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The presence of heavy metals in water and the muscle tissue of grayling (*Thymallus thymallus)* of the Una River

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Abstract

Heavy metals belong to a group of substances, which, after reaching a natural habitat, can manifest various negative effects. This paper analyses heavy metals (Cu, Cd, and Pb) in the Una river and fish samples, whereby individuals of grayling (Thymallus thymallus) were used for analysis. The samples were taken at three locations at the Una river: Ripač, Hatinac, and Grmuša. Alongside with the examination of the content of heavy metals, quality of water was tracked, including the following parameters: temperature, pH, electrical conductivity, dissolved oxygen, suspended matter, BPK_5, KPK – Cr, total nitrogen – N, total phosphorus P, sulphates, chlorides, nitrites – NO_2, nitrates – NO_3, fluoride, and heavy metals (Cu, Cd, and Pb). Besides the content of heavy metals, proteins, fats, water, carbohydrates, and mineral matter were found in the grayling fillets. Amount of heavy metals (Pb, Cu, and Cd) were under maximum allowed concentration (MAC).

Key words: heavy metals, water, fish.

Introduction

European grayling is a salmonid species exposed to different and long historical life development, even before Pleistocene (Parkinson et al., 1999; Koskinen et al., 2001; Weiss et al., 2002; Froufe et al., 2003; Gum et al., 2005). In Austria, for example, because of the danger of its extinction, it was proclaimed "endangered animal species" in 1997, although that is the area of the European zone of grayling (Uiblein et al., 2001). Pollution of surface waters with heavy

metals is a serious ecological issue, because some toxins, even in small concentrations as polluters, are non-biodegradable and can have toxic action, even far away from the source of pollution (Tilzer and Khondker, 1993). Although some metals like Fe, Cu, and Zn are important micronutrients, they can be harmful for the physiology of living organisms in greater concentrations (Kar et al., 2008; Nair et al., 2010). The greatest interest is shown for these elements because of frequent soil contaminations, water, and food chain contaminations as well (He et al., 2005). Mazet et al. (2005) proved presence of cadmium and lead in samples of fish from a wider range of locations alongside the river stream, and their concentrations did not exceed values defined by European regulations. In the study done by Alić et al. (2004), lead (Pb) content in the muscle tissue of fish (mean) upstream of the city of Bihać was 0.29 mg/kg, and downstream 0.34 mg /kg, copper (Cu) upstream 0.82 mg/kg and downstream 1.33 mg/kg and the content of cadmium (Cd) upstream 1.00 mg/kg and downstream 1.64 mg/kg.

Material and Methods

Fishing and sample taking was done in March 2013. Samples of water and fish for analysis were taken at three different locations: Ripač, Hatican, and Grmuša, a total of 3 samples of water and 12 fishes were analysed. Determining physical and chemical parameters of the quality of the water sample was done according to the standard methods (APHA, 2000). At spot (In-situ) temperature, oxygen saturation, pH value, and electrical conductivity were measured. Basic chemical composition of the fish meat (proteins, fat, carbohydrates, water, and ashes), as well as the analysis of water, was done in the laboratory of the Biotechnical Faculty, University of Bihać, by using standard AOAC (2000) methods: Fish and Marine Products, Ed. 2000. Researched locations are shown in Table 1. The water and fish samples were delivered in a portable, cooled device to the laboratory, where they were prepared for the analysis. In order of determine heavy metals, destruction of samples of the grayling muscle tissue was done by digesting in the "Multiwave PRO", Anton Paar microwave. 6 ml of concentrated HNO_3 and 1 ml of HCL was added to the 0.3 gram sample, and then burned by a given temperature programme. Heating and burning of the mixture was done for 10 minutes, and after that, the samples were cooled and diluted.

Tab. 1. Digestion programme in Multiwave Pro

Power (Watt)	Ramp (min)	Hold (min)	Fan
650	5	10	1
1400	10	10	1

Analysis of heavy metals was done by using the method of atomic absorption spectrophotometry, "Analytical Methods" FP-3 Analysis of Meat and Meat Products (2000.). The analysis was done using "Perkin Elmer" Aanalyst-800, an atomic absorption spectrophotometer.

Tab. 2. Suggested conditions for analysis on AAS

Element	Flame	Wave length	Burner	Kalibra.meto	Stock Stand.Solut
Pb	Air-acetylene	283.3 nm	10 cm	Linear/nulu	Lead 1000 mg/l
Cu	Air-acetylene	324.8 nm	10 cm	Linear/nulu	Cooper 1000 mg/l
Cd	Air-acetylene	228.8 nm	10 cm	Linear/nulu	Cadmium1000 mg/l

Results and Discussion

The water examined from the upstream to the downstream of the Una River has approximately uniform values of electrical conductivity, and their range was from 392 µS/cm to 477 µS/cm, which classifies them in the first class quality surface waters. Studies done so far on the stream of the Una River show the values under limits (600 µS/cm) with the tendency of increasing values outside of the cities.

