–2007. The content of total acids, depending on the application of biostimulative fertilizers, ranged from 1.68% (Biochikol 020), over 1.70% and 1.79% (control and Tytanit) to 1.82% (Astonik SL). The mentioned values are in the range of the obtained values of this study, with a significantly lower content of total acids recorded in this study on fruits analyzed from fields treated with biostimulative fertilizer *Veramine*.

In many studies, the content of total acids in raspberry *Polka* cultivars in the conventional cultivation method ranged from 0.8% to 1.87% [9]; [4]; [7], which is also significantly less than the value of total acid content in fruits analyzed in this study from fields treated with biostimulative fertilizer.

Antioxidant properties of raspberry Polka fruit

Table 2. Average values of antioxidant properties of 1 ofka faspoerty fruit					
Treatment	Vitamin C	Total phenols content	Total flavonoids	Antioxidant capacity	
	(mg/100 g FW)	(mg/g DW, presenting as	content (mg/g DW)	(µmol Fe2+/g DW)	
		gallic acid)			
Veramine	$20,60 \pm 0,29^{\circ}$	$12,67 \pm 0,42^{a}$	$4{,}21\pm0{,}10^{\mathrm{a}}$	$15,\!47\pm0,\!36^{a}$	
Control	$22,18 \pm 0,10^{b}$	$12,25 \pm 0,18^{\rm ab}$	$3,87 \pm 0,07^{\rm b}$	$14,18 \pm 0,28^{b}$	
ANOVA	p ≥ 0,05	p ≤ 0,05	p ≤ 0,05	p ≤ 0,05	

Table 2. Average values of antioxidant properties of Polka raspberry fruit

Based on the results of one - way analysis of variance (One - Way ANOVA), a significant effect of treatment (foliar fertilizer with biostimulative effect) on some of the antioxidant properties of *Polka* raspberry fruit, i.e. the content of total phenols, total flavonoids and antioxidant capacity of *Polina* fruit. ($p \le 0.05$) A higher average content of vitamin C in the fruits of raspberry cultivar *Polka* was registered in the fruits from the control field compared to the fields treated with biostimulative fertilizer [7].

Significantly higher content of vitamin C compared to these studies was published by Atonik SL, Biochikol 020 PC and Tytanit (in the study of the impact of three types of fertilizers with biostimulative effect on fruit yield and quality of three Polish raspberry varieties: *Pokusa, Polka* and *Poranna rosa* during 2006 - 2007.

The content of vitamin C in this study ranged from 43 mg / 100 g FW (Tytanit), over 47.00 and 48.00 mg / 100 g FW (Astonik SL and Biochikol 020) to 59.00 mg / 100 g FW in fruits from the control field. In the analysis of fruits of raspberry cultivar *Polka* grown organically and conventionally [7], obtained similar results in this study when it comes to vitamin C content.

Vitamin C content in cultivar *Polka* grown organically was 21.9 mg / 100g FW, and *Polka* grown in conventional ways 25.3 mg / 100g FW.

Based on the Tukey test, no major differences in total phenolic content was observed between fruits analyzed from the *Veramine*-treated field and the control field, (p> 0.05). Higher values of total phenol content compared to these studies were published in the study by [9]. The content of total phenols in this study for freeze-dried raspberry cultivar in organic cultivation was 18.64 mg GAE / g DW, and in conventional cultivation the content of total phenols was 15.17 mg GAE / g DW.

Observing the content of total flavonoids in the dry matter, a higher content was registered in fruits from fields treated with *Veramine* (4.21 mg / g DW), compared to control fields (3.87 mg / g DW). [11] in their research assessing the vegetative growth and fruit chemistry of some raspberry and blackberry varieties in southern Poland reported that the content of total flavonoids in three experimental years in fresh matter of *Polka* fruit ranged from 19.90 mg / 100g FW for 2011. year, 27.77 mg / 100g FW for 2012, 22.71 mg / 100g FW for 2013.

Antioxidant capacity in the tested samples of dried raspberry fruit was determined by the so-called. FRAP (Ferric Reducing / Antioxidant Power). The obtained values indicate that the higher FRAP value, and thus the best reduction ability, was measured in the dried fruits of the *Polka* variety from the fields treated with *Veramine* (15.47 μ mol Fe2 + / g DW), compared to those from the control fields (14.18 μ mol Fe2 + / g DW).

Table 3. Average values of the fruit weight of the Polka raspberry variety				
Treatment	Fruit mass (g)			
Veramine	$3,51 \pm 0,46^{a}$			
Control	$3,11 \pm 0,45^{b}$			
ANOVA	$p \le 0.05$			

The mass of the fruit of the raspberry variety *Polka*

Based on the data from Table 3, a statistically significant difference was found in the average weight of the fruit depending on the treatment. It has been shown that the factor of different types of foliar fertilizers with biostimulative effects affects the fruit weight of raspberry cultivar *Polka* ($p \le 0.05$). A higher fruit weight was found in the experimental fields treated with *Veramine* (3.51 g), while in the controls it was 3.11 g.

Significantly higher values of fruit weight expressed at the weight of 100 fruits were published by [10] when examining the influence of three types of fertilizers with biostimulative action (Atonik SL, Biochikol 020 PC and Tytanit). Obtained values of the mass of 100 fruits of the *Polka* variety in studies published by [12]; [13]; who examined the impact of the application of biostimulative fertilizer based on algae extract (Goemar BM 86) on the development and fruit

quality of dicotyledonous raspberry cultivars were consistent with the values obtained in this study.

4. CONCLUSION

After the presented results of the analysis, and the comparison of treatment and control analysis, it can be concluded that raspberry plants treated with *Veramine* are characterized by lower content of total acidity (1.39%), dry matter (12.71%) and vitamin C content (20.60) mg / 100g fresh weight) compared to the control treatment. This study showed that *Veramine* had a positive effect on the weight of the raspberry fruit and the remaining chemical and antioxidant properties that were investigated in this paper in relation to the control treatment. Based on the above, it can be concluded that the application of this fertilizer has given enviable results compared to the control variant, and it is recommended that raspberry producers use this fertilizer in production.

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THE EFFECT OF A HEALTHY DIET ON THE PREVENTION AND CONTROL OF TYPE 2 DIABETES

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Key words: diabetes, prevention measures, healthy diet, risk factors

ABSTRACT:

Diabetes mellitus is a chronic non-communicable disease that occurs as a result of a number of factors, of which lifestyle or bad lifestyle habits are the leading cause of type 2 diabetes. Type 2 diabetes occurs in middle age, so it is very important to take care of what is consumed and in what quantities. With a quality and balanced diet, type 2 diabetes can be prevented and later controlled. In this paper, I will analyze people with diabetes who are overweight (BMI greater than or equal to 25) and their diet until the onset of type 2 diabetes, as well as whether they follow a diabetic diet. Due to poor or no preventive examinations, a very common example in practice is that people, in addition to being overweight, have a genetic predisposition and a lot of stress. With my work, I will try to point out the importance of proper nutrition as well as the importance of a healthy lifestyle.

1. INTRODUCTION

A healthy diet is important for several reasons and necessary for all living beings and has several roles such as: food should provide energy that serves to sustain life, enables breathing, circulation, work of the heart and other organs in the body; energy obtained from food is used, among other things, for human movement and mechanical work; food also provides the body with building material for building new tissues during growth and development, as well as for constant tissue regeneration. Interest in healthy eating is growing. While some just want to eat healthy, others reach for vegetarianism or a macrobiotic diet. The basics of a healthy diet, i.e. the essential rules of a healthy diet are: variety, moderation and quality. Any healthy eating strategy or diet is based on the above three laws.

The diversity of the diet implies the intake of all the ingredients needed by the body, namely: carbohydrates, fats, proteins, minerals and trace elements, vitamins, ballast substances and water. Any diet that does not respect these laws is harmful to health in the long run.

Diet moderation refers to the intake of appropriate, neither too much nor too little amounts of certain nutrients, that is, the intake of those amounts of food that correspond to our work and physical activity.

The quality of nutrition is reflected in the selection of those foods that have a higher biological value, a greater variety of nutrients, greater freshness and less pesticide pollution, etc.

The highest quality food choices include fruits and vegetables, whole grains (whole grains), fish and poultry. Low-quality foods are white sugar and white flour, pork and fatty beef, sodas and snacks, etc.

Also, the quality of nutrition is reflected in maintaining the appropriate ratio of carbohydrates (55%-65%), fats (20%-25%) and proteins (15%-20%) [1].

2. MATERIAL AND METHODS

The goal of this research is to find out with the help of the data obtained from the research whether there is a connection between a healthy diet and healthy lifestyle habits for changes in body weight and blood sugar in people with type 2 diabetes. The study aims to examine whether and to what extent changes in diet and daily exercise affect weight loss and maintaining relatively good blood sugar levels in people with type 2 diabetes.

80 respondents participated in the research. The subjects are patients of the family medicine clinic of the Banja Luka Health Center, with elevated body weight and blood sugar values.

The research was conducted in the period from February 10, 2022 to March 10, 2022. Measurement of initial body weight values, blood sugar values and calculation of body mass index were performed in the family medicine clinic at the beginning and at the end of the research. While the test subjects themselves controlled their blood sugar before and after eating at home.

Subjects were asked to follow the diet from table 1. All subjects were given a copy of this table and verbal instructions about the importance of following this diet.

Note: all research participants are on continuous oral antiglycemic therapy.

Food ingredient	Description	Purpose	There are	Share in the diet	Give preference
Carbohydrates	Better known as sugars	Energy security	Sugar, flour, pasta, potatoes, rice	55% -65%	Whole grains
Fats	All oils and fats	Energy supply, melting of vitamins D, E, K, A.	Oils, fats, butter, cheeses, seeds	20% - 25%	Olive and fish oil Omega 3 fatty acids
Proteins	Proteins, amino acids	Building materials	Meat, milk, legumes, eggs	15% -20%	from white poultry meat, from
Minerals and trace elements	Sodium, potassium, calcium, selenium, zinc, copper, iodine, chromium, iron, etc.	Ensuring cellular function and structure	Meat, whole grains, vegetable s and fruits		Fruits and vegetables
Vitamins	A, D, EC, C, B complex, etc.	regulates processe s in cells and metabolis m, antioxida	Fruits, vegetable s, meat, milk, etc.		Fruits and vegetables

Table 1. Foods that should be preferred in the daily diet and purpose.

Data were statistically processed using Microsoft Office Excel 2010. Descriptive analysis, correlation and T-test were used for statistical calculation of data [2,4].

3. RESULTS AND DISCUSSION

80 respondents participated in the research, of which 40 (50%) were men and 40 (50%) were women (Graph 1). The median age is 60 years, from 35 to 90 years (Graph 2).



Graph 1. Gender structure of respondents

The graph clearly shows that an equal number of male and female respondents participated in the research.



Graph 2. Age of respondents

Table 2 shows the mean body weight of the subjects before adhering to the correct diet (at the initial measurement).

mean value	
average value	97.28
Maximum value	116
The most common value	95
Variation interval	30
Mean absolute deviation	38.87
Standard deviation	6.23
Coefficient of variation	6%

Descriptive analysis of the research shows that the body weight range of the respondents is 30 kg. The percentage of variability is 6%, which means that the variability of the subjects' body weight is very weak. The maximum value of the subject's body weight is 116 kg, and in comparison with the normally defined body weight, it can be concluded that this is a very high value of the obtained body weight, while the most frequently measured body weight of the subject is also 95 kg.

Table 3 shows the mean body weight of the subjects after following the appropriate diet (control measurement at the end of the study).

Table 3. Mean values of body mass				
mean value				
average value	91.03			
Maximum value	108			
The most common value	93			
Variation interval	28			
Mean absolute deviation	37			
Standard deviation	6			
Coefficient of variation	7%			

Descriptive analysis of the research shows that the body weight range of the respondents is 28 kg. The percentage of variability is 7%, which means that the variability of body weight in the subjects is very weak. The maximum value of the subject's body weight is 108 kg, and in comparison with the normally defined body weight, it can be concluded that this is a high value of body weight, but significantly less than the initial maximum body weight of the subject. the measured weight of the subject is 93 kg.

The first correlation analysis included the body mass index (BMI) values of the subjects before adhering to the proper diet (at the beginning of the study) and the body mass index (BMI) of the subjects after adhering to the proper diet (control measurement at the end of the study).

A Pearson-on correlation coefficient of 0.942 was obtained, which indicates that the correlation between the body mass index before starting a proper diet and immediately after starting a proper diet is very strong and positive. This can also be seen on the scatter diagram (Graph 3).



Graph 3. Scatter diagram

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The research examined whether there is a relationship between changing one's diet or following a healthy diet and body weight, assuming that there is no relationship. T-test was used.

The calculated value of the T-test statistic is 8.4 and the table value with significance level α = 0.01 is 2.64. Since the table value is less than the calculated p value of <0.01, we conclude that there is a statistically significant difference.

There was a decrease in body weight in subjects who followed a proper diet.

Table 4 shows the average blood sugar level of the subjects before the proper diet (at the initial measurement).

Table 4. Mean blood sugar values				
mean value				
average value	10.42			
Maximum value	14.8			
The most common value	9			
Variation interval	6.9			
Mean absolute deviation	0.0			
Standard deviation	1.52			
Coefficient of variation	15 %			

Descriptive analysis of the research shows that the range of blood sugar values in the subjects is 6.9 mmol/l. The percentage of variability is 15%, which means that the variability of the blood sugar values in the subjects is relatively weak. The maximum blood sugar value is 14.8 mmol/l, and in relation to the normally defined blood sugar value, it can be concluded that this is a very high blood sugar value obtained, while the most frequently measured blood sugar is also high and amounts to 9 mmol/l.

Table 5 shows the average value of the blood sugar of the subjects after a proper diet (control measurement at the end of the study).

Table 5. Mean blood sugar values				
mean value				
average value	7.55			
Maximum value	9			
The most common value	8			
Variation interval	2.7			
Mean absolute deviation	0.0			
Standard deviation	0.65			
Coefficient of variation	9 %			

Descriptive analysis of the research shows that the range of blood sugar values of the subjects after a proper diet is 2.7 mmol/l. The percentage of variability is 9%, which means that the variability of the blood sugar values in the subjects is very weak. The maximum value of blood sugar after a proper diet is 9 mmol/l, and in comparison with the normally defined value of blood sugar, it can be concluded that it is an elevated blood sugar value, but significantly lower compared to the blood sugar at the beginning of the study, while the most often measured blood sugar value in subjects after a proper diet is also high, but lower compared to the blood sugar value at the beginning of the study and is 8 mmol/l.

Another correlational analysis compared blood sugar values before following a proper diet and after following a proper diet. A Pearson-on correlation coefficient of 0.715 was obtained, which indicates that the correlation is directly expressed and positive. This can also be seen on the scatter diagram (Graph 4).



Graph 4. Scatter diagram

The study examined whether there was a relationship between blood sugar levels in subjects with type 2 diabetes and dietary changes, assuming there was not. T-test was used.

The calculated value of the T-test statistic is 8.42 and the table value with significance level α = 0.01 is 2.64. Since the table value is smaller than the calculated p value of <0.01, we conclude that there is a statistically significant difference, i.e. there is an impact of dietary changes on blood sugar reduction.

Table 6 shows the mean value of blood sugar of the subjects who, along with a proper diet, took a light walk every day for 30 minutes (control measurement at the end of the study).

Table 6. Mean blood sugar values	
mean value	
average value	6.61
Maximum value	7.3
The most common value	7
Variation interval	2.3
Mean absolute deviation	0.0
Standard deviation	0.45
Coefficient of variation	7 %

Descriptive analysis of the research shows that the range of blood sugar values of subjects who, along with a proper diet, exercised and walked lightly every day for 30 minutes, was 2.3 mmol/l. The percentage of variability is 7%, which means that the variability of the blood sugar values in the subjects is very weak. The maximum blood sugar value is 7.3 mmol/l, and in comparison with the normally defined blood sugar value, it can be concluded that this is a relatively high blood sugar value, but lower compared to the blood sugar of subjects who do not walk lightly. 30 minutes, while the most commonly measured blood sugar value is high but also lower and amounts to 7 mmol/l.

The third correlation analysis compared the blood sugar values of the subjects who followed a proper diet but did not exercise by walking for 30 minutes and the blood sugar values of the subjects who exercised. A Pearson-on correlation coefficient of 0.64 was obtained, which indicates that the correlation is directly expressed and positive. This can also be seen on the scatter diagram (Graph 5).



Graph 5. Scatter diagram

The study examined whether there was an association between the blood sugar values of subjects with type 2 diabetes and a daily 30-minute light walk, assuming that there was no association. T-test was used.

The calculated value of the T-test statistic is 5.6 and the table value with significance level α = 0.01 is 2.71. Since the table value is less than the calculated p value of <0.01, we conclude that there is a statistically significant difference, that is, that there is an effect of a light walk lasting 30 minutes on blood sugar reduction.

Our research proved that middle-aged and elderly respondents have increased body weight. Research has statistically proven that a healthy diet affects weight loss in people with type 2 diabetes. We have statistically proven that a healthy diet along with physical activity in the sense of daily light walking for 30 minutes leads to a lowering of blood sugar in people with diabetes. type 2.

4. CONCLUSION

Based on the research results, the following can be concluded:

- people older than 35 have a problem with increased body weight and elevated blood sugar levels,
- the same number of men and women with increased body weight and elevated blood sugar levels,
- there is a significant difference in the measured body weight before and after following a proper diet, i.e. there is an impact of dietary changes on weight loss in people with type 2 diabetes,
- there is a significant difference in the measured blood sugar values before and after adhering to a proper diet, i.e. there is an impact of dietary changes on the reduction of blood sugar in people with type 2 diabetes,
- there is a significant difference in the measured blood sugar values of subjects who, in addition to dietary changes, also practiced daily light walking for 30 minutes, i.e. there is an effect of daily light walking for 30 minutes on lowering blood sugar in people with type 2 diabetes.

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CONTINUOUS ENVIRONMENTAL IMPROVEMENTS

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Keywords: environmental protection, accountability, PDCA continuous improvement

ABSTRACT:

The paper presents continuous improvements in environmental protection. This is one of the critical aspects of the overall quality of environmental protection. It is important to understand that protection is not a project to be implemented, and then forgotten. Environmental protection must be continuously implemented and improved by introducing, implementing and maintaining an integrated management system. Modern tendencies in management are that employees are not given simple, standardized tasks and methods, but rather goals, and they have the freedom to choose the way to achieve those goals. In this way, the modern meaning of the PDCA cycle moves from classical harmonization to higher levels of performance. This requires a higher level of education and training of employees, development, individualization and creativity. In order to achieve this, it is necessary to involve all employees in the realization of the established goals.

1. INTRODUCTION

Bosnia and Herzegovina, as well as the surrounding countries, have constant environmental problems. These problems arise due to improper planning. Everything related to political decisions is a wrong path, and a path without a goal and a vision. Environmental issues must be addressed by an expert team at the state, entity and cantonal levels. This is the most important part of all management systems. There are different approaches at all levels of government and none of them takes into account the requirements of the standards [1].

ISO standards show that there may be unique approaches but they need to be implemented in organizations in order to fully understand the requirements and individual nuances related to environmental protection. In organizations that deal with environmental protection, in which the

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requirements have not been implemented or measurable and achievable goals have not been set, it is not known who is responsible and for what area. That is why we have legal illegal landfills throughout Bosnia and Herzegovina.

The beginning of ISO 14000 dates back to 1990 with the formation of the Business Charter for Sustainable Development (BCSD), an organization of 50 business leaders interested in ecology and development. The basic idea of their work is that economic development can take place only in a healthy living space [2].

At the United Nations Conference on Environment and Development in Rio de Janeiro in 1992, world leaders agreed on a comprehensive strategy for sustainable development. This strategy aligns global needs and ensures a healthy and preserved world for future generations.

There are three known Rio conventions [3]:

- a) The United Nations Convention on Biological Diversity,
- b) The United Nations Convention to Combat Desertification, and
- c) The United Nations Framework Convention on Climate Change.

Each of the three conventions represents a way and a contribution to the goals of sustainable development. All three conventions are intrinsically linked, have a common area of action in the same ecosystems but at the same time have a unique approach to their thematic areas [3].

In March 2021, the Law on Environmental Protection entered into force in the Federation of Bosnia and Herzegovina ('Official Gazette of the Federation of Bosnia and Herzegovina 15/21''). Accordingly, by-laws have been drafted to date. It is very important to note that the Regulation on projects for which environmental impact assessment is mandatory and projects for which the need for environmental impact assessment is decided has been adopted ('Official Gazette of the Federation of Bosnia and Herzegovina No. 51'').

2. ISO STANDARDS

The purpose of this international standard is to provide organizations with a framework for environmental protection and responses to changes in its condition in balance with socioeconomic needs. They specify the requirements that enable the organization to achieve the intended outcomes, set for its environmental management system.

Achieving a balance between the environment, society and the economy is considered essential in order to meet current needs without compromising the ability of future generations to meet their own needs. Sustainable development, as a goal, is achieved by balancing the three pillars of sustainability [4].

The ISO 14001 standard specifies requirements for the environmental management system, which an organization can use to improve its environmental performance. This is for organizations that want to manage their responsibilities for the environment in accordance with positive legal regulations and contribute to sustainable development. The implementation of these requirements achieves the goals and projected outcomes of the environmental management system, which provide value for the environment of all stakeholders and society as a whole. Consistent with the organization's environmental policy, the intended outcomes of the environmental management system include improving environmental performance, meeting compliance obligations, and achieving environmental goals [1].



Figure 1. ISO standard requirements [4]

Figure 1 shows the framework introduced in the international standard integrated into the PDCA model, which can help new and existing users understand the importance of a systems approach. The PDCA model can also be represented by an image in the form of a Deming cycle.



Figure 2. PDCA Deming cycle [5]

The PDCA or Deming cycle can be briefly described as follows [6]:

- ✓ Plan: Establish the environmental goals and processes necessary to obtain results in accordance with the organization's environmental policy.
- ✓ **Do:** Implement processes as planned.
- ✓ Check: Monitor and measure processes in relation to environmental policy, including its commitments, environmental objectives and operational criteria, and report on results.
- ✓ Act: Take actions for continuous improvement.

3. UNDERSTANDING AND BENEFITS OF ISO 14001

Organizations dealing with environmental protection in Bosnia and Herzegovina are in crisis, problems, limitations or lag behind the requirements of the European Union. Organizations must anticipate the arrival of some of the invisible factors and make timely improvements. The goal of this type of radical improvement is to get the organization out of the existing crisis, problem or limitation. These improvements are used to manage risks and overcome upcoming crises, problems or constraints that management must anticipate.

The process of improvement in an organization cannot begin with continuous expansion of improvement until the organization is trained to do so. It is the transition of the organization from the position it is in to respond to expected or current changes in and around itself.

Most utility companies in Bosnia and Herzegovina are in this state. It is necessary to bring an expert team to these organizations, which will perform process reengineering with their

competencies, and introduce an integrated management system to achieve the set goal, which is the application of European standards and declarations. In other words, the desire is to introduce zero-waste technology.

Utility companies intending to implement ISO 14001 are advised to make an assessment of the existing environmental management system, and to identify activities that meet ISO 14001 requirements and shortcomings that need to be addressed, especially in cases where illegal dumps are legalized.

It is very important to understand that ISO 14001 is a standard of environmental management, not a standard of environmental work. Therefore, state authorities must define environmental policy for all organizations and ensure that they [6]:

- respond to the nature, scale and environmental impacts of its activities, products and services,
- to be involved and engaged in the continuous improvement and prevention of pollution,
- to participate and engage in compliance with relevant environmental laws and regulations and other requirements applicable to the organization,
- to provide a basis for establishing and reviewing environmental goals and efforts,
- to be documented, implemented and maintained and communicated to all employees,
- to be available to the public.

The main benefit of the introduction of an integrated quality management system, i.e., the implementation of the standard requirements is the use of raw materials, both industrial and municipal waste, to obtain alternative energy. Organizations that introduce and implement an environmental protection system have a number of advantages [7]:

- easier implementation of risk management is ensured, especially when it comes to endangering the ecosystem,
- more rational management of available resources,
- special benefits are received from the European Union,
- the possibility of damage to the environment is prevented,
- insurance costs are reduced in case of incidents,
- better relations are established with all partners, neighbors and the wider community,
- increases the self-awareness of all employees, the culture of the organization and behavior towards nature and the environment in general,
- significant benefits are gained in expanding into new markets and retaining existing ones, and

• it is committed to an ecologically positive approach in its own work, development and production and the provision of services.

4. CONCLUSION

Very few municipalities in Bosnia and Herzegovina have solved the problem of municipal and industrial waste supply. All previous plans in Bosnia and Herzegovina regarding the creation of regional landfills have either been incorrectly implemented or have not been implemented at all. In order for municipalities to solve this problem, it is necessary to develop structural plans and comply with and implement the requirements of ISO standards, directives and conventions.

ISO 14001 is applicable in any organization that wants to achieve the following:

- ✓ To implement, maintain and improve the environmental management system.
- \checkmark To ensure compliance with the stated environmental policy.
- \checkmark To show such harmony with others.
- \checkmark To request certification from an external organization.
- \checkmark To do self-determination and self-declaration of compliance with standards.

In order to achieve this, it is necessary to educate management staff with the requirements of ISO standards and introduce an integrated management system.

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AFLATOXIN WITH AN ASPECT TO ANIMAL FEED

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Key words: mycotoxins, aflatoxins, feed, ELISA

ABSTRACT:

Molds of the genus Aspergillus are among the most common causes of spoilage of food and feed, and in adverse conditions can produce mycotoxins as toxic secondary metabolites. Aflatoxins are a group of mycotoxins that often contaminate various cereals, most often corn, and by entering the food chain due to animal feed contaminated with animal feed, they can also be present in food of animal origin. Aflatoxin contamination can affect all processes from cultivation and harvesting, through storage to processing, although only storage in high humidity conditions is generally mentioned. The appearance of aflatoxins is conditioned by climatic conditions, geographical position, agricultural processes and raw material processing processes. In order to prevent the formation of aflatoxins, it is necessary to first prevent the growth of mold at all stages of production, collection, transport, processing, storage and sale of foods on which mold can develop. Constant monitoring of aflatoxin levels in food and animal feed is also required. Methods for the determination of aflatoxins are: ELISA method, gas chromatography, liquid chromatography, high performance liquid chromatography and mass spectrometry. The aim of this study was to examine the presence of aflatoxin B1 in animal feed in the area of USC. The obtained results will show us whether the measures are observed before the harvest, during the harvest and after the harvest, and whether we are sure of this biological danger when consuming meat, eggs and dairy products.

1. INTRODUCTION

Aflatoxins often contaminate cereals, so systemic control of mycotoxins in food and feed is necessary to avoid negative health effects as well as economic losses in agriculture. Early

Bihać, 09 - 10 June 2022.

detection of aflatoxins in feed reduces the possibility of their transfer to the meat of the animals we consume or their products [1]. In order to prevent the contamination of agricultural products with mycotoxins, the modern production of healthy and high-quality products must be based on the principles "from farm to table", the HACCP (Hazard analysis and critical control points) system, i.e. hazard analysis, prevention and control of critical points, and elimination of potential hazards in the technological process of production [2]. Mycotoxins are secondary products of the metabolism of some types of molds, which, due to food contamination, are a very serious problem for human and animal health. Ingestion of mycotoxins can cause numerous toxic responses, from acute toxicity (pulmonary mycotoxicosis, organic dust syndrome, toxic mold syndrome, etc.) to, more often, chronic health problems, including immunosuppression or even carcinogenesis. Protein supplements, enzymes and additives, as well as milk, milk products, meat and meat products can also be contaminated with mycotoxins, if the feed was contaminated with molds. Molds that most often grow on stored food are Penicillium, Aspergillus and Mucor, while species from the genera Alternaria, Helminthosporium and Chaetomium contaminate crops during the growing season in the field [3]. Due to climate change, aflatoxins will appear more and more often in parts of the world with a moderate climate, and at the same time, in tropical regions, due to high temperatures, some aflatoxicogenic species could die out, which would have a positive effect on agriculture there. The same authors point to the conclusion that the effect of climate change on the colonization of crops by molds and the production of mycotoxins should be evaluated individually because each type of mold grows in its optimal conditions of air temperature and water activity and creates toxic metabolites, which makes this problem even more complex [4]. Systemic control of mycotoxins in food is necessary to avoid negative effects on the health of both humans and animals, and at the same time to avoid economic losses in agricultural and animal production [5].

For the purpose of determining aflatoxin, different orientation (screening) and confirmatory analytical methods are used, which can further be divided into qualitative and quantitative. Orientation methods include analytical procedures that can be shown in a documented, traceable manner to have been evaluated and to have a percentage of false negative results of less than 5% at the concentration level of interest. Of the orientation methods in the determination of mycotoxins, the enzyme-linked immunosorbent assay (ELISA) method is most often used. The development of the ELISA method enabled the implementation of fast, reproducible and sensitive analytical methods, suitable also for the determination of aflatoxin. Literature data indicate that when it comes to aflatoxin determination, ELISA has numerous advantages over other analytical methods. These advantages mainly relate to the speed of the method, the possibility of analyzing a large number of samples in a short period of time, high specificity, simplicity, low cost and the use of harmless reagents. The quality of ELISA kits, however, can

vary from manufacturer to manufacturer, and there are differences even in different series of kits from the same manufacturer.

Compared to other parts of the world, the European Union (EU) has the most extensive and welldeveloped regulations defining MRLs for AFB1 in different types of food and feed. In addition, numerous countries (candidates for EU membership) have also adopted regulations regulating the presence of these mycotoxins in food and animal feed, including all items from the currently valid regulations in the EU.

The methods of sampling and analysis that competent authorities should carry out for the purpose of controlling aflatoxin levels are prescribed by Commission Regulation No. 401/2006, as amended and amended by 2002/32/EC, and are significantly different from those prescribed in other countries of the world. The aforementioned Directive defines MRL by types of fertilizers and fodder mixtures, while the regulations of some countries in this context specify more limit values depending on the purpose for which the feed will be used. Considering the origin of AFM1 from AFB1 in milk and milk products, from the point of view of human health, the strictest criterion for AFB1 is applied to fertilizers intended for feeding dairy cattle (MRL= 5 $\mu g/kg$) [4]. The maximum permissible levels considered generally safe in food and feed are listed in Table 1.

Food/animal feed	AFB ₁	
Food intended for human consumption	(μg/kg)	
Maize and cereals as fertilizers in the production of animal feed	20	
Maize and grains for growing breeding cattle, pigs and poultry	100	
Maize and cereals for feeding pigs	200	
Maize and cereals for fattening cattle, pigs and poultry	300	

*(Commission Regulation 1881/2006 and 165/2010; Commission Directive 2003/100/EC) [7].

In the Federation of Bosnia and Herzegovina, the control of mycotoxins in food products is carried out based on the Rulebook on the Maximum Permitted Amounts of Certain Contaminants in Food ("Official Gazette of Bosnia and Herzegovina", No. 37/09) [8].

The aim of this work was to examine the presence of aflatoxin B1 in feed in the area of USK. The obtained results will show us whether the pre-harvest, harvest and post-harvest measures are respected and whether we are safe in terms of this biological hazard when consuming meat, eggs and dairy products.

Even in small quantities, mycotoxins pose a danger to animal and human health. Humans and animals ingest mycotoxins through food, inhalation or through the skin. Aflatoxins are one of the most toxic mycotoxins. They are formed by strains of mold of the genus Aspergillus, and the

temperature suitable for their growth is from 26 to 38° C and the moisture content of the substrate is greater than 18%. Aflatoxins are thermostable, soluble in organic solvents, and are very sensitive to alkaline solutions and acids, and are almost insoluble in water. In general, mycotoxins have hepatotoxic, dermonecrotic, immunosuppressive, nephrotoxic, carcinogenic, neurotoxic, and estrogenic effects. Contamination with mycotoxins can result in significant economic losses in livestock production, that is, in the production of meat and meat products, and above all, their presence in foodstuffs can indirectly negatively affect human health. Suppression, i.e. protection of food and animal feed from mycotoxins must be carried out throughout the entire food chain "from cultivation to table".

The rulebook on the implementation of analytical methods and the interpretation of results defines the conditions for the application of analytical methods, and each method must be previously tested through the determination of validation parameters and must provide accurate and precise data, be specific enough to determine very low concentrations of mycotoxins, i.e. have low detection limits. The immunoenzymatic method (ELISA-Enzyme-Linked Immunosorbent Assay) is mostly used as a simple and fast method, economically and environmentally acceptable with the possibility of analyzing a large number of samples [9].

2. MATERIAL AND METHOD

Data on the content aflatoxin of the samples animal feed were made in the laboratory of the Veterinary Institute of Bihać, accredited (BAS EN ISO/IEC 17025:2006) with the standard and ELISA Enzyme Linked Immunosorbent Assay. A total of 54 samples were analyzed in a period of 6 months. The method of sampling and preparation of the sample was carried out according to the instructions from the regulations on sampling and analysis methods of the amount of mycotoxins in food Official Gazette of Bosnia and Herzegovina, No. 37/09 and amendments to Rulebook 68/12 and 62/17. The feed samples that were analyzed were: beef feed, food for laying hens, food for pigs, maize/barley, wheat feed flour, wheat, barley, beef feed, corn, chicken starter, food for growing chickens and others.

Enzyme-linked immunosorbent assay is a common analytical biochemical test, first described by Engvall and Perlmann, 1971. The assay uses a type of enzyme-linked immunosorbent assay (EIA - Environmental Impact Assessment) to detect the presence of a ligand (usually a protein) in a liquid sample, using antibodies. directed at the protein being measured. Sample preparation and extraction: grind the sample and store it at room temperature until analysis. Weigh 5.0+/-0.1 g of sample in a 50 ml screw centrifuge and add 25 ml of 33% methanol and mix for 2 minutes. Allow the contents to settle for 15 minutes at room temperature and filter the sample through a Whatman filter. In a 15 ml centrifuge, dilute the sample 1:2 (v/v) with 33% methanol, 1 ml of extract + 1 ml of diluted methanol and test the sample. All reagents in the kit are kit and lot specific and must not be mixed with one another. The kit is stored in a refrigerator at $+2^{\circ}C$ to +8°C and can be used until the expiration date, indicated on the label on the box. Before use, all components from the kit should reach room temperature, so it is necessary to take the kit out of the refrigerator an hour and a half to two hours before use [8]. All microtiter plate applications were in duplicate.

