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PROCEEDINGS

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(EDITORS)

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FOREWARD

The Balkan Scientific Conference of Biology is organized on the occasion of the 45th anniversary of the Faculty of Biology, University of Plovdiv.

The organizers of the conference wish to facilitate networking among Balkan scientists and exchange of thoughts and ideas in area of biology.

The colaboration among scientists from various Balkan universities shall contribute to our professional development, uniting our efforts in developing joint scientific projects and contracts.

All the papers and posters of eight sections of biology presented at the conference, appear in these two parts of the Proceedings. The editors have organized them into related topics.

The editors

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CONTENTS OF HEAVY METALS IN BONE AND MUSCLE OF FISH IN THE WATER OF THE RIVER BREGALNICA

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ABSTRACT. This study represents the results of research of the contents of some heavy metals (Pb, Zn, Cu, Cd and Fe) in bone and muscle of fish. The research has been done on four measuring points (Zletovo, Strmoš, Bučište and Žiganci). The determination of the contents of heavy metals in water and animal tissue was done by the standardized methods. The results have been statistically processed, looking for the correlation between the content of heavy metals in the water and in the analysed material. These measuring points differ in the degree of pollution, which was confirmed by our research. The results indicate a correlation between contents of heavy metals in water and analysed materials in relation to the measuring points and difference in the value in relation to the measuring points.
KEY WORDS: heavy metals, water, fish, contamination, river Zletovica, correlation

INTRODUCTION

The term "heavy metals", although not easily defined, is widely recognized, and used. It is commonly adopted as a group name for the metals and metalloids which are associated with pollution and toxicity, but also includes some elements which are essential for living organisms at low concentration.

"Toxic metals" is an alternative term to "heavy metals" but is rather emotive and applicable only to the non-essential elements, such as Pb, Cd, Hg, As, Tl and U; it is not appropriate for biologically essential elements, such as Co, Cu, Mn, Se and Zn. The term "trace metals" is becoming a popular preference to "heavy metals" but has

yet to attain such common usage. The widely recognized term "heavy metals" will therefore be retained in this paper.

"Pollution" is, in some ways, a term easier to define than "heavy metals", but it is commonly confused or used interchangeably with the term "contamination". Although several interpretations of the terms "pollution" and "contamination" exist, the definition given by Holdgate is widely accepted. This states that *pollution* is "the introduction by man into the environment of substances or energy liable to cause hazards to human health, harm to living resources and ecological systems, damage to structures or amenity, or interference with legitimate uses of the environment". Other definitions use the term "contamination" where the anthropogenic inputs do not appear to cause obvious harmful effects and "pollution" is applied only to situations where toxicity has occurred. However, this is unsatisfactory because the effects of the "contaminant" may not be fully understood at the time. In Holdgate's definition, "pollution" covers any concentration of a potentially harmful substance, whether or not adverse effects are observed. In practice, the term 'contamination' and 'pollution' are frequently used interchangeably, although pollution is usually more pejorative.

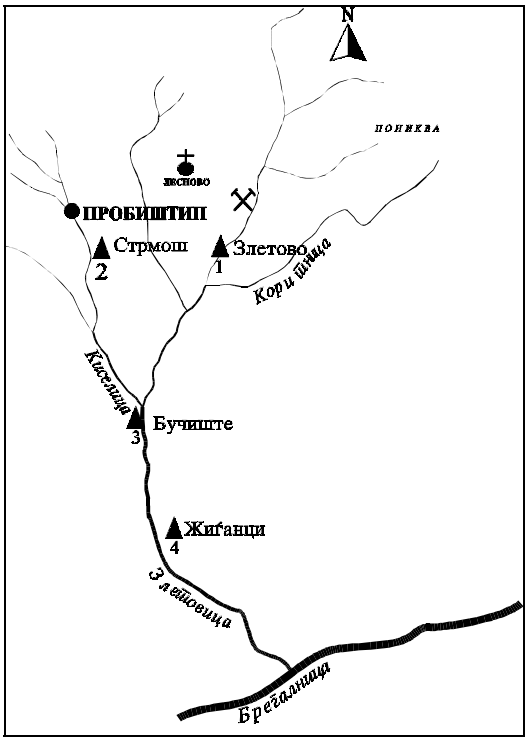

Figure 1. The location of measuring points

MATERIALS AND METHODS

The method used for determination of heavy metals in water is by Allen et al. (1986).

