HEAVY METALS IN WATER AND MUSCLE TISSUE OF TROUT (*Salmo trutta*) IN THE RIVER UNA

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ABSTRACT

This paper presents the results of research on the content of heavy metals in water and fish of the River Una. During 2012 and 2013 water and fish samples were taken from four locations, and research was conducted in two seasons: autumn 2012 (I season) and spring 2013 (II season). The caught fish at all locations belonged to the species of trout (*Salmo trutta*). The content of Pb, Cu and Cd was determined by means of an atomic absorption spectrophotometer "Perkin Elmer" AAnalyst - 800, using a flame photometry technique. The content of Pb, Cu and Cd was below the level of maximum allowable concentrations (MAC). The research of heavy metal presence in the water of the River Una indicated the value of Pb lower than 0,0010 mg/l, Cu lower than 0.4 mg/l, and Cd lower than 0,1 mg/l.
Heavy metals in fish muscle tissue were directly related to the pollution of river. Lead content in muscle tissue of fish caught in the River Una was the highest in samples which were caught at the fourth location, with 0,205 mg kg^{-1}, while the highest copper content was identified in the same location, 0,1280 mg kg^{-1}. The content of cadmium in fish muscle tissue samples was below 0,1 mg kg^{-1}. The heavy metal content in the tested samples of water and fish the River Una was below the allowable limit. These results indicate that the pollution of the River Una did not reach a significant level.

Keywords: cadmium, lead, copper, fish, atomic absorption spectrophotometry

INTRODUCTION

Ecotoxic metals are metals that are "toxic" to the living world in its dissolved phase. The used term heavy metals was not entirely suitable, because some light metals, e.g. beryllium, are toxic and some heavy metals, e.g. iron, are not toxic. It is more correct to use the term metals in trace or trace metals because their natural concentrations are very low (<1 μL^{-1}), and is found in nature only in traces. However, when their concentrations raise (mostly anthropogenic), and become dangerous for the living world of the aquatic environment, the term *ecotoxic metals* is the most appropriate. Metals are an important factor in any aquatic environment since, in many cases, the biodiversity of the aquatic ecosystem depends on them. Sewerage system and waste water from industrial plants are discharged directly into the watercourse, without previous purification. Also, without separation of solid waste, electrical appliances are disposed together, so that, through residual water, heavy metals reach to brooks and rivers, which burden water and organisms of the aquatic environment. This method of waste disposal can lead to environmental exposure to harmful substances, such as, among others, heavy metals[1,2,3]

The pollution of surface water by heavy metals is a serious environmental problem especially because some of them are toxic at low concentrations, do not break down and have a cumulative effect. Although some metals, such as Fe, Cu and Zn, are essential micronutrients, they can be harmful to the physiology of living organisms in higher concentrations[4,5]. Heavy metals as pollutants are non-biodegradable, and can become toxic even when they are away from the source of pollution[6].

Heavy metals in traces, which have been extensively studied in recent decades, include Copper (Cu), Lead (Pb), Cadmium (Cd), Zinc

(Zn), Iron (Fe), Manganese (Mn), Arsenic (As), Mercury (Hg) and Selenium (Se). These elements are showing the greatest ecological interest due to frequent contamination of soil, water and food chain. Furthermore, determining the amount of heavy metals in the tissues of animals gives an insight into environmental pollution and possible immunopathological changes and intoxication of animals[7]. Environment is an essential element of human existence. This is a result of the interaction of natural elements such as earth, air, water, climate, biosphere with elements created by human activity[8]. All these interactions and effects of existential conditions and opportunities for further development of society to protect the environment, mainly contaminated areas, must be identified; evaluate the extent of damage and identify the causes that are produced by these imbalances. It is necessary to maintain the quality of the environment by reducing the negative effects of human activities. Potentially toxic metals resulting from anthropogenic activities cause serious disturbances in ecosystems[9,10].

In fish samples from a number of locations along the river course, Mazet et al.,[11] proved the presence of cadmium and lead, where their concentrations did not exceed the value defined in European Regulation (European Regulation R466/2001)[12]. The amount of lead in fish muscle tissue samples was correlated with the concentration of polychlorinated biphenyls being explained with different population density and urbanization. In the research of [13], the content of Pb in fish muscle tissue (middle value) upstream from the city of Bihać amounted to 0,29 mg/kg and downstream of the city of Bihać 0,34 mg/kg, Cu, upstream 0,82 mg/kg, downstream 1,33 mg/kg and the content of Cd upstream 1,00 mg/kg and downstream 1,64 mg/kg.