Implementation: take the required amount of reagent

- 1. Add 200 microliters of distilled water to the blank wells (blank test),
- 2. Add 50 microliters of the zero standard to the maximum binding well,
- 3. Add 50 microliters of the standard to the appropriate wells,
- 4. Add 50 microliters of the sample to the appropriate wells,
- 5. Add 50 microliters of enzyme conjugate to each well except blank wells,

6. Add 100 microliters of anti-aflatoxin antibody to each well except blank wells, using a multichannel pipette. Cover the plate with cling film and aluminum foil. Mix the plate gently by hand,

7. Incubate for 20 minutes at room temperature in the dark and

8. Wash the microplate with 200 microliters of wash solution, a total of 5 times. After rinsing, pour the contents of the plate into a suitable container. Make sure that all wells are well filled with washing buffer for each wash. After the last rinse, dry the plate well with absorbent paper and make sure that there are no air bubbles in the wells.



Figure 1. Sample preparation for analysis (Source: author)

3. RESULTS AND DISCUSSION

In cooperation with JU "Veterinarski zavod Bihać" in the area of USK, 54 different types of feed were analyzed. The table shows the results of the analysis.

Table 2. Analysis results						
Ordinal number	Sort	MAQ (maximum allowed quantity mg/kg)	Result mg/kg			
1.	The feed samples that were analyzed were	0.005	<0.00010			
2.	///	///	///			
3.	Beef feed	0.02	0.00015			
4.	Food for laying hens	0.02	0.00021			
5.	Food for pigs	0.02	< 0.00010			
6.	Maize/barley	0.005	< 0.00010			
7.	Wheat feed flour	0.02	<0.00010			
8.	Wheat	0.02	< 0.00010			
9.	Barley	0.02	<0.00010			
10.	Beef feed	0.02	<0.00010			
11.	Corn	0.02	<0.00010			
12.	Chicken starter	0.02	<0.00010			
13.	Food for growing chickens	0.02	0.00018			
14.	Food for growing chickens	0.02	<0.00010			
15.	Food for laying hens	0.02	<0.00010			
16.	Food for bulls	0.02	<0.00010			
17.	Feed for beef cattle	0.02	<0.00010			
18.	Food for laying hens	0.02	<0.00010			
19.	Soy	0.02	<0.00010			
20.	Corn	0.02	<0.00010			

21.	Oats	0.02	<0.00010
22.	Wheat	0.02	<0.00010
23.	Fodder mixture	0.02	<0.00010
24.	Corn	0.02	<0.00010
25.	Mixture for dairy cows	0.005	0.00021
26.	Barley in the grain	0.02	<0.00010
27.	Corn	0.02	<0.00010
28.	Wheat	0.02	<0.00010
29.	Mixed dressing	0.02	0.00016
30.	Concentrate for laying hens	0.02	<0.00010
31.	Concentrate for lambs	0.02	<0.00010
32.	Oats for cattle feed	0.02	<0.00010
33.	Starter	0.02	<0.00010
34.	Additive for pig fattening	0.02	<0.00010
35.	Food for dairy cows	0.005	<0.00010
36.	Complementary food	0.02	<0.00010
37.	Chicken feed	0.02	<0.00010
38.	Feed for fattening	0.02	<0.00010
39.	Corn flour	0.02	<0.00010
40.	Corn	0.02	<0.00010
41.	Livestock meal	0.02	<0.00010
42.	Starter	0.02	<0.00010
43.	Food for piglets	0.02	0.00020
44.	Mixture for feeding sows	0.02	<0.00010
45.	Calf feed	0.01	<0.00010

46.	Food for dairy cows	0.005	<0.00010
47.	Finisher	0.02	<0.00010
48.	Starter for lambs	0.01	<0.00010
49.	Starter	0.02	<0.00010
50.	Beef feed	0.02	<0.00010
51.	Food for lactating cows	0.005	<0.00010
52.	Food for laying hens	0.02	<0.00010
53.	Pig fattening	0.02	<0.00010
54.	Grover chickens	0.02	<0.00010
55.	Feed for beef cattle	0.02	<0.00010

The maximum concentration of mycotoxins in agricultural and food products is legally regulated. For AFB1, which is considered the most toxic of all mycotoxins, the permitted value, i.e. the maximum permitted amount in animal feed, with the exception of supplementary and complete feed mixtures, is 0.02 mg/kg (ppm) ("Official Gazette of BiH", no. 37/09, EU 574/2011). The results of this study (Table 2) showed that all samples didn't exceed the established limits of 0.02 mg/kg set by the Feed Regulation and Directive 2002/32/EC as amended by Commission Regulation (EU) 574/2011 [10]. Many authors reported concentrations that exceeded established limits. In Iran, the concentration of AFB1 in hay was higher (10%; 4/40) than the EU limit [11]. In Tanzania, Mohammed et al. [12] found that AFB1 was present in 65% (13/20) of the feed samples. An interesting study that evaluated the presence of mycotoxins in relation to the geographical area, reported that more than half of the samples collected in Europe exceeded the legal limit of quantification; in the Asia-Pacific region, AF contaminations were more frequent [13], and a higher content was found in feed samples collected during the winter season than those collected in the summer months [14].

The most extensive research was conducted by the company Biomin, on as many as 15,596 samples of animal feed from Europe, in the period from 2009 to 2017. High values of around 40% were recorded in 2011, 2012, 2013 and 2014. In other research years, the values were below 20% [15]. Mwakosya et al. [16] reported that all feed collected from producers, suppliers and consumers tested positive for aflatoxin B1. They found that 91% of the samples collected had aflatoxin B1, which exceeded the internationally acceptable limit for animal
feed safety. Similarly, Magembe et al. [17] reported that the majority of samples in the surveyed villages (100%) in Kilosa were unsafe for consumption as they exceeded 20 μ g/kg. A rapid monitoring test, such as the rapid AFB1 ELISA described in this paper, could greatly benefit local producers and manufacturers by providing an affordable tool for a quality management system that protects animal and human health.

4. CONCLUSION

Even in small quantities, mycotoxins pose a danger to animal and human health. Humans and animals ingest mycotoxins through food, inhalation or through the skin. Aflatoxins are one of the most toxic mycotoxins. They are formed by strains of molds of the genus Aspergillus, and the temperature suitable for their growth is from 26 to 38°C so the moisture content of the substrate is greater than 18%. Aflatoxins are thermostable, soluble in organic solvents, and are very sensitive to alkaline solutions and acids, and are almost insoluble in water. In general, mycotoxins have hepatotoxic, dermonecrotic, immunosuppressive, nephrotoxic, carcinogenic, neurotoxic, and estrogenic effects. Contamination with mycotoxins can result in significant economic losses in livestock production, that is, in the production of meat and meat products, and above all, their presence in foodstuffs can indirectly negatively affect human health. Suppression, i.e. protection of food and animal feed from mycotoxins must be carried out throughout the entire food chain "from cultivation to table".

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ECOCRITICAL QUESTIONING OF BOSNIAK ORAL LITERATURE

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Key words: oral literature, ecocriticism, Bosniak oral tradition, nature

ABSTRACT:

Nature is reflected in the oral literature of Bosniaks in various ways. It is not only a cause for the creation of songs and stories, but, through motifs and themes, it is an integral part of oral literature. Questioning the connection between nature and the oral literary tradition is not new in literary criticism. However, it seems that little has been written about this connection from the aspect of ecocriticism. Therefore, the objective of this paper is the ecocritical questioning of oral traditions, that is, the analysis and interpretation of examples in which the effort of the collective to influence the protection of the environment, flora and fauna is recognized. With the help of the descriptive and comparative method, and method of working on the text, the paper confirms the initial assumption that selected examples of oral traditions recorded in the Bosniak community convey knowledge about the importance of preserving nature, i.e., the environment. The paper contributes to a better understanding of the relationship between oral literature and nature.

1. INTRODUCTION

In the book *Readings from Bosnia and Herzegovina*, Ludvik Kuba, a famous Czech melographer, musicologist, painter and travel writer, writing about his personal experience in Bosnia in 1893, recorded the following:

"I never knew in advance how I would find a song. The song is a bird, a fish, a butterfly, a cloud. It is present everywhere, but hidden. And when it appears, it easily disappears again. The one chasing it is truly in a difficult position. It protects itself from him like the girl protects herself from a boy in the Bosnian song:

Girl: I am the sun.
Boy: And I'm a cloud in the sun.
Girl: I am a pearl.
Boy: And I'm a thread on clothes.
Girl: I'm a sweet basil.
Boy: I'll pluck the stalk of basil.
Girl: I will become a fish.
Boy: And I a diver, I'll catch a fish.
Girl: I will become a golden cup.
Boy: I'll raise a glass to my lips'.

(Kuba 1998: 41)

This excerpt from the book, which Munib Maglajlić pointed out is one of the "most interesting books about Bosnia and Herzegovina written by a foreigner" (Maglajlić 1983: 209), is important for the ecocritical questioning of oral literature for at least two reasons. The first is that Kuba, describing the search for a Bosnian song, shed light on the relations between man, nature and oral literature and showed that writers had long noticed the connection between the aforementioned phenomena in this area. Another reason lies in the fact that the example of oral poetry that the travel writer included in his text, which is the result of the creativity of oral singers, reflects a world in which animate and inanimate nature are one. Therefore, it can be concluded that the questioning of the way nature is reflected in oral literature is not a phenomenon that is only related to the 21st century, but it has continuously appeared since the first critical and theoretical works on oral literature, and that nature is present in the most diverse ways in oral songs and stories on the territory of Bosnia and Herzegovina.

The aim of this paper is the analysis and interpretation of Bosniak oral traditions, in which the community's efforts to influence nature protection can be recognized. The paper first presents ecocriticism and the characteristics of oral literature and oral traditions using the method of description, and then analyzes and interprets selected examples. In addition to the mentioned method, the paper also uses the comparative method and the method of working on the text. Finally, results of the ecocritical questioning of oral traditions are presented. The initial hypothesis of the work is that Bosniak oral traditions transmit knowledge about the importance of preserving nature and the environment.

2. ECOCRITICISM

There are many definitions of ecocriticism. This paper will begin with the one presented by Cheryll Glotfelty in the introduction to the first collection of ecocritical discussions, *The Ecocriticism Reader: Landmarks in literary theory* (1996). According to Glotfelty, ecocriticism

is studying the relationship between literature and the physical environment. In addition to ecocriticism, ecopoetics, environmental literary criticism and green cultural studies are also used as synonyms. In the broadest sense, ecocriticism tries to answer the questions of the role of the physical environment in novels, whether writing about nature can be characterized as a genre, how literacy has influenced the relationship of man to nature, how the concept of wilderness has changed over time, and what influence can ecology have towards the study of literature.Despite the wide scope of research, ecological criticism starts with the basic premise that human culture is connected to the physical world, it influences it, but also it is influenced by it. Comparing the literary theory and ecocriticism extends the concept of the world refers to society (sociosphere) and ecocriticism originates from William Ruecert, who presented the application of ecology and ecological concepts in the study of literature in the essay *Literature and Ecology: An Experiment in Ecocriticism*. Unlike Ruecert, Cherill Glotfelty views ecocriticism more broadly (Glotfelty 1996).

In order to understand the ecocritical questioning of literary texts, the text *Theory from the Fringes: Animals, Ecocriticism, Shakespeare* by Simon C. Estok is of great importance, in which the author, among other things, briefly presents the ecocritical objectives: "Firstly, ecocriticism committed to introduce changes by analyzing the functions of depicting the natural environment in written documents (literary or other). Secondly, it committed to making connections. Thirdly, it committed to adhere to pluralism and embrace other activist theories" (Estok 2007: 85).

3. ORAL LITERATURE

Oral literary creativity is labelled differently in scientific literature: folk literature, oral literature, folk creativity, folklore, folklore literature, oral tradition. The mentioned terms are connected, first of all, with the effort to emphasize the essence of this art, but also the specificity by which it differs from written literature. In this paper, the term oral literature is used in the sense described by MajaBošković-Stulli in the text *On the Terms of Oral and Popular Literature and Their Names*. Bošković-Stulli begins from the criteria of oral artistic communication and notes the following:

"From the aspect of literature, oral literature is an aesthetic (literary) message resulting from natural (contact) communication – separated from the context of life, but bearing witness to it indirectly." (Bošković-Stulli 1983:52)

According to Snežana Samardžija, the term oral literature refers to the oral existence, duration and transmission of works. Indirectly, this definition includes other characteristics of the work that an individual creates in front of an audience. According to this author, the term oral literature is interesting because it contains a contradiction:

"Thus, in relation to written literature, one can justifiably speak of oral literature, but the very combination of the terms 'oral' and 'literature' (from the word 'book') connects categories that are excluded, and the term 'oral literature' can be seen as an oxymoron." (Samardžija 2021: 8)

Oral literature preceded written literature, and with the appearance of letters it continued to exist in parallel with written literature. In relation to written literature, oral literature has a special type of communication (oral), which was reflected in the creation, transmission and preservation of oral literary works.

In addition to a special way of functioning, works of oral literature also have extra-literary functions. They can also belong to practical life, that is, they can transmit certain knowledge and be a lesson, an activity that makes work easier or as a pastime that fills free time in a community. Oral literary works can be 'an expression of political and social opinion, as a form of oral journalism'' (Bošković-Stulli, Zečević 1978: 11).

Songs and stories are realized in direct contact between the poet/storyteller and the audience. Their formation is also influenced by the audience, and it can be said that works of oral literature are the result of the creativity of several individuals. For Vid Latković, the fundamental characteristic of oral literature is the fact that several individuals, often belonging to different time periods, participate in shaping oral literary works. Works of oral literature are collective creations (Latković 1982: 7).

Unlike songs and stories of written literature, oral songs and stories are not materially fixed and can change with each new performance. Each new interpretation, depending on the creativity of the participants of the oral tradition, can be different from the previous one. Therefore, a greater number of variants appear in oral literature.

Oral stories and songs easily cross national, ethnic, linguistic and any other borders. For example, the plot pattern about children whose parents leave them in the forest because they did not have enough money to feed them is widely spread internationally and is known to many European nations. In the Aarne-Thompson-Uther catalog of oral stories, it is marked as motif 327A. In addition to being attractive to oral storytellers, this plot pattern was also interesting to writers, so they shaped it as a fairy tale that belongs to written literature. The plot pattern about Hansel and Gretel can also be recognized in poems, for example in the poetry of NasihaKapidžić-Hadžić (cf. Dervišević 2022).

There is no doubt that the oral literary tradition of Bosniaks in general is important for written literature in these areas. For Muhsin Rizvić, oral literature represents "the central current, matrix and determinant, spiritual and aesthetic self-realization of the overall development of Bosniak

literature since its beginning" (Rizvić 2005: 76). Its importance, especially the oral lyric song – sevdalinka, was recognized not only by literary scholars but also by writers.

The famous Bosnian writer Skender Kulenović, in his essay *From the Emerald of Una*, writing about the image of the world in which Hamza Humo's poetic sensibility was born and shaped, notes the following: "This world is terribly sensuous: and its fantasy of paradise is sensuous. And under the stone of the patriarchal taboo, he expressed himself in the most common love fluid – he sang, in his ancient word, one of the most refined and dramatic love lyrics in the world" (Kulenović 1996: 78).

3.1. Oral traditions

Oral traditions represent an independent genre of oral prose. These are stories that are based on belief in the reality of what is being told. The composition and style are simple, often oneepisode. These stories usually talk about historical memories, the origin of phenomena and things, and mythical creatures.

For Hermann Bausinger, the essential characteristics of tradition are indefiniteness and vagueness, impressiveness instead of precision as in fairy tales, and vagueness instead of solid outlines. It expresses the inexplicable and exceeded everyday norms through the forms that folk belief and traditional motif patterns have already prepared. The author states that the discussions, the sinister and mysterious elements in tradition are not only experienced but also overcome and banished by magic. Based on the questioning of oral traditions, Bausinger singles out three origins of these stories. The first source is experience or subjective observation, the second source is occurrences and objective events such as war conflicts, unusual natural phenomena and murders, while the third source includes any objective reality that requires an explanation, for example rocks with an unusual appearance (Bausinger 2018).

One of the works important for the study of traditions is certainly the text *Categories of Oral Prose Literature* by Carl Wilhelm von Sydow, which provides a concise overview of the prose categories of oral literature. Following von Sydow's findings, we can distinguish three forms of oral traditions today: memorate, chronicle and fabulate. Memorates are personal experiences of an individual. Chronicles are various memories presented as claims. Fabulates are short stories based on folk belief (Sydow 2018).

The examples in this paper, apart from von Sydow's division, are also classified based on the division proposed as a draft at the meeting of the Commission for Oral Traditions in Budapest in 1963. It is the division of traditions into etiological and eschatological, historical and cultural-historical, and finally supernatural beings and forces (mythical). The fourth and last group within the framework of traditions consists of legends and stories about gods and heroes. Since the Bosniak tradition does not recognize legends and stories about gods and heroes, as this group is

defined in other traditions, the fourth group of traditions in the Bosniak setting is represented by hikaje (hićaje) – stories about the life of God's prophets.

4. INTERPRETATIONS

Among the oral traditions presented by Aiša Softić in the *Anthology of Bosniak Oral Stories*, there are examples through which the oral tradition transmits knowledge about the importance of preserving the environment, flora and fauna. This way, oral tradition demonstrates respect towards nature with stories, but also encourages care for the world in which we live. These are the stories entitled *When the earth, stone and every grass spoke, The Serpent* and *On the Origin of Alija Derzelez's heroism*. This part of the paper will analyze the aforementioned examples, and then extract parts in which knowledge about the importance of the environment is recognized, followed by the determination of the role of these parts for the content of the example as a whole.

4.1. When the earth, stone and every grass spoke

During the 1950s, Ahmed Đonlagić from Draganić near Glamoč told the recorder Alija Nametak an oral tradition about an unusual stone on which footprints can be seen. The narration is short and without a detailed explanation, but precisely describes the area where the reason of the tradition is located.

"On the left side, one hundred meters from the road going to Glamoč from Livno, between the bridge and the stone (of Omeraga Bašić), there is a stone like two tables and on it the footprints of a four-year-old child. The people say that once upon a time the earth, and stone, and every grass spoke, that everything was soft, so the footprints remained."

This example is an etiological tradition, that is, a tradition whose content refers to the origin of the Earth, everything on the Earth, but also the cosmos. The topic of etiological traditions is also known in other oral traditions. In the Bosniak region these traditions are influenced by Islamic teachings. Belief in the truth of the content is recognized in the statement "the people say" and by highlighting the area where there is an unusual stone that is the incentive for the creation of the story.

Based on the content of this tradition, it can be concluded that the people passed on the ancient inherited belief about earth and stone as living matter, but also the belief that once upon a time all phenomena could communicate with words. The insight in the recorded material provided the conclusion that it is not uncommon in Bosniak oral tradition to tell stories about stones (Softić 1998). In this regard, let us mention that according to the *Dictionary of Symbols*, the content of which is referred to by Aiša Softić, the stone has a special place in oral tradition. According to the legend of Prometheus, the stone retained a human scent. In Islam, the stone in the true sense of the word is the Black Stone in the Ka'ba in Mecca. They call it the right hand of God (yemin Allah). By placing hand on that stone or kissing it, the believer swears allegiance, an act called istiham (achievement/covenant). On Judgment Day, this stone will testify in favor of the believers who came on pilgrimage (Softić 2002: 46).

In the content of the tradition told by Ahmed Đonlagić in 1957, the effort of the tradition to convey not only belief but also knowledge about the universal connection of phenomena in the world is recognized.

4.2. The Serpent

Among the traditions recorded by the associates of the National Museum of Bosnia and Herzegovina, and taken over by Aiša Softić in her selection of oral stories, there is also an example entitled *The Serpent*. Through the content of this tradition, the oral tradition opposes the killing of serpent and indirectly calls for their care. Namely, the serpent saved the ark of God's prophet Noah from flooding.

"The serpent, they say, should not be killed because it blocked Prophet Noah's vessel from sinking." When there was a flood, Noah placed all kinds of animals and various seeds in the vessel. "And mice began to gnaw the wood of the vessel and water began to enter the vessel. The serpent then curled up, but asked God to allow it to feed on the sweetest blood." As the sweetest blood is between the eyes, it asked to be allowed to bite every animal and human between the eyes. However, God did not allow it to do that, but rather told that it can bite people wherever she can, but for their hands and feet.

This tradition, by its characteristics, is an etiological tradition as defined by Nada Milošević-Dorđević. For this researcher of oral literature, etiological traditions "interpret the origin of celestial bodies, phenomena in nature and society, explain the characteristics of men, animals and plants, and their very existence serves the tradition as proof of the truthfulness of the interpretation. That is why etiological traditions are retrospective. (...) Pictorial explanations adhere to patterns of behavior, morals, and customs adopted in tradition in order to explain the unknown by the known" (Milošević-Đorđević according to Softić 2002: 43).

The oral tradition suggests that serpents should not be killed because they helped save the world during the flood that the holy books write about. The narration is in accordance with the religious atmosphere of the environment that transmits the narration. Through this tradition, inherited beliefs and knowledge are transmitted, which have the function of preserving the diversity of the animal world. The preservation of serpents is supported by their role in preserving life on Earth during the described event, through which the oral tradition tried to prevent their destruction.

It is notable that even today, Bosniaks narrate etiological traditions that convey beliefs and knowledge about the importance of animals and encourage care for them. According to one of them recorded in 2022 in Bihać, pigeons should be taken care of because they are birds dear to God. Namely, after the flood, a dove flew from the vessels of God's servant Noah, searching for land. When he returned with an olive branch, it was a sign that land was near. Furthermore, pigeons have red legs because they have been in the water for a long time. ⁶ The content of this and the previously mentioned tradition encourages listeners to take care of animals, which reflects the cognitive character of the tradition.

4.3. The Origin of Alija Đerzelez's heroism

Based on the oral literature material found so far, the oldest recorded example of the legend about the origin of the heroism of the famous Bosniak hero Alija Derzelez is found in the collection of *Folk Songs of the Bosniaks in Bosnia and Herzegovina* by the organizer and collector of folk treasures, Kosta Hörmann. In the *Appendix* to the first book of songs, K. Hörmann records stories about Derzelez as a note to the song, which will Aiša Softić later include in her selection in the *Anthology of Bosniak Oral Stories*. This example is a mythological tale centered on gaining strength with the help of fairy milk.

The weak and bald Alija is mocked and insulted by children and adults. Running away from the people, Alija came to the forest. In the deserted forest, he hears a child's cry and sees a newborn in the grass, heated by the sun. He cuts off a raw branch and makes a shade for the child. Then a fairy appeared and as a reward for protecting her child granted him his wishes – to be strong and a hero that no one could overcome, and to have a good horse. After the fairy has nursed him twice, he gains strength and then comes to the horse.

According to its poetic characteristics, the story about the heroism by Alija Derzelez corresponds to the fabulate, as it is presented in the works on oral literature. Namely, fabulates are stories that have 'a solid and stable narrative that is transmitted from beginning to end with the same compositional scheme along with changing secondary elements such as the place and time of events, personalities, etc.'' (Marks according to Softić 2002: 19). Since the recorders, collecting oral stories, recorded examples that have the same plot patterns as the mentioned example from Hörmann's collection, but with different names of the heroes, it can be concluded that this is a fabulate. One of the stories was recorded by the teacher and children's writer Hamdija Mulić at the beginning of the 20th century, and another example was recorded by Radmila Filipović, an associate of the National Museum of Bosnia and Herzegovina, in 1956.

Belief in the truth of the tradition is recognized in the statement: "This is what they say about the origin of Alija's heroism". With this beginning, the narrator refers to the oral tradition and

⁶ As told by Ismet Hajrulahović (1979) from Bihać. Recorded by Amira Dervišević.

suggests to the listeners that the content that follows is true. The cross-linking of the text with the toponyms Vučija Luka, Sarajevo and At Mejdan affects the reception of the content as possible. In addition to the belief in truthfulness, the cognitive character is taken as a criterion for defining the concept of tradition, which is reflected in the fact that traditions convey some knowledge. The cognitive character of this story is presented indirectly through the statement: 'Suddenly he remembers something and cuts off a raw fir branch, raw for the first time, and sticks it next to the child, to shield him from the heat of the sun...'' From the content of the story, we learn that the hero had never beforecut the green branches, but rather collected and cut the dry ones, which can be understood as an instruction to the listeners on how to treat trees. This part of the tradition. Since the hero of the Bosniak epic tradition is at the center of the story, it can be concluded that his attitude towards nature is a guide to how other people should treat their environment. Thus, the tradition conveys knowledge about the importance of preserving greenery and forests because, as we read from the content of the story, they are a space that we share with supernatural beings – fairies.

The importance of this statement was also emphasized by Esma Smailbegović in the study *Folk tales of Sarajevo*. Namely, when questioning the oral traditions about Alija Derzelez, the author noticed that these accounts provide numerous and precise information about his life at a time when he had not yet become a celebrated hero. In these notices we find information about his family, occupation, appearance and character. He was weak, unpleasant and already bald at an early age. "He was pious and very good and never cut down young trees and young branches," notes Smailbegović (1986: 73). This statement affects the formation of the main character. With a more detailed description of his relationship to nature, he becomes closer to the listeners. Since this is the famous hero of the Bosniak epic, the oral tradition formed an example that should be followed.

5. CONCLUDING REMARKS

In regard to the ecocriticism as presented in the texts of Cheryll Glotfelty and Simon C. Estok, the paper analyzed the etiological and mythological oral traditions recorded in the Bosniak community. All examples possess poetic qualities of the type to which they belong. Belief in truthfulness is manifested by statements that refer to tradition such as 'the people say'', 'they say'' and 'this is what they say about the origin of Alija's heroism''. The cognitive character of tradition is manifested in the transmission of knowledge, and in the selected examples of knowledge, they concern the preservation of animal and plant life. In etiological traditions, oral tradition conveys inherited beliefs and knowledge that once upon a time, stone and earth were alive and all phenomena spoke, that serpents helped the world survive the flood, and that caring for forests is a characteristic of good people. The selected sections have the function of

explaining unusual phenomena (*When the earth and stone and every grass spoke*), strengthening religious feelings (*The Serpent*) and characterization of the character (*The Origin of Alija Derzelez's heroism*). After the analysis and interpretation of selected examples of oral traditions, the initial assumption that the oral tradition transmits knowledge about the importance of the environment, plants and animals through the content of stories was confirmed. It is important to point out that with these traditions, the oral tradition encourages the listeners/readers to care for the world to which they belong.

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IMPACT DETERMINATION OF JABLANICA MUNICIPAL WASTEWATER ON NERETVA RIVER WATER QUALITY IN THE HIGH WATER LEVEL SEASON BY WATER QUALITY INDEXES (WQI) COMPARISON

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Key words: Water quality index, waste water, bacteriological load, Neretva river

ABSTRACT:

Aim of this article is to determine impact of Jablanica municipal wastewater on Neretva river water quality. Samples were collected in a period of four years during high water level season at upstream and downstream points. Quantitative estimation implied determination of important chemical and microbiological parameters. Water quality indices (WQI) have been calculated and comparative analysis was performed. The study also determined and analysed bacteriological load. It was found that there is no statisticaly significant chemical and bacteriological impact on the water quality of the Neretva River.

1. INTRODUCTION

Neretva river is the largest and hydrologically richest tributary of the Adriatic Sea in the Balkans. It springs in the area of Borča, at 1227 m altitude, below the mountain Jabuka in the south - eastern part of Bosnia and Herzegovina [1]. Regionally, the Neretva River belongs to the territory of Herzegovina, with a length of 230 km, of which the last 22 km belong to Croatia. The Neretva system hydrographically drains the largest part of the Adriatic basin, and the catchment basin is about 10,500 km². The area of the Neretva river basin is of hydrologically specific limestone-dolomite composition, and it is characterized by rich groundwaters, underground streams and sinkholes. The upper streams of Neretva river have the character of a mountain river

with a large elevation difference, many overfalls, narrow valleys and large mechanical power [2]. The water in upper catchments of Neretva river water is of the first class quality.

In the middle stream Neretva river passes through a series of manmade reservoirs, arable land and settlements. Original features of the river have been strongly modified by anthropogenic influence (Variščić, 2004). The impact is mutual because altered properties of the river affect humans, as the water is used for energy production, tourism, food production, irrigation and other needs.

Sewage systems include planning, design, construction and use of facilities, devices and facilities for reception and transport, as well as treatment of polluted water and their discharge into the water recipient or subsequent use. It is part of a technical system that builds on the human need for water in every segment of life and activities, which ensures quality collection and disposal of wastewater, and their treatment (UNDP, 2006).

Degradation of physical and chemical quality of water as a consequence of anthropogenic activity is often gradual and subtle adaptation of aquatic ecosystems. These changes cannot always be easily observed until dramatic changes occur in ecosystem status (Scheffer *et al.* 2001). Regular monitoring of the biological, physical and chemical constituents of aquatic ecosystems can serve to detect extreme situations in which the ability to return ecosystems to normal condition extends beyond their capabilities. Urban wastewater includes sanitary water that contains physiological waste as well as wastewater from other household uses. These waters also include atmospheric wastewater that is formed as a surface runoff from urban areas. An important characteristic of these waters is a high proportion of organic substances that can be degraded microbiologically into gases and substances with bad smell and appearance [5] (Bitton, 2005). Global concerns that fresh water will become a scarce resource in the future have set monitoring processes and applied water quality assessment methodology as the most important issues of the modern age [7].

While water monitoring for different purposes is well defined, overall water quality is difficult to estimate from a large number of samples and parameters (Chapman, 1992) [8]. Analysis of monitored parameters, individually or in groups according to common characteristics, provides partial information on overall water quality (Pesce & Wunderlin, 2000). Traditional approaches to water quality assessment are based on the comparison of experimentally determined parameter values with existing local norms. However, they do not provide an overall spatial and temporal profiles presentation of overall water quality (Debels *et al.*, 2005) [9]. In order to overcome all these difficulties, the Water Quality Index (WQI) was developed. It is a mathematical instrument that transforms a large number of data into one value (Štambuk - Giljanović, 1999), which in a simple and understandable way determines the quality and possible use of water (Bordalo *et al.*, 2001) [11]. It is used to understand water quality issues, integrating complex data and providing

results that describe the situation and estimate water quality trends, and provides important data in terms of increasing support for work to improve water resources quality (Cude, 2001). Various variants of the water quality index have been described in the literature, as well as many comparative efficiency studies. Some of the approaches to the water quality index that have been frequently used in the public domain for water quality assessment are: United States National Health Foundation Water Quality Index, Canadian Water Quality Index, British Columbia Water Quality Index, Oregon Water Quality Index, Florida Water Quality Index. This paper aims to evaluate quality of water using Neretva's Water Quality Index (NWQI) developed in 2012 (Riđanović, *et. al.*, 2012). It was also aimed to determine impact of bacteriological water quality parametres on WQI values.

Direct sewage outflow into Neretva river as a receive can have multiple negative impacts on water quality, which is obvious from hygienic and aesthetic aspects. Wastewater can affect receiver waters in a variety of ways. Pollution in the narrow sense refers to degradation of water quality by physical, chemical and biological pollution, to the extent that water becomes harmful to human health [14].

Jablanica municipality is located just downstream from the Jablaničko lake reservoir. Jablaničko lake receives water from the municipality of Konjic after sewage treatment as well as wastewater from smaller settlements on the lake's coastline. The lake is surrounded by arable land and is used for tourism. Fluctuations in water levels of the reservoir are large and the lake often has a problem with algae blooms. Immediately after discharge from the generators, the Neretva River flows through the municipality of Jablanica. This paper investigated impact of wastewater from the municipality of Jablanica on the water quality of the Neretva River. Wastewater is discharged directly into the river without any prior treatment.

2. MATERIALS AND METHODS

The municipality of Jablanica discharges wastewater into the Neretva River at two outlets: Pipeline of unknown profile, located on cp. (cadastral parcel) No. 2311 K.O. Jablanica, at the locality "Bare", determined by the following approximate coordinates $43^{0}39'10,18$ " N and $17^{0}45'35,65$ " E; and pipeline of unknown profile, located on cp. No. 2309 K.O. Jablanica, on the site "Stadion", determined by the following approximate coordinates $43^{0}39'28,62$ " N and $17^{0}45'47,28$ " E. For reseasch purposes, sampling sites were determined on the profile of the Neretva River upstream from the outlet "Bare" and downstream from the outlet "Stadion". Sampling sites are shown in Picture 1.



Picture 1: Sampling sites (source: Google Maps)

Coordinates of sampling sites are presented in Table 1.

Coordinates Sample site	Ν	Е			
upstream	43°39.697'	17°45.899'			
downstream	43°39.109'	17°45.385'			

Table 1: Coordinates of sampling sites

Samples were collected for chemical and microbiological analysis over a period of 4 years (2017 – 2020) in the high level water season in accordance with applicable norms [15-17]. For determination of water quality index (WQI), we are measured oxygen saturation (%), pH (1), temperature (0 C), total P (mg/l), total N (mg/l) and conductivity (μ S/cm). Water Quality Index values with bacteriological impact (bWQI) values were determined. In the first step subindex (SI) was calculated for all parameters above, according to the established methodology [18]. Water quality classification based on WQI values is presented Table 2.

Table 2. Water quality classification				
WQI	Classification			
10 - 59	Very bad			
60 - 79	Bad			
80 - 84	Acceptable			
85 - 89	Good			
90 - 100	Excellent			

Table 2:	Water	quality	y classi	fication
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Bacteriological load has been estimated by compiling data on total coliforms, enterococci and E. coli concentrations. Calculated Water Quality Index values for upstream and downstream sampling sites were compared and statistical analysis was conducted to determine significance.