The water for analysis is first filtered and then 250 ml of it are taken out with a pipette. To this 250 ml we add 0.5 ml H_2SO_4. Samples prepared like this are taken to a sandy bath so that the water can evaporate until an ash gray, whitish sediment is obtained.

The sediment is dissolved with warm distilled water, transported quantitatively into Keldal's dish for combustion, then 2 ml of HNO_3 is added and 1 ml of $HClO_4$, and again it evaporates until it is dry. The sediment is again dissolved with warm distilled water, it is filtrated in a 100 ml dish, it is filled up to the mark with distilled water and is stirred.

The reading of the concentration of heavy metals in water is performed by an atomic absorber, type PERCIN ELMER – 5000.

The value read by the atomic absorber is divided by 2.5 in order to get the concentration of heavy metals in mg/l.

Method for determination of the contents of heavy metals in animal tissue

The examined tissue is measured on an analytical scale, then it is put in a porcelain bowl and onto a cooker until it is by gradual evaporation of water. The tissue prepared in this way is put into an oven at 470^{o}C, for 12 hours. Then it is taken out from the oven, it is cooled and the whitish ash is dissolved with 10% HCl. It should be transported carefully in a glassware of 25 ml which is filled with distilled water up to the mark. The reading of the heavy metals concentration is done by absorption spectrophotometer with respective wave length for each element.

RESULTS AND DISCUSSION

Table 1. Average annual values of the contents of heavy metals in the water of the river Zletovica [mg/l]

Measuring point	Lead (Pb)	Zinc (Zn)	Cadmium (Cd)	Copper (Cu)	Iron (Fe)
Zletovo	0.040	0.088	0.004	0.046	0.357
Strmoš	0.274	3.913	0.043	0.100	1.919
Bučište	0.687	0.923	0.028	0.062	0.668
Žiganci	0.190	0.254	0.023	0.056	0.360

Lead

In contaminated aquatic systems almost the whole of the lead is firmly connected to the sediment and only a small portion is dissolved in water. The reception and the accumulation of lead in aquatic organisms from water and sediment are under the influence of various external factors such as temperature, saltiness, pH-value etc. Lead is accumulated mostly in the liver, kidneys, bones and gills of fish. The organisms incorporate lead from the environment, usually in proportion to the level of contamination.

Table 2. Min, max and average annual values of the content of Pb (lead) in the bone and muscle of the fish [mg/kg] of fresh weight

Measuring point	bone			muscle		
	min	max	mean	min	max	mean
Zletovo	4.17	7.23	6.21	1.27	2.21	1.96
Strmoš	31.45	40.79	38.26	8.19	14.42	12.48
Bučište	28.05	36.18	32.19	4.79	8.23	7.96
Žiganci	23.70	31.74	29.04	3.48	6.98	5.75

The absolute minimal values of the content of lead in the bones of fish during the research period vary in the limits of 4.17 mg/kg fresh weight at the measuring point Zletovo as the relatively same environment, to 31.45 mg/kg in Strmoš, whereas the absolutely maximal values are from 7.23 mg/kg (Zletovo) to 40.79 mg/kg (Strmoš). The average annual values of the content of lead in fish bones vary from 6.21 mg/kg (Zletovo) to 38.26 mg/kg (Strmoš).

The situation is similar in fish muscles where the lowest minimal (1.27 mg/kg), the maximal (8.19 mg/kg) and the lowest average annual values (1.96 mg/kg) of lead content in fish muscles were measured at the measuring point Zletovo which is also the place where the lead content is the lowest (0.041 mg/l), i.e. lower than MCL for the I and II category of water. The highest absolutely maximal value was measured at the measuring point Strmoš (12.48 mg/kg), and relatively high average annual values were measured at measuring points Strmoš (12.48 mg/kg) and Bučište (7.96 mg/kg) where the lead content in the water is higher than 0.100 mg/l and is above MCL for III and IV category of water (Table 1).

Zinc

Zinc belongs to the group of heavy metals which are of essential importance for the living organisms. It is contained in some enzymes e.g. carboanhydrase, and in some other enzymes like enolase, peptidase etc. it represents an activator.