Tab. 3. Physical-chemical analysis of the Una River water

	Parameters	Unit of measure	Location Ripač	Location Hatinac	Location Grmuša
1	Temperature	ºC	10.80	12.40	11.30
2	pH	/	7.37	8.04	7.76
3	Electrical conductivity	µS	392.00	477.00	405
4	Dissolved oxygen	mg/l	7.30	8.20	8.30
5	Total suspended matter	mg/l	0.17	0.89	0.65
6	BPK$_5$	mg O$_2$/l	0.62	1.04	0.67
7	KPK - Cr	mg O$_2$/l	2.94	4.78	0.78
8	Total nitrogen- N	mg/l	1.34	4.27	2.04
9	Total phosphorus- P	mg/l	0.43	1.974	1.03
10	Sulphates	mg/l	27.253	30.177	31.321
11	Nitrites- NO$_2$	mg/l	0.021	0.157	0.319
12	Nitrates– NO$_3$	mg/l	2.762	3.481	3.015
13	Chlorides	mg/l	4.035	5.028	5.971
14	Fluorides	mg/l	0.249	0.439	0.423

The main issues in water are connected to organic pollution. Chemical oxygen consumption in the tested water, as a measurement of the presence of organic matter, has a range from 2.94 to 4.78 mg O2/l and it points to low organic matter content. Values of BPK$_5$ show the consumption of oxygen spent on dissolving organic matter, hence it represents measure of the water pollution. All tested waters had the value of consummated oxygen under 2.0 mg/l, which classifies them in the first class of waters. Tested parameters do not exceed permitted values.

Tab. 4. Chemical analysis of the grayling (*Thymallus thymallus*) muscle tissue

Una - Locations	Proteins (%)	Fats (%)	Water (%)	Mineral matters (%)	Carbohydrates (%)
Ripač	17.76	3.31	77.22	1.31	0.39
Bakšaiš	17.05	3.56	77.70	1.27	0.42
Vrkašić	17.14	4.03	77.01	1.25	0.55

When it comes to results of the analysis of the fats content in grayling from the location Ripač, its amount was 3.31% increasing to 4.03% in grayling from the location Vrkašić, which fits into the information found in literature (FAO, 1994.). According to FAO (1994.), the average share of proteins, which are considered to be of high quality because of their amino acid content, ranges from 18.8 to 19.1 %.

However, variations of shares of proteins in fish have a greater range (16-21%), provided that the minimal shares found in literature sometimes were 6% (Kovačević, 2001). Bojčić et al. (1982) give an average share of 20%. The tested grayling samples have a lower share of proteins (17.05%-17.76 %) that fits into other studies (Bećiraj, 2009), and the values are lower than the average in the literature. This can be explained by the fact that proteins are used as energy in this case, because the location Ripač has the lowest temperature (10.8°C, Table 3) that affects the occurrence of hydrobionates negatively. In the water of relatively fast flow, it is harder for fish to find feed that has enough carbohydrates and fat.

Hence, graylings from this location require more energy for life activities than graylings from the other two locations, which have a slower flow and more organic matter, which is confirmed by parameters of BPK and HPK in Table 3. Content and quality, that is, amount of feed that is available to fish, quality of ecosystem affect the amount of proteins in fish (Kovačević, 2001). The share of water in our grayling samples ranged from 77. 01%-77. 70%, based on which we can conclude that there is an agreement with literature (Alibabić, 2005. Bećiraj, 2009.) According to Alibabić (2005), average mineral matter in grayling was

1.32%, whereas in our samples this value ranged from 1.25 % on the location of Vrkašić (downstream) to 1.31 % on the location of Ripač (upstream).

According to the explained results, we can conclude that our results agree with the citations in the literature, which confirms the statement that the quality of the fish meat depends on the quality of water that they live in. Carbohydrates content in the analysed grayling ranged from 0.39% to 0.55%, which is consistent with the study by Bećiraj (2009), whose value was 0.44%.

Tab. 5. Concentration of heavy metals in the Una River water

Una-Locations	Pb (mg/kg)	Cu (mg/kg)	Cd (mg/kg)
Una-Ripač (upstream)	0.000	0.0021	0.004
Una-Hatinac (downstream)	0.000	0.0108	0.021
Una-Grmuša (downstream)	0.000	0.0116	0.018

Results of the analysis of heavy metals do not exceed (MAC) in any of the water samples of the Una River. The value of lead (Pb) was not identified. Copper (Cu) content on the location of Ripač (upstream) ranged from 0.0021 to 0.0116 mg/l on the location of Grmuša (downstream). Vidaček et al. (1999) have questioned the content of heavy metals in the waters of Karašica-Vučica. Values obtained for copper (Cu) varied from 0.0033 to 0.0213 mg/l, lead (Pb) from 0.0046 to 0.0405 mg/l, cadmium (Cd) from 0.002 to 0.0039 mg/l, which is in accordance with our results, except the values of lead (Pb) that were not identified in our results. According to the study by Veladžić et al. (2017) in the samples of water, in both periods, the value of lead (Pb) was identified only on the locations of Vrkašić and in concentration less than 0.001 mg/l, whereas in our study lead was not present. The content of copper (Cu) in the Una River in both periods was lower than 0.4 mg/kg, cadmium (Cd) less than 0,1 mg/kg, and our data from all three locations show much lower values for the concentrations of copper and cadmium, which confirms that there has not been greater pollution of the Una River.