3. RESULTS AND DISCUSSION

Water quality index (WQI) and modified water quality index (bWQI) were calculated according to subindex values of chemical and microbiological parameters. Table 3 shows WQI and bWQI values as well as results of microbiological analysis.

1										
Year	W	WQI bWQI		Total coliforms (100 ml)		Enterococci (100 ml)		<i>E. coli</i> (100 ml)		
	upst	dwst	upst	dwst	upst	dwst	upst	dwst	upst	dwst
2017	91,38	90,37	42,26	85,84	1200	1100	1500	6200	5200	420
2018	92,70	90,04	42,49	84,57	6000	3000	3000	500	2000	500
2019	92,70	93,38	71,39	42,61	8000	8000	600	1600	1300	2800
2020	94,02	94,02	42,73	42,73	37000	110000	5000	1700	3000	9000

Table 3: Values of water quality indexes and microbiological load

upst-upstream; dwst - downstream

According to the absolute WQI values we can state that the water quality in the Neretva river from the aspect of tested chemical parameters belongs to the water of excellent quality. All calculated values of WQI exceed the value of 90. However, including E. coli content in calculation of WQI value, the water quality declines from excellent to very bad, displaying values of 42,73; 42,49 and 42,26 for bWOI. One upstream sampling site showed bad quality of water, scoring bWQI of 71,39. Downstream, the water quality values change from excellent to very bad with two measurements having values of 42,61 and 42,73; along with the acceptable 85,84, and good quality with 84,57 for two other sites, after including E. coli in calculation of the WQI value. For 2017 and 2018 we notice an improvement in water quality from very bad to acceptable and good, if upstream and downstream values of bWQI are compared.

Comparison of the upstream and downstream values for WQI and bWQI with the t-test, shows that the difference is not significant with p < 0.05 for both indices. We notice the same pattern for microbiological parameters, ie the difference between upstream and downstream values is not significant for all three parameters tested - total coliforms, enterococci and *E. coli* with p <0.05.

3.1 Bacteriological load

Figures 1, 2 and 3 respectively, show significant bacteriological load in river Neretva, with all values exceeding prescribed normatives for water safety.



Figure 1. Concentration of total colifom bacteria (100 ml)

High levels of bacteriological indicators of water quality indicate possible presence of pathogenic organisms that may cause serious water-borne infections. Presence of pathogenic bacteria highly correlates with high values of bacterial indicators [19].



Figure 2. Concentration of enterococci (100 ml)

Previous research has shown that high concentrations of fecal coliforms highly correlates with increased concentrations of nitrogenous compounds and phosphorus, which as essential nutrients for growth aquatic organisms facilitate significantly increased algal production leading to algal bloom and eutrophication [18].



Figure 3. Concentration of E. coli (100 ml)

For recreational purposes, the number of fecal coliforms above 2000 microorganisms in 100 ml and *E. coli* more than 130 microorganisms in 100 ml, can lead to the emergence of serious infectious diseases (WHO, 1996). Epidemics related to swimming / bathing in water occur sporadically, through the transmission of diseases ranging from gastroentritis, acute diseases of the respiratory system, and irritation of the ears, eyes and skin due to contact with water contaminated with household sewage.

4. CONCLUSION

Based on the obtained research results, the following conclusions can be reached:

- The overall water quality in the Neretva river through the municipality of Jablanica from a chemical point of view expressed as WQI suggests that the water is of excellent quality.
- Bacteriological impact expressed as bWQI in some cases greatly changes the character of water quality in the category of very bad and bad.
- The difference between upstream and downstream values of WQI and bWQI is not significant, that implies the impact of municipal wastewater from the municipality of Jablanica on water quality in the Neretva river is not notable.
- All absolute values of bacteriological parameters exceed, many times, the allowed values, hence the river Neretva is under high bacteriological load.
- Recreational use of river Neretva, unfortunately poses a significant health risk.
- Although the Neretva river is under a high bacteriological load, the impact of wastewaters from the municipality only of Jablanica is not significant, because the water of the Neretva river suffered high bacteriological pollution before collecting wastewaters from Jablanica.
- The obtained results are good basis for further research in order to directly determine causes of pollution of the Neretva river and suggest measures for reduction or complete elimination of them.

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BENEFITS OF INTRODUCTION AND IMPLEMENTATION OF ISO 14001 STANDARD IN THE MEAT INDUSTRY

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Key words: ISO 14001, environmental management system, benefits, internal effects, external effects

ABSTRACT:

The international standard ISO 14001 is the best known standard of the ISO 14000 series and specifies the requirements for all organizations, of any type or size, to implement an environmental management system. The number of ISO 14001 certificates is growing every day all over the world, although somewhat weaker in BiH, but it is increasingly being introduced and implemented by companies in BiH. The Environmental Management System (EMS) is a practical tool for environmental initiatives that can ultimately generate financial benefits through competition or improving organizations: reducing costs and increasing revenue. These benefits have prompted companies to implement the ISO 14001 standard, which is today the most popular environmental management standard, with more than 300,000 certified companies worldwide. The implementation of the ISO 14001 standard brings benefits to companies that adopt it that can be classified into external and internal benefits. These benefits are visible, among other things, through measures to improve environmental protection, profitability, efficiency, improve image, improve customer satisfaction and improve employee satisfaction. According to most literature citations, benefits and advantages are classified into three groups that can be manifested through different effects: internal effects such as cost reduction, improved environmental protection, increased productivity, increased profit margin, improved internal procedures, improved employee morale, and external marketing effects such as improving corporate image, increased market share, increased customer satisfaction, increased time and

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delivery to customers. In addition, there are environmental benefits such as improved relations with communities, improved relations with the authorities. This paper analyzes the benefits of the introduction and implementation of ISO 14001 standard in the food industry with an emphasis on the meat industry, and presents key quantitatively measurable indicators of improving internal and external effects.

1. INTRODUCTION

Environmental issues have become a global concern in recent decades. Most of the environmental problems and challenges relate to the continuous consumption of materials, energy and water by companies, resulting in the depletion of these resources. In addition, the uncontrolled emission of toxic gases, waste and effluents into soil, water and air by companies also has a negative impact on the environment. EMS (Environmental management system) is a set of systemic and comprehensive mechanisms designed to assist organizations in introducing good environmental practices into their overall business strategy to reduce the negative impact on the natural environment and improve business environmental management. By applying EMS, the company can more easily identify, assess and find an appropriate solution to environmental problems arising from its own facilities [1]. According to [2] Environmental Management System (EMS) represents the organizational structure, responsibilities, practices, procedures, processes and resources for the establishment and implementation of environmental policy, where it acts as a voluntary instrument in controlling the operational impact of companies on the environment. On September 1, 1996, the International Organization for Standardization (ISO) published the ISO 14001 standard, structured on the basis of ISO 9001; standard in the field of environmental protection. The recent 2015 edition highlights the enhanced approach compared to the 2004 edition on improving performance by minimizing water and energy consumption, as well as producing fewer emissions and waste, introducing a life cycle perspective and developing external and internal communication strategies [3]. The benefits of the introduction and implementation of ISO 14001 are multiple, of which the following stand out: internal benefits - organizational improvement, environmental performance and cost reduction with raw materials; external benefits - improving the image, expanding the market of relationship benefits — environmental consumers and investors; and awareness, competitiveness, and customers [4].

2. ENVIRONMENTAL MANAGEMENT SYSTEM, ISO 14001

An environmental management system is a set of management tools and principles designed to create the administrative procedures that an organization needs to integrate environmental issues into its day-to-day business practices [5]. ISO 14001 is an internationally accepted standard that sets requirements for an environmental management system. It helps organizations improve their environmental performance through more efficient use of resources and waste reduction, gaining

competitive advantage and stakeholder trust, stressing the need for commitment to the environment. ISO 14001 provides a framework that ensures that an organization meets its environmental goals through consistent control of operations [6]. The ISO 14001 standard is based on the Plan-Check-Do-Review-Improve cycle. The plan cycle deals with the initial stages in which firms become compliant with ISO 14001. The verification cycle deals with checking and correcting errors. The do cycle is a general overview of the complete process by the top management of the company. The last cycle is a cycle of rotation of reviews and improvements that never ends, so companies are constantly looking for ways to develop their EMS [7]. The latest version of the ISO 14001: 2015 standard places emphasis on sustainable development and achieving a balance between the environment, society and the economy. Key improvements in standards include strategy alignment, increased governance, effective communication, improved environmental protection, and monitoring of environmental issues throughout the life cycle of products and services [8]. ISO 14001: 2015 covers the following topics (areas) related to the environmental management system: organization context, leadership, planning, support, operations, performance evaluation, improvement [9]. The international standard ISO 14001 requires the organization to: establish an appropriate environmental policy, identify environmental aspects arising from past, present or planned actions, products or services and to determine the importance of their impact on the environment, and to determine appropriate legal and other requirements oblige the organization. The application of the standard is not legally prescribed, but is voluntary [1]. China and Japan are undisputed leaders in the absolute number of certificates worldwide, with 20 and 18 percent of the total, respectively. Spain ranks third in the number of certificates issued and the country in the world with the highest intensity of ISO 14001 certification [10].

2.1 Motives as a foundation for the implementation of ISO 14001

The need to achieve results is an individual's desire to succeed, while the motivation is the desire to achieve success or avoid failure [11]. [4] in their research they focus on ISO 14001 and distinguish between three types of motives that lead companies to implement the ISO 14001 standard: ethical, competitive and relational. Ethical motives are a response to feelings of environmental responsibility, competitive motives stem from the search for competitive advantage, and relational motives stem from the desire of companies to legitimize and improve the relationship that exists between different stakeholders in the company. According to the literature, for ISO 14000, the main external force driving companies to seek certification is customer pressure, which includes meeting customer requirements, finding solutions to customer problems, and meeting customer expectations [10]. Key success factors for EMS implementation: support and commitment of managers, employee awareness, training and involvement, internal motivations for EMS certification, collaborative management with effective communication [12]. All managers and employees, especially those belonging to the

commercial department, perceive the norm as a differentiating element in the field of distribution and are of the opinion that ISO 14001 can bring greater benefits and greater financial performance for the organization [13]. According to some authors, motivations can be divided into four categories: market drivers, social drivers, financial drivers and regulatory drivers. ISO 14001 is based on the Plan, Do, Check, Act (PDCA) cycle and this methodology promotes the process of continuous improvement of organizational effectiveness, enables organizations to develop their environmental performance and demonstrate compliance with existing laws and increase their stakeholder relations [14].

2.2 Impact of meat industry production complexes on the state of the environment

There are two main types of solid waste in the meat industry - inedible products such as bones, fat, heads, feet, skin, hair and offal and packaging materials, mainly paper, plastic and metal. Wastewater arises from many activities such as washing livestock, corpses and offal, cleaning equipment and the working environment, personal hygiene of workers and washing trucks. As for wastewater, it is important to emphasize that it contains several types of contaminants such as blood, fat, manure, undigested stomach contents, meat and meat extracts, dirt and cleaning agents. The main indicators of wastewater are the amount of wastewater discharged and the load of pollutants that is generated; both depend on the type of meat and meat products produced and the technological environment [9]. The meat sector is one of the leading polluters in the food industry. Regardless of the perspective, the environmental impacts of the meat chain affect three dimensions - climate change in relation to global warming potential, acidification potential and eutrophication potential; consumption of natural resources (mainly water and energy) and pollution of the environment with various types of waste and discharge of wastewater. Life Cycle Assessment (LCA) is an environmental tool that takes into account greenhouse gases (GHG) emitted from all stages of agricultural and food production. Mapping the process along with setting the scope and boundaries is to clarify which part of the meat chain is analyzed from a "farm to fork" perspective. It usually covers farms, slaughterhouses and meat processing plants, but can be extended to the retail and use of meat products in the household. The collection of inventory data is the most important part, because the uncertainty of this data can cause an inaccurate calculation of various environmental indicators. The interpretation of the results is in direct correlation with the boundaries as well as the quality of the collected data. Finally, as a result of the LCA study, mitigation strategies can focus on the primary sources of environmental impact in the meat chain. Adequate control of waste and wastewater through ISO 14001 as a monitor can provide increased production results and more efficient environmental policy, as key purposes of the standard [15]. The best solutions include the introduction of best environmental practices in areas with livestock farms, proper location and construction of local livestock farms, reducing the amount of biological waste, disposing of waste in the safest way, reducing water and improving wastewater quality [9]. The importance of ISO 14001 in the meat

industry is reflected in the regulation of wastewater and emissions, and the amount of slaughterhouse waste. After the regulation, it is necessary to monitor the situation in the mentioned phases on a daily basis, respectively periodically.

2.3 Benefits of ISO 14001 implementation in the meat industry

The main focus of ISO 14001 is on the structure, implementation and maintenance of a formal environmental management system. ISO 14001 (environmental management system) is expected to be a factor in the business success of companies in the future. For a company to be sustainable, it must reduce pollution for the benefit of the environment, stimulate economic growth and integrate social responsibility into its system. Improving the company's position in the market, moving from conventional to sustainable practices, improved relationship with society due to better environmental performance and improvement in waste processing are the goals of ISO 14001 standards [16]. The literature suggests clear benefits and motives from the implementation of the ISO 14000 standard (increased sales, cost reduction, access to international markets, better communication with suppliers, improved employee relations, among others) [10]. Obtaining accredited certification according to ISO 14001 gives commercial value to companies or organizations including reducing greenhouse gas emissions, waste management while ensuring better control of business risk and competition [9]. Businesses have the opportunity to identify a common environmental performance indicator (EPI) to achieve effectiveness, based on the following eco-indicators: biodiversity, food production, average global surface temperature and atmospheric carbon dioxide concentrations, human population and resource depletion. The use of indicators as a management tool demonstrates the effectiveness of the organization to ensure clear working and environmental conditions [16]. Hwee-Nga (2009) stated that the existing literature has documented the perception that ISO 14001 certification leads to increased competitive advantage, regulation, increased financial performance, and improved reputation and reduced operating costs. According to Ferrón-Vílchez (2016), in terms of environmental performance, companies characterized by adopting ISO 14001 and, at the same time, monitoring their environmental activities are more likely to have improvements in their environmental impact than companies that harmonize standards adoption, and low monitoring or do not adopt ISO 14001 and manage environmental monitoring [4]. Gavronski, Ferrer and Paiva (2008) highlighted four groups of benefits: productivity benefits (reduced resource use, optimized process flows, reduced production costs, better employee motivation), financial benefits (ability to obtain investment funds from government organizations, access to special credit with reduced interest rates, reduction of insurance premiums), market advantages (competitive advantages, positive effects on the market and with customers, opportunity to set an example to suppliers) and social benefits (improved corporate image for society in general, reduced environmental responsibility, improved cooperation of environment protection authorities) [17]. ISO 14001 contributes to the managerial efficiency of

the company, increases staff motivation, reduces pollution and builds customer loyalty and trust in the company. The implementation of ISO 14001 increases the awareness of employees about relevant environmental regulations, the organizational image of the company and improves mainly EMS for solving environmental problems for efficiency. In terms of quantitative indicators, the amount of water consumed, acoustic noise, the amount of emitted gases, environmental risks, solid and liquid waste generated and total energy consumption are taken into account. The percentage ratio of these significant aspects gives the actual state of these companies for their EMS effectiveness [16]. In terms of energy and resource consumption, Đekić et al. (2014) concluded that the application of ISO 14001 leads to savings in water and energy consumption based on research on 20 Serbian food companies. Hasan and Chan (2014) recorded reduced waste and material and energy use based on the opinions of 170 ISO 14001 certified organizations. Aslo, Castka, and Prajogo (2013) reported reductions in pollution and energy consumption [18]. The benefits found by certified companies can be classified into three categories: internal benefits demonstrated: organizational improvement, environmental performance and cost reduction with raw materials; external benefits such as improving the image, expanding the market of consumers and investors; and benefits of relationships such as environmental awareness, competitiveness and customers [4]. Internal benefits can be divided into several groups; organizational benefits: quality of management, quality of training, working conditions and safety, quality of environmental information, compliance with the law, encouraging innovation, improved procedures, strategic environmental responsibility review, financial benefits (cost savings from reducing materials, energy and waste) and benefits for people (increased employee motivation, improved skills, better company image among employees, forum for dialogue between staff and management). External benefits can also be divided into different groups: commercial benefits (acquiring new customers / businesses and satisfying existing customers, gaining competitive / marketing advantage, staying in business, developing more environmentally friendly products), environmental benefits (improved environmental efficiency, legal compliance), increased energy and material efficiency, reduced pollution) and communication benefits (positive public image, better customer relations, better cooperation and relations with regulatory and administrative bodies, improved communication with stakeholders, setting an example to other companies in the sector). Others are also worth mentioning: internal business (increased efficiency, well-defined responsibility, increased environmental awareness, standardization of environmental management), corporate governance (fewer complaints, improved profitability, resource savings and reduced waste, increased social recognition), marketing effects increased market share, customer trust, improved corporate image) and relationships with suppliers (better relationships with suppliers, tighter control over suppliers, promotion of ISO 14001 certification among suppliers, improved environmental awareness of suppliers. The following advantages can also be pointed out: productivity benefits (reduction of resource use, optimization of process flows, reduction of production costs, better

motivation of employees), financial benefits (possibility of obtaining investment funds from government organizations, access to special loans with reduced interest rates), market advantages (competitive advantages, positive effects on the market and with customers, opportunity to set an example to suppliers) and social benefits (improved corporate image for society in general, reduced environmental responsibility, improved cooperation of environmental authorities) [17]. The benefits that ISO 14001: 2015 provides to organizations include: reduced waste management costs, lower distribution costs, better corporate image, savings in energy and material consumption, and overall improvement in environmental performance. The positive impact of ISO 14001 and EMS may also depend on how much the firm is willing to invest in resources and time to improve its environmental performance. Global export-oriented firms are more likely to realize product and process innovation than those firms that are mainly focused on regional / local markets. Certified companies have a positive impact on innovation and commitment to the company's environmental goals [7].

3. CONCLUSION

The amount of pollutants harmful to the environment is increasing every day and it is necessary to update their source and quantity, as well as to take corrective actions and measures in order to neutralize them. Regulating the amounts of carbon monoxide, methane, nitrogen oxides, sulfur dioxide and other pollutants is extremely important for the preservation of the natural environment. This can be achieved with the right methodology based on the legal regulations of ISO 14001, with a solid scientific basis. Through extensive scientific research, the importance of this standard in preserving and protecting the environment has been proven, which is mostly reflected in: improvement of organizational flows, general cost reduction, improvement of the company's image, raising the environmental awareness of company employees. ISO 14001 is a voluntary management tool, i.e. the application of the ISO 14001 standard is not legally regulated, however, statistically speaking, the frequency of presence on all continents is evident; the areas with the highest ISO 14001 certification are East Asia and the Pacific (189,505) and Europe (120,595). The country with the highest number of certificates is China (137,230), followed by Japan (27,372), Italy (26,655), United Kingdom (16,761), Spain (13,771) and Germany (9,444) [8]. In Bosnia and Herzegovina, ISO 14001 certification is at a relatively low level, primarily due to the high costs of introduction and implementation. The environmental awareness of employees in Bosnian-Herzegovinian companies is at a satisfactory level in many spheres of production processes, while in the meat industry there is much room for improvement, especially in regulating the amount of slaughterhouse waste and rendering, and wastewater management. The introduction and implementation of ISO 14001 standard would significantly affect the regulation of critical production points, which would result in improved environmental performance, and to balance the already disturbed ecological balance in the long run.

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ECOSYSTEM SERVICES ASSESSMENT AS A TOOL FOR EFFECTIVE NATURAL RESOURCES MANAGEMENT

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Key words: ecosystem services, ES, natural resources, management, TESSA methodology

ABSTRACT:

Human activities have a great impact on the natural environment resulting in ecosystem degradation and biodiversity loss. These changes have heavily influenced nature's functioning and in turn the benefits that it provides to people – the ecosystem services (ES). Recognising that these changes ultimately affect us, there is a growing interest among stakeholders from different sectors (nature protection, agriculture, forestry, fishery, water management, economy) in ES concept. As the importance of ES is gaining wider recognition, there has been a need for tools that could potentially provide information to decision makers on ES supply under current and future anthropogenic pressure. Therefore, the explicit ES assessment methods are considered as main requirements for the implementation of the ES concept into natural resources management planning and related decision making.

The purpose of this paper is to present customised TESSA methodology for ecosystem services assessment applied in 6 pilot sites in the Croatia-Serbia cross border region. The methodology emphasises the importance of comparing estimates for alternative states of a protected site (for example, before and after application on management measures) so that decision-makers can understand the importance of such measures, and hence the benefits for human well-being that may be lost through the degradation or gained by conservation.

1. INTRODUCTION

Ecosystem services encompass all-natural products and processes that contribute to human wellbeing and personal and social enjoyment derived from nature [1]. Based on the CICES (Common International Classification of Ecosystem Services) approach, ecosystem services are categorized into three groups: (i) provisioning services, which involve outputs from ecosystems that can be exchanged, traded, and directly consumed or used by people in various activities; (ii) regulatory services, referring to ecosystem outputs that are not consumed but influence the performance of individuals, communities, and their activities; and (iii) cultural services, which represent non-material ecosystem outputs with symbolic, cultural, or intellectual significance [2]. The relationship between ecosystem services and the conservation status of habitats and ecosystems, as well as their connection to socio-economic well-being, is evident.

Recent European-scale studies have demonstrated that habitats in favorable conservation status exhibit greater biodiversity and have a higher potential to provide regulating and cultural ecosystem services compared to habitats in an unfavorable conservation status. Positive interactions between biodiversity and ecosystem services have been observed, such as increased biomass production in forests with higher tree species richness. Additionally, enhanced crop genetic diversity has shown to be beneficial in pest and disease management, and it has the potential to improve pollination services and soil processes [3].

The concept of ecosystem services holds substantial potential in enhancing existing natural resources management approaches, offering valuable information to decision-makers about ecosystem services supply under current and future anthropogenic pressures. Therefore, the assessment of ecosystem services is considered a fundamental requirement for implementing the ecosystem services concept into natural resources management planning and related decision-making processes. Various methods are available for assessing ecosystem services, differing in their aims, ranging from mapping and modeling the supply and demand of ecosystem services to appraising their economic and non-economic significance through valuation techniques [4].

This paper aims to present a straightforward and user-friendly customized TESSA methodology for assessing ecosystem services, which was applied in six pilot sites within the Croatia-Serbia cross-border region. The TESSA methodology offers a practical and effective approach to conduct ecosystem services assessment, supporting conservation efforts and informed decisionmaking in the study area.

2. METHODOLOGY

This research employs the Toolkit for Ecosystem Service Site-Based Assessment (TESSA) to evaluate the ecosystem services provided by wetland habitats in the Croatia-Serbia cross-border region. TESSA serves as a practical and comprehensive guide, aiding in the identification of significant ecosystem services at a specific site of interest and outlining the requisite data for their measurement [5].

TESSA exhibits broad applicability, extending its utility to a wide range of sites and catering to diverse user groups. These users encompass conservation practitioners, natural resource

managers (e.g., forestry, fisheries, water managers), protected areas managers, land use planners, the extractives industry, utilities companies, non-governmental organizations (NGOs), public bodies, and landowners. Thus, TESSA presents a versatile and accessible tool that facilitates ecosystem service assessments across various contexts and stakeholders, fostering informed decision-making and effective management practices.

The TESSA methodology comprises the following procedural stages:

- 1. Preparatory Activities: The process initiates with preparatory activities encompassing the deliberate selection of the assessment site, formulation of assessment objectives, and identification of pertinent stakeholders to be engaged from the outset.
- 2. Preliminary Scoping Appraisal: This phase entails a comprehensive survey of the assessment site, its services, and activities that exert an impact on the site. It necessitates the explicit definition of ecosystem types, ecosystem services, and principal pressures. Notably, the identification and evaluation of threats and activities at the site that may influence the provision of ecosystem services are integral aspects of this step. Information gathering is accomplished through multiple meetings with key stakeholders or through a unified workshop involving all key stakeholders.
- 3. Determination of Alternative State: This critical stage involves identifying the potential alterations the site may undergo as a consequence of policy or management changes under consideration. The alternative state may arise due to factors such as land conversion or policy shifts, for instance, the lifting of quota controls on fishing or the construction of a dam. This aspect demands significant time and meticulous contemplation.
- 4. Planning the Full Assessment: The process necessitates the selection of specific ecosystem services for assessment, considering the available capacity and time constraints.
- 5. Data Collection at Assessment Sites: This step involves reviewing existing data, giving careful consideration to its relevance, accuracy, and reliability, as well as collecting new data from the assessment sites.
- 6. Analysis and Communication of Results: The final stage entails the analysis and effective communication of the assessment findings. Proper interpretation and clear communication of the results are paramount, particularly if the assessment is intended to influence decision-making processes.

3. RESULTS AND DISCUSSION

The customized TESSA (Toolkit for Ecosystem Services Site-based Assessment) methodology has been implemented in six pilot sites, three located in Serbia (Bara Trskovača, Bosut forest,
and Special Nature Reserve Zasavica) and three in Croatia (POR Podpanj and fishponds Donji Miholjac, North part of Middle Danube, and Spačvanski basen). These pilot sites are situated within the Middle Danube floodplain, encompassing the Croatia-Serbia cross-border region (see Figure 1). They represent remnants of the once expansive and continuous floodplains within the Danube basin, distinguished by their exceptional biodiversity and the extensive range of ecosystem services they offer. The project area includes diverse habitats, such as old riverbeds, backwaters, wet meadows, floodplain forests, and meadows. These ecosystems play a vital role in providing habitats for numerous plant and animal species. Additionally, they serve various functions, including food production, flood regulation, water purification, tourism, recreational opportunities, as well as cultural and spiritual experiences. Despite their significant societal contributions, wetland habitats in this region face considerable pressures resulting from inadequate water management, intensive agriculture, the spread of invasive alien species, poaching, and overexploitation of natural resources.

Consequently, the primary objective of the ecosystem services assessment was to enhance the protection and sustainable utilization of wetland habitats in the pilot sites. The entire assessment process was conducted in a participatory manner, involving relevant stakeholders representing water management, forestry, nature protection, local government, and civil society sectors from both countries.

During the preliminary scoping appraisal, ecosystem types, ecosystem services, and primary pressures were identified for each pilot area. Ecosystem types were classified based on the ecosystem typology outlined in Maes J. et al. (2013), combining Corine Land Cover (CLC) classes for spatial explicit mapping, adjusted with European Nature Information System (EUNIS) habitat types where necessary. Ecosystem services were classified according to the Common International Classification of Ecosystem Services (CICES), offering a flexible and hierarchical classification adaptable to specific situations and needs [2]. Matrix showing general relationships between habitat types and ecosystem services were used to decide on which services to measure during the full assessment.



Figure 1. Location of the pilot sites

An essential aspect of the preliminary scoping appraisal was the identification of threats and activities occurring at the sites that may impact the delivery of ecosystem services. Each threat was assessed concerning timing, scope, and impact, employing the scale presented in Table 1.

		8 11
Timing	Scope (% of site affected)	Impact (degree of change in
		next 10 years)
1. Likely in long term	0. Little of area (<10%)	1. Low (<10%)
(beyond 10 years)	1. Some of area (10- 49%)	2. Moderate (10- 30%)
2. Likely in short term	2. Most of area (50- 90%)	3. High (>30%)
(within 10 years)	3. Whole area (>90%)	
3. Happening now		

Table 1: Guidance for estimating timing, scope and impact of threats occurring at the site [5]

Based on the existing land cover and identified threats affecting the pilot sites, a comparison was made between the current state and an alternative state that could potentially be achieved within 10 years. The stakeholders and experts opted for a "positive" alternative situation when the driving force for change is nature conservation. This positive alternative state primarily entails an increase in the scope and/or quality of certain ecosystem services within the pilot area, as illustrated in Table 2 for the Bosut forest pilot site. The "negative" alternative state (without conservation measures applied) served as the basis for the development of the Action Plan for the improvement of ecosystem services in the pilot areas for the period 2020-2030.

positive enanges, bosat forest [0]					
Land cover category	Current state %	Alternative state %	Land cover changes in %		
311 Broad-leaved forest	83,2	82,0	-1,2		
324 Transitional woodland/shrub	8,1	6,0	-2,1		
411 Inland marshes	3,2	6,2	+2,0		
511 Water courses	4,3	4,3	0,0		
512 Water bodies (oxbovs)	0,3	1,00	+0,7		
Antropogenic (112+122+211+242)	0,9	0,5	-0,4		
Total	100 %	100 %			

 Table 2: The share of certain classes of land in the current and possible future state of land cover impacting by positive changes, Bosut forest [6]

During the phase of the preliminary assessment, the importance of each land cover class for the delivery of ecosystem services (biodiversity, wild goods, cultivated goods, cultural goods, water-related service - flood protection, and climate regulation) was scored on a scale from 0 to 5, as demonstrated in Table 3 for the Bosut forest pilot site.

 Tabela 3: Results of preliminary assessment of the significance of a particular class of land cover for the delivery of ecosystem services in Bosut forest [6]

Corine land cover class	Biodiversity	Wild goods	Cultivated goods	Cultural goods	Water related service	Climate regulation
311 Broad-leaved forest	5	5	4	5	5	5
324 Transitional woodland/shrub	3	3	4	2	3	5
411 Inland marshes	5	4	4	5	4	5
511 Water courses	4	3	2	3	4	3
512 Water bodies (oxbovs)	5	3	3	3	3	2
Antropogenic (112+122+211+242)	1	1	3	2	0	0

Exppalantion: Importance of the land cover classess: $_{,0}^{,\circ}$ = not relevant; $_{,1}^{,\circ}$ = very small relevance; $_{,2}^{,\circ}$ = small relevance; $_{,3}^{,\circ}$ = mderate relevance; $_{,4}^{,\circ}$ = high relevance; $_{,5}^{,\circ}$ = very high relevance

In subsequent steps, all identified ecosystem services were mapped in both the current and alternative states. Figure 2 and 3 illustrate water-related services in the current and alternative state for the Bosut forest.



Figure 2. Water related service - current state (Bosut forest)



Figure 3. Water related service – positive alternative state (Bosut forest)

The results of the assessments were utilized to draft a realistic and feasible Action Plan for the enhancement of ecosystem services in the pilot sites for the period 2020-2030.

4. CONCLUSION

The evaluation of ecosystem services provided by wetland habitats within the scope of this study indicates that the TESSA methodology represents a cost-effective and user-friendly guidance tool suitable for implementation by conservation practitioners. The principal advantage of this methodology, when compared to alternative approaches, lies in its incorporation of the "alternative state" concept for a given site (e.g., pre and post-conversion to agriculture). This feature enables decision-makers to comprehensively assess the net consequences of such changes and, consequently, the potential benefits to human well-being that may be lost through the alterations or gained through conservation efforts.

Comprehending the impacts of present and potential changes in the condition of natural resources and their management at specific sites is of great significance in promoting well-informed planning decisions, supporting biodiversity conservation, and enhancing the delivery of ecosystem services. This study effectively illustrates how ecosystem services can be positively altered through the implementation of conservation measures and defines the desired situation achievable through a clear set of actions.

The use of geospatial visualization to depict changes in ecosystem service delivery resulting from management measures has proven to be a potent tool in facilitating the decision-making process when formulating the Action Plan for the enhancement of ecosystem services in the pilot areas. Such visualization aids in conveying the potential outcomes of various management strategies and contributes to more informed and effective decision-making.

Acknowledgement

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BIODEGRADATION OF SYNTHETIC WASTEWATER CONTAMINATED BY HEAVY METALS IN THE LABORATORY BIOREACTOR

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Key words: biodegradation, synthetic wastewater, heavy metals, activated sludge

ABSTRACT:

Contamination of water with heavy metals has a serious impact on the environment and human health. Increased concentrations of heavy metals are mainly associated with industrial discharges, but insignificant amounts of heavy metals can also be found in municipal wastewater.

In this research, synthetic wastewater was prepared with the addition of heavy metals of different concentrations (Fe, Pb, Cd, Zn and Co), so that the concentration of heavy metals in wastewater is 4-5 times higher than the maximum allowable concentration of heavy metals in water prescribed by the Decree on Conditions for Discharge of Wastewater into the Environment and Public Sewerage Systems (Official Gazette of the Federation of BiH, No. 96/20).

Biodegradation was performed in a laboratory bioreactor with a volume of 2 L, under the aerobic conditions. For the biodegradation of synthetic wastewater, activated sludge from wastewater treatment plants was used, in which mixed bacterial cultures were prepared by the technique of accumulation of nitrificants and denitrificants. Biodegradation was monitored for 6 days. In synthetic wastewater, an increased initial concentration of organic matter expressed as COD was measured, and it was 286 m /L, as well as an increased concentration of ammonia - 108 mg / L, and phosphorus – 34.4 mg / L. The concentration of organic matter, ammonia and phosphorus after the completion of the biodegradation process was significantly reduced, and the values were reduced to 1.422 mg /L (ammonia) and 13 mg /L (phosphorus).

1. INTRODUCTION

By biological processes we mean the removal of pollutants by the action of microorganisms. Microbiological activities can remove dissolved organic constituents (carbon and energy sources), inorganic constituents (nitrogen and phosphorus source) and suspended particles remaining after primary wastewater treatment [1].

In secondary wastewater treatment, depending on the origin, biological aerobic and anaerobic methods based on different ratio of microorganisms to dissolved oxygen are used to remove dissolved ingredients.

Biological treatment using aerobic activated sludge process has been in practice for well over a century [2].

The activated sludge process in the treatment of wastewater involves blowing oxygen or air into raw, unsettled sewage and this process uses a mass of microorganisms (usually bacteria) to aerobically treat wastewater.

Organic contaminants in the waste waterprovide the carbon and energy required to encourage microbial growth and reproduction; nitrogen and phosphorous are sometimes added to promote this growth. The wastewater is thoroughly mixed with air in an aeration tank, and the organic matter is converted into microbial cell tissue and carbon dioxide [3].