Table 3. Min, max and average annual values of the content of (Zn) zinc in the bone and muscle of the fish [mg/kg] of fresh weight

Measuring point	bone			muscle		
	min	max	mean	min	max	mean
Zletovo	28.43	42.12	39.91	5.14	8.96	7.26
Strmoš	104.73	138.81	123.45	22.04	29.19	25.42
Bučište	76.22	89.93	84.26	12.24	18.91	16.23
Žiganci	58.14	71.33	64.36	10.52	18.45	14.96

The absolute minimal values of the content of zinc in the bones of fish during the research period vary in the limits of 28.43 mg/kg fresh weight at the measuring point Zletovo as the relatively same environment, to 104.73 mg/kg in Strmoš, while the absolutely maximal values are from 42.12 mg/kg (Zletovo) to 138.81 mg/kg (Strmoš). The average annual values of the content of zinc in fish bones vary from 39.91 mg/kg (Zletovo) to 123.45 mg/kg (Strmoš).

The situation is similar in fish muscles where the lowest minimal (5.14 mg/kg), the maximal (8.96 mg/kg) and the lowest average annual values (7.26 mg/kg) of zinc content in fish muscles were measured at the measuring point Zletovo which is also the place where the zinc content is the lowest (0.088 mg/l), i.e. lower than MCL for the I and II category of water. The highest absolutely maximal value was measured at the measuring point Strmoš (29.19 mg/kg), and relatively high average annual values were measured at measuring points Strmos (25.42 mg/kg) and Bučište (16.23 mg/kg)

where the zinc content in the water is higher than 3.913 mg/l and is above MCL for III and IV category of water (Table 1).

Cadmium

Cadmium belongs to the group of heavy toxic metals. Its mechanism of action is based on the high affinity to SH-groups disrupting the synthesis of nucleic acids and the processes of oxidative phosphorilation. It inhibits the effect of the alkaline phosphatase and numerous enzymes that contain SH-groups.

Table 4. Min, max and average annual values of the content of (Cd) cadmium in the bone and muscle of the fish [mg/kg] of fresh weight

Measuring point	bone			muscle		
	min	max	mean	min	max	mean
Zletovo	0.13	0.34	0.24	0.024	0.072	0.056
Strmoš	2.78	5.92	4.28	1.43	3.45	2.16
Bučište	1.23	2.74	1.92	0.36	0.92	0.61
Žiganci	0.78	1.86	1.02	0.19	0.68	0.48

The absolute minimal values of the content of cadmium in the bones of fish during the research period vary in the limits of 0.13 mg/kg fresh weight at the measuring point Zletovo as the relatively same environment, to 2.78 mg/kg in Strmoš, while the absolutely maximal values are from 0.34 mg/kg (Zletovo) to 5.92 mg/kg (Strmoš). The average annual values of the content of cadmium in fish bones vary from 0.24 mg/kg (Zletovo) to 4.28 mg/kg (Strmoš).

The situation is similar in fish muscles where the lowest minimal (0.024 mg/kg), the maximal (0.072 mg/kg) and the lowest average annual values (0.056 mg/kg) of cadmium content in fish muscles were measured at the measuring point Zletovo which is also the place where the cadmium content is the lowest (0.004 mg/l), i.e. lower than MCL for the I and II category of water. The highest absolutely maximal value was measured at the measuring point Strmos (3.45 mg/kg), and relatively high average annual values were measured at measuring points Strmoš (2.16 mg/kg) and Bučište (0.61 mg/kg) where the cadmium content in the water is higher than 0.043 mg/l and is above MCL for III and IV category of water (Table 1).

Copper

Copper belongs to the group of essential microelements incorporated into a number of metalloenzymes. It is distributed throughout the body, but it is mainly deposited in the liver, brain tissue and blood.

In our research of the content of copper in bones and muscles of fish living in the waters of the river Zletovica and its tributaries, it has been concluded that values in bones are higher. The result values concerning respective sites of research are similar to the rest of the elements.