MATERIALS AND METHODS

Sampling of water and fish in the River Una was carried out in September 2012 and March 2013.

Water and fish samples of the River Una were taken for analysis at four different locations: Ripač, Orljani, Bakšaiš and Vrkašić.

Table 1. Locations of samples

Una locality	The position of locality	located (E:N)
Una – Ripač	(46,5 km from the source)	15,9534380 44,7632863
Una – Orljani	(Sunce, 55,5 km from the source) - above Bihać	15,9070863 44,8003857
Una- Bakšaiš	(60,5 km from the source) – the city of Bihać	15,8640207 44,8283788
Una-Vrkašić	(62,5 km from the source) - below Bihać - after collectors	15,8451380 44,8358962

After sampling, the water and fish samples were submitted to a portable cooling device in the laboratory, where we performed preparation of samples for analysis. The analysis was performed by atomic absorption spectrophotometry. Analyses were performed on the atomic absorption spectrophotometer "Perkin Elmer" AAnalyst - 800.

Atomic absorption spectrophotometry is an optical method based on measuring the absorption of electromagnetic rays by atoms in the ground state. The atoms in the ground state disappear by thermal dissociation[14]. In atomic absorption, concentration can be determined from measurements of atoms light absorption in the ground state during irradiation with

an appropriate excitation source. The flame emission concentration can be determined from the intensity of the radiation which is emitted at a fraction of atoms that have passed in the excited state[15]. For the most routine analysis a flame temperature around 2400°C is required, which can be achieved by, for example mixtures of air - acetylene. The sample for analysis in liquid state is introduced into the flame by spray, resulting in a dispersion of fine liquid drops[14]. The analysis performed by atomic spectroscopic methods almost always requires simple and complex sample preparation. These steps of sample preparation are generally the most critical part of the analysis, because they are responsible for most errors[16]. In order to determine heavy metals, destruction of samples (fish muscle tissue) was done by microwave digestion in the mixture of nitric acid and hydrogen peroxide, in accordance with the instructions for handling apparatus of microwave digestion (Milestone, Sart D). In 1,5 g of sample, we added 7 ml of conc. HNO_3 and 1 ml of H_2O_2, and then we burnt it at 200 ° C, on 1000 W. The first heating was carried out for 10 minutes, then 10 minutes of burning, and finally the cooling process which showed the flow diagram on display of the oven. The conditions under which the analysis of heavy metals was conducted are shown in Table 2.

Table 2. Recommended conditions for analysis on AAS

Element	Flame	Wavelength	Burner	Calibration methods	Stock Stand. Solution
Pb	Air-acetylene	283,3 nm	10 cm	Linear / zero	Lead 1000 mg/l
Cu	Air-acetylene	324,8 nm	10 cm	Linear / zero	Cooper 1000mg/l
Cd	Air-acetylene	228,8 nm	10 cm	Linear / zero	Cadmium1000mg/l

RESULTS AND DISCUSSION

To determine the pollution degree of any part of the ecosystem including water, it is necessary to determine whether pollution can harmfully affect human health or to determine the limit value of the amount of harmful substances intake, in our case of heavy metals in water and fish of the River Una, under which, based on scientific research, there is no or the least possible risk of adverse effects on human health and/or the environment as a whole. The results are presented in Table 3,4,5 and 6.

Table 3. The results of the analysis of heavy metals in water of the River Una (September 2012)

Una locality	Ripač	Orljani	Bakšaiš	Vrkašić
Pb (mg/l)	0,0000	0,0000	0,0000	< 0,001
Cu (mg/l)	< 0,4	< 0,4	< 0,4	< 0,4
Cd (mg/l)	< 0,1	< 0,1	< 0,1	< 0,1

Table 4. The results of the analysis of heavy metals in fish of the River Una (September 2012)

Una locality	Ripač	Orljani	Bakšaiš	Vrkašić
Pb (mg kg^{-1})	0,0000	0,0013	0,0000	0,2050
Cu (mg kg^{-1})	0,8001	0,9090	1,1350	0,1280
Cd (mg kg^{-1})	< 0,1	< 0,1	< 0,1	< 0,1

The research results did not exceed in any sample MAC (maximum allowable concentrations). In water research samples of both periods, the values of Pb were identified only on location Vrkašić in the concentration of less than 0,001 mg/l. In both periods, the content of copper Cu in the River Una was less than 0,4 mg/l, while the value of Cd was below 0,1 mg/l.