The process of activated sludge can also include the process of nitrification.

The process is based on the nitrogen cycle: it represents one of the most important nutrient cycles in terrestrial ecosystems.

Carbon and nitrogen are the main sources of life, but their uncontrolled input into watercourses through wastewater discharges causes environmental quality problems. Pollution needs to be controlled in order to reduce carbon and nitrogen concentrations at a certain level and improve the quality of the environment. Nitrification and denitrification processes are used to remove nitrogen.

Heavy metals have a negative impact on the environment and the health of the population due to their toxicity. In view of the toxicity and in order to meet regulatory safe discharge standards, it is essential to remove heavy metals from wastewater both to decrease the amount of wastewater produced and to improve the quality of treated effluent, before it is released into the environment [4].

2. MATERIAL AND METHODS OF WORK

In this research, synthetic wastewater was prepared with the addition of heavy metals of different concentrations (Fe, Pb, Cd, Zn and Co), so that the concentration of heavy metals in wastewater is 4-5 times higher than the maximum allowable concentration of heavy metals in water. Wastewater contains mostly organic matter that is easily degraded by microorganisms. The

parameters in the synthetic wastewater sample were regularly monitored: temperature, pH, conductivity, oxygen, ammonia, nitrites (NO₂), nitrates (NO₃), phosphorus (PO₄³⁻), COD (mgO2/L) and heavy metals of different concentrations (Fe, Pb, Cd, Zn and Co). Physico-chemical parameters of wastewater was determined using standard methods for testing water and wastewater [5] and Regulation on conditions for discharging wastewater into the environment and public sewerage systems [6]. The parameters and methods by which the analysis was performed are shown on Table 1.

Parameter	Unit	Method
Colour		
Flavour		
Temperature (°C)	⁰ C	Electrochemical
pH		Electrochemical
Conductivity(µS)	μS	Electrochemical
Oxygen saturation (%)	%	Electrochemical
Dissolved oxygen (mg/L)	mg/L	Electrochemical
Evaporated residue (mg/L)	mg/L	
Nitrites (mg/L)	mg/L	Spectrophotometric
Nitrates (mg/L)	mg/L	Spectrophotometric
Ammonia (mg/L)	mg/L	Spectrophotometric
Phosphor (mg/L)	mg/L	Spectrophotometric
$COD (mgO_2/L)$	mgO ₂ /L	Spectrophotometric
Heavy metals	mg/L	AAS

Table 1. Parameters and methods of wastewater analysis

The processes of biodegradation was conducted in a laboratory reactor with working volume of 2 liters. Aerobic conditions were achieved by aeration reactor contents and bringing the air with the help of aquarium pumps, with mixing of the reactor contents using a stirrer placed in the reactor.

3. RESULTS AND DISCUSSION

The paper presents the results before, during and after the biological treatment of synthetic wastewater. The procedure took place in laboratory conditions for 6 days and on that occasion the results of the analysis were examined. A bioreactor with a volume of 2 L was used with the implementation of aeration conditions, more precisely with an aeration pump in order to improve the activity of microorganisms involved in the process treatment. As already mentioned, a sample of synthetically prepared wastewater contaminated with heavy metal solutions was used. Table 2 lists the basic parameters of activated sludge quality.

Parameter	Value
Depositing substances	60/1000
(ml sediment)	
Dry matter (mg/L)	1.6
Ammonia (mg/L)	5.44
Nitrites (mg/L)	0.84
Phosphorus (mg/L)	11.2

Table 2. Chemical properties and physical indicators of quality of activated sludge

Table 3 presents the results of the analysis of individual wastewater parameters before the implementation of the biological treatment process as well as the maximum allowable values for their release into the natural recipient. Insighting the table, we can conclude that the analyzed sample significantly exceeds the allowable values of certain parameters such as ammonia whose initial concentration is 108 mg /L, which is even 100 times higher than the maximum allowable concentration. Significantly higher concentrations of heavy metals were also induced by the addition of their solutions to the wastewater sample.

Parameter	Value	Limits	
Flavour	Strong odour	-	
Temperature (°C)	17.9	30	
pH	5.81	6.5-9.0	
Conductivity(µS)	921	-	
Turbidity (NTU)	5.17	-	
Oxygen saturation (%)	20.1	-	
Dissolved oxygen (mg/L)	8.9	-	
Evaporated residue (mg/L)	920	-	
Ammonia (mg/L)	108	10	
Nitrites (mg/L)	34.25	-	
Nitrates (mg/L)	13.3	10	
Phosphorus (mg/L)	34.4	2.0	
COD (mgO ₂ /L)	286	125	
Fe (mg/L)	5.310	2.0	
Pb (mg/L)	1.018	0.5	
Cd (mg/L)	0.237	0.1	
Zn (mg/L)	4.057	2.0	
Co (mg/L)	4.123	1.0	

Table 3. Chemical properties and physical indicators of quality of synthetic wastewater

Table 4 lists the chemical parameters and physical indicators of quality of synthetic wastewater during biodegradation in the bioreactor over 6 days. The first measurement was performed after half an hour, and the next after an hour from the beginning of the process, resulting in slightly increased values such as temperature and conductivity while oxygen saturation as well as

dissolved oxygen are slightly decreased. After 144 hours (6 days) as last results were measured we observed increase in conductivity levels up to 913 μ S, also increase in oxygen saturation levels up to 27.7 as well as increase in dissolved oxygen levels, while temperature levels begin to drop which is closely related to increase in oxygen levels. Through the overall measurements of the pH value we observe that there is no significant deviation from the initial one.

Table 4. Chemical properties and physical indicators of quality of synthetic wastewater during biodegradation in the laboratory bioreactor

Parameter	1	2	3	4	5	6	7
Flavour	Strong	Strong	Strong	-	-	-	-
Temperature (°C)	27	30	25	18	18	19.5	19
pН	6.5	6.7	7.0	6.5	6.5	5	6.3
Conductivity(µS)	864	790	763	904	759	989	913
Oxygen saturation (%)	20.1	18	17.5	22	23	31.4	27.7
Dissolved oxygen	8.6	7.1	7	9.9	10.2	13.5	11.9
(mg/L)							

1-Value after 30 minutes; 2-Value after 1 hour; 3-Value after 2 hours; 4-Value after 24 hours; 5-Value after 48 hours; 6-Value after 5 days; 7-Value after 6 days

The concentration of ammonia and phosphorus after the completion of the biodegradation process was significantly reduced, which indicates the efficiency of the process. From the initial 108 mg/L (ammonia) and 34.4 mg/L (phosphorus) the values were reduced to 1.422 mg /L (ammonia) and 13 mg /L (phosphorus) (Graph 1).



Graph 1. Concentration of ammonia and phosphorus (mg/L) during 6 days of biodegradation

The concentration of nitrites and nitrates was also monitored for 6 days of the procedure. During the first few hours we can notice that there is a significant increase in nitrate concentration, which we understand as a consequence of the nitrification process where ammonia is oxidized to nitrates and therefore they currently accumulate, after which their concentration continues to drop after 24 hours. The nitrite concentration also increases slightly during the first hour of the procedure, after which it decreases and reaches a value of 0.12 mg /L at the last measurement (Graph 2).



Graph 2. Concentration of nitrite and nitrate during 6 days of the biodegradation procedure

On the graph 3 we can observe that COD value has dropped significantly, which indicates that the oxidation of organic matter progresses over time, which is supported by the provided aeration condition in the bioreactor. However, the oxidation process would have proceeded much faster in the absence of toxic substances, in our case, heavy metals which prevented the oxidation from taking place much faster than achieved.



Graph 3. Concentration of organic matter expressed as COD

Graph 4 shows a significant decrease in Fe concentration (mg/L) during the process. After 2 days (48 hours) the concentration decreased from the initial 5.310 to 2.374 mg/L. After 5 days (120 hours) we measured again and the concentration was 0.259 mg/L. The last measurement was performed after 6 days (144 hours) and noted a concentration value of 0.169 mg/L, which indicates that we achieved the desired result and the value within the permitted release into recipients, which for Fe is 2 mg/L. Unlike other observed heavy metals whose concentration was not within the values of the maximum allowable concentrations of heavy metals for discharge into natural watercourses [6]. After the biodegradation process and the last measurement the concentration of Pb was 0.908 mg/L which is not within the allowable value of 0.5 mg/L for Pb.



Graph 4. Concentration of Fe, Pb, Cd, Zn, Co (mg/L) in synthetic wastewater during 6 days of biodegradation in laboratory bioreactor

Summarizing the results from the beginning throughout all 6 days of the biodegradation process we observe a decrease in the value of organic matter expressed as COD as well as reduced values of heavy metal solutions Fe, Cd, Zn, Co, except Pb whose concentration still exceed the maximum allowable concentrations for discharge into natural recipients, even after the last day of the decomposition process. COD values are within the permitted values, but these values would have dropped significantly if the presence of heavy metals did not interfere with the oxidation of organic matter.

4. CONCLUSIONS

Biodegradation processes which include processes with activated sludge are economical and environmentally friendly wastewater treatment processes that are also highly efficient in the first place. These treatment processes are primarily recommended for the removal of biodegradable substances from wastewater because they have shown the highest level of efficiency there. In our case, the presence of toxic substances, more precisely heavy metals, had an inhibitory effect on the activity of activated sludge microorganisms and on the slow oxidation of organic matter. Processes in which activated sludge is used as a combined group of microorganisms for wastewater treatment are preferably processes involving waters that are less contaminated or those that contain biodegradable substances in their composition.

Based on the results of wastewater testing, a decrease in parameters was determined. There was a decrease in the concentration of ammonia, nitrite (NO₂), nitrate (NO₃), phosphorus (PO_4^{3-}), as

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well as heavy metals (Fe,Cd, Zn and Co), except Pb which is extremely toxic. The oxidation process would take place much faster if there were no toxic substances, in our case heavy metals that prevented the oxidation from taking place much faster than was achieved.

Due to the increased concentration of phosphorus and after 6 days of biodegradation, it is recommended to prolong the time of biodegradation or add substrates such as zeolites or calcites that bind phosphorus, so that the phosphorus concentration is in accordance with legal regulations. Increased phosphorus concentration above the maximum allowable concentration even after 6 days of biodegradation is possible due to the presence of heavy metals in wastewater.

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PROGRAM OF MEASURES FOR PRESERVATION AND IMPROVEMENT OF THE ENVIRONMENT OF RURAL SETTLEMENTS

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Key words: environment protection, rural development

ABSTRACT:

The outflow of population from the countryside to the city is a general trend in Serbia, and the reasons are above all we can find it in the decline of the rural economy, poor infrastructure equipment and lack of conditions for basic living standards.

Analyzing the communal problems of the rural area, a shortcoming is noticed rural water supply management system, lack wastewater collection and treatment infrastructures and non-coverage municipal waste removal service. All these communal problems result negative impact on the quality of life of the inhabitants of rural settlements and negative environmental impact.

Measures that would contribute to the preservation and improvement of the environmental condition of rural areas must be created in accordance with sustainable development. By defining the real situation in rural areas, mechanisms of action could be identified through environmental models of sustainability that would reduce weaknesses and shortcomings.

At the same time, defining the real situation and relations in the Serbian rural area is a contribution to the development of methodological research in this area.

1. INTRODUCTION

For vital economic, social and environmental viability of nations, sustainable rural development is very important. That is the process of improving the quality of life and economic well-being of people which are living in rural areas, often relatively isolated and sparsely populated areas [1].

Rural development has tradiotionally focused on the expoloitation of land through the agriculture and forestry, and useing all natural resources such as water, soil, biomass, etc. Changes in global production and increased urbanization have changed the character of rural areas. Resource extracton and agriculture as dominant economic drivers have replaced by tourisam, niche manufacturers and recreation [2]. Approach to development from a wider perspective has created greater focus on a broad range of development goals rather than merely creating incentive for agricultural or resouce based businesses. For development rural region, emphasis on locally produced economic development strategies has a big role [1], but also there are education, infrastructure (physical and social) and enterpreneurship [3].

Urban regions has many similarities, which is in contrast to rural regions hose are highly distinctive and with large variety of rural development approaches used globally [4]. Wherever they live, people should have a satisfactory quality of life, which defined by the World Health Organization as "an individual's perception of their position in life in the context of the culture and value system in which they live and in relation to their goals, expectations, standards and concerns" [5].

Analyzing the communal problems of the rural area, can be notices the lack of a water supply management system for the rural population, the lack of infrastructure for the collection and treatment of wastewater and the lack of coverage of the municipal waste collection service. All these communal problems result in a negative impact on the quality of life of the inhabitants of rural settlements and a negative impact on the environment.

An additional problem of rural areas is the improper construction of septic tanks and the mass phenomen of turning wells into septic tanks. In this way, it directly affects the pollution of groundwater, which is an invaluable resource.

In such places with a smaller number of people, where there is no wastewater management system, it is easier to access and create natural wastewater treatment systems (ecosystem processors, artificial wetlands) that prove to be very effective. Water purifiers based on phytoremediation of the type of artificial wetlands are characterized by low construction costs, easy maintenance, and high efficiency. By planting plantations of plants from the group of agro-energy crops such as Miscanthus x giganteus instead of the usual marsh plants, these purifiers get an additional role, so in addition to the function of water purification, they simultaneously produce biomass. Miscanthus biomass is a renewable source of energy and is most often used to obtain biofuel or thermal energy, but it is also widely used in other economic activities. Due to the simultaneous development of the process of water phytoremediation and biomass production, there is no waste that would be generated at the end of each growing season in the case of planting common wetland plants. In this way, the use of agro-energy crops contributes to the sustainability of the purifiers, that is, enables their functioning in accordance with the principles of sustainable development.

2. MEASURES FOR IMPROVEMENT RURAL SETTLEMENTS

Data on population density (number of inhabitants per 1 km²) show that the uneven spatial distribution of the population in settlements and regions of Serbia in the last inter-census period (2002-2011) continues to increase, despite the fact that the total population is decreasing. It is another in a series of our demographic and development paradoxes that especially affects rural settlements in underdeveloped municipalities and regions of Serbia. In the period between the last two Censuses, at the level of Serbia, the rural population decreased from 43.6 % (2002) to 40.6 % (2011) [6].

Based on the data on the age structure of the population in Serbia according to the type of settlement in the regions, accelerated aging can be seen as one of the important factors influencing the depopulation of villages. Villages are declining not only because young people from villages are moving to cities, but also because the old population is dying much more than they are being born [7].

The outflow of population from the countryside to the city is a general trend in Serbia, and the reasons can be found primarily in the decline of the rural economy, poor infrastructure and lack of conditions for basic living standards.

In rural areas, technical solutions in the field of wastewater management should be based on ecosystem solutions. Economy, simplicity and possibility of application at the place of origin are an advantage for their wide application in the treatment of municipal wastewater.

2.1. Ecosystem procesor

Nature has ability to self-renew water, regular, uncontrolled consumption and pollution no gives the system enough time to do its job. For that reason, it is necessary apply and succeed already established natural processes. Purification system water ecosystem processes rely on natural processes. Function absorption and decomposition of harmful substances in water top plants and microorganisms.

Artificial wetlands are not a new concept, because the first such purifiers were constructed in the middle of the 20th century. According to estimates from 2009, around 10,000 wastewater treatment plants of this type operate in the European Union [10]. The application of ecosystem processors began in the 80s in Europe and America, they were introduced in Slovenia in 1990, in Croatia in 1997, and to date they have undergone numerous changes, improvements and variations through research and application, so the possibility of application is very wide. This type of treatment plant, with an adequate technical solution, can be used for the treatment of wastewater from one lonely facility to a settlement of five thousand inhabitants. There are examples of application for settlements with over 20,000 inhabitants.

Ecosystem processor (ESP) [8] is a multifunctional system that by simulating natural processes performs:

- 1. wastewater treatment,
- 2. biomass production for energy needs,
- 3. creates a new habitat for plants and animals and
- 4. performs additional oxygen production.

Constructed ecosystem processors for water purification have been developed on the basis of knowledge of the structure and function of natural aquatic ecosystems, primarily wetlands. The more we understand the processes in natural wetlands, the more efficiently we will construct the ecosystem processor.

As a result of extensive research and practical application of ecosystem processors, it has been developed through various solutions of design, characteristics, functioning and maintenance in accordance with the specific requirements of purification. A special type of constructed ecosystem processor on the river bank in which water from the river was pumped into the main reservoir and then discharged into three pools with different plant species that performed the purification process is given in Picture 1. The purified water was then returned to the river. This system has proven to be effective in cases of torrential inaccessible smaller streams and canals.

Different types of terrain but also wastewater provide the possibility of diversified types of ecosystem processors. It means new possibilities for application in different capacities, localities, special requirements and through improving the efficiency of purification and integration with other natural and anthropogenic ecosystems (Picture 2).



Picture 1. Type of ecosystem processor



Picture 2. Work on ecosystem processor

Each ecosystem processor for water purification consists of a pool of appropriate design, which contains water, substrate and plants. These components are manipulated during the construction of ecosystems, while others (microbial communities and aquatic invertebrates) are mostly formed naturally.

Ecosystem processors are the habitat of many invertebrates and vertebrates. Invertebrates participate mainly in the decomposition of organic matter detritus. The larvae of many insects live in water and consume organic matter. The larval state can last for several years. Some are significant predators. From the vertebrates are inhabited by fish, amphibians, reptiles, birds and mammals. Visually, these processors are an unusually rich environment. Color complexity, the shape of the size and line of water and vegetation that follow the natural contours of the terrain allow for a complete fit into the ambience.

2.2. Waste and wastewater management

Waste represents a huge loss of resources in the form of materials and energy, and inadequate waste management. Most villages in Serbia do not have an organized system for collecting and disposing of waste, but the population manages, most often burning waste. Disposal can have serious environmental consequences. Landfills, occupy significant ones surfaces and can cause air, water and soil pollution, while uncontrolled incineration of waste material can to lead to the emission of dangerous pollutants into the air [9].

Mesures for improve rural area in filed of waste management are:

- Organized collection with the possibility of primary waste selection,
- Provision of disposal containers,
- Regular waste collection by the competent utility companies.

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Waste management should be integrated, and design solutions and wastewater management should be solved by the principle of minimum practical size (household, group of households, settlement, community, source ...), which reduces the mutual flows of water and waste. Rural wastewater management systems should be adapted to local conditions in sparsely populated areas with a specific terrain configuration. Techno-economically, the most optimal technological solution, in rural areas, is wastewater treatment for groups of housing and social standard facilities using a system that includes: waterproof septic tank combined with additional treatment and biotreatment techniques.

In the field of wastewater management, and in order to improve the state of the environment, the proposed measures to be implemented relate to:

- Measures for collection and treatment of waste (sanitary and fecal) rural water settlements, by building decentralized wastewater treatment systems and establishing a management system,

- Measures of pre-treatment of processing wastewater in rural areas, which come from local processing industries, food production and cooperative activities,

- Measures for recycling material residues (agro residues, animal residues, process sludge, etc.), which completes the process as end products products energy resource – biogas.

2.3. Education and improve awareness of environmental protection

Improved the capacity of good quality education in rural areas, could helps to keep rural population. Young people are moveing to urban areas for better opportunities in education and employment. Education is considered a vital element in the development of a society, a system, and a county.

If educated young people stayed in the countryside and engaged in agriculture and other jobs related to rural areas, the whole system would be improved.

In addition, increasing the awareness of the current population about environmental protection plays an important role in protecting, preserving and improving the environment. Conducting regular education and promotion of good agricultural and production practices, approaching the topic of adequate management of waste, wastewater and pesticides, are just some of the topics that should be given more attention. The problem must be solved systematically, from the competent institutions at the state level to the regional and local ones.

3. CONCLUSION

Rural areas are in the previous period it experienced serious stagnation in terms of infrastructure communal arrangement. Bearing in mind the depopulation of rural areas and the weak the economy cannot be expected to create the conditions for construction of large utility systems.

The aim of this paper was to propose a different approach to wastewater treatment, that is, the treatment of wastewater at the place of their origin, rounding off the complete process. In that way, avoiding investments in capital utilities infrastructure, contributes to the preservation of water quality through minimal investment individual households or groups of households into small decentralized ones purifiers. In accordance with the principles of sustainable development, wastewater is necessary treat as close as possible to the place of their origin, which leads us to decentralized, local small capacity systems with supporting infrastructure.

We proposed construction of decentralized wastewater treatment systems based on ecosystem principles in accordance with legal regulations, water management conditions and principles of construction of ecoremediation treatment systems.

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THE DARK SIDE OF ARTIFICIAL LIGHT

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ABSTRACT:

In the recent past, Earth inhabitants had a completely dark night and sky full of stars at night, while in the last twenty years, only the inhabitants of less-developed countries could enjoy the starry sky, who had no possibility to install sources that would threaten the night sky by overuse of artificial lighting.

Specifically, today we are faced with light pollution, which is primarily the result of the scattered light of cities and other light emissions from artificial sources, mainly from urban areas and areas where economic operators (factories, warehouses, etc.) are concentrated. Much of the light flux from these sources, and therefore energy, is scattered and lost in the sky, which makes the night sky brighter and the celestial objects in its background become invisible. Light pollution is now considered a complex problem that has a detrimental effect on the environment with a very wide range of consequences.

The harmful effects on the environment are primarily reflected in disturbances in astronomical observations, inducing hormonal disorders in humans working at night, in plants disturbing vegetation cycles in plants, disorienting birds in space, sea turtle cubs and some other animal species, which all together directly affects at their survival, often causing changes in the habitat of endangered animal species. This has led to the development of scotobiology, which as a branch of biology deals with the benefits of darkness and helps to establish a safe level of brightness, duration and color of night illumination to avoid the harmful effects of light pollution on the living world.

Given that there is a need to protect the environment as a whole, and human health as well, from light pollution, it is necessary to take appropriate measures to educate the public about the

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potential adverse effects of light pollution while at the same time improving legislation in this area of environmental protection, because only through valid legal framework for the adoption of regulations on protection against light pollution, it is possible to reduce, if not completely eliminate, the harmful effects of light pollution in the environment.

1. INTRODUCTION

For decades, air, water and soil pollution have been the most important challenges in environmental policy, while light pollution has remained scientifically, culturally and institutionally in complete darkness. Due to the fact that the emission of artificial light has dramatically increased in the last few decades, there is an urgent need to investigate the physiological, ecological and socioeconomic impact of dark and night loss on the living environment, as well as finding solutions for technical and institutional lighting improvement in order to reduce harmful effects.

Artificial light began to be used by humans before Paleolithic times or 2.5 million years ago, when hollow stones or shells were filled with moss soaked in animal fat and lit to produce their first lamps. The tallow, which was first burned in these lamps, was later used with natural wax to make candles until the 19th century, when they were replaced by stearin produced from tallow and paraffin, which began to be widely produced from oil at the end of the century.

At the beginning of the 19th century, the light flame of burning carbon gas replaced candles and oil lamps, and began to be produced on an industrial scale, thus spreading artificial light first in large cities and later beyond. In the middle of the same century, the production of kerosene began, the use of which in lighting artificial light conquered even the most remote rural settlements.

Shortly afterwards, lighting, along with electric propulsion, was the main area of application for electrification after the construction of the first DC power plants for public use and the first AC power plant, and the electric light bulb is one of the most important symbols of technical progress since its early 19th century concept until today.

Although the invention and widespread use of artificial light are one of the most important technological achievements of man, the disappearance of night darkness is increasingly recognized as harmful. Namely, night lighting can have serious physiological consequences for humans, ecological and evolutionary consequences for animal and plant populations and can shape entire ecosystems. However, the possible harmful effects of light pollution have not yet been fully investigated. In response to climate change and energy shortages, many countries, regions and communities are developing new lighting programs and concepts with a strong emphasis on energy efficiency and greenhouse gas emissions. Given the dramatic increase in artificial light at night (0-20% per year, depending on the geographical region), there is a need to introduce measures to prevent and protect against light pollution that go beyond energy

efficiency and include human well-being, structure and functioning of ecosystems and interconnected socio-economic consequences. Such a shift in policy requires a healthy transdisciplinary understanding of the importance of night darkness and its loss to the people and natural systems on which we depend. This requires knowledge of appropriate lighting technologies and concepts that are environmentally, socially and economically viable, as otherwise society may encounter global detrimental effects of artificial light with unpredictable outcomes.

2. ON LIGHT POLLUTION

Until recently, light, i.e. the visible part of the electromagnetic spectrum, although the most important type of radiation for humans, along with thermal radiation, has not been treated as something that can adversely affect the environment and pollute it.

Once upon a time the Earth inhabitants had a completely dark night and sky full of stars at night, while today, only the inhabitants of less-developed countries could enjoy the starry sky, who had no possibility to install sources that would threaten the night sky by overuse of artificial lighting. Much of the artificial light emitted from various sources (urban settlements, industry, roads, etc.) is scattered and lost in the sky, making the night sky brighter and celestial objects in its background becoming less visible or completely lost. Namely, astronomers were forced to relocate their observatories outside urban areas, whose lighting, illuminating the atmosphere, drastically reduced the contrast between the natural darkness of interstellar space and the dim light of distant astronomical objects, due to increased sky brightness. This side effect is due to excessive artificial lighting and scattering of visible and invisible light on various particles in the atmosphere (gases, water vapor, pollen, dust) and causes harmful consequences for humans and the environment as a whole.

However, biologists were the first to approach the systematic study of this phenomenon, so F. J. Verheijen [1] in the late fifties of the last century was the first to mention the so-called light traps, and twenty years later he used the English term photopollution, which today is mostly replaced by the term light pollution.

Although astronomers were the first to recognize changes caused by artificial light in the form of increased sky brightness, it was not until the beginning of this century [2-4] that multiple negative effects of artificial lighting on the environment, human health and social well-being were identified. It was quickly established that the consequences of light pollution can be biological, energy, economic, social, and ultimately aesthetic.

Scientists have paid more attention to the research of light pollution and its consequences for the last 30 years, which has resulted in the adoption of laws and other regulations prescribing measures to reduce and prevent the negative consequences of artificial lighting as a new form of environmental pollution. In this way, a modern definition of light pollution was reached,

according to which light pollution is considered to be any change in the level of natural light at night caused by the introduction of light produced by human activity. In a broader sense, these are changes in the level of natural light at night caused by the emission of light from artificial light sources that adversely affects human health and endangers traffic safety due to glare, direct or indirect light radiation, interferes with life and/or migration of birds, bats, insects and other animals and disturbs the growth of plants, threatens the natural balance, interferes with professional and/or amateur astronomical observation of the sky and unnecessarily consumes energy and distorts the image of the night landscape [5].

Organized confrontation with light pollution on a global scale was started by the *International Dark Sky Association (IDA)* founded in the USA in 1988 as a non-profit organization, which today brings together numerous civil society members from more than 70 countries. IDA was the first who define light pollution *as any harmful effect of artificial light, including increased sky brightness, blinding, illumination outside the area to be illuminated, overexposure, reduced visibility at night, and scattering of light energy [6,7].*

Satellites began to be used in the 1990s to investigate the problem of increasing the night sky brightness caused by the introduction of artificial light. Satellite images of the night side of the globe made it possible, with the help of light scattering theory, to calculate the increase in the brightness of the night sky caused by light sources recorded in these satellite images. As a result of these studies, a new Atlas with light pollution maps based on observations and collected data was released in 2016, which indicated a significant increase in light pollution compared to previous studies [8]. This atlas showed for the first time how light pollution affects the world population on the basis of statistically calculated values of pollution levels that are also visually shown, Figure 1.



Figure 1. Map of Europe's artificial sky brightness [8]

The results of these studies showed that about 83% of the world's population lives under lightpolluted night skies where the night sky brightness at the zenith is more than 14 microcandelas per square meter (μ cd m⁻²) or individually, more than 99% of American and European populations live under light-polluted skies. Also, due to light pollution, the Milky Way (Figure 2) is not visible to a population representing more than one-third of humanity, including 60% of Europeans and nearly 80% of North America [8].



Figure 2. Milky Way over Rocky Mountains, Colorado, USA [9]

Furthermore, the possibility of seeing the Milky Way from their own homes on clear nights is excluded for all residents of Singapore, San Marino, Kuwait, Qatar and Malta, and for 99%, 98% and 97% of the population of the United Arab Emirates, Israel and Egypt. Bearing in mind the average number of inhabitants per unit area, the same study calculated that the countries with the largest part of their territory from which the Milky Way is not visible due to light pollution are Singapore and San Marino (100%), Malta (89%), Qatar (55%), Belgium and Kuwait (51%), Trinidad and Tobago and the Netherlands (43%) and Israel (42%) [8].

2.1 Impact of light pollution on the living world

For millions of years, the change of day and night has been a fundamental determinant of the ecosystem, and the life cycles of the entire plant and animal world, including man, have been adapted to their natural daily changes. So even at night, which is not completely dark because there are several sources of natural light, even in the most remote parts of the Earth, there is life that is adapted to just such conditions.

A constellation of eminent scientists from various scientific disciplines (mathematics, astronomy, philosophy, archaeology, etc.) from Socrates, Plato, Aristotle, Ibn Al-Haytham, J. C. Maxwell, Huygens, Newton, Young, Einstein [10], all the way to our contemporaries, studied the characteristics of light as electromagnetic radiation in the visible and invisible part of the spectrum [5] and its noble effect on the living world on Earth.

Unfortunately, today there is a lot of scientific evidence [11-15] of the negative and harmful effects of light pollution and the resulting disruption of the natural cycle day / night on flora and fauna because artificial light at night has become one of the most significant changes in the human environment. Today, many harmful effects of light pollution are still not fully explained or are poorly understood, because the identified impact on the growth and survival of the organism is very complex due to the fact that these effects result from the way artificial light at night changes interactions with other species in the form of indirect effects and the way in which it changes the physiology of the individual in the form of direct or immediate effects.

These adverse effects can take various forms, such as reduced nesting of birds in overly lit areas, orientation weakening of migratory birds, declining populations of many insect and bat species, reproductive disorders of certain fish species, plant vegetation disorders, declining numbers of individuals and plant species in certain areas. due to the decline in the number of nocturnal pollinators or the complete extinction of certain animal and plant species. Artificial disturbance of the natural cycle day / night, in addition to the flora and fauna, can of course cause serious harmful psycho-physiological and even health effects on humans.

2.1.1 Impact of light pollution on plants

Plants, like animals, are sensitive to light, its color, intensity and duration of exposure. Light is a vital requirement for plants due to the fact that it is needed for photosynthesis as well as information for plants related to germination, budding, leaf and flower formation, affects flower color and induces flowering induction, etc., all based on absorption lights of appropriate wavelengths that comply with their development requirements. This mainly refers to the part of the light spectrum of wavelengths between 400 and 700 nm (Figure 3), which is also known as photosynthetically active light and corresponds to a more or less visible part of the spectrum, i.e. the part to which the human eye is sensitive [16,17].

Plants have different behavioral responses to different wavelengths of light, where flowering, germination, and photosynthesis associated with exposure to different parts of the visible light spectrum. Namely, photoreceptors in plants use light to obtain and feel information about the season and even the time of day, which directly affects or controls seed germination, stem growth or elongation, leaf spread, flower development, avoiding shade, etc. Although light is dominant factor in the life of plants because it affects their physiology, excessive and uncontrolled exposure to artificial light can cause harmful consequences.

In most cases, the intensity of light pollution is not enough to affect the process of photosynthesis, but changing the perception of the day/night cycle due to artificial increase in day length, directly disrupts their biological daily rhythm or so-called circadian cycle [18].



Figure 3. Electromagnetic spectrum [17]

Due to such a conditioned disorder of plant physiology, phenomena such as promoting the spread of leaves, and thus increased exposure of plants to air pollutants, or greater susceptibility to water stress are possible. For the same reason, in urban areas, delays in leaf drop from deciduous trees near street lights can be observed (Figure 4), as well as the early onset of spring bud burst, thus increasing the risk of exposure to frost and pathogens.



Figure 4. A tree that keeps the leaves longer on the illuminated side. This light can shorten the tree's life [19]

Another form of indirect harmful effects of light pollution on plants is related to plant pollination [20, 21]. It is known that pollination of plants takes place day and night, but there are plants whose pollination takes place mainly at night thanks to nocturnal pollinators, and which attract with their seductive scents and abundant amounts of nectar. Unfortunately, with the appearance of light pollution or street lamps near these plants, pollinators (e. g. moths and others) are attracted to light and do not pollinate plants, because they are attracted to a light source that is usually their mortal enemy, Figure 5.

Another form of indirect harmful effects of light pollution on plants is related to plant pollination. It is known that pollination of plants takes place both day and night, but there are plants whose pollination takes place mainly at night thanks to nocturnal pollinators. Plants attract nocturnal pollinators with their seductive scents and abundant amounts of nectar. Unfortunately, with the appearance of light pollution or street lamps near these plants, pollinators (e. g. moths and others) are attracted to light and do not pollinate plants, because they are attracted to a light source that is usually their mortal enemy, Figure 5.



Figure 5. Indirect impacts of artificial light at night on plant communities and pollinators [22]

Figure 5 shows the cascading action of artificial light at night on plant communities and pollinators, where solid lines indicate direct or immediate effects, while dashed lines indicate indirect effects. The direct negative impact of light pollution on nocturnal pollinator communities is transmitted to plants, reducing their reproductive success, which has consequences for day pollinators, as it reduces the amount of available food resources.

2.1.2 Impact of light pollution on wild animals

The fundamental importance of light on animals is primarily reflected in its role in encouraging daily and seasonal activities such as feeding, food storage, reproduction, nesting and migration. In the case of excessive light in the environment, animals have a biological rhythm disorder that regulates wakefulness, sleep and metabolic activity through hormones, as well as circadian rhythm disorders that control physiological processes and patterns of behavior through which animals adjust their biological daily rhythm to daily variations, i.e. 24-hour variations in the environment.