Tab. 6. Concentration of heavy metals in the grayling muscle tissue (*Thymallus thymallus*)

Una- Locations	Pb (mg/kg)	Cu (mg/kg)	Cd (mg/kg)
Una - Ripač	0.000	0.034	0.006
Una - Hatinac	0.010	0.052	0.012
Una - Grmuša	0.000	0.076	0.014

The content of lead (Pb) was only identified in the sample of Una-Hatican (city zone) and it was 0.010 mg/kg. Copper (Cu) value ranged from 0.0034 mg/kg to 0.076 mg/kg, meanwhile the values of cadmium (Cd) ranged from 0.006 mg/kg to 0.014 mg/kg. In the study by Alić et al. (2004) done on the muscle tissue of trout (Salmo trutta m. fario L.) of the fish caught in the Una River, average amount of lead (Pb) was determined upstream from 0.29 mg/kg to 0.34 mg/kg downstream, which was not in accordance with our results.

This, however, confirms citations of literature (Hadžibeganović et al., 1985; Tomašević et al., 1988), that the river of upstream and in city zones is less polluted with smaller concentrations of heavy metals. Concentrations of copper (Cu) in the muscle tissue of a trout (*Salmo trutta* m fario L.) was 1.08 mg/kg, in the studies of Alić et al. (2004), which is greater than the values that we obtained in all three locations of Una River. As it was stated by Alić et al. (2004), values of cadmium (Cd) in the muscle tissue of trout caught in the Una River was 1.32 mg/kg, which is not in accordance with our study and has higher values. In the muscle tissue of river trouts, the content of Cd was less than 0.01 mg/kg (Uthe and Blieg, 1971; Tomašević et al., 1988). In very contaminated areas, such as the River Severn in Great Britain, higher content of that metal in the fish meat was confirmed, as it was 2.5 mg/kg (Tomašević et al., 1988). Mazet et al. (2005) state that the concentration of Cd and Pb analysed in fish of the Drome River is lower than limit values described in the literature.

Conclusion

According to the tested physical and chemical parameters of water, the Una River can be classified in the first-class quality of waters at all three locations. Chemical content of the fish meat depends on the species, sex, age, season, feed availability, quality of biotope, physical activities, and the behaviour of fish. Since Una is an upstream (Ripač), mostly cold water, with a relatively fast flow, grayling from this location find it necessary to have more energy for life activities (less % of proteins) than other grayling from the other two locations (downstream). The concentration of heavy metals in the water and the muscle tissue of grayling from the Una River does not exceed maximum allowed concentration of heavy metals. Concentration of heavy metals in the muscle tissue is in a direct connection with river pollution. The Una River is not significantly polluted, which is confirmed by the results of our study of copper, cadmium, and lead concentrations in the grayling muscle tissue. Our results showcase that the concentrations of heavy metals are very low, lower than the limit values described in literature and that there has not been a significant pollution of the water stream of the Una River, as it was previously mentioned.

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Присуство тешких метала у води и мишићном ткиву липљена (*Thymallus thymallus*) из ријеке Уне

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Сажетак

Тешки метали припадају групи материја које након доспијавања у животну средину могу испољити различите негативне ефекте. Овим истраживањем обухваћена је анализа тешких метала (Cu, Cd и Pb) у води ријеке Уне и узорцима риба, при чему су за анализу кориштене једнике липљена (*Thymallus thymallus*). Узорци су узети на три локалитета ријеке Уне: Рипач, Хатинац и Грмуша. Упоредо са испитивањем садржаја тешких метала, праћен је и квалитет воде и то следеће параметре: температура, pH, електроводљивост, отопљени кисеоник, суспендиране материје, BPK_5, KPK - Cr, укупни азот - N, укупни фосфор P, суфати, хлориди, нитрити - NO_2, нитрати – NO_3, флуориди и тешки метали (Cu, Cd и Pb). Такође су поред садржаја тешких метала у филеу липљена одређени: протеини, масти, вода, угљикохидрати и минералне материје. Количине тешких метала (Cu, Cd и Pb) биле су испод максимално дозвољених концентрација (МДК).

Кључне ријечи: тешки метали, вода, риба.