Table 5. The results of heavy metals in water of the River Una (March 2013)

Una locality	Ripač	Orljani	Bakšaiš	Vrkašić
Pb (mg/l)	0,000	0,000	0,000	< 0,001
Cu (mg/l)	< 0,4	< 0,4	< 0,4	< 0,4
Cd (mg/l)	< 0,1	< 0,1	< 0,1	< 0,1

Table 6. The results of heavy metals in fish of the River Una (March 2013)

Una locality	Ripač	Orljani	Bakšaiš	Vrkašić
Pb (mg kg^{-1})	0,0000	0,0000	0,0000	0,1560
Cu (mg kg^{-1})	0,4010	0,5020	0,7630	0,0650
Cd (mg kg^{-1})	< 0,1	< 0,1	< 0,1	< 0,1

Vidaček et al.,[17] explored the content of heavy metals in Karašica-Vučica water basin. Concentration values of Cu results ranged from 0,0033 to 0,0213 mg/l, Pb from 0,0046 to 0,0405 mg/l, Cd from 0,0002 to 0,0039 mg/l, being consistent to our results. According to the Regulations on maximum levels for certain contaminants in food (Official Gazette of BiH 37/09)[18], the values of heavy metals Pb, Cu and Cd in fish muscle tissue samples did not exceed allowed concentrations. In trout muscle tissue samples (*Salmo trutta*) overfished in the period of September 2012, the content of Pb at the locality of Orljani (55,5 km from the source-over Bihać) was 0,0013 mg kg^{-1}, while the value at the locality of Vrkašić (62,5 km from the source below-Bihać-after collector) was increased to 0,2050 mg kg^{-1}. The determined amount of Cu in the muscle tissue of trout (*Salmo trutta*) ranged from 0,8001 mg kg^{-1}, the locality of Ripač (46,5 km from the source) to 1,1350 mg kg^{-1}, the locality of Bakšaiš (60,5 km from the source - the city of Bihać). The amount of Cd in all fish samples was less than 0,1 mg kg^{-1}.

In samples of muscle tissue of trout (*Salmo trutta*) fished in the period of March 2013, the value of Pb was slightly lower and was identified only at the locality of Vrkašić and was 0,1560 mg kg^{-1}. Determined Cu amount in the muscle tissue of fish ranged from 0,4010 mg kg^{-1} to 0,7630 mg kg^{-1}. The minimum value of copper was found at the locality of Orljani, while the largest at the locality of Bakšaiš. The amount of Cd in all samples of fish muscle tissue was less than 0,1 mg kg^{-1}. According to the research by Alić et al.[13] in the trout muscle tissue (*Salmo trutta*) harvested in the River Una, the average amount of Pb was 0,32 mg kg^{-1}, being higher when compared to our results; the amount of Cu in muscle tissue of trout (*Salmo trutta*) was 1,08 mg kg^{-1}, which is lower than the value we found on the locality; the value of Cd in the muscle tissue of trout (*Salmo trutta*) harvested in the River Una was 1,32 mg kg^{-1}, not being in accordance with our research and had a higher value.

CONCLUSION

During the periods of the years 2012 and 2013, the content of heavy metals in water and trout (*Salmo trutta*) of the River Una in observed locations did not exceed the maximum allowed concentrations of heavy metals (Official Gazette BiH37/09)[18].

- The analysis of heavy metals Pb, Cu and Cd in water and fish of the River Una showed that the content was higher downstream

from the town of Bihać compared to the content upstream from the town of Bihać.

- From the analysis, it can be seen that the heavy metal content in 2012 and 2013 was lower than the content of heavy metals determined in the year 2004[13].
- Our results indicate that metal concentrations were very low and that there was no significant pollution of watercourses of the River Una.

It is necessary to closely monitor concentrations of metals during a long period and immediately respond to any changes caused by anthropogenic influence. Only by determining precisely and systematic monitoring of the concentrations of trace metals, we can react on time and preserve this great treasure of ours.

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