Numerous results of research conducted so far on the harmful effects of light pollution on the living world have shown that these effects have a very wide range, both in their form and in the number of taxa including plants [23], insects [24], amphibians [25], birds [26] and mammals [27,28]. Although light pollution affects both nocturnal and daily animals, it has been found to pose a greater threat to animals that are active at night. According to the International Dark Sky

Association, the animals most affected by light pollution are sea turtles, bats, Atlantic salmon, zooplankton, certain species of butterflies, owls, mice, etc. [29].

Given the fact that all bats are nocturnal animals, as well as about 90% amphibians, about 60% mammals and invertebrates and about 30% primates [30], it is evident that these animals have serious problems during light nights and ultimately their survival depends on taking the necessary measures to prevent light pollution in the environment. Some of the examples that will be mentioned here can serve as different effects of light pollution on different animals.

2.1.2.1 Insects

According to a 2017 study by German scientists [31], Europe has lost almost 80% of 'its' insects in less than 30 years, causing a domino effect and the disappearance of more than 400 million birds. It is important to note that in addition to birds, hedgehogs, lizards, fish, amphibians also depend on insects as a food source, and the possible domino effect should be investigated in relation to these species. It is important to point out here that this decrease in the populations of certain insects, among which the most endangered species are butterflies (lat. Lepidoptera), bees, wasps, ants, hornets (lat. Hymenoptera) and beetles (ladybirds, fireflies), is caused not only by excessive deforestation, using pesticides, mineral fertilizers and urbanization that brings lighting and participates in light pollution. In addition to the most well-known causes of harmful effects on insect life, light pollution is not given due attention, especially if we take into account that about 60% of all insect species are classified as nocturnal (nocturnal) and a third of them die from harmful effects of artificial light [32]. Possible harmful effects of light pollution on insects are numerous and can be classified according to the changes they cause in terms of physiological processes and patterns of behavior to those that cause: a) eating disorders, b) communication and reproduction disorders, c) temporal and spatial disorientation, d) increased attractiveness to light sources leading to danger, etc. [33,22-24].

2.1.2.2 Aquatic organisms

Natural light and its intensity are associated with the life of many aquatic species and the entire structure of aquatic environments. Thus, at night, the only natural light that spreads underwater comes from natural sources, and these are the stars, the Moon, and the light of aquatic organisms that glow in the dark (bioluminescence). Therefore, any, even the slightest light pollution, changes the intensity, color's and frequencies to which aquatic organisms may be exposed, and this can influence the behavior of aquatic organisms by changing their natural circadian cycle [34]. Light pollution of water systems is due to the fact that today more than half of the world's population lives within 100 km from the sea shores, lakes or rivers, and half in their immediate vicinity. This indicates that this proximity is the cause of exposure of water systems to artificial

light emitted from urban areas, hotel settlements, recreation centers, industrial plants, roads and the like.

An example of the harmful effects of light pollution on aquatic organisms is the change in zooplankton behavior. Namely, like many other aquatic invertebrates, zooplankton move vertically in the water column, and with this vertical migration at night, in order to avoid their fish predators, it feeds on surface by phytoplankton. However, in the conditions of present light pollution, the illumination of its migration path lasts both night and day, and therefore the number of migrating zooplankton and the amplitude of vertical migration decreases. This not only causes a decrease in the zooplankton population, but also leads to an increase in the phytoplankton population on the water surface. In the long run, these changes could have consequences for the balance of aquatic ecosystems and, due to changes in the prey / predator ratio, affect the food chain and water quality [34,35].

The way in which artificial light or light pollution can adversely affect fish can serve as a series of examples of research about harmful effects of Atlantic salmon (lat. *Salmo salar* L.), which is a conserved and economically important species and its ecology and behavior have been studied for a long time. Thus, recent research has shown that salmon exhibit behavioral changes in response to artificial light, although the physiological processes behind the observed behavioral changes have not yet been fully established [36].

It is known that in natural light salmon migration correlates with sunset, but in the presence of artificial light, e. g. from street lighting, their migration becomes accidental which can jeopardize its chance of survival. In addition, it has been found that in rivers where salmon spawn, artificial light at night can be detrimental to younger salmon as it becomes more exposed to predators.

Of course, there is evidence that some species other than salmon have experienced the harmful effects of light pollution, such as trout fish and sea trout fish, barbell fish, grayling fish, eel, etc. [37].

2.1.2.3 Amphibians and reptiles

Because of the detrimental effects of artificial light, many amphibians have shown both physical and behavioral disorders, including a disruption of their ability to know when to return home and reproduce that calls into question their reproduction.

Since most amphibian species are nocturnal and water-dependent, it has been found that light pollution causes orientation disorders and they may have difficulty migrating during the mating and breeding stages, which may result in reproductive difficulties. It has been noted that it is for this reason that populations of many amphibian species face declining numbers, although the harmful effects of light pollution occur in other areas of their lives and artificial light is a major problem [37].

Most salamanders, for example, which are also nocturnal animals and have about three hundred species that live exclusively in humid environments on all continents except Antarctica and Australia, are not phototactic animals and run away from light. Artificial night lighting near their habitat can affect their physiology and behavior, although the results of research to date have not been sufficient to explain whether and what the possible impact of artificial light is on their population.

Among reptiles, and in the animal world in general, sea turtles are one of the most dramatic examples of how artificial light on beaches can disrupt their behavior. Namely, females of many species of sea turtles return to the same beaches for decades and lay their eggs in the sand, where they were born. Since these beaches are brightly lit at night today, it happens that the light bothers the females and they stop nesting on them and look for a new habitat. If females lay eggs on these beaches, it happens that young turtles that lay during the night due to the reduced danger of predators, instinctively move towards the sea following the reflection of the moon on its surface, and very often, disoriented by artificial light sources, instead of the sea, move towards the coast where they perish on roads etc. [33,37,38].

2.1.2.4 Birds

Birds, like insects, play a very important role in their ecosystems and are vital for agricultural production as well as for the movement of nutrients in natural systems. Unfortunately, birds are not spared the harmful effects of light pollution, especially those that migrate and hunt at night with the help of moonlight and starlight, because artificial lights can disorient them and divert them towards dangerous urban brightly lit "traps". Namely, when the bird is attracted by the artificial light of urban areas and once in the city, there is a big problem of navigating the space, and the glass facades of skyscrapers and bright lighting of buildings are often fatal traps. Such stray birds are usually not able to leave the city during the day, nor can they recognize the dangerous glass surface on which they see attractive reflections of trees or indoor ornamental plants behind the glass. Their rapid flight towards these reflections, or plants on the other side of the glass, usually leads to severe injury or death. Experts estimate that about 100 million birds die each year in the United States alone due to collisions with glass facades of buildings, and some studies indicate that glass surfaces are more dangerous to birds than any other human activity [39].

One of the most vulnerable groups facing the threat of global extinction are birds because about 40% of their 11,000 species are declining, and some of the reasons for this decline are related to light pollution affecting bird disease transmission, ability to move during migration, numerous fatal collisions with buildings in urban centers, lighthouses at sea, wind turbines, etc. [30,39,40].

2.1.2.5 Mammals

In the world of wildlife, where many species are exposed to light pollution and experience its harmful effects on a daily basis, mammals are not immune, so bats, raccoons, coyotes, deer, elks, etc., have long since begun to show changes in behavior when it comes to their nutrition, due to the harmful effects of excessive lighting. Visual impairment of certain species due to the harmful effects of artificial light leads to an increased risk of their exposure to natural predators and increased mortality within the population.

The harmful effects of light pollution on the behavior of mammals in the wild are also reflected in the increased risk of predators, which directly affects the reduction of certain populations. Behavioral changes in animals can also affect changes in their nutrition and have effects not only on individual species but also on entire ecological communities. For example, if a predator has a long time to hunt due to the presence of artificial light, then it is given the opportunity to expand the number of species of its prey. At the same time, for a species that has become a "new" prey to a particular predator for these reasons, the risk of its survival increases and the population in that habitat decreases.

Bats make up almost a quarter of the world's mammals, with more than 1,300 species, and are a very important group of mammals that are adversely affected by light pollution. This is supported by the fact that they are a very important link in the natural regeneration of tropical forests, pollination of a number of plants that bloom at night and control the number of active insects at night. In addition to all this, it is important to note that bats are one of the slowest-breeding mammals because they have only one young per year, and the infant mortality rate is more than 50%, so the populations of some species are very difficult to recover. Although bats live extremely long (up to 30 years), they are one of the most endangered species due to the influence of various environmental factors, including light pollution [41].

The results of a recent study [42] challenged the popular myth that bats are attracted to light, and it has been found that bat activity decreases in areas that are illuminated at night.

This is in contrast to previous assumptions that bats are helped by public lighting because it allows them to feed more easily on insects that accumulate around lighting fixtures. Light pollution interferes with their orientation in space, and bothers them when hunting insects, so often in urban areas, you can see bats flying around lighting fixtures with swarms of insects, but they, unfortunately, then do not have a feast, but in fact fight, to catch anything. This can, of course, cause malnutrition in bats, which in turn often leads to negative effects on the speed of reproduction of this species [43].

Migratory bats, which cover great distances between their summer and winter habitats, attract many and varied light installations during migration, which causes them to deviate from the usual routes, and at the same time causes the body to weaken due to loss of excess energy.
Unfortunately, there are a number of examples that could illustrate the different forms of harmful effects of light pollution on other species from the group of mammals (wolves, lions, bears, coyotes, possums, raccoons...), but the most common harmful effects for all of them are declining reproduction, difficulties in finding food due to too much light, increased exposure to predators, all together leading to population decline and even biodiversity.

These few examples of the harmful effects of excessive and unnecessary use of artificial light at night are enough to point out the seriousness of artificial light as an environmental burden with harmful effects on the biorhythm of living organisms, especially those with increased nocturnal activity. In order to prevent the harmful effects of artificial light on living organisms, scientists have expanded the field of scotobiological research, so now scotobiology is no longer a biological branch focused on the benefits of darkness for the living world, but also deals with harmful effects of artificial light at night on plant and animal physiology, biochemistry and behavior, including humans [44].

2.1.3 The impact of light pollution on man

Humans, like plants and animals, are susceptible to the harmful effects of light pollution given that in modern society a very large number of people are exposed to artificial light sources during the night, and night activities and night shifts have become a way of life for modern society. Thus, and due to the growing consumer demands of society for the availability of goods and services during 24 hours, the number of hours that the average person spends under artificial light is increasing every day. Due to man's excessive exposure to artificial light, his natural biological cycles are disrupted, which were established during evolution in the conditions of the natural change of day and night. This is also the reason why the natural rhythm of biological cycles in the human body is very slow and very difficult to adapt to changes in the usual distribution of light and changes in day and night. Namely, biological rhythms are time cycles within which many normal functions of the human body take place, including periods of sleep and activity, then behavior and most physiological and endocrine processes, Figure 6. These daily or 24hour rhythms within the body, similar to those described in plants and animals, controls the main so-called a circadian clock located in the area of the hypothalamus in the brain and adjusts its work according to the natural change of day and night, i.e. light and darkness [45]. Circadian rhythm in the human body includes all physiological and psychological changes, and changes in individual behavior, related to the change of day and night, i.e. during one whole day and lasts about 24 hours. This rhythm is regulated by the internal biological clock, which determines the basic physiological activities - feeding and sleeping pattern, body temperature, blood pressure, heart rate, brain wave activity, hormone and urine production, hormone secretion, blood sugar levels, regeneration of our cells, etc.



Figure 6. Circadian cycle scheme in man [46]

Thus, for example, a person who wakes up around 7 am has the best wakefulness around 10 am, his reaction time is the shortest around 3 pm, the highest cardiovascular efficiency and physical fitness is at its peak around 5 pm, and the highest blood pressure and body temperature are around 7 pm. After that, the preparation of the organism for sleep begins at around 9 pm, because then the secretion of the hormone melatonin begins, and at around 2 am after midnight the organism is in the deepest sleep. As early as around 4.30 am, the body has the lowest body temperature, and around 7 am it starts waking up and after that the secretion of melatonin stops. All these times are approximate and vary from individual to individual and are valid in the case when the organism is not exposed to light pollution, or when the circadian rhythm is not disturbed by the action of artificial light. Although the circadian rhythm varies from individual to individual, in humans it cannot be shorter than 23.5 or longer than 24.6 hours without detrimental effect on health.

The results of numerous studies [11-15] indicated a large number of potentially harmful effects of short-term and/or long-term exposure to artificial light during the night, ranging from traditional industrial, municipal and domestic sources to exposure via TV screens, personal computers, mobile phones and the like devices, without which modern man cannot imagine everyday life. All of these sources, some less, some more, cause circadian rhythm disorders,

which are associated with sleep disorders such as insomnia and late sleep syndrome, as well as depression, hypertension, attention deficit disorder, obesity, diabetes and cardiovascular disease. Another important biological function that is disrupted by the presence of artificial light at night is the production of melatonin since melatonin is a powerful antioxidant and anticarcinogen and is responsible for regulating metabolism and the immune response. This is evidenced by the results of research [47] on the impact of exposure to artificial light at night as a possible etiological factor in the development of human cancer, since darkness during the night stimulates the secretion of this hormone, while daylight as artificial, slows its secretion. As melatonin is involved in many processes (sleep control, circadian rhythm, retinal physiology, cancer development and growth, immune system, removal of free radicals, etc.), its secretion disorder can result in a weakened immune system associated with increased incidence of cancer (breast,

prostate, colon...) and other diseases and other physical and mental disorders [44,47]. The *International Agency for Research on Cancer (IARC)* at the *World Health Organization*

(WHO) in its last published monograph in 2019, as well as earlier in 2007, published results [43] according to which it classified night work as a carcinogen in group 2A - *probably a carcinogen for humans* based on limited evidence of cancer in humans, sufficient evidence of cancer in experimental animals.

This is particularly important for people working in night-time activities, such as the health, industrial, transport, trade and services sectors, where approximately one of 5 workers worldwide is engaged in the night shift [48]. In these activities, it is necessary to implement measures that would reduce night work to the minimum, as well as to conduct regular medical examinations of all employees in shift work, i.e. those who work at night. It is known, according to the *World Health Organization*, that 30-50% of all cancer cases can be prevented [49], so prevention offers the most cost-effective long-term cancer control strategy. The most important thing is to provide conditions for the implementation of national policies and programs to raise awareness, reduce exposure to cancer risk factors, including artificial light at night, and provide people with available information and support they need to adopt healthy lifestyles.

3. CONCLUSION

Although artificial light was recognized as an environmental factor in the late 1950s, it was not until the beginning of this century, when multiple negative effects of artificial lighting on the environment, wildlife, human health and social well-being were identified, that the effects of light pollution were systematically studied. In the past period, many harmful effects of light pollution have been identified, which can be biological, energy, economic, social and even aesthetic in nature.

This paper attempts to briefly present the possible harmful effects of light pollution on the living world of the environment, with special emphasis on the harmful effects on human health. In

addition to the basic concepts of light pollution, examples of scientific evidence of the negative and harmful effects of light pollution and the resulting disturbance of the natural cycle day/night on flora and fauna are presented. Although artificial light at night has become one of the most significant changes in the human environment, many harmful effects of this environmental factor on the growth and survival of organisms in conditions changed by artificial light at night, and thus resulting in the changes in the interaction of organisms with other species, either directly or indirectly, as well as changes in the physiology of individual plant and animal species, were not fully explained.

Since light pollution alters the natural metabolism of the circadian clock day and night in plants, insects and animals, humans are not spared its harmful effects on health. This is of course confirmed by scientific research and there is evidence of deteriorating human health at both epidemiological and physiological levels, which is associated with exposure to artificial light at night, which includes insomnia, sleep disorders, mood swings, early diabetes, obesity and even increased risk of some cancers.

Based on the above, it is possible to conclude that it is necessary to take measures as soon as possible and provide conditions for the implementation of these measures through national policies and programs, to raise awareness of the importance and dangers of artificial light and light pollution and to ensure the information and support for people who need to adopt healthy lifestyles without consequences for the environment and their own health.

Measures to protect against light pollution should include protection against unnecessary and harmful light emissions into space, in order to protect components and the living world of the environment and improve the life quality of present and future generations. It is necessary to take into account that no measure should be in conflict with the regulations in the field of occupational safety and health, especially for people working in activities where night work is common.

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THE INFLUENCE OF TYPE OF SOIL ON THE LEVEL OF MINERAL SUBSTANCES IN HERZEGOVINA TOBACCOS

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Key words: mineral substances, tobacco, varieties, red soil, alluvial soil, influence

ABSTRACT:

Mineral substances have an important part in the chemistry of tobacco and their share in the composition of entire dry substance is approximately 11 - 30%. They are visible through the ashes content that is left after the burning process. The level of mineral substances is particularly important factor in regulating the burning process of tobacco and the quality of tobacco is inversely proportional with the quantity of ashes. The highest content of mineral components have tobaccos dried in shades, then tobaccos dried in sun and the lowest level have tobaccos dried with hot air. Type of tobacco, soil, climate, the position of the leaf on the stem, way of drying tobacco etc, are influencing the level of mineral substances In order to examine the influence of the soil type on the ashes content in Herzegovina tobacco, the research was carried out on two localities: Pocitelj hill (medium deep red soil, Tera rossa) and Pocitelj valley (deep alluvial soil) near the Neretva River in the town of *Čapljina* in BiH. Tobacco was dried traditionally in the sun. The content of total mineral substances was analyzed in the samples of three tobacco varieties (Ravnjak, VH i VH-32) per insertion. The results showed that the content of total mineral substances in all three varieties and in all insertions was in the accordance with the literature data and that the influence of soil type and variety has different levels of significancy.

1. INTRODUCTION

Minerals are significant part of tobacco chemistry, and their share in the content of total dry matter ranges from 11 to 30% (Butorac, J. 2020). They are expressed through the ash content that remains after the tobacco is burned. The content of mineral substances is a particularly

important factor in regulating the combustion process of tobacco, and thus its quality. A balanced mineral fertilization is essential for a quality tobacco leaf (Barney et al, 1989). The content of mineral substances in the tobacco leaf is influenced by the position of the leaf on the stem, ie. insertion - insertion, soil type, pH value, genotype and way of fertilization (Karaivazoglou et al 2007, Raymond et al 2008, Golia et al 2009,). The influence of N, P and K fertilization on the mineral composition of tobacco leaves was evaluated in field conditions during three vegetation seasons. The amounts of added N, P and K had a very significant effect on the concentration of Ca, Mg, B, Zn, Cu, Mn and Al and a significant effect on the concentrations of other mineral components of the leaves were similar to those for the added N, P and K (Peterson et al, 1969). According to research conducted by Palaniyandi et al (2009), nitrogen had a direct effect on Ca, Mg and B, and inversely on Zn and Cu. Potassium had an inverse effect on Ca, Mg, B, Zn and Cu. Although not all coefficients were significant, K was inversely correlated with all eight mineral elements.

Kissling (1925), Shmuk (1948) and Bruckner (1959), among others, whose works point to the high ash content in tobacco, studied the mineral substances in tobacco.

Kažić and Mitrović (1940), Deželić, Šunjić and Viličić (1949), Ivanović (1960) and Đemidžić -Alić (1980) studied the mineral substances in the Herzegovinian type of tobacco. Their works show that the lower classes of tobacco and tobacco products contain more ash, and that the quality of tobacco is inversely proportional to the amount of ash. The studies of Kažić and Mitrović (1940) indicate that the lowest ash content is in Herzegovinian tobacco, ie. varieties Ravnjak with medium-sized leaves, which is the highest quality goods.

The most important components of the mineral complex of tobacco are potassium, calcium and magnesium, while others are present in much smaller quantities. These elements are mostly in the form of salts. Potassium salts have a beneficial effect on tobacco combustion. Tobacco burning is less favourably affected by calcium salts, while magnesium salts have an adverse effect on flammability. Chlorine has a particularly negative effect on the burning of tobacco if it is present in large quantities (Butorac, J. 2020). Tobacco-dried tobaccos have the highest content of mineral components, followed by sun-dried tobaccos, and hot air-dried tobaccos. (Butorac, J. 2020).

2. MATERIAL AND METHODS

Three varieties of tobacco were planted on two different types of soil, medium-deep red soil and deep alluvial soil. Two sites were selected for this experiment, and they are as follows: -Počitelj hill - medium-deep red soil -Počitelj field - deep alluvial soil by Neretva river.

At both selected sites, three varieties of tobacco belonging to the Herzegovinian type were planted: Ravnjak, VH 32 and Veliki Hercegovac.

Experimental site

Three varieties were planted in three repetitions of two rows and two marginal rows, which made 20 rows in total (9 plots plus 2 protective rows), 150 m^2 or a total of 500 plants on medium deep red soil and also on deep alluvial soil along the Neretva, which totals 300 m^2 of area and 1000 plants of all varieties including protective rows. Since both plots are not large, planting was done by hand.



Figure 1. Experimental sites Počitelj field and Počitelj hill

Fertillization

During ploughing, 500 kg of NPK was added 7:14:21 per hectare (7.5 kg per plot of 150 m²) with the addition of nitrogen - KAN with planting another 50 kg/ha (0.75 kg per plot of 150 m²).

Insertion and drying

Insertion and drying have a standard procedure for Herzegovinian tobacco types. Tobacco is dried naturally, in the sun. After drying of all the insertions, when the moisture content of the tobacco leaf was suitable for manipulation, tobacco samples were taken for analysis. A total of 54 samples were taken, and for each variety, on both types of soil, a sample from the lower, middle and upper insertion was selected in three replications. The content of mineral substances was determined by gravimetric method in the laboratory of the Tobacco factory in Mostar.

Also, soil samples were taken from both sites and analysed for basic fertility parameters by standard methods in the reference laboratory of the Federal Institute of Agropedology in Sarajevo. The results of the basic parameters analysis of soil parameters at both sites are shown in the tables 1. i 2.

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Depth	pH in H ₂ O	pH in 1M	CaCO ₃	Humus	total N	P_2O_5	K ₂ O
		KCl	(%)	(%)		mg/100g	mg/100g
						0 0	
0 - 20	7,90	7,30	14,35	2,75	0,15	1,6	19,50
30 - 50	7,95	7,30	14,85	2,03	0,12	1,0	14,7
60 - 80	8,10	7,60	65,30	2,01	0,06	2,1	9,2

Table 1.: Basic parameters of soil fertility from the site of Počitelj field (deep alluvial soil)

Table 2.:	Basic parameters	of soil fertility from	the site of Počitelj Brdo	(medium-deep red soil)
	1	2	5	

Depth	pH in H ₂ O	pH in 1M KCl	CaCO ₃ (%)	Humus (%)	total N	P ₂ O ₅ mg/100g	K ₂ O mg/100g
0 - 20	7,55	6,80	-	4,50	0,24	10,7	40,00
20-40	7,60	6,80	-	3,81	0,20	5,0	40,00
50 - 70	7,50	6,50	-	2,79	0,14	10,0	40,00

The results of the analyses on the ash content were statistically processed using the analysis of variance and the Tukey test.

3. RESULTS AND DISCUSSION

The content of mineral substances in Herzegovinian tobacco expressed over the ash content as the mean value \pm standard deviation (SD) by insertion height is shown in Tables 3, 4 and 5. Graphs 1, 2 and 3 show the ash content in tobacco leaves by insertion and statistical significance of the influence of variety, soil and their interaction on the ash content, with different letters indicating statistical significance.

	-	(
SOIL TYPE/VARIETY	VH	VH32	RAVNJAK		
RED SOIL	26.440 ± 0.02 b	27.160 ± 0.04 a	22.467 ± 0.47 c		
ALLUVIUM	30.087 ± 0.01 a	$27.547 \pm 0.40 \text{ b}$	19.180 ± 0.13 c		

Table 3.: Ash content in tobacco leaves for lower insertion (%)



Graph 1. Ash content in tobacco leaves for lower insertion

Visoki hercegovac variety had the highest average ash content in the lower insertion on alluvium, and the lowest average ash content was recorded in samples of Ravnjak variety on alluvium. The obtained values are in accordance with the literature data.

The analysis of variance determined a statistically very highly significant influence of the variety on the ash content in lower insertion and the interaction of soil and variety as experimental factors, while the same was not established in soil as an experimental factor.

The Tukey test revealed a statistically very significant difference in the ash content of the lower insertion between all three varieties, both on alluvial soil and on red soil.

		()
SOIL	VH	VH32	RAVNJAK
TYPE/VARIETY			
RED SOIL	$18.737 \pm 0,02$ c	20.070 ± 0.05 a	$19.643 \pm 0,47$ b
ALLUVIUM	19.280 ± 0,01 a	16.457 ± 0,21 b	$15.727 \pm 0,11$ c

Table 4.: Ash content in tobacco leaves for medium insertion (%)



Graph 2. Ash content in tobacco leaves for medium insertion

The average ash content of medium-sized leaves shows that the samples of the variety Visoki Hercegovac 32 on red soil have the highest values, while the lowest values were recorded in the variety Ravnjak on alluvial soil. All obtained values are in accordance with the literature data. Analysis of variance showed that the influence of variety, soil and interaction in medium insertion on the ash content was statistically very highly significant.

The Tukey test showed that a statistically highly significant difference in the ash content of the middle insertion was found between all three varieties on red soil. The same difference was found in all three varieties and on alluvial soil. A statistically highly significant difference in the content of this parameter was found in all three varieties from alluvial soil in relation to red soil.

			·
SOIL TYPE/VARIETY	VH	VH32	RAVNJAK
DED COH	15 552 . 0.0051	15.000 . 0.0((1	16 610 - 0.00
RED SOIL	17.553 ± 0.005 ba	17.823 ± 0.066 ab	16.613 ± 0.32 c
ALLIVIIM	17.410 ± 0.01 cb	18.027 ± 0.037 a	17.413 ± 0.28 bc
THEE CONTONN	17.410± 0,01 00	10.027 ± 0.057 d	17.415 ± 0.2000

Table 5.: Ash content in tobacco leaves for upper insertion (%)



Grafikon 3. Ash content in tobacco leaves for upper insertion

The highest average ash content in the samples obtained in the leaves of the upper insertion was found in the variety Visoki Hercegovac 32, on alluvium, while the lowest average value for this parameter was registered in the variety Ravnjak, on red soil. And in the samples of the above readings, the obtained values are in accordance with the literature data.

The established variant of the analysis showed a statistically very significant influence of the variety, in soil and interaction the same influence that is statistically significant, in the above reading, on the analysed parameter. Tukey test revealed a statistically significant difference in ash content, in the upper insertion, between varieties Ravnjak and varieties VH and VH32 on red soil, while the difference between varieties VH and VH32 on the same soil can not be considered statistically significant. In the alluvium, the difference between the ash content, in the upper insertion, in Visoki Hercegovac and Ravnjak is not statistically significant, while for the same parameter the difference between VH32 and VH, and between VH32 and Ravnjak is statistically highly significant.

Only the difference in the observed parameter, in the upper insertion, in the variety Ravnjak, between red soil and alluvium is statistically highly significant, while the differences in the other two varieties between different soil types are not statistically relevant.

4. CONCLUSION

In the lower insertions of all three varietys, the influence of soil as an experimental factor on the ash content was not established, while the variety significantly influenced the ash content from both localities.

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Bihać, 09 - 10 June 2022.
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The ash content in the leaves of the middle insertions, as well as the upper insertions, was found to be very highly influenced by all three experimental factors, ie. variety, soil and their interaction. The obtained results are in accordance with the literature data so this research proved that the soil, as well as the variety and position of the leaves on the stem, ie the height of insertion, have an impact on the mineral content of all three varieties belonging to Herzegovinian tobacco. Since the quality of tobacco and the ash content are inversely proportional, it can be concluded that this research has shown that for the Herzegovinian type of tobacco as regards to the mineral content, the leaves of medium insertions have the highest quality and most wanted on the market.

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NATURE BASED SOLUTIONS AS A TOOL FOR SUSTAINABLE DEVELOPMENT

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Key words: nature-based solutions, wastewater treatment, circular economy

ABSTRACT:

Nature-based solutions (NBS) are innovative approach inspired and supported by nature that are used for solving different environmental challenges. They use complex processes of nature, improving overall local environmental conditions, such as its ability to store carbon and regulate water flow, to achieve desired outcomes such as reduced disaster risk, improved human well-being and socially inclusive green growth. In comparison to conventional way of dealing with environmental challenges, NBS are cost-effective, simultaneously provide multiple benefits: environmental, social, and economic bones and help build resilience to climate change and other natural and anthropogenic risk. EC pointed out four goals that can be addressed by naturebased solutions: (i) enhancing sustainable urbanisation; (ii) restoring degraded ecosystems; (iii) developing climate change adaptation and mitigation and (iv) improving risk management and resilience.

This paper present advantages of applying nature-based solutions for enhancing sustainable urbanisation with the focus on advanced wastewater treatment based on one of IFAS (Integrated fixed biofilm activated sludge).

1. INTRODUCTION

Climate change, loss of biodiversity and degradation of ecosystems are interlinked and have devastating consequences for socio-economic stability, health, and human well-being. One promising path to transformative change, which is increasingly explored both in research and in practice, involves working with and enhancing nature. In practice, this involves applying

innovative approaches that are inspired and supported by nature, known under the concept of nature-based solutions (NBS) [1].

According to European Commission [2] "nature-based solutions are cost-effective, simultaneously provide environmental, social and economic benefits and help build resilience. Such solutions bring more, and more diverse, nature and natural features and processes into cities, landscapes, and seascapes, through locally adapted, resource-efficient, and systemic interventions."

NBS are actions which are inspired by, supported by, or copied from nature. They use the features and complex system processes of nature, such as its ability to improve overall local environmental condition, store carbon and regulate water flow, reduce the effects of urban heat islands, achieve desired outcomes: reduced natural and anthropogenic disaster risks, improved human well-being and socially inclusive green growth. NBS are energy and resource-efficient, and resilient to change, but to be successful they must be adapted to local conditions,

European Commission [3] recognised four principal goals that can be addressed by nature-based solutions:

- Enhancing sustainable urbanisation NBS can stimulate economic growth and at the same time improve the environment, making cities more attractive, and enhance human well-being.
- Restoring degraded ecosystems NBS can improve the resilience of ecosystems, enabling them to deliver vital ecosystem services and to meet other societal challenges.
- Developing climate change adaptation and mitigation by providing more resilient responses and enhance the storage of carbon.
- Improving risk management and resilience. NBS can lead to greater benefits than conventional methods and offer synergies in reducing multiple risks.

In comparison to conventional way of dealing with environmental challenges, NBS are costeffective, simultaneously provide environmental, social, and economic benefits and help build resilience. They can be cost more effective, especially if the principle of LCC (Life Cycle Cost) is implemented.

What is more, the implementation of NBS through the deliberate inclusion of ecological processes within human-influenced environments to deliver desired ecosystem services can play a vital role in achieving the Sustainable Development Goals at both global and local levels [4].

One of the most appreciated characteristics of NBS are their co-benefits and multifunctionality. Despite being designed for specific purpose (inter alia urban drainage), NBS can deliver several ecosystem services at the same time (e.g., treatment of various sources of pollution, evaporative

cooling, and biodiversity). A single element can serve in isolation but also in synergy for a building and a wastewater treatment plant at the same time [5], therefore multifunctionality makes NBS an important concept for cities to achieve sustainable resource management.

According to data provided by World Business Council for Sustainable Development [6] conventional infrastructure spending amounts about 3.8% of global GDP, equivalent to US\$2.6 trillion in 2013, and could grow to US\$3.4 trillion per year through 2030. Therefore, governments are interested in identifying cost-effective alternatives to grey only infrastructure or technology-based infrastructure to tackle challenges arising from biodiversity loss, climate change, more frequent natural disasters and the anthropogenic riska caused by rapid urbanisation. Nature-based solutions have demonstrated financial advantages due to a reduction in initial capital expenses, on-going operational expenses [3]. Additionally, they are superior if the LCC principles are applied.

The purpose of this paper is to present opportunities for applying nature-based solutions for enhancing sustainable urbanisation. Advanced WWTP is used as an example.

2. NATURE-BASED SOLUTIONS FOR ENHANCING SUSTAINABLE URBANISATION

Currently, 73% of Europe's population live in cities which is projected to increase to 82% by 2050, resulting in over 36 million new urban citizens [7]. This will pose a range of challenges, including a growing demand for resources and waste production. To cope with these challenges, the transformation of cities into sustainable systems is required. Consequently, the implementation of nature-based solutions, as a holistic approach, could be solution for sustainable urbanisation. According to Maksimović [8] and Božović et al [12] the following urban challenges can be addressed by NBS:

- reduction of water and air pollution as well as reduction of risk from urban heat islands;
- increasing the resilience of cities to several types of extreme weather conditions caused risks such as pluvial floods and droughts;
- improving public health, comfort of life and aesthetic values in cities; (4) increasing energy efficiency by using local natural resources;
- reducing socio-economic problems and
- improving biodiversity and urban, suburban interactions.

A stated by Atanasova et al. [5] current management of resources in cities can be considered as linear and single-functional. Water resources are imported and delivered to consumers, after use and treatment, they are discharged into environment as residual. For changing linear to multi-functional concept, substantial changes in the infrastructure design and management are needed.

However, there are a number of easy-to-use approaches for the implementation of nature-based solutions in cities. For example, heat stress in cities can be addressed by increasing green spaces and using green walls and green roofs which could reduce temperature up to 10°C in Mediterranean areas. Additionally, these approaches can also contribute to reducing flood risk and air pollution hazards, reducing energy demand in buildings (by 10-15%) and improving both indoor and outdoor quality of life [3] and human health in general, euPOLIS project [13].

Having in mind all mentioned above, nature-based solutions for solving urban problems constitute a critical element in addressing sustainable development goral SDG 11 - sustainable cities and communities. However, to be effective in solving problems of urban communities nature-based solutions require multisectoral and multidisciplinary approach as well as the willingness to allow new institutions to form as innovative solutions emerge [5], [9].

2.1 Nature-based solutions for wastewater treatment in urban areas

Conventional technologies for wastewater treatment (WWT) technologies originate from the 19th century and with certain modifications are applied in most existing and newly designed wastewater treatment plants (WWTPs) in the world.