The absolute minimal values of the content of copper in the bones of fish during the research period vary in the limits of 1.56 mg/kg fresh weight at the measuring point Zletovo as the relatively same environment, to 14.23 mg/kg in Strmos, while the absolutely maximal values are from 3.23 mg/kg (Zletovo) to 26.74 mg/kg (Strmoš). The average annual values of the content of copper in fish bones vary from 2.77 mg/kg (Zletovo) to 19.48 mg/kg (Strmoš).

Table 5. Min, max and average annual values of the content of (Cu) copper in the bone and muscle of the fish [mg/kg] of fresh weight

Measuring point	bone			muscle		
	min	max	mean	min	max	mean
Zletovo	1.56	3.23	2.77	1.04	1.98	1.36
Strmoš	14.23	26.70	19.48	4.72	8.43	6.22
Bučište	11.78	18.14	14.49	1.96	5.14	3.98
Žiganci	8.73	14.26	11.04	1.13	2.76	1.73

The situation is similar in fish muscles where the lowest minimal (1.04 mg/kg), the maximal (1.98 mg/kg) and the lowest average annual values (1.36 mg/kg) of copper content in fish muscles were measured at the measuring point Zletovo which is also the place where the copper content is the lowest (0.046 mg/l), i.e. lower than MCL for the I and II category of water. The highest absolutely maximal value was measured at the measuring point Strmoš (8.43 mg/kg), and relatively high average annual values were measured at measuring points Strmoš (6.22 mg/kg) and Bučište (3.98 mg/kg) where the copper content in the water is higher than 0.100 mg/l and is above MCL for III and IV category of water (Table 1).

Iron

Iron as a microelement has a considerable physiological role for animal organisms. It is found mostly in red blood cells as a blood pigment - hemoglobin. It is also contained in myoglobin which has a structure similar to the structure of hemoglobin. One part of iron is deposited in the liver in the form of albumen - ferritin.

Table 6. Min, max and average annual values of the content of (Fe) iron in the bone and muscle of the fish [mg/kg] of fresh weight

Measuring point	bone			muscle		
	min	max	mean	min	max	mean
Zletovo	16.21	25.68	22.78	5.74	12.18	9.26
Strmoš	37.43	48.19	42.58	7.94	15.49	12.48
Bučište	34.14	47.26	40.93	5.13	9.36	7.92
Žiganci	21.58	33.45	29.73	6.18	14.78	10.14

The absolute minimal values of the content of iron in the bones of fish during the research period vary in the limits of 16.21 mg/kg fresh weight at the measuring point Zletovo as the relatively same environment, to 37.43 mg/kg in Strmoš, while the absolutely maximal values are from 25.68 mg/kg (Zletovo) to 48.19 mg/kg

(Strmoš). The average annual values of the content of iron in fish bones vary from 22.78 mg/kg (Zletovo) to 42.58 mg/kg (Strmoš).

The situation is similar in fish muscles where the lowest minimal (5.13 mg/kg), the maximal (9.36 mg/kg) and the lowest average annual values (7.92 mg/kg) of iron content in fish muscles were measured at the measuring point Bučište. The highest absolutely maximal value was measured at the measuring point Strmoš (15.49 mg/kg), and relatively high average annual values were measured at measuring points Strmoš (12.48 mg/kg) and Ziganci (7.92 mg/kg) where the iron content in the water is higher than 1.919 mg/l and is above MCL for III and IV category of water (Table 1).

CONCLUSIONS

The content of lead in the waters of the river Zletovica is in correlation to the content of lead in the bone and muscle of fish with correlations coefficient $\rho = 0.71$ and $\rho = 0.72$ respectively.

The content of zinc in the waters of the river Zletovica is in correlation to the content of zinc in the bone and muscle of fish with correlations coefficient $\rho = 0.80$ and $\rho = 0.71$.

The content of cadmium in the waters of the river Zletovica is in correlation to the content of cadmium in the bone and muscle of fish with correlations coefficient $\rho = 0.80$ and $\rho = 0.83$.

The content of copper in the waters of river Zletovica is in correlation to the content of copper in the bone and muscle of fish with correlations coefficient $\rho = 0.45$ and $\rho = 0.54$.

The content of iron in the water of the river Zletovica is not in correlation to the content of iron in the bone and muscle of fish with correlations coefficient $\rho = 0.10$ and $\rho = 0.19$.

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