Primarily due to the unpleasant smell, which spreads around such assets, these plants are located far from the city. The cost of building such systems is too high, particularly due to the high cost of building trunk sewers tunnels and pumping stations for transporting wastewater to a remote plant. Furthermore, the conventional plants require high operating costs, due to high energy consumption needed for air compressor operation and transportation of water from the long distances.

The time has come to replace these 19th century technologies with new ones for the 21st and 22nd centuries, not only because of savings in construction, operating and maintenance costs, but also because of other benefits and qualities that drive it [8] and [10].

For example, in a traditional outdoor pond with activated sludge, bacteria move freely in space, and with increased rainfall in the combined sewer system, they are carried away, thus it takes time to re-establish the necessary balance of water treating microorganisms, activated sludge etc. On the contrary, in IFAS technologies fixed biofilm plants use natural 'carriers' such as roots of aquatic plants or artificial ones located in so called reactors, on which a concentrated-fixed biofilm is created, with a richer spectrum of microorganisms that decompose organic waste much faster, take up less space and require less energy for blowing air into the reactors compartments. This systems reduces the required volume of the reactor (aeration basin), and thus the required footprint (land) for the plant and energy consumption for compressor operation, while significantly reducing operating costs. The visual difference between these two technologies is shown in Figure 1



Figure 1. Traditional and new technologies for WWT (Source: Makimović, Č., 2018), based on Biopulus technology [10]

2.2 Advantages of nature-based solutions for wastewater treatment in urban areas

According to Maksimović [8] and Biopolus [10], the advantages of nature-based solutions for wastewater treatment in urban areas are as follows:

- They fit harmoniously into the concept of cities of the future with decentralized units (metabolic hubs) for energy production, food, recycling of wastewater and other elements of the circular economy;
- Occupy significantly less land area for construction compared to conventional treatment systems. This frees up land for the construction of new housing and other assets;
- The smell is no longer felt, which increases the market value of city land and facilities;
- Nature-friendly treatment technology that does not create unpleasant smell and noise and can look very pleasant, thus eliminating the negative perception of such plants;
- The possibility of building such systems in urban areas, significantly reduces the cost of building trunk sewers that divert wastewater to remote conventional plants.
- Due to the shorter length (travel time), the septic contents creation/decomposition and formation of aggressive chemical compounds that damage the pipes and the structure of the building are significantly reduced;
- There is no need for protective space (buffer) around the plant (diameter of about 1 km, often more);
- Several types of treated effluent can be obtained with different degrees of purification, which can change over time depending on the need (for example, nutrients are retained in summer, water is used to irrigate green areas, in winter the nutrients are removed, and the water is used to replenish the underground aquifer). Wastewater treated in this way can be a new resource for reducing drinking water demand;
- Sludge, except for biogas-energy, can be used for compost, extraction of enzymes, biopolymers, cellulos etc., which can be a source of significant income and attract investors to invest their own funds in WWTP as a profitable business;

- Flexible design: compact and modular, meaning it can be expanded and scaled-up to the desired size and outflow parameters and
- Economic advantages: (i) construction of such facilities is up to 20-25% lower compared to conventional water treatment systems, (ii) operating costs are at least 35% lower compared to conventional systems.

2.3 The Metabolic Network Reactor (MNR) technology for wastewater treatment in urban areas

MNR technology for wastewater treatment is created by company Biopolus. It is patented 3rd generation Integrated Fixed-film Activated Sludge (IFAS) water treatment technology.

The main principle behind the MNR technology is a well-known natural phenomenon, where microbial biofilm develops on the roots of aquatic plants. Biofilm is formed on a combination of aquatic plant roots and artificial 'root' as shown in Figure2. The artificial roots mimic natural plant roots, maximizing the surface area to which microbial communities, can attach enabling optimized water treatment [10].



Figure 2. Formation and characteristics of fixed biofilm of metabolic network reactor at synthetic root [10]

Specially selected plants have a crucial role in the engineered ecosystem of MNR technology. Plant types can range from exotic tropical varieties to local native plants. Both the functionality and the aesthetic value of the plant is taken into consideration during designing phase. Additional selection criteria include the local climate of the facility and the needs and budgetary restrictions [11].



WATER TREATMENT & RECYCLING

Figure 3. Wastewater treatment and recycling in Metabolic Network [10]

This technology is applicable to several types of wastewaters in which organic matter dominates, such as household water, food and beverage industry, sugar factory. It is designed to be modular and expandable. It provides conditions for integrating water treatment and recycling with a wide range of other functions including food production, energy recovery technologies, and community functions as presented at Figure 3). Being odour free and compact, it can create garden-like atmosphere, fitting into any urban environment through creative architecture (Figure 4).



Figure 4. MNR reactor [10]

Over 200 WWPT have already been constructed with MNR technologies and its predecessors developed under the coordination of the fourth author.

3. CONCLUSION

Nature-based solutions are proven to be efficient in solving different environmental challenges, particularly in urban areas. They provide opportunities for multidimensional and multifunctional solutions in cities and create conditions for circular economy and urban green growth.

The Metabolic Network Reactor technology for wastewater treatment (MNR) is innovative water treatment technology that demonstrate successful application of nature-based solutions concept in cities. On one hand, MNR technology is easy for operation by being modular compact and odour free. On the other one, it is economically advantageous both in construction and operational phase which make it attractive nowadays for many cities striving towards sustainability.

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ECOCRITICISM AND CONTEMPORARY LITERATURE FOR CHILDREN

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Keywords: children's literature, eco-ethics, ecocriticism, ecosystem, nature protection

ABSTRACT:

The danger of ecosystem damage and efforts to promote nature conservation have contributed to the growing popularization of eco - ethics. Eco or ecological ethics as a framework of different ethical orientations is part of the movement for the protection of nature and the diversity of its life forms. This is a specific area of philosophical research and teaching on the establishment of ethical norms as a criterion of moral behavior towards the living world and ecosystems in nature. Man today is in imbalance with nature and it is necessary to restore a harmonious relationship in order to improve the quality of human life.

In contemporary children's literature in Bosnia and Herzegovina, the topics of ecosystem preservation and environmental protection are increasingly present. Knowledge is mostly learned and acquired in childhood through all forms of social action, so children's literature seems to be a suitable medium for the transfer of knowledge. In this article we are questioning the strategies of contemporary authors for children in promoting environmental protection and the ways in which the authors integrate environmental protection messages into the text.

1. FROM ETHICS TO ECOCRITICISM

The word ethics comes from the Greek word *ethos* – custom, habit, character, residence, atmosphere. The Greek word *ethikos* comes from *ethos*, *etho* (to adopt something, to get used to). It is also mentioned in the texts of Homer and Hesiod in which it is used to explain the way in which a living being has become accustomed to its environment. The word *morality* has its origins in the Latin language, from the word *mos/mores*, which has the same meaning as the word *ethos* (custom, behavior, rule). Ethics and morality have a very long history, and can be traced back to prehistoric times, that is, from the beginning of collective life when people in the community began to be interested in collective values. During history, some of the values have

passed into legal norms and legal framework, and others are still respected as morally correct values. Morality is the practice of correct behavior, that is, a set of basic rules or ways of behavior that a certain society considers acceptable and permissible. Ethics is the basis of all human relationships, and its role is to preserve the integrity and dignity of every human being, as a prerequisite for a good life. At the end of the 19th and the beginning of the 20th century, the question of ethics, moral philosophy, came to the forefront of questioning in all scientific disciplines and social spheres.

However, with more pronounced questioning of the culture of contemporary society, the issue of human action precisely in the context of looking at the creation of "good" or "evil" is the focus of very conflicting and conflicting scientific analyzes and interpretations. For example, medical ethics has been constantly discussed since Hippocrates, but the progress of biomedicine and the development of scientific technologies also caused the emergence of bioethics as a link between humanistic values and natural sciences. It is about the idea of how to live, what is considered acceptable or unacceptable, what deserves admiration and what is despised, what is good and what is bad. Ethics determines the understanding of human duties and obligations, in the context of relationships with oneself and others. Ethics and the ethical environment clearly direct emotional responses, create standards of human behavior, shape them and clearly influence them (Blacburn 2001: 12). Ethics as a set of rules defines good and bad behavior. It is actually a set of rights and duties related to: human virtues (honesty, empathy, solidarity, loyalty), the right to life and personality, the study and development of ethical behavior, the obligation of everyone not to commit bad acts...

Singer (2000: 350) believes that ethics is a theory of human relations, and "the orders of ethics are basically orders to do good to people, and perhaps to all beings who are able to feel". The question of ethics is related to all aspects of social action, so today we consider issues of business ethics, ethics of social networks and privacy protection, cyber-ethics, political ethics, the concept of media ethics, ethics of religion, education, bioethics, ecoethics, etc. Today, there is not a single area or segment of human life that is not linked to ethics. Recently, another discipline is particularly relevant in modern science, and that is eco-ethics, that is, the question of ethics and the protection of the human environment.

Ecological ethics or eco-ethics is a discipline that seeks to encourage discussions about the extension of man's moral obligation to animals, plants, certain areas of nature or life in general. Eco-ethics is formed by various theories arising from dissatisfaction with traditional ethics, which does not provide a satisfactory concept of the relationship between man and nature. Not only eco-ethics but also eco-history as an independent discipline is particularly interested in issues of environmental pollution, ozone reduction, global warming, in fact concern for everything that is a consequence of human activity and development. Man's careless attitude towards the environment, based on anthropocentrism for almost two centuries, has led to a series of global environmental problems, culminating in an environmental crisis. Population growth,

urbanization, industrialization, increase in the amount and types of waste and numerous other factors have led to air, water and soil pollution, influenced climate change, reduced snow and ice cover, soil impoverishment and other negative aspects in all parts of the ecosphere.

An important factor that contributes to the negative impact on the environment is the irresponsible attitude of man, i.e. the absence or insufficient level of environmental awareness. Therefore, it is very important to develop a sense of responsibility, which becomes a key concept of eco-ethics:

"All positions critical of anthropocentrism have in common that they appeal to a broader understanding of responsibility. It is not only about responsibility for people, including 'future generations' (H. Jonas), but about 'boundlessly expanded responsibility towards everything that lives' "(A. Schweizer) (Tićac, Marinović 2012: 56).

One of the authors who was among the first to speak about issues of environmental protection is the American politician and environmentalist Al Gore, who believes that it was our civilization that brought us to the brink of ecological disaster. The consequences of global changes in the ecosphere affect every individual, but every individual also affects the change of the global environment to a greater or lesser extent through their work, consumption, or lifestyle. The premise of establishing a balance with the ecological system is "establishing a balance within ourselves, between what we are and what we do" (Gore 1994: 40).

The increasingly intense interest in environmental issues in many academic disciplines led to the emergence of ecocriticism - an academic field within literary studies. With the development that ran parallel to the emergence of modern environmental politics, ecocriticism became the product of a historical and theoretical model that also includes the rising tide of ecological awareness in the XXI century. *Literary ecology* and/or *ecocriticism* is a scientific discipline that studies the ways in which relations between man and the environment are reflected in literary works. The growing number of ecocritical writings, this relatively young discipline, ranges from the cultural history of human ecological systems to the critical analysis of the meaning of concepts such as man and nature. Within this field, numerous sub-discourses have emerged that explore environmental justice, gender, ethics, different articulations of critical theory, the relationship between the natural sciences and the humanities, and multiple versions of activist politics.⁷

One of the first theorists of ecocriticism, Cheryll Glotfelty in her study *The Ecocriticism Reader*, noted that environmental critics investigate how nature and the natural world are imagined through literary texts, just as feminist criticism examines language and literature from a gendered

⁷ The development of ecocriticism takes place in three stages or three waves. In the first stage of ecocriticism, an effort was made to observe nature in such a way as to dehistoricize it, to seek a return to nature and to celebrate nature as it is, wild and spontaneous. The spread of ecocritical thought led to the emergence of the second stage, which directs human action within ecology, where we talk about the relationship to animals and plants and the ecological concepts of gender and race. The third stage of ecocriticism is expanding beyond the Anglo-American area where it was originally focused and pointing to the global problems we face.

perspective or as Marxist criticism in his reading of the texts he brings an awareness of the modes of production and economic class (1996: VIII). Glotfelty cites ecological criticism as the simplest definition of ecocriticism, *ecological criticism* - research into the relationship between literature and the physical environment (Glotfelty 1996: XVIII-XIX).

The creator of the term *ecocriticism* is William Rueckert, who initiated it in the article *Literature and Ecology: An Experiment in Ecocriticism* (1978). Ecocriticism in Rueckert's definition implies the application of ecology and ecological concepts in the study of literature - from which Cheryll Glotfelty distances herself, applying the term ecocriticism in a broader sense - to the research of the relationship between literature and the physical world, while also referring to synonymous terms - ecopoetics, environmental literary criticism, green cultural studies (Marjanić 2006: 165).

As we have already emphasized, ecocriticism is a movement born from the desire to improve the environment and the way people relate to the environment. This idea or movement was not realized through science, but through writing and literary work. Although a relatively new direction, ecocriticism develops in several waves, and different characteristics are united in the fact that man is in constant interaction with nature, and the consequences of their interaction are visible both for man and for the environment. Therefore, there are frequent examples of contemporary authors integrating thoughts about the importance of nature conservation into their works. They start from the assumption that the young reader will adopt eco-ethical ideas and have an affirmative opinion on nature conservation issues.

The fact is that the relationship between people and the environment has been depicted in literary works since ancient times. For decades, humanists have been studying environmental issues, approaching them using the tools of literary, historical, and cultural analysis. However, not only do these themes appear in literature for adults, but they are more and more common as themes in literary texts for children. Environmental literature for children and young people provides creative and imaginative narratives that can encourage young people to think about their own relationship with nature. Such literature most often thematizes contemporary environmental issues, but also provides insight into ecocatastrophe, global warming, anthropocentrism, sustainability and other important aspects of life on Earth.

2. LITERARY ECOLOGY AND CHILDREN'S LITERATURE

If we want children to flourish, we need to give them time to connect with nature and love the Earth before we ask them to save it. (Sobel 1999: 1)

Literary ecology was initiated by Joseph W. Meeker's study *The comedy of survival: studies in literary ecology* (1974), which offered a new (green) reading of literature from an ecological

perspective and tried to shed light on the link between literature and natural phenomena. Thus, Meeker's concept of literary ecology implies the research of biological themes and relationships that are manifested in literary works, as well as an attempt to discover the role of literature in the ecology of the human species. The above-mentioned paradigm is also defined by the phrase *green literature or literature of green culture* (Marjanić 2006: 165).

Although the formal practice of ecocriticism — sometimes called green studies — is considered a more recent addition to literary theory (second half of the 20th century), there has been a marked increase in writing about the environment and its importance in contemporary culture. Literary ecology or literature as cultural ecology, developed as a separate direction of ecocriticism, within German, English and American studies. Its creator is considered to be Hubert Zapf, a German cultural theorist. Zapf's cultural-ecological concept of literature is based on an analogy with a metaphorical ecosystem. Literature has an ecological power necessary for the development, creativity and self-renewal of culture, overcoming ideological and pragmatic simplifications, demarcations and sharp divisions. The ecological power of literature is manifested in the fact that literature connects what is in culture with dominant discourses and systems of power (politics, morality, economics, law, science, ideology, etc.) separated, instrumentalized or simplified and re-incorporates into culture what is from it has been eradicated, but it is crucial for its vitality and self-renewal (Čeh Steger 2021: 224).

Within the cultural-ecological function of literature, theorists (Zapf 2008; Čeh Steger 2021) distinguish three different functions that represent starting points for staging the relationship between culture and nature. The triple cultural-ecological model of literature consists of a critical function (cultural-critical metadiscourse). In it, simplified rigid images of the world and hierarchical dualisms are symbolically articulated and critically reflected upon. Then there is the anti-discursive function (imaginative anti-discourse) - in which what was excluded from the dominant systems and discourses or marginalized is put in the foreground. The last is the reintegrative function (reintegrative interdiscourse) - which leaves room for creative processes of integration of separate discourses, for analogies between life and aesthetic processes, for revitalization of processes and for re-establishment of the relationship between culture and nature. In the study *Kulturökologie und Literatur: Beiträge zu einem transdisziplinären Paradigma der Literaturwissenschaft (Cultural ecology and literature: Contributions to the transdisciplinary paradigm of literary studies)* Zapf (2008: 35-37) believes that literature has the power to overcome established boundaries between culture and nature, to open new views on the multiple nature of life and to renew cultural and literary creativity.

Children's literature, first of all in preschool education, but much more in school teaching, has always, and even today, gained importance precisely through ethical criticism, because its connection with the interpretation and analysis of literary works constantly and always includes (i) ethical components. It requires discussing the ethical, sociological and other preoccupations of the text, the role of literature in society, its (non)influence on the individual and current social

changes, etc. All this in no way conditions the importance and role of the aesthetic nature of literature, nor, specifically in schools, does it marginalize the aesthetic component of education (Pašić Kodrić, Pečenković 2020: 31-32).

Since childhood is a period in which the acquisition of knowledge and new experiences is a part of everyday life, it seems that literature is also a suitable medium for transmitting this knowledge. But it is quite wrong to see literature only as a means of imparting knowledge. On the contrary, understanding the pedagogical function of literature as primary is completely wrong. Contemporary science of literature tries to reconcile the aesthetic and ethical dimensions of the work. The history of literary theory abounds in efforts to define literature and the literary. Within these efforts, the importance of literature from a social and cultural aspect in terms of educational mediation, literature as a 'possible world' and a potential mirror of reality, i.e. a world suitable for the imaginary practice of empathy or at least a possible encounter with the Other, literature as a means of sharpening ethical judgments of readers, etc. (Peternai Andrić 2016: 90).

Literature starts from reality, the topics it talks about are based in reality, the problems it deals with are real, the questions raised in literary works are real, and the solutions that are put forward or contested are real. However, the attachment of literature to reality is not of such a nature that we could understand literary works as statements about real life or as preserved testimonies about the historical circumstances of their creation. (Solar 2005: 18)

Literature helps to stimulate ethical thinking, responsibility and compassion, enables insight into primary values and the development of non-cognitive abilities such as compassion and empathy (Pašić Kodrić, Pečenković 2020: 151). Empathy between the child and the natural world should be the main goal of children's environmental education because that time is characterized by a lack of differentiation between self and other. That cult of connection can be the emotional foundation for a quality ecological concept that everything on Earth is connected and that people are in an inseparable relationship with nature, animal and plant life, as well as everything that surrounds it.

Although awareness of environmental importance has intensified in the last few decades, it has been present in human life since the earliest days. In literature, the awareness of the importance of nature has been present since its beginnings, pastoral motifs in antiquity, then the romantic obsession with nature, the Victorian novel in which the problems of industrialization and pollution are present, and modern literature which is connected with science fiction and predictions of an apocalyptic future created by man's carelessness towards the environment . All of the above resulted in the development and establishment of ecocriticism as a modern methodology in the study of literature. Messages about the importance of preserving nature can be found in poetry one of the first South Slavic poets for children, Jovan Jovanović Zmaj, from the 19th century.

Wherever you find nice places, plant a tree there! And the tree is grateful so he will reward. It will reward abundantly shade, its fruit. It will reward either you either your brother. (Jovanović Zmaj 1988: 123)

Explicit messages about the preservation of the natural environment in Zmaj's poetry are burdened with a significant function of didacticism, which is understandable considering that children's literature in the 19th century was considered a means of educating the youngest generations. Contemporary authors strive to offer poetry that will have an aesthetic dimension as well as an ethical one. In the book *School Mischief* (2012), Amir Talić dedicated a cycle of poems to medicinal herbs (*Learning herbs*), trying to teach the youngest readers about the medicinal properties of certain plant species from an anthropocentric point of view. In that cycle there are also songs: *Chamomile and Pomegranate, Mint, Sage, Thyme, Mallow, Comfrey, Linden* and many others.

In Muhidin Šarić's book *Cvrkutanka* (2021), the author also titled a cycle *Flower* in which the lyrical subject sings about the beauty of flower species. It contains eight poems including: *Wheat, Rose, Cactus, Lilac, Rose...* In the poem *Wheat*, the importance of this plant is celebrated:

Her leaf are/ taut strings,/ the whole field it is a great harp/ on which the wind for people and birds/ it plays from the miles/ the song of abundance. Why is wheat a flower? Because it feeds the world. Šarić (2021: 28)

Through their poetry, both authors try to connect the world of childhood and the world of nature as places that are primordial, pure and innocent. It is precisely the infantile connection with the natural world, which diminishes during growing up, that writers for children try to connect more closely with maturation. Therefore, in his collection at the beginning of the Honey Song cycle, Talić will send more explicit messages to the readers: "The bee is the great secret of our planet. In the world of global pollution, it is the most endangered insect. If the pollution becomes critical and the bee disappears as it did in America, the fate of man is questionable and life on Earth in general. Let's protect nature." (Talić 2012: 67).

2.1. Eco Bunny by Mirsad Bećirbašić

In order to draw attention to the importance of nature conservation and environmental protection, writer Mirsad Bećirbašić published the picture book *Eco Bunny* in 2008. Bećirbašić incorporated the educational intonation of the story of Eco Bunny into children's responsibility for the environment and expressed it in a playful and imaginative way. Eco Bunny is a hero with whom children identify and in whom they recognize a friend whom they want to protect.

This hero cares for the well-being of all those who live in the forest: he shares with the oak tree the pain caused by the noise from the nearby road, he grieves with the dandelion when its fluttering crown is blown away by the breeze in an unknown direction, he shares joy with the quails when their chicks hatch. Out of joy, Eco Bunny began to sing songs. In order to be a better singer, Eco Bunny visited the meadow to get to know the meadow flower, because the song must be as true as possible. But there is no sign of flowers in the meadow. The meadow was littered with trash left behind by children playing. There was "waste of various kinds, from bags, cans, bottles". Eco Bunny was sad: "But here they played, here they breathed clean air, and this, like any other part of the planet, is their home - noted Bunny. - I have to draw their attention, I have to show them how sad and miserable I feel right now" (Bećirbašić 2008).

He called for the help of the windbreaker to move all the garbage that the children left on the meadow to the playground, so that they would see that garbage should not be left behind. Arriving at the playground, the children first had to clean up all the garbage and finally understood the message of the Bunny: "They understood the message that every part of the Earth is part of their home" (Bećirbašić 2008). The message that this author sent to all the little ones is that everything is more beautiful and fragrant in pure nature, and "that sfragnant, that soul, besides me, the rabbit, you and all the other children keep" (Bećirbašić 2008).

By direct address, the author tries to directly influence the consciousness of the youngest readers. This picture book assimilates all the necessary functions that a quality picture book for children should contain: the cognitive function enables the reader to develop and upgrade his knowledge about nature and its relationships, the experiential function offers the reader an insight into knowledge that the child has not encountered (e.g. a child from an urban environment has never experienced life in nature), the aesthetic function develops a sense of beauty and evokes emotions, and the fun function, which is also extremely important, because if the fun function is not present, the others will not be satisfied either. Because in a fun way, it is necessary to build a proper relationship with the book in children. And Bećirbašić managed to combine all the functions in the fluffy story of Eco Bunny: he tried to act on the children's emotions and direct them to the right path. Through picture books, the child learns to recognize different feelings in himself and others, and connects them with situations in the world of the text and situations from real life.

Perceiving ecological problems, integrating them into the world of literature can serve as a starting point for developing ecological awareness among readers. "Children between the ages of nine and thirteen, in matters concerning the environment (compared to personal and interpersonal matters), most believe that they can contribute to solving environmental problems by feeling their own responsibility. In older children, such a belief is, of course, weaker" (Dietmar 1996: 324).

Dealing with the issues of the relationship between children's literature and ecology, the Croatian theorist Karol Visinko (1999) believes that considering the age of the readers, there are three levels of inclusion of literary art in the process of environmental education. It is the lowest level that contains the least poetic or aesthetic. Cognitive types of picture books and various illustrated children's books can be included in this literature. At the next level are literary and artistic texts on ecological topics, which can be burdened with a significant dimension of didacticism and pedagogy. The emergence of literary art as one of the components of ecologically developed consciousness represents the highest level of the relationship between literature and ecology, which is especially realized in children's stories.

2. 2. Why This Monster Grows by Fuad Tabak

Fuad Tabak in the literature of Bosnia and Herzegovina confirmed himself as a playwright in the nineties of the XX century. Master of physics, Tabak translated the perception and understanding of nature and natural phenomena into artistic engagement by writing his radio and theater plays in which he talks about issues of environmental preservation. Physics explores the fundamental questions of nature and influences other sciences, art and philosophy, so Tabak's environmental activism will be the basis for creating artistic texts for the youngest. They can implicitly read messages about how important it is to respect nature and what are the consequences of our irresponsible behavior towards it.

The book of drama texts *Why This Monster Grows* (2017) contains three drama texts (*Fairy Tale about Fairy Tale, In the Land of the Terrible Censor and Magical Forest*) and three plays (*Blue Planet 1, 2, 3*). In them, the author through "existential-anthropological optics" shares the experience of his childhood and its symbolism "as the root of his own existence, as man's recapture of his own experience, as an extension of his spontaneous and free life as a 'space of freedom' that opens up new 'spaces of freedom' of their readers" (Fejzić 2017: 10). Two years later, Tabak will translate the dramatic and theatrical texts into fairy tale narratives and publish them in the collection Tales of a Magical Forest (Fairy tales and fables for children and adults).

The title story *Why This Monster Grows* metaphorically through fairy tales tells about the struggle of boys and characters from fairy tales: Little Red Riding Hood, Peter Pan, Captain Hook, Snow Queen and others with the help of forest animals and an the Oak, against the terrible

monster that threatens to destroy the beauty of nature in which plants and the animals live more and more closely, in a meadow full of dirt and waste:

"The old oak tree, which has been there, on the edge of the meadow, since who knows when, looked sadly at the papers and plastic bottles scattered around. There were also a few cans and even one shoe with a torn sole from which a mud-smeared sock was sticking out. It was not usual for oaks to be tired, and especially not for them to sleep during the day. But our oak tree was tired. Maybe he was tired of looking at all that mess and maybe that's why he felt the need to take a nap or at least to sink into a dream about some other times in which he enjoyed the beauties that surrounded him". (Tabak 2019: 73)

Through joint efforts and united against the danger that threatens everyone, the characters from the fairy tale save the forest from monstert, and Tabak introduces the youngest readers to the importance of recycling, processing, collecting and sorting waste.

The girl and boy from the Blue Planet story are also worried about the fact that the meadow where they were once happy was full of garbage: "The meadow was full of garbage. Papers, plastic and paper bags, some rags and even an old sweater"(Tabak 2019: 98). The idyll of a boy and a girl is joined by three elves: the first one in pearl-green clothes who takes care of living creatures that live in water... in rivers, seas, ponds; a tall elf in a dark green suit takes care of the living things that live on the surface of the earth and in the earth; and the third - in sky-blue clothes, who takes care of those who need air. The elves were sad and scared, because nature is changing - plants get sick, forests disappear, rivers are cloudy and the air is polluted. The boy ran across the meadow until he stepped on a broken glass bottle and cut his leg. The elves tried hard to find the plant that helps stop the bleeding that grew in abundance in the meadow, but it was gone. The boy had to go to the infirmary, and the elves worriedly remained in the meadow, determined to restore nature to its former glory.

In these fairy tales, the author explicitly warns and draws attention to the dangers of disrupting the ecosystem. Industrialization and the negligent attitude of man towards nature have a negative effect on the quality of the environment and human health. Man today is out of balance with nature, and it is necessary to restore a harmonious relationship with nature in order to improve the quality of human life. Therefore, Tabak appeals to the youngest readers, but also draws the attention of adults to the necessity of changing man's consciousness, his attitudes towards the world and future generations (Pašić Kodrić, Pečenković 2020: 200).

3. CONCLUSION

Today, the concept of ecology is one of the most important in political, social and cultural, educational and philosophical discourse. The discourse on the environment (or ecological

discourse) has intensified due to the growing threat of human-caused environmental disasters. In modern times, we witness the very frequent use of the prefix "eco", which indicates that the term with which it is used means natural, unspoiled, clean and healthy. Ecology propagates living in harmony with nature and raising awareness of the problems of environmental pollution, while eco-ethics seeks to arouse the responsibility of man towards nature and encourage the moral practice of doing good and conscientious behavior towards the environment.

Literature can thus serve as an initiator of change, whereby it is possible that from the earliest period, children from the margins act on their "adult" environment and dictate a new code of conduct. In addition to linguistic, cognitive, emotional and social goals, the environmental story is a means of spreading global knowledge as a way of life. Preservation of nature and landscape is not only an intangible value, but a prerequisite for a quality life for every individual. Whether they do it through a poetic text, through picture books or narrative types adapted to older age, the authors whose works we have analyzed in this paper, such as Fuad Tabak, Mirsad Bećirbašić, Muhidin Šarić or Amir Talić, strive to influence their readers by offering them, in addition to art, a part science and ethics that lead to a better and more beautiful life. Therefore, contemporary authors strive to integrate the awareness of nature conservation into their narratives and encourage the love of nature and the feeling of belonging as an inseparable part of nature in the youngest readers.

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FOREST MANAGEMENT









COMPARATIVE ANALYSIS OF THE AVAILABILITY OF FOREST WOOD PRODUCTS FOR THE NEEDS OF THE WOOD INDUSTRY IN THE AREA OF ZENICA - DOBOJ CANTON IN THE PERIOD 2017. – 2021. OPPORTUNITIES AND USE

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Key words: wood industry, wood products, coniferous and deciduous wood, logs, roundwood, pulp / firewood, Bosnia and Herzegovina Zenica - Doboj Canton

ABSTRACT:

The paper analyzes the availability of forest wood products for the needs of the wood industry in Zenica - Doboj Canton. This study seeks to examine the potential development of wood industry in the Zenica - Doboj Canton on the basis of the availability of forest resources, and it was analyzed the production of forest wood assortments of the only producer, the company JP ŠPD ZDK d.o.o. Zavidovići (forest user). The most important species of forest trees in this area are fir, spruce, white and black pine, beech, oak, and other deciduous trees. The largest amount of coniferous wood was produced in 2018 and amounted to 193,273 m^3 , and the smallest in 2020 when it amounted to 156,027 m³. The percentage share of coniferous logs (assortments for the wood industry) was the lowest in 2017 when it was 59%, and the highest in 2021 with a percentage share of 64%. The largest amount of deciduous wood was produced in 2017 and amounted to 170,841 m³, and the smallest in 2020 when it amounted to 126,259 m³. The percentage share of hardwood logs (assortments for the wood industry) was the lowest in 2017 when it was 35%, and the highest in 2018 with a percentage share of 40%. The largest amount of total wood mass was produced in 2017 when it amounted to $344,253 \text{ m}^3$, and the lowest in 2020 when it amounted to $282,286 \text{ m}^3$. The percentage of logs varied from 47-53%, technical roundwood from 6-8%, and pulp / firewood from 39-47%. The average demand for coniferous logs by the wood industry in the ZDK area varied from 46-57%, and deciduous from 45-61% (installed primary wood processing capacities are about 618,000 m³). Due to the significant

amount of cellulose wood and firewood produced, opportunities are opened for the use of forest biomass and the production of pellets, briquettes and other wood products.

1. INTRODUCTION

According to preliminary data from the Second National Forests Inventory on Large Areas in Bosnia and Herzegovina, forests and forest land cover 3,231,500 ha, which is about 60% of its total area. In the structure of forests and forest lands, high forests are represented on about 51% of the total forest area, coppice on about 39%, shrubs on 4%, and barren lands and clearings on about 6% (Lojo et al., 2011). Total area of forests and forest lands in Zenica – Doboj Canton cover 179,477.67 ha (59%). Of the total area, 69.12% belongs to high forests with natural regeneration, 15.22% for coppice forests, 9.18% for forest crops and plantations, and 6.48% for bare forests, shrubs and other unproductive areas. 21,946.40 ha of land is under mines. The average annual harvest volume is 434,000 m³ (gross), or about 362,000 m³ net wood mass (JP ŠPD ZDK, Forest Management Plans).

Forests provide multiple ecological, economic and social benefits, such as wood and non-wood forest products and ecological functions, necessary for the survival of mankind. The modern approach to forest management, with the use of the resources they provide us, implies the preservation of their biodiversity, and the preservation of the stability of the functions of forest ecosystems.

Due to the growing demand for wood and wood products globally, illegal loggings pose a significant threat as it contributes to the process of forest disappearance and degradation. This endangers biodiversity and disrupts the sustainable use of forests, including the survival of businesses that comply with relevant legislation. These facts strengthen the social awareness for a more rational use of natural resources.

The wood industry is of special strategic importance for Bosnia and Herzegovina. The wood industry is the only industry in BiH where exports exceed imports. This branch is very promising and employs a large number of people in BiH.

According to Delić et al. (2016) forestry and wood industry have an extremely long tradition in Bosnia and Herzegovina, and the number of employees in 1990 was about 110,000, or 13.6% of the total number of employees at that time. According to Šaković (1996), forestry and wood industry were the main carriers of economic development in 41% of municipalities in BiH, with a share in the gross product of municipalities over 50%.

According to Delić et al. (2011) the demand for forest wood assortments depends on the installed capacities for wood processing, the supply of forest wood assortments and technical - technological factors for wood processing. According to the strategy for development of the wood industry in the Federation of Bosnia and Herzegovina (2015) for the period 2016 - 2025 (Stjepanović et. al., 2018,), 115 sawmills for wood processing were registered, with installed

capacities of 618,000 m³ per year and number of employees of 695.

2. METHODOLOGY

The aim of this paper is to determine the analysis of forest wood products supply by quantity and quality for the period 2017-2021 (JP ŠPD ZDK). The results of the research can serve as information for the development of forestry and wood industry sector in Zenica - Doboj Canton area in the coming period. The key factors in the relations between forestry and wood industry in Zenica - Doboj Canton area are the company JP ŠPD ZDK d.o.o. Zavidovići as a user of forests and the main producer of forest wood assortments and companies of primary and final wood processing who are buyers of these wood assortments. Throughout the work, wood assortments are grouped into two categories: conifers and deciduous trees (according to the type of wood), and according to technical quality into three categories, logs, technical roundwood and cellulose / firewood. Data are presented through volume (m^3), and through percentage share.

3. RESULTS AND DISCUSSIONS

In table 1. it is shown the average annual harvest on a forest management basis (gross and net mass).

	8	(0	/		
Ord.	Forest management area	Area	Average annual harvest plan		
num.	Porest management area	(ha)	Gross mass	Net mass	
1.	Olovsko	32.371,38	119.202,2	100.335,0	
2.	Gornjebosansko	29.741,79	115.486,0	96.101,0	
3.	Kakanjsko	19.386,42	50.905,4	41.592,0	
4.	Krivajsko	83.134,09	126.368,0	105.614,0	
5.	NUU	14.843,99	22.233,4	18.532,0	
Total		179.477,67	434.194,9	362.174,0	

Table 1: Average annual harvest (gross and net mass)

As previously stated the average annual harvest volume is 434,000 m³ (gross), or about 362,000 m³ net wood mass. Installed wood processing capacities in Zenica - Doboj Canton area amount to 618,000 m³. In the graph 1. it is shown the net volume cuttings in the period of 2017. - 2021.



Graph 1. The net volume cuttings in the period of 2017 - 2021





Graph 2. Quantity of coniferous wood mass produced in the period 2017 - 2021 (m³)

The largest amount of coniferous wood was produced in 2018 and amounted to 193,273 m^3 , and the smallest in 2020 when it amounted to 156,027 m^3 .

In the graph 3 it is shown the percentage of coniferous wood mass produced in the period 2017-2021.



Graph 3. Percentage of coniferous wood mass produced in the period 2017-2021

The percentage share of coniferous logs (assortments for the wood industry) was the lowest in 2017 when it was 59%, and the highest in 2021 with a percentage share of 64%. Graph 4 shows the amount of produced deciduous wood in the period 2017-2021.



Graph 4. Quantity of deciduous wood mass produced in the period 2017 - 2021 (m³)

The largest amount of deciduous wood was produced in 2017 and amounted to 170.841 m^3 , and the smallest in 2020 when it amounted to 126.259 m^3 .

In the graph 5 it is shown the percentage of deciduous wood mass produced in the period 2017-2021.



Graph 5. Percentage of deciduous wood mass produced in the period 2017-2021

The percentage share of hardwood logs (assortments for the wood industry) was the lowest in 2017 when it was 35%, and the highest in 2018 with a percentage share of 40%.

Graph 6 shows the total amount of produced wood (conifers and deciduous) in the period 2017-2021.



Graph 6. Quantity of total amount of produced wood (conifers and deciduous) in the period 2017-2021

The largest amount of total wood mass was produced in 2017 when it amounted to $344,253 \text{ m}^3$, and the lowest in 2020 when it amounted to $282,286 \text{ m}^3$.

In the graph 7 it is shown the percentage of total wood mass (conifers and deciduous) produced in the period 2017-2021.

Bihać, 09 - 10 June 2022.



Graph 7. Percentage of total wood mass (conifers and deciduous) produced in the period 2017-2020

The percentage of logs varied from 47-53%, technical roundwood from 6-8%, and cellulose / firewood from 39-47%.

Installed capacity of wood industry in the Zenica - Doboj Canton amounts to $340,000 \text{ m}^3$ of conifers and deciduous for $278,000 \text{ m}^3$. Graphs 8 and 9 show the average demand of the wood industry for coniferous and deciduous wood in Zenica - Doboj Canton area.



Graph 8. Supply / installed capacities for coniferous wood



Graph 9. Supply / installed capacities for deciduous wood

The average supply for installed capacities for coniferous logs by the wood industry from the area of Zenica - Doboj Canton varied from 46-57%, and hardwood from 45-61% (JP ŠPD ZDK).

4. CONCLUSIONS AND RECOMMENDATIONS/IMPLICATIONS

The most important species of forest trees in Zenica – Doboj Canton area are fir, spruce, white and black pine, beech and oak. The most important conclusions can be summarized as follows:

- Timber industry and forestry in Zenica Doboj Canton are promising industries with a significant share in gross domestic product.
- The largest amount of coniferous wood was produced in 2018 and amounted to 193,273 m³, and the smallest in 2020 when it amounted to 156,027 m³. The percentage share of coniferous logs (assortments for the wood industry) was the lowest in 2017 when it was 59%, and the highest in 2021 with a percentage share of 64%.
- The largest amount of deciduous wood was produced in 2017 and amounted to 170,841 m³, and the smallest in 2020 when it amounted to 126,259 m³. The percentage share of hardwood logs (assortments for the wood industry) was the lowest in 2017 when it was 35%, and the highest in 2018 with a percentage share of 40%.
- The largest amount of total wood mass was produced in 2017 when it amounted to 344,253 m³, and the lowest in 2020 when it amounted to 282,286 m³. The percentage of logs varied from 47-53%, technical roundwood from 6-8%, and cellulose / firewood from 39-47%.
- The average demand for coniferous logs by the wood industry in the ZDK area varied from 46-57%, and deciduous from 45-61%.

- Due to the significant amount of wood pulp and firewood produced, opportunities are opened for the use of forest biomass and the production of pellets, briquettes and other wood products.
- In order to improve the situation in forestry and wood industry, it is necessary to develop precise development strategies for these two closely related industries. It is necessary to improve the mutual relations of these two sectors by defining the quantity and structure of production of forest wood assortments, modernization of equipment for production and processing of wood, and professional training of staff in these two economic industries.

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FOREST MANAGEMENT IN REDUCING CARBON DIOXIDE EMISSIONS

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ABSTRACT:

Climate change, as a consequence of (un)rational anthropogenic activities, has started a "war" against the whole world without a shot being fired. The inevitable growing trend of CO_2 concentrations in the atmosphere, taking into account the fact that forest ecosystems are a natural pools of large amounts of carbon dioxide both above and under ground, with the possibility of increased storage capacity, led to emphasizing the importance of forests in the context of climate change mitigation and adaptation. Aim of this paper is to describe the role of global forests in reducing atmospheric carbon, with emphasis on forest management practices in order to preserve current carbon stocks within forest ecosystems, increase carbon storage, as well as further carbon inclusion in the environmental policies, with a slight look at the concept of carbon neutrality.

1. INTRODUCTION

The definition says that man are living beings who have a developed brain capable of abstract thinking, speech, empathy, problem solving and many other characteristics. Yet, to what extent would man be all that he is without the environment and/or natural resources? By environment we mean everything that surrounds us including living environment, flora and fauna, as well as man, which are in constant correlation connected by numerous joint actions. All the inhabitants of the natural environment have adapted to it and use it in a way to provide themselves with habitat, food and everything else necessary for any living organism to survive. On the other hand, man, as the most developed and most rational being went a step further and found a way to put the environment, together with all of its segments under his control in order to fulfill all more

or less necessary tendencies and desires of mankind, which is not so surprising since man itself is a being of need and he directs all his activities, whether conscious or not precisely towards fulfillment of those needs. Progress of mankind in scientific and technological terms at the expense of natural resources has certainly brought many benefits that have changed the world for the better, making people's lives easier by giving us the opportunity for a better and more comfortable life where almost everything is available. Still, everything comes at a price.

Enjoying uncontrolled progress at the expense of natural resources until the second half of the last century no account was taken of the fact that natural systems are not inexhaustible sources of matter and energy and that emissions from numerous human activities can have an extremely adverse impact on the environment, as well as long lasting effects on the natural system of our planet. Every day we witness climate change, lack of drinking water, endangerment of ecosystems and biodiversity, industrialization, excessive use of fossil fuels, deforestation, urbanization, population migration, earthquakes and many other negative impacts. Plants and plant ecosystems largely depend on climatic conditions and any change in the ecosystem directly affects the vegetation. Proof of this is visibility of climate change impacts and climate variations on forest ecosystems around the world. On the other hand, forests are very powerful tool in fight against climate change, given the many opportunities for sinking of large carbon amounts within forest ecosystems. Today, the world is unfortunately in a situation of ecological deficit, where the population's needs for natural resources exceed its supply, which can lead to a serious problem of depletion of natural resources, as well as increasing CO₂ emissions into the atmosphere. Carbon dioxide is a key greenhouse gas, and therefore its concentration in the atmosphere is crucial in shaping the global climate. Coping with climate change will require changes and adjustments to forest management strategies and delaying them will increase costs as well as difficulties in implementing those cahanges. What certainly goes in favor of the situation is that forests are sources, but also reservoirs of carbon dioxide which makes them scientists best friend in fight against climate change.

2. THE ROLE OF FORESTS IN REDUCING CO₂ EMISSIONS INTO THE ATMOSPHERE

Forests represent crucial global communities. They cover approximately 30% of world's land are, which includes most of terrestrial biodiversity. They represent carbon pools by sequestrating carbon, play a key role in regulating climate and water regimes and have many other ecosystem roles and services of key importance (Morin, X., et al., 2018). Carbon dioxide (CO₂) is crucial greenhouse gas and all changes in carbon cycle at global level that affect atmospheric concentration of CO_2 are inevitable factor in climate formation. Forests play an important role as sources but also as carbon dioxide reservoirs. A source of greenhouse gases is considered to be any process or activity that releases greenhouse gases into the atmosphere. Deforestation, as well

as forest degradation are one of the largest sources of greenhouse gases. That way, carbon that has been "trapped" in forests in the form of CO_2 is released into the atmosphere, along with other greenhouse gases such as methane. According to estimates, 17% of global greenhouse gas emissions are caused by deforestation and forest degradation (FAO Forestry paper 172, 2013). On the other hand, a carbon tank is considered to be any reservoir that stores or "captures" carbon from the atmosphere in form of CO_2 . During growth, forests act as carbon dioxide reservoirs and are responsible for removing large amounts of CO_2 from the atmosphere. Forest vegetation, together with forest soil stores about half of the earth's carbon, with possibility of potentially greater storage than the current one. Forests absorb CO_2 through process of photosynthesis, store it as carbon, and release it through processes of respiration, decomposition or combustion. Forest's ability to store carbon depends on the rate of forest growth, as well as forest's ability to retain as much carbon as possible. Vigorous young forests, which are in phase of growth and development, absorb large amounts of carbon quite quickly. Older forests and forest vegetation usually already contain certain amounts of carbon stored in their stocks and these stocks are very slowly, if at all replenished (FAO Forestry paper 172, 2013).

Trees absorbe and stores carbon dioxide (CO_2) from atmosphere through a series of processes. Absorbed carbon dioxide is converted into sugars by process of photosynthesis and sugar containing carbon become cellulose, hemicellulose and lignin. In other words said, carbon converted into other substances is basic building block for creation of new cells and compounds that together make up the wood mass, ie wood. By using fossil fuels man are releasing about 6.5 billion tons of CO₂ into the atmosphere every year and by cutting down forests outside the principles of forestry science and profession, about additional 1.5 billion tons (Appenzeller 2004). Combustion of fossil fuels releases large amounts of the most important greenhouse gas carbon dioxide, which appears in the atmosphere today in much larger quantities compared to the period before the 18th century, that is, before life filled with needs imposed by industrial way of life. During its life cycle manicured forest retains large amounts of carbon dioxide and thus mitigates the adverse effects of the greenhouse effect. If the net annual growth of forest exceeds the amount of removed wood mass, ie if we have a positive trend of annual increase in wood stocks, the amount of carbon stored increases as well. Carbon of the aboveground phytonutrients is retained both in living and dead forms of trees and in addition there is a volume of carbon stored in roots and soil. Also, after felling large part of carbon inside trees remains locked in form of long-lived forest products, which increases the incorporation of carbon in built environment.

The amount of total carbon stored in terrestrial ecosystems is approximately 3170 GT (gigatons), of which almost 80% is found in soil (Ontl, T.A., et al. 2012), making soil the second largest carbon pool after the ocean. It is estimated that CO_2 uptake through the process of photosynthesis by terrestrial vegetation results in soil storage rate of about 3 GT carbon per year (ONTL, T.A.,

et al. 2012). The biomass of wood created in process of photosynthesis, in the absolute dry state, contains about 50% of carbon bound from CO_2 , ie one half of dry weight of wood is carbon. For example, pedunculate oak per 1 m³ of produced wood (raw biomass) binds 285 kg of carbon, which for pedunculate oak forests averages 2 to 3 tons per hectare. A similar amount is obtained for beech, while for species such as willow, poplar, fir and spruce it is about 40% lower, while for pine it is up to 30% lower. Carbon binding depends more on habitat quality than on tree species. Low-density tree species grow faster so for the same habitat quality on average equal amounts of bound carbon are obtained. However, the differences in carbon sequestration within stands of different ages are large. If we observe developmental stages of forest, largest amounts of growth lasts (Prpić, B., 2004). The amount of stored, as well as the amount of released carbon varies significantly depending on the type of forest. However, some generalizations are possible due to the relative similarities of individual forests within specific biomes such as e.g. tropical, temperate and boreal forests.

Most tropical forests are important carbon stores, as they typically retain large amounts of carbon, averaging about 110 tonnes per hectare. Somewhere around half of the amount of carbon located in tropical rainforests is stored in vegetation, which is a fairly high percentage and much higher than in any other biome. Residual carbon is found in tropical forest soils, which have a modest carbon content (compared to other biomes), due to rapid decomposition of dead biomass caused by hot and humid conditions, as well as rapid rinsing of minerals from the soil (Gorte, R. W. 2007).

Forests within temperate zone are characterized by great variety of species, including hardwood species such as oak, maple and beech, as well as softwood species such as pine or Douglas fir, as well as many other species. However, whether we look at any type of temperate forests individually, or look at them as a whole, temperate forests have much less species diversity than tropical forests. Therefore, they do not have the ability to retain the same amount of carbon as tropical forests, making that percentage much lower. Average temperate forest has a carbon storage capacity of an average 70 tonnes per hectare. More than one third is stored in vegetation, while remaining two thirds are stored in soil. Higher proportions (but smaller amounts) of carbon in the soils of temperate forests compared to tropical forests are due to slower process of decomposition of matter. Many forests within the temperate zone are managed with aim of producing commercial wood and wood products. This way of management can have a great impact on carbon storage (Gorte, R. W. 2007).

In terms of carbon, boreal forests contain higher amounts of this element compared to temperate but also tropical forests, averaging more than 180 tons per hectare. Less than one-sixth is located in vegetation, while the other 84% is located in soils of boral forests, which is almost three times

higher than in temperate and tropical forests, as well as higher in general compared to any other biome except wetlands. Carbon accumulates in large quantities in soils of borrelian forests, primarily due to the extremely slow process of decomposition of matter caused by short summers, as well as high acidity of coniferous soils, which inhibit decomposition processes. The high carbon content of boreal forest soils is of great importance for the carbon cycle (Gorte, R. W. 2007), therefore it is necessary to think twice before starting any activities that could disrupt the soils of boreal forests, which would lead to carbon release. In general, Europe's manicured forests all their life span between natural rejuvenation and care from young to mature forests, are in the stage of optimal biomass production, so their impact on climate change mitigation is fully proportional to what is expected of forest in terms of carbon sequestration.

3. CARBON NEUTRALITY

The world's forests store enormous amounts of carbon. According to FAO estimates (2006), forests store 283 gigatons (Gt) of carbon in their biomass alone, which, with the addition of carbon stored in soil, dead wood and debris, makes more than atmospheric carbon (FAO Forestry paper 152, 2012). However, lack of balance between release of carbon into the atmosphere by anthropogenic combustion of fossil fuels and deforestation, along with carbon uptake by terrestrial and ocean systems, has nevertheless led to an increase in CO_2 pressure in the atmosphere. Carbon neutrality as a last-minute salvation plan presents a model of actions that strikes a balance between the amount of CO2 released and the amount of CO2 removed, encouraing net zero carbon dioxide emissions. The concept is conceived as a continuation, ie the next step after the Paris Agreement and the movement currently, according to the National Public Utilities Council, has 137 countries in the race for carbon neutrality. Although all 137 countries have not yet set a precise targeted year for their neutrality policies, most have focused on 2050., or 2060. In addition to some countries indecisivness, others already have a clear plan and vision. An example of this is the European Union whose high goals include achieving the title of the first continent to eliminate as many emissions as it produces by 2050., followed by a step further, which implies removing more CO₂ emissions from the atmosphere than is emitted. One way to achieve this is by compensating emissions produced in sectors. Emissions from one sector can be compensated by reducing emissions in another sector through use of renewable energy sources, energy efficiency, as well as use of low-carbon technologies. Although well thought out in theory, in practice it will surely be more difficult to achieve.

By 2020., average global concentration of CO_2 in the atmosphere reached a record 415ppm (parts per million), which is a large increase from pre-industrial levels in the 1850s, when it was 285 ppm (Chen, M., J., 2021). Numbers, as well as consequences in forms of drought, floods, extinction, new insects and diseases, loss of biodiversity, rising ocean levels and many others are large, but so is the task that will require a lot of effort, work and change when it comes to

countries that have committed themselves to this crucial undertaking for the survival of mankind. With all this said, it is more than clear that climate change and its consequences are our harsh reality, which could cost us the life style we are used to, if not life at all, if the set measures and goals do not turn from paper to reality. Ironically, fossil fuels that the Earth has formed in its interior for millions of years, and whose mass use is one of the main reasons why the world is currently in the situation it is in, have become one of the factors why the carbon neutrality model must become a reality. Anthropogenic actions have depleted almost half of fossil fuel resources, and following current rates of use and exploitation, the quantities needed to meet the needs of mankind, according to estimates by the BP Statistical Review of World Energy (2016), oil and natural gas may last approx. 50 years more, and coal for approx. 100. So, if compassion for ourselves does not force us to change our habits, energy crisis certainly will. Although there is enough renewable energy to meet the energy needs of the human population, such as energy in the form of biomass, geothermal energy, water and solar energy, tides and other similar sources, they all entail questionable accessibility to larger masses of people, given high prices and complicated procedures for establishing such energy systems. Salvation remains in hope of understanding the seriousness of the situation and observing fossil fuels, not through the market price, but in the form of an assessment of the potential damage caused by carbon dioxide emissions, which is much higher.

The most important component when planning activities to achieve carbon neutrality is certainly the reduction of carbon dioxide emissions. Carbon dioxide emissions can be reduced in several ways, some of which are: replacing fossil fuels with renewable energy sources (carbon free energy) such as e.g. nuclear energy or hydropower, industrial capture, removal, storage and use of carbon, recycling of solid waste, ie reuse of the same, reduction of energy consumption, as well as increased efficiency of energy used (Chen, M., J., 2021).

Even though all of these solutions are effective, establishment and management of forests in a way to improve the removal and storage of carbon from the atmosphere have been identified as major ways and opportunities to mitigate increase in anthropogenic greenhouse gas emissions and reduce global warming rates. In support of all this is the fact that the importance of forests at the global level in terms of reducing greenhouse effect is indicated by many more or less binding European documents. They all share a common position clearly expressed in Article 21 of the 2003. Ministerial Conference on the Protection of Forests in Europe, which states "Take concrete measures to contribute to the overall reduction of greenhouse gas concentrations in the atmosphere and promote sustainable forest management in accordance with with the United Nations Framework Convention on Climate Change and the Kyoto Protocol."

Keeping global warming below 1.5° to avoid the effects of climate change requires removal of huge amounts of CO₂ from the atmosphere, together with drastic measures to reduce current and

future emissions. The Intergovernmental Panel on Climate Change (IPCC) estimates that about 730 billion tonnes of CO₂ (730 pentagrams of CO₂) must be removed from the atmosphere by the end of the century (IPCC, 2018). This figure is equivalent to the total CO₂ emitted by the United States, the United Kingdom, Germany and China since the beginning of the Industrial Revolution. No one knows how to capture so much of CO₂, but one thing is for sure, forests will have to play very important, if not crucial role in all this. Locking carbon into ecosystems has been proven to be effective, safe and economically viable. In addition, increasing forest covered areas brings with it other benefits, souch as biodiversity protection, water regimes management, all the way to creation of new jobs. The IPCC estimates that increasing the total area of the world's forests, forest lands and woody savannas could store about a quarter of atmospheric carbon needed to limit global warming. Numerically, that would mean increasing forest area by 24 million hectares every year until the end of 2030.

4. FOREST MANAGEMENT IN RETURNING BALANCE

Forestry, in addition to the fact that with its activities, ie nurturing forest ecosystems creates CO_2 storage tanks, there is also a contribution in the form of possibility of using biomass-based fuels that could replace petroleum products and coal. Need to reduce deforestation and intensity of felling, is certainly imposed, as well as need to establish new ones, while managing existing forests with an emphasis on maximum carbon assimilation. Natural forest regeneration is the cheapest and technically simplest option.

In addition to the aforementioned activities of establishing new forests and "carbon" plantations, agricultural and forestry systems, urban forests, together with the full range of management and cultivation options represent a chance for even greater carbon sequestration with appropriate measures to restore degraded forests, defining different breeding and thinning systems, favoring species that have a greater ability to asimilate carbon, fertilization, fire protection, and many others. Immature forests across Europe, North America and East Asia serve as carbon "reservoirs" and must be under careful human intervention because half of the biomass they accumulate is carbon (Schoene, D. and Netto, M., 2005).

In fight against climate change, the most optimal areas for planting trees are tropical and subtropical regions. Trees in these areas grow quickly, growth involves carbon absorption and sequestration and land is relatively cheap and accessible. Well-managed forests not only help cool the planet, but also alleviate poverty in low-income regions, conserve biodiversity, and support the United Nations Sustainable Development Goals, especially goals: 1 - No poverty, 6 - Clean water and sanitation, 11 - Sustainable cities and communities, 13 - climate action and 15-life on land. Forest management practices in order to combat carbon increasing in the atmosphere, without violating the basic objectives of forest management such as: providing

sufficient quantities of wood and non-wood forest products, preserving forest vegetation and soil, improving water and air regimes, using forests for spiritual, recreational, and many other purposes, all within the framework of sustainable development, can be roughly grouped into 3 categories (Brown, et al., 1996):

- Management of pre stored carbon
- Managing storage of new amounts of carbon
- Encouraging the use of wood based products

4.1. Management of pre – stored carbon

Forests have many functions. These functions are reflected through wood or non-wood forest products, all the way to importance of forests for human health by regulating air and water regimes, recreation, religious and spiritual activities. One of the main functions is production, which enables use and exploitation of forest products, but also the function of carbon sequestration and storage, which is often neglected compared to the production role, which can be attributed to intangibility, ie inability to phisicylly see apopted carbon, compared to other forestry products such as e.g. logs. However, as stated in Chapter 3, world's forests, according to FAO estimates (2006), store 283 gigatons (Gt) of carbon in their biomass alone, which, with the addition of carbon stored in soil, dead wood and debris, makes more than atmospheric carbon (FAO Forestry paper 152, 2012). In order to preserve stored carbon, it is necessary to implement a number of measures, primarily related to stopping forest deforestation and degradation in order to increase agricultural land. If we take into account growing trend of the human population, we encounter a new problem that indicates, above all, the imbalance in the division of land necessary for the life of all of us. From 1 billion of world's population in 1800. by 2020., that number has risen to 7.9 billion, and with UN forecasts by 2050. that number will rise to almost 10 billion, which means an increase in agricultural land, at the expense of forested areas, to meet the needs of the population, primarily in the form of food products. Today, the world's forests cover 31% of the world's land area, while 37.6%, according to FAO estimates, are classified as agricultural land. In addition to deforestation for purpose of forming agricultural areas, high expectations from forest ecosystems when it comes to need for wood and wood products for socio-economic development creates additional pressures on forests, as well as stored carbon within them, making them vulnerable. In order to avoid such situations, one of the ways to preserve the amount of carbon stored within existing ecosystems is to expand protected forest areas, where in addition to protecting biodiversity, emphasis would be placed on protecting those forests that sink large amounts of carbon. Therefore, carbon would be safe in such forests. Forcing policies and programs to reduce deforestation globally, as well as maximizing agricultural production in current areas, would certainly make a major contribution to conserving stored carbon stocks.

4.2. Managing storage of new amounts of carbon

Aboveground phytonutrient carbon is retained in both living and dead tree forms. In addition, there are volumes of carbon stored in the roots and soil. After felling, a large part of the carbon inside the trees remains locked in the form of long-lived forest products, which increases the incorporation of carbon in the built environment. Storage of new amounts of carbon means an increase in carbon sinks in the aboveground and underground parts of vegetation, soil, as well as long-lived wood products. Increasing the productivity of forest stands, changes in the way forests are managed, as well as aphorestation, would increase the possibility of sinking larger amounts of carbon. One of the following ways is to establish plantations on non-forested land. In that case it is important to emphasize that this type of solution in form of plantation is not a permanent solution when it comes to solving the problem of storaging new amounts of carbon. The reason for this is the fact that plantations, unlike natural forests are much poorer in terms of carbon storage due to regular renewals, which releases stored carbon back into the atmosphere every 10-20 years, while natural forests accumulate carbon for many decades. However, this type of plantation, even with small contribution to carbon storage, is certainly a support to the domestic economy.

4.3. Encouraging the use of wood – based products

Encouraging greater use of wood aims to increase the incorporation of carbon in the built environment, or in other words said, transfer and installation of wood-based materials together with the carbon stored inside it, instead of using fossil fuel-based materials. The replacement approach is suitable for several reasons, one of which is the long-term "locking" of carbon over a period of tens or even hundreds of years, as well as reducing fossil fuel consumption due to replacement with wood products or materials, which makes this approach not only based on increasing carbon sinking, but also the use of carbon in the form of various products. Forcing greater use of wood-based products and materials offers multiple ways to reduce carbon and can replace the use of fossil fuels up to several times. E.g. wood obtained from a stand of the first generation after afforestation can first be used for production of construction materials due to needs of construction industry in replacing one of the previously used products. Then, when construction materials life cycle ends, wood biomass can be converted into bio - energy, ie bio fuel, which will replace fossil fuels for the second time. At the end of the first rotation period, the cycle can be repeated and in successive management cycles each substitution will result in a net carbon mitigation effect (Linder, M., et al. 2007). This approach will certainly largely depend on the market prices of fossil fuels, as well as the price of the wood to be exploited.

5. CONCLUSION

If we go back chronologically a little over a century ago, even then we can find traces of concern in the research of scientists related to carbon dioxide emissions and global warming. However, beginning of understanding the seriousness of the situation took place in the 1990s, after certain reactions from the IPCC, the Kyoto Protocol and later the Paris Agreement. Unfortunately, all of the above, looking at today's situation in the world, has not borne much fruit and has not left many options when it comes to finding a solution to current situation. The best solution is to reduce the amount of atmospheric carbon to amounts close to those of the pre-industrial period, which would require more than the concept of carbon neutrality. Therefore, it is no longer enough to just balance emissions and removals, but it is necessary to have higher removals than emissions. The biggest problem in releasing harmful emissions is use of fossil fuels, whose cessation of use and transition to renewable energy sources are key steps to achieve carbon neutrality, which would be the greatest step of humanity towards restoring its natural balance.

Global warming together with climate change in our time are our reality as well as a threat to the existence of life on planet Earth. As a result there is a growing awareness in society about the urgency of developing environmental policies which should result by climate change mitigation. However, the development of policies, laws, and other written acts is often ahead of the ability of researcher and researchers to provide an integrated, concrete solution to alleviate such complex problem. In addition, the situation is further aggravated by the need to balance trade-offs between sustainable management, economic viability, land value, availability of resources, employment opportunities, and other socio-economic factors.

Forests represent communities of critical importance globally covering approx. 30% of the world's land area, which includes most of the world's biodiversity. They represent carbon rezervoares, play a key role in regulating climate and water regimes and have many other key roles and services of ecosystems. As such, they are humanity's greatest asset in combating climate change. For forestry sector, tackling climate change will require changes and adjustments to forest management strategies and delaying them will increase costs as well as difficulties in implementing those changes. Wide range of opportunities along with potential solutions to improve current situation offered by forest ecosystems will require improving forest's ability to absorb and store carbon, as well as encouraging greater use of wood through installation in built environment. Researches, new methods, systems for monitoring maximum potentials and forest abilities, as well as its limitations, limiting the need for agricultural land, economic methods of valuation forests who conserve and store carbon, changes and adjustments of forest management strategies, maximized use of species with greater possibility of carbon assimilation, taking into account questionability of plantation production and promotion of wood products in order to replace some other, less environmentally friendly products are some of the forestry segments

whose progress can mean a big step for humanity when it comes to reducing carbon dioxide emissions.

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LEGAL AND ECONOMIC REGULATIONS IN ENVIRONMENTAL PROTECTION









ASSESSMENT OF KRUŠNICA RIVER WATER QUALITY IN THE SPRING-SUMMER SEASON BASED ON PHYSICAL AND CHEMICAL CHARACTERISTICS OF WATER

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ABSTRACT:

Monitoring the aquatic ecosystem is critical since it allows us to obtain a set of data that indicates the ecosystem's state. The analysis of physico-chemical parameters, which are decisive factors, is part of the water quality assessment process. The aim of this study was to determine the state of water quality from the Krušnica River in the spring-summer season based on the analysis of physico-chemical parameters. For the needs of the analysis of physical and chemical parameters, water samples were taken from the upper, middle and lower course of the river Krušnica. Temperature, pH, oxygen concentration, oxygen saturation, chemical oxygen demand, electrolytic conductivity, biological oxygen demand, suspended solids, chlorides, sulfates, ammonia and nitrites were among the physico-chemical parameters analyzed. Based on the results of physico-chemical parameters of water from the Krušnica River and on the basis of the Decision on the characterization of surface and groundwater, reference conditions and parameters for water assessment and water monitoring (2014), the analyzed parameters indicate a high or good ecological status, which indicates the absence of anthropogenic influence or very little influence of anthropogenic action, where these conditions do not have a negative impact on the biological world.

1. INTRODUCTION

The term water quality means water whose components are at an optimal level and allow unhindered growth of plants and animals [1]. Many authors [2,3,4] draw attention to the importance of aquatic biotope monitoring, based on which we can arrive at a series of data indicating the state of the ecosystem. The productivity of aquatic organisms depends on the physico-chemical characteristics of water, as well as on the sufficient amount of nutrients necessary for the growth of living organisms [5]. Climate change as well as anthropogenic activities lead to major changes in the hydrological cycle and deterioration of water quality, which is a key issue for the sustainable development of human beings [6]. Seasonal variations, and natural processes such as temperature and rainfall can significantly affect water quality [7]. Monitoring the physicochemical parameters of water plays a key role in the assessment of the aquatic ecosystem [8]. The aim of this study was to assess the quality of water from the Krušnica River based on the analysis of physico-chemical parameters in the spring-autumn season.

2. MATERIALS AND METHODS

2.1 Lokality

The Krušnica River is located in Bosanska Krupa and is a right tributary of the Una River. It springs near the village of Gudavac at an altitude of 200 m and is 6.5 m long [9]. For the physico-chemical analysis of water on the longitudinal profile of the river Krušnica, three localities were selected: upper course / spring - Vranjska (N 44 $^{\circ}$ 51'29.95" E 16 $^{\circ}$ 10'11.40"), middle course - Zalug, lower course / estuary - Pazadžik (44 $^{\circ}$ 53'40.6 " N 16 $^{\circ}$ 9'34.4 " E) (Figure 1).



Figure 1 – Lokalitet istraživanja

2.2. Water sampling

Water samples from the Krušnica River for the purposes of this research were taken in accordance with the regulations on water sampling at the three mentioned localities. About 1000 ml of water from the Krušnica River was taken from all three localities into plastic bottles at a depth of 20-30 cm.

2.3. Physico-chemical parameters

Physico-chemical analysis of water from the river Krušnica included the analysis of 12 parameters, of which the values of water temperature, pH, electrical conductivity, oxygen concentration were immediately determined in the field, while other parameters were determined in the laboratories of the Biotechnical Faculty.

Parameter	Measuring unit	Analytical methods					
Temperature	°C	Temperature probe, <i>in situ</i>					
pH-value	-	Electrochemical in situ					
O_2 concentration	mg/l	Electrochemical in situ					
Electrical conductivity	μS/cm	Conductomentric in situ					
Total suspended solids	(mg/l)	Gravimetric, filtering through a filter, pore diameter 0.45 µm					
Oxygen saturation	%	Calculation method					
BOD ₅	(mg O ₂ /l)	Five-day biochemical oxygen demand at 20 oC					
KMnO4 demand	(mg O ₂ /l)	Vol. Kubel-Tiemann					
Chlorides - Cl	(mg/l)	Ion chromatography					
Nitrates- NO ₂	(mg/l)	Ion chromatography					
Ammonia – NH_4^+	(mg/l)	Spectrophotometric					
Sulfates	(mg/l)	Ion chromatography					

Table 1. Parameters determined according to standard methods [10].

3. RESULTS AND DISCUSSION

 Table 2. The results of physical and chemical parameters from the Krušnica River from all three localities in the spring and autumn seasons.

Danamatan	Measuring	Spring		Midstream		Estuary	
rarameter	unit	spring	autumn	spring	autumn	spring	autumn
Temperature	°C	9,7	10,0	9,8	10,0	10,5	10,2
pH value	-	7,74	7,60	7,81	7,3	8,0	7,8
O ₂ concentration	mg/l	10,6	9,4	10,5	8,6	11,0	7,8
Electrical conductivity	μS/cm	451	418	449	432	446	436
Total suspended solids	(mg/l)	0,05	0,027	0,02	0,028	0,01	0,15
Oxygen saturation	%	93,56	83,2	92,67	76,2	98,2	69,3
BOD ₅	$(mg O_2/l)$	1,32	0,94	2,33	1,32	2,59	1,45
KMnO4 demand	$(mg O_2/l)$	5,0	6,95	5,0	7,58	4,2	9,16
Chlorides - Cl	(mg/l)	21,4	17,83	21,4	17,83	17,83	21,4
Nitrates- NO ₂	(mg/l)	0,0003	0,00126	0,00037	0,00128	0,00041	0,00086
Ammonia– NH ₄ ⁺	(mg/l)	0,08	0,09	0,14	0,11	0,12	0,11
Sulfates	(mg/l)	11,58	4,22	10,62	4,86	15,7	4,86

The recorded value of temperature from all three localities in the Krušnica River ranged from 9.7 to 10.5 0 C, which corresponds to the climate of the area. The lowest value was measured in the spring at the source of the Krušnica River (9.7 0 C), while the highest temperature values were

recorded at the estuary of the river in the spring (10.5 0 C). Water temperature as one of the key factors, controls metabolic and reproductive activities as well as life cycles [11]. In the case of an increase or decrease in temperature, and large fluctuations, the metabolic activities of aquatic organisms can be accelerated, slowed down or stopped completely [12]. The pH values at all localities are slightly alkaline, except for the middle course, where the recorded values in the spring were 7.3. Slightly elevated pH values indicate a higher intensity of photosynthesis, with water being insufficiently saturated with CO₂ due to faster assimilation of CO₂ from water compared to assimilation from the atmosphere [13]. The pH value of water recorded at springs in the municipality of Bužim is slightly lower compared to the results of local research, except for the pH value from the estuary [14]. According to the Decision on surface and groundwater characterization, reference conditions and parameters for water status assessment and water monitoring (2014), the recorded pH values at all sites indicate a high ecological status [15]..

One very important indicator of water quality is dissolved oxygen. Dissolved oxygen determines the amount of gaseous oxygen dissolved in water that plays a fundamental role in the life of organisms [16]. In our research, the recorded values of dissolved oxygen ranged from 8.6 to 11.0 mg / 1. Slightly higher values of dissolved oxygen compared to our research were recorded in July at Vrelo Bosne where it was 12.5 mg/l [17]. Based on the Decision (2014) and the recorded values of dissolved oxygen, the ecological status of water in the upper and middle stream is high, while in the lower course during the summer is recorded good ecological status.

Electrical conductivity is a useful physical indicator of a water's ability to conduct electricity and is an important tool for assessing water purity [18]. The recommended value of electrical conductivity of drinking water according to [19] is 1500 μ S / cm, and according to [20] 1000 μ S / cm, while the values of electrical conductivity of water for livestock and poultry according to [21] is <1000 μ S / cm, at why the recorded values of electrical conductivity at all sites in our research are satisfactory in relation to the allowable values.

Suspended solids in water describe the concentration of inorganic salts and a small amount of dissolved organic matter. The increased concentration of suspended solids is harmful to aquatic organisms, especially if it is of organic origin because it consumes dissolved oxygen in water. The values of suspended solids in the urban rivers of Bangladesh ranged from 75 to 145 mg / L [6], with the values being much higher than the results of our research.

The recorded oxygen saturation values in our studies ranged from 98.2 to 69.3%. The values of oxygen saturation in Vrelo Bosne ranged from 105.2 to 126%, and are slightly higher compared to the results of our research [17], compared to the results of our research, they are higher.

 BOD_5 is an important indicator of aquatic ecosystem pollution by wastewater and industrial pollutants [22]. Biological oxygen demand is the amount of dissolved oxygen required by aerobic organisms to decompose organic matter at a temperature of 20 ^{0}C and for five days. In our studies, the values of BPD₅ ranged from 0.94 to 2.59 mgO₂/l, and according to the Decision

(2014) and based on the analyzed parameter, the ecological status of the tested water varies from high to good [15].

The consumption of KMNO₄ at all analyzed localities of the Krušnica River ranges in the interval of $4.2 - 9.16 \text{ mgO}_2/\text{l}$, where these values indicate a good ecological status [15].

The high concentration of chloride in water indicates the presence of organic matter, probably of animal origin [23]. Namely, chloride ions are necessary for the life of plants and animals, they are found in the form of sodium chloride (NaCl) [24]. Slightly higher values of chloride concentration were reported by [25] in the river Bosna (63.46 - 169.5 mg / l) in relation to the results of our research.

Very low concentrations of nitrite were recorded in all analyzed water samples from the Krušnica River, which is satisfactory in terms of drinking water. Very similar results of our research were recorded by [14] analyzing the quality of water from springs in the municipality of Bužim.

The amount of ammonia in water depends on the pH value and temperature of the water. Elevated values of ammonia in water indicate fecal contamination [26]. Ammonia can undergo oxidation by microorganisms and be converted to nitrites or nitrates [27]. Based on the results obtained in our research on ammonia, a high ecological status can be noted [15].

High concentrations of sulfate in water are probably the result of the decomposition of phytoplankton and aquatic macrophytes or are the result of oxidation of sulfides and sulfites to sulfates in the presence of photosynthetic sulfur bacteria [28, 29]. In our research, low values of sulfate concentration were recorded in all samples of water from the Krušnica River.

4. CONCLUSION

Based on the conducted research and analysis of physico-chemical parameters of water from the Krušnica River, it can be concluded that the ecological status of waters from the Krušnica River depending on the flow ranges from high to good. Minor deviations of the analyzed parameters in the middle and lower course of the River Krušnica are the reason why part of the Krušnica River passes through the populated and urban zone, or indicate the possibility of mild fecal contamination. Ultimately and based on the obtained values of analyzed parameters and the notation of high and good ecological status of water from the Krušnica River, it can be concluded that there is no or very small anthropogenic changes in these sites, whereby these conditions do not have a negative impact on the living world.

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GREEN ACCOUNTING AND ENVIRONMENTAL AUDIT

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Key words: social responsibility, environmental protection, sustainable development, green accounting and environmental audit

ABSTRACT:

The end of the 20th and the beginning of the 21st century were marked by climate changes, which warned that natural resources are not inexhaustible as they seemed and that it is necessary to act globally to monitor the use of natural resources and to develop mechanisms for the service of environmental protection. Based on these facts, rapid economic growth is accompanied by more frequent and advanced technological changes. Therefore, the question asked is what do individuals and society as a whole unity can do to prevent or minimize everything that inevitably comes to us. Traditionally, the focus of the enterprise has been exclusively on making a profit with the least possible costs and risks, and social responsibilities, which include also environmental protection, have often been ignored. The use of the traditional accounting system becomes insufficient to cover the social benefits and social costs arising from the activities of business entities in both the private and public sectors. Nowadays companies are abandoning such a concept and for this purpose, they accept a completely new concept of accounting, known as "green accounting", while in parallel with it there are developments of special forms of audit whose primary task is to assess the implementation of sustainable development policy from the social, economic, environmental and technological aspects as well. The policy of improvement and protection of the environment as well as adequate environmental management is an economic necessity for the successful operation of business systems in a modern environment, and the audit is expected to adjust its tools to the degree of compliance of activities and processes with specific requirements arising from eco-regulations and standards of business policy.

In this paper, by using the method of analysis and synthesis, as well as the method of description, all the features of accounting and environmental auditing are represented with the most

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important conclusions reached by the analysis of secondary literature and experiences of countries in the region in conducting the observed type of audit.

1. INTRODUCTION

"Green" accounting is a relatively young discipline that has a special focus on the improvement and protection of the environment. It aims to achieve long-term effects from which each individual will have multiple benefits. In terms of accounting as a factor that greatly affects the use of natural resources, it focuses on obtaining information on the flow of energy, water, and waste materials, as well as other financial information related to profit and costs. We are witnessing the increasing danger to our planet, which is reflected in the inhumane use of natural resources, the unfavourable impact of climate change, and inadequate production and consumption. Climate is considered a global public good that affects all social processes. in order to prevent all negative substances that will have lasting consequences on humanity, sustainability and social responsibility have become an essential part of the business world. In order to adjust the impact of business on the environment, it is necessary to use natural resources rationally, reduce the emission of negative substances into the air and soil, and collect and recycle waste separately. With the introduction of the term "green" accounting, business entities included the costs of environmental protection in their financial statements, that is, they devoted themselves to the appearance of an eco-balance sheet. The result of these applications should not call into question the company's financial condition. Such operations are followed by an environmental audit, the main task of which is to assess whether the business system complies with ecoregulations and standards prescribed by law.

This paper includes the analysis of "green" accounting and its connection with all other sectors in the company. On the basis of this, we will refer to the audit of the environment, which has become the fundamental task of the eco manager.

2. ACCOUNTING AND GREEN ACCOUNTING

This part of the paper presents the theoretical framework of the concept of accounting and the emergence of green accounting, as well as the impact that individuals and companies can have on the ecosystem. Sustainable development plays a major role in every scientific discipline. Economists narrow and limit sustainable development by reducing economic goals to growth and efficiency, social goals to fair distribution and poverty reduction, and ecological sustainability is sought to be achieved only by managing natural resources.[1] The key factors that influence the preservation of the environment are the awareness and education of the population, the development of technology in the direction of ecology, and legal regulations.

2.1. The concept and importance of "green" accounting

Historically speaking, the definition of accounting has changed and supplemented over time. What they have in common is that accounting is one of the most important sectors in every company. Accounting as a theory can be defined as logical reasoning based on the establishment of principles that provide a framework in which accounting practice operates, but also a guide for the development of new practices and procedures.[2] Regardless of what activity the company is engaged in, it is necessary to keep prescribed records of economic activities. When it comes to accounting information, it should be said that it serves as a basis for making business decisions and as a means of control.[3] Such information, in addition to management, is also important for other interested users such as: the government, customers, creditors, investors, etc.

The advancement of technology, which greatly changed the company's development strategy, led to the fact that the management of the company, in addition to the basic rules, must also respect the rules of environmental protection. In other words, when performing the basic activity, the principle of sustainable development and the quality of the future life of civilization should be chosen. For the principle of sustainable development, we can say that it is a phenomenon that advocates meeting the current needs of generations without having a negative impact on the needs of future generations. In addition to classic *owes- claims* accounting, the need for environmental protection accounting has emerged, the main goal of which is to protect the environment. As part of environmental protection accounting, the term "green accounting" is used (picture 1). Green accounting integrates environmental costs into the business financial results of a company. It not only shows the development of the company in terms of economic benefits but also reflects the environmental costs that tje company needs to pay for economics benefits. [4] It is also called responsibility accounting.

The purpose of this accounting is the assessment of the economic effects of the application of relevant regulations, standards, contracts, protocols and other international, European, national and other measures, whose task is to improve and protect the environment and save and replace non-renewable and environmentally harmful resources.[5]

This accounting primarily emphasizes the tools that will be used to convey information about the use of natural resources and to what extent such operations cause environmental protection costs, which will selflessly, in addition to profit, promote the preservation of non-renewable resources.

Managerial accounting for environmental protection deals with the preparation, analysis and use of information that is necessary for eco-management, namely: information on the consumption of energy, water, raw materials and materials, and incurred costs as well as savings in the area of environmental improvement.



Picture 1. The area of environmental accounting [4]

2.2. Accounting for environmental costs

The costs of environmental protection are the costs of investment in the improvement and protection of the environment, which, viewed through financial statements, should leave a short-term and long-term effect on society. According to measurability, these costs are divided into:

- convection costs
- hidden costs
- unpredictable costs
- costs of relations with interest groups and costs of image.

Convection costs include the costs of raw materials and materials, capital, etc. The most common hidden costs can be costs arising from the planning, information, testing, recycling and business control stages.

On the other hand, there are costs that are not easily measurable and unpredictable and may appear in the form of penalties and expenses imposed by the state. Costs that are not easily measurable include the costs of relations with interest groups and include the costs of relations with customers, suppliers, employees and the public sector.

3. AUDIT OF THE ENVIRONMENT

3.1. Concept and types of audits

When we talk about the concept and meaning of the word "revision", we can say that it originates from the Latin word "revidere", which means to review, and accordingly, revision is nothing but a subsequent review. The audit represents a systematized process of objectified acquisition and creation of evidence on economic events and results with the aim of establishing alignment between existing business reports and pre-set criteria and delivering it to interested users [6].

Based on the definition of audit, we can talk about the types of audit and view it as:

- internal audit
- external audit
- state audit

Internal audit belongs to the internal control of the company and represents a competent, independent and consulting activity with the aim of controlling the processes and procedures established by the management.

The external audit is applied to the complete set of financial statements (balance sheet, income statement, statement of changes in capital, statement of cash flow and notes. State audit refers to the audit of public expenditures. [7]

3.2. The concept and development of environmental auditing

The exceptional growth of interest of individuals and companies in caring for the environment has caused the increasing involvement of companies in social responsibility projects. One of such projects is the implementation of an environmental protection audit, the primary goal of which is to focus on the analysis and auditing of the effects of business operations on the environment. Such analyzes refer to the work of employees, detailed control of facilities and machines, but also the ways of using, storing and disposing of resources.

The audit of environmental protection has some features that should be highlighted [8]:

- It is a mechanism that companies and insistences use to highlight their progress in environmental sustainability.
- For sectors that generate more waste or residue of all kinds (emphasizing any industrial or chemical process), it serves as a valid measurement for public administration.
- In addition to the compliance standards imposed by various laws in enforcement, it also enables companies to voluntarily offer a more conscious image of the ecosystem and, therefore, convey a set of brand values to the public.

• This type of audit must also be carried out periodically, due to the necessary monitoring that is needed to study the effects caused to a certain ecosystem.

According to the old and still prevailing understanding, the connection between the economy and environmental protection are essentially opposites.

It is considered that environmental protection means a decrease in profitability for companies and increased costs for consumers, and that profitability simultaneously implies the use and destruction of natural resources.[9]

However, day by day, these attitudes are changing more and more in favour of environmental protection, which is greatly helped by the revision of environmental protection. There has been the formation of large international corporations for sustainable development, and we are increasingly talking about a "green" consumer mentality and the creation of an "ecological image". That is why the companies themselves are focusing more on environmental protection, which is greatly helped by the environmental audit. It represents a part of social auditing, a new branch in which the essence of auditing and accounting information is related to the environment, that is, to the natural environment. By strengthening environmental audit. [10] The Council of the European Union defines environmental audit as a management tool consisting of a systemic, documented, objective and regular evaluation of the environment in order to control the practices that have an environmental impact and to assess their conformity with the entity's policies. [11]

By conducting an audit of environmental protection [12]:

- a better reputation and a positive image of the subject in the business environment is achieved, and more credibility is given to the information related to the impact on the environment provided in the basic financial reports or special reports on the environment.
- there is a reduction of risks related to the implementation and compliance with environmental regulations, provisions and protocols.
- the risk of legal disputes related to incorrect presentation and interpretation of environmental information presented to users is reduced
- the process of making business decisions by investors is accelerated, and assumptions and conditions are created for quick and easy access to the dedicated means of support of ecological funds.
- the quality of information on the environmental reporting system is improving.
3.3. Internal and external environmental auditing

Internal audit of the environment should primarily be seen as an extended arm of management, whose task is to satisfy the information requirements of the management of a certain business system in the realization of goals and tasks defined by the protection policy and tasks defined during the protection and improvement of the environment. In doing so, the principles, instruments and techniques of internal audit are taken into account, with the special task of realizing the obligations arising from the accepted policy towards the environment [13].

The goal of the environmental audit is to provide relevant information that is necessary for the management to make decisions, and a report on the conducted audit is submitted to the management. Also, the results are published publicly so that they are available to all interested users.

ADVANTEGES OF INTERNAL ENVIRONMENTAL AUDIT	ADVANTEGES OF AN EXTERNAL ENVIRONMENTAL AUDIT
Enables the avoidance or minimization of responsibility for harmful environmental impact	Contributes to raising the image and better reputation of the business system in the environment
Becomes the basis for the systematic reduction of insurance premiums	Gives more credibility to information published in basic financial reports or special reports in the environment
Provides an information base for making business decisions that are important for increasing financial results, as well as improving relations with the environment	Leads to a reduction of risk in relation to compliance with eco-regulations and the application of other eco- regulations and protocols
Improves the impact of the EMS and ensures a better relationship with the environment	Significantly reduces the risk of disputes, and due to the potentially incorrect presentation of information about the environment to users or their misinterpretation
It leads to better results in the field of risk management	Accelerates the making of business decisions by investors, and creates prerequisites for quick and easy use of dedicated funds of eco-funds
Ensures a higher level of satisfaction of customers and other users of services due to the consideration of their requests and the implementation of eco- communication	Contributing to the improvement of the overall relationship in EMS
Contributes to raising the image and better reputation of the business system in the environment	Encourages audit development
It refers to more efficient performance of internal work processes and cost savings	Improves the quality of the information in the environmental reporting system

Table 1. The advantages of internal and external environmental audits [12]

4. CONCLUSION

New age problem lies in inhumane attitude toward the environment. In order for individuals as well as business entities to change their immoral attitude towards nature, it is necessary for every polluter to pay for the economic damage caused to the ecosystem. Green accounting as a part of modern accounting aims to provide management information that will be useful for ecomanagement. The task of all levels of management is to make decisions that will be in accordance with relevant regulations, standards and protocols in order to protect the environment. The purpose of the environmental audit is to help protect the environment and minimize the risks of business activities to the environment. From the perspective of the company itself, the goal of the environmental audit is to check whether the company has complied with environmental regulations and requirements and achieved previously set environmental goals. An environmental audit can also provide key information to the company's management about areas of risk, but also about progress towards the achievement of set strategic goals. An effective environmental audit can identify non-compliance with regulations and identify areas of poor management and control, which can otherwise result in environmental incidents and damage. On the other hand, properly conducting an environmental audit can highlight significant operational efficiencies and create cost savings. Companies are focused on financial information precisely because of the costs that would arise from new knowledge and processes. Opinions are divided in the world, American and British companies are increasingly focused on the ecosystem, and European companies on finance. Every company should achieve an eco-balance in terms of improving the understanding of environmental costs that will have a positive effect on capital with the comparative expansion of knowledge and data connection with other sectors in the company. This could be achieved by a single law and environmental protection standards.

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MENAGMENT RESPONSIBILITY

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Key words: ISO 9001, ISO 2600, business responsibility, societal responsibility, quality

ABSTRACT:

Product quality is the standard by which a product is measured against products of a similar kind, ie the level of quality of something. To satisfy the customer, the customer's reception of the product directly affects how the quality of the product or service is determined. The introduction of a quality management system is a strategic decision of the organization, which is why the implemented quality management system should contribute to improving the characteristics of the organization's management system. In order to ensure product quality, it is important to pay special attention to projects in ensuring socially responsible operation of modern business systems, whose role of project management has changed radically over the past two decades due to the undeniable number of projects and their more complex business strategies. Project management organizations highlight seven key themes in ISO 26000 on social responsibility, which are strongly related to project management. Projects have an important impact on the establishment of ongoing operations and are important tools for ensuring the strategic development of business systems. Four different links and interdependencies between project management and systems theory highlighted by ISO 26000 are highlighted: (1) systematic treatment of project management, (2) systematic treatment of projects as complete works, (3) systematic treatment of projects as part of strategy, (4) systematic treatment of projects throughout the life cycle. This is where the responsibility of the management arises, both in terms of the product being of satisfactory quality, and in terms of the conditions of the workers and their work, and to be ready to take on the consequences of working in the company. It is tied to legal regulations and moral legality, and is an important part of business relationships. Business responsibility is an indispensable part of business, and its components are the responsibilities and types of managers, responsible communication, and social responsibility in general. By simply following the ethically imposed rules of social and business responsibility in

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all business segments, it ensures satisfactory results, making it an imperative of every successful company.

1. INTRODUCTION

For modern-day consumers, many aspects of the products the financially support are of the utmost importance to them. "Will it satisfy their basic needs? Will they have to return the said product because it has proven to be hazardous or harmful for either them or their environment? Is the product worth the price?" All these questions go into the examination of if the product or service is of high enough quality, thus also if the organization or the manufacturer can provide their best to the customer. To be able to satisfy the modern-day customer and therefore their most basic demands, one must implement standards into their firms to ensure only the best outcome for their business. That also means being responsible and reliable to the workers of the said company, to not harm the environment and to make sure to terminate any sort of corruption possible in the earliest of stages. During the mid to late 20th century, the International Organization for Standardization (ISO) defined certain standards any company, organization or stakeholder must oblige to, so to create a better working environment and secure the returning of the customer and not the product.

2. QUALITY – DEFINITIONS AND IMPORTANCE OF ISO 9000 STANDARDS

There are many ways of understanding what quality means in a professional and working setting, but the most important aspect of it being in organisations and the industry itself. To make something worth of calling "high-quality", the process requires time and effort from all sides included as well as certain financial sacrifices for future gain. To meet the set expectations, all parties involved must follow standards set by the International Standard Organisation, with special focus on the ISO 9000 standard family, as well as ISO 45000, ISO 14000, and ISO 26000. [1] ISO 9000 standards focus on quality as a group of traits and virtues of a product that will ultimately satisfy the customer. Every product and/or service has to have a set of values that need to be met in order to be considered of quality, because of which the standard set ISO 9000 was coined: to ensure the satisfaction of the customer while making sure there is no foul play from the manufacturers. ISO 9000 standard's main focus is the basics of QMS, as well as seven main principles of quality management which are considered the most important and basic structural principles for any type of ensuring of quality. [2] To make something of high quality can be applied to both products and services in type of labour from the most high-qualified intellectual works to the simplest of tasks. By the definition of BAS EN ISO 9000, quality is "the degree to which a set of inherent characteristics meets the requirements". [3] Monitoring the development of quality management means that the subsystem adapts to the existing quality requirements in the organization according to the quality loop. Achieving ISO 9001 by the company usually indicates that the company has an established sound quality system, and that quality and customer satisfaction are the core values of the company. ISO 9001 is considered the minimum standard that an organization that takes quality seriously should meet, and many organizations require their subcontractors to be ISO 9001 certified. [1]

According to the traditional approach, control is the initial stage of quality according to which some products or production processes, either from the intermediate stages or at the end, are subjected to control in order to determine whether they meet the established specifications. Today, quality management is a set of operations (programming, coordination, and execution) aimed at improving quality. There are varying ways to check and improve products or services. An audit is an independent examination of financial information of any entity, whether profit oriented or not, irrespective of its size or legal form when such an examination is conducted with a view to express an opinion thereon. [4] To control one body of work, auditing is integral and it can be either in the form of inter-organisational audit from the manufacturers themselves, or external by certified profesionals. Internal auditing has heavily changed throughout the history, mostly because of the industrial revolution and mass-production that ensured afterwards. With the development of factory production and the division of labor, quality control becomes the responsibility of a special group of employees - controllers. [5] With further development, the organizational structures of the company are also changing. Quality control is transferred to the competence of a special department, then it is transferred to other business functions, and with the advent of the ISO 9000ff series of standards since 1987, it has grown into an integrated guality assurance and management system. External guality control is carried out by authorized organizations in order to achieve the quality of goods and services in accordance with applicable national standards and regulations. Although organizational quality control can be performed differently, there are basically always three points of quality control: input quality control, quality control in the production process and final control and testing. [5]

3. MENAGEMENT – DEFINITION AND SOCIO-ENVIRONMENTAL RESPONSIBILITIES

Management is the core part of any organization, whether it is a business, non-profit organization or government agency. Management includes activities to set the strategy of the organization and coordinate the efforts of its employees (or volunteers) to achieve their goals by applying available resources, such as financial, environmental, technological and human resources. In modern business conditions, in the age of rapid globalization and disregard for both human and environmental sacrifices companies make to secure profit and to be able to achieve massproduction of their product, it is important to recognize the characteristics of the business environment in order to adopt appropriate strategies for future business and establish a management system to internal and external satisfaction, without causing too much harm to any

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party involved. In doing so, one should keep in mind the present of one's business is very uncertain, which further complicates business operations and requires rapid and continuous changes and the possibility of implementing them, thus taking in mind any and every risk that could emerge from the growing production standpoint. [6] In such a business environment, management has a very responsible and demanding task of establishing a successful business by setting adequate goals and using appropriate resources, be it by putting strategies of keeping the work environment safe on paper or putting the work in practice with a qualified team. Management involves identifying the mission, goal, procedures, rules, and manipulation of a company's human capital to contribute to the company's success. [7] This implies effective communication: the business environment (as opposed to the machines which work on demand) implies human motivation and implies some kind of successful progress or outcome of the system, for which proper motivation and award systems are needed to secure a spinning wheel of work and inter-industrial organization. The top management team develop plans and strategies as part of the core management system with the goal of continuous improvement and innovation of internal organization and business results, with the ultimate goal of achieving business excellence. For this to function, management must:

- Create and maintain awareness of the organization as well of the importance of meeting, and if possible overcoming the requirements, needs and expectations of customers and other requirements identified by the processes,
- Establishes a vision, mission, policy, strategic and operational planning that includes quality policy and objectives
- Establishes QMS as a means to achieve the goals of organizations that meet customer requirements
- Conducts regular reviews of QMS in order to determine its efficiency, effectiveness in achieving business goals of organizations and the establishment of measures, and improvement and innovation of both systems and goals
- Invests efforts and resources to improve adequate resources for the realization of products and / or services and to achieve the goals of the organization.

The collaboration of ethics and economics in the 20th century developed the scientific discipline of business ethics. The first ideas of ethical business have started circulating during the late 1960s and relate to some social affairs in the economy, such as workers' rights to adequate wages, satisfactory working conditions and fairness in business relations, as well as rising awareness of the environmental damage factories produce. Nowadays, the issue of the possibility of correct action, based on ethical principles, is gaining new meaning and importance globally

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and regionally. After the growing prevalence of moral errors and deviations, as well as financial scandals of corporations, many organizations are placing increasing emphasis on the adoption of codes of ethics and social policy of corporate responsibility. [7] Ethics is set as a necessary component of the activities of any organization. Successful global and domestic organizations have become aware of the fact that in the competitive arena, business ethics enable growth and development, thus increasing efficiency and productivity. Numerous studies show that business ethics and profitability are not mutually exclusive concepts, and companies that have a business ethics system in their own business system show better results than those that have not. Business ethics is a way of conceiving, concluding, communicating and performing work in simultaneous harmony with spiritual sociological biological and natural in accordance with the spiritual, sociological, biological and natural laws of man and the environment. A person, who by their decisions in any way endangers his workers, customers or competitors, behaves immorally and unethically, is by these laws and ethics considered incompetent to lead and manage an organization and is seen as a threat to the well-being of the company. [8] From a management perspective, ethics sets standards for what is good or bad in leadership and decision-making. Ethical problems arise when the decisions of individuals or groups can harm or benefit only the higher-ups. Standards for ethical or socially responsible behavior are included in each individual as well as in the organization itself, its attitudes, norms and beliefs, its own ethical standard, ethical decision-making framework and moral development greatly influence an individual's ethics. Within the organization itself, there is an organizational culture that unites the overall picture of values in the organization, and there is a formal organizational system that affects the values and behavior according to the established organizational framework and reward system. [9] Because the organization does not operate alone without contact with the environment, it is also influenced by external stakeholders, such as the state, customers and the market in general, which can affect the standard of ethics or social responsibility.

3.1. ISO 26000 - Social responsibility

Based on the principles of sustainable development, social responsibility is becoming a priority topic for discussion within companies and among all stakeholders. It is an effort to contribute, together with employees, the continuous growth of sustainable economic development in order to achieve targeted improvements in the quality of life. As a result, the International Organization for Standardization (ISO) has developed ISO 26000 guidelines for corporate social responsibility. [10] The task of this standard is to lead and help organizations move from good intentions to good practices, based on international consensus, with the aim of encouraging the application of best practices of social responsibility worldwide. Thus the term social responsibility was adopted in the 1970s. Initially, the focus was on business organizations and therefore today the term CSR is often used, ie corporate social responsibility. The term SR (Social Responsibility) is used in the development of the ISO 26000 standard, because the ISO

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26000 guidelines are applicable in all types of organizations. The need for a social responsibility standard was identified by ISO / CAPOLCO, the Committee on Consumer Policy in 2001. In 2004, an international conference was held to discuss the need to adopt a standard for social responsibility. The draft ISO 26000 standard was put to a vote in September 2009 and lasted until February 2010. The ISO 26000 standard does not contain requirements but guidelines as to how to lead an organization and is not given as a certificate to businesses. [11]

The main points of the ISO 26000 standard have had an impact on the way companies operate, how they treat their employees and how they assess and limit the impact of their activities. Although society has made progress in many areas over the past decades, the guidelines of the ISO 26000 standard remain relevant in addressing modern-day challenges and further activities in the business world. Due to the 2020 COVID outbreak pandemic, many organizations have put into perspective their style of work as well as what social responsibility means in a way to ensure every workers safety as well as the safety of their customers and consumers of their products. [12] That also meant to take a closer look into what the business world does to the natural environment as well, by taking in consideration how to stop spread of certain catastrophes that have happened in the early 2020s, like the mass burning of the Amazon rainforest and the rising levels of smog in bigger city areas.

The following issues should be taken into account when applying the recognized approach: human rights, labor practices, environment, fair operational practices, consumer issues and community involvement, and local development. This International Standard provides guidance for all types of organizations, regardless of their size or location, regarding: concepts, terms and definitions related to social responsibility; trends and characteristics of social responsibility; principles and practices related to social responsibility; basic topics and issues related to social responsibility; by integrating, implementing, and promoting socially responsible behavior throughout the organization. Through policies and practices, the organization contributes, in the sphere of its influence; identifying and engaging stakeholders; and communicating commitment, performance and other information related to social responsibility. [7]

ISO 26000: 2010 or EN ISO 26000: 2020 (Guidance on social responsibility) version released in November 2020, marked the first ten years without any changes in the said standard. ISO 26000, the international standard for social responsibility, is one of the most widely used and recognized ISO standards. Over the past decades, the ISO 26000 standard has proven to be more than a guide to "doing the right thing". It is important that human rights stand out in the standard both as a principle and as a main theme, which helps to identify risk situations related to human rights, conduct in-depth analysis and resolve complaints. [10] The standard brings together international expertise on social responsibility: what it means, what issues organizations need to address in order to act in a socially responsible way and what is the best practice in introducing

social responsibility. The ISO 26000 or EN ISO 26000 standard is intended to help organizations contribute to sustainable development. Its aim is to encourage them to go beyond mere compliance with the law, recognizing that compliance with the law is a fundamental duty of every organization and an essential part of its social responsibility. It also aims to promote a common understanding in the field of social responsibility and to complement, not replace, other documents and initiatives for social responsibility.

The guidelines provided by the ISO 26000 standard are [10]:

- Recognition of social responsibility and involvement of participants,
- Ways of integrating socially responsible behavior into the organization,

• Seven key principles of social responsibility, which include: Responsibility, Transparency, Ethical behavior, Respect for the interests of participants, Respect for the rule of law, Compliance with international standards of conduct, Respect for human rights

• The seven basic subjects and issues related to social responsibility are: Organizational management, Human rights, Work practices, Environment, Good operational practice, Consumer questions, Participation and community development.

The aim of using the ISO 26000 standard is as follows [10]:

• Assist organizations in addressing their social responsibilities with respect cultural, social, environmental and legal differences and conditions of economic development.

• Provide practical guidance on the operationalization of social responsibility.

• Assist in identifying and connecting with stakeholders and strengthening credibility of reports and claims of social responsibility.

- Emphasize results and improve performance.
- Increase trust and satisfaction in organizations among their customers and other participants.

• Achieve compliance with existing documents, international agreements and conventions and existing ISO standards.

- Promote common terminology in the field of social responsibility.
- Expand awareness of social responsibility

3.2. Management responsibility

Project management planning should focus on coordinating team members using a corporate social responsibility strategy, as well as taking in mind as to how decrease both the stressful work environments during production as well as harmful emissions. This includes social awareness at all stages of the project in order to achieve continued economic and environmental sustainability. Guidelines and laws on sustainable development provide a general framework for project managers to ensure that they are committed to global transparency, sustainable development and accountability. The ISO 26000 standard provides guidance to managers so to ensure that they enhance their commitment to improving overall ethical standards and sustainability practices worldwide, as well as putting human ethical practices into action with minimal damage caused to the social and natural environment. Managers must publish their individual sustainability results by integrating and following guidelines for reporting on corporate social responsibility and sustainability. Global reporting on these activities is called value-added reporting because they add value to projects by allowing the world to publicly monitor and evaluate international business activities and their adherence to corporate social responsibility guidelines. Therefore, in order to review their organizational performance and continuous progress related to compliance with international laws of corporate governance and sustainable development, managers must do intensive research as to how to implement sustainable practices and to continuously work on value-added reporting. [13] Managers can monitor and report arbitrarily on their own social responsibility project management or in integrated company reports, while also putting into perspective their flaws and what can be fixed in the following phases of development. As new international initiatives will go with severe and high penalties for companies that do not apply the standard of social responsibility, it is assumed that there will be greater sanctions and penalties for managers who have failed to apply social responsibility. The integration of CSR into strategic plans as well as project plans provides a strict set of environmental, social and social guidelines that would help meet the needs of all stakeholders in a sustainable way. [14] To implement CSR into everyday working places also means following the guidelines for taking better care of the environment in the sense of stopping the further deforestation, mass chemical emissions and pollution through sustainable practices. It is therefore essential that all project plans clearly and in detail explain how each phase of the project will comply with global CSR regulations to inform all stakeholders including customers, suppliers, distributors and partners about the implementation of a social sustainability strategy for project management. Then everyone would realize how socially aware the organization is, which will greatly help them become and remain sustainable in the future, as well ensure great successes in the business world. This would further down cause a domino effect of sustainable and ethical practices that both manufacturers and stakeholders would follow to gain trust from the general public and increase potential profits.

4. CONCLUSION

Many manufacturers and corporations would describe themselves as of high quality and able to give the best services to both the worker and the consumer, but those claims mustn't be without proof. To ensure no laws are being broken and no person was left harmed, the ISO standards have been implemented into the modern industrial world. With the rise of globalization and internet connections, for businesses to keep existing without the risk of losing workers and therefore jobs, they must implement as many of the most important standards as possible into their working practices so to keep their business afloat. That means focusing especially on the CSR standard known as ISO 26000, which means paying attention to sustainability, ethics and respecting of human rights, while also taking care of nullifying any possible pollution their production process may cause. By implementing the guidelines the standards set, organizations and businesses are set to flourish and better themselves in the following years of the 21st century.

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