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Heavy Metal Concentrations in Predator Fish

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Abstract: Waters can be polluted by heavy metals which are accumulated and concentrated by fish therefore they show the degree of environmental pollution. The aim of this study was to determine concentrations of heavy metals in water, mud and fish organs to determine whether these concentrations are allowed and in accordance with normative provisions and considering the pollution by heavy metals if the fish meat is hygienically safe food of animal origin. Concentrations of heavy metals (lead, chromium, manganese, iron, copper and zinc) were determined in water, mud and different organs (liver, kidney, intestine, milt and skin+muscle) of pike (*Esox lucius*) and European catfish (*Silurus glanis*) by Energy Dispersive X-Ray Fluorescence method (EDXRF). Statistically significant difference was determined between the concentrations of heavy metals in mud and water ($p<0.05$) as well as in fish organs ($p<0.05$). The obtained results show that the highest concentrations of heavy metals were determined in liver and the lowest ones in skin and muscle i.e., in edible fish parts. In accordance with normative regulations of the European Union and the Republic of Croatia, the determined values are lower than the maximally allowed concentrations of heavy metals in fish muscle. When the pollution by heavy metals is taken into account, it indicates that the researched fish meat is hygienically safe food of animal origin.

Key words: Heavy metals, pike (*Esox lucius*), European catfish (*Silurus glanis*), water, mud, Croatia

INTRODUCTION

Water pollution is the consequence of natural causes as well as of a range of anthropological activities. It is manifested through the change in basic indicators of water quality and they are microbiological and physicochemical including heavy metal concentrations. A lot of data mainly obtained based on quality control and biomonitoring of waters and organisms living in them, fish among others also confirm that (Rashed, 2001).

Small quantities of harmful substances don't necessarily cause perishing but after being consumed, they can be cause of fish diseases. Small quantities of harmful substances, heavy metals among them can make fish as the food of animal origin harmful to human health because of the capacity of accumulating in tissues and their bioaccessibility (Odzak *et al*., 2000). Fish meat is rich in easily digestible proteins which makes it especially valuable food of animal origin. Heavy metals enter water systems from the atmosphere by erosions of geological substances or anthropologic activity (Alam *et al*., 2002). The most frequent sources of heavy metal pollution originate from the industry, traffic and utility waste and they can also originate from some herbicides. Heavy metals are dangerous for living organisms because of their perseverance, high toxicity and their tendency of accumulation in ecosystems.

They aren't decomposed in water or detoxified by metabolic processes. Metal toxicity is greatly affected by physicochemical characteristics of water. Firstly, these are pH value, concentration of diluted oxygen, concentration of magnesium and potassium ions and others.

Lead enters surface waters from mines, lead industry, lead alloys and lead compounds. It mostly accumulates on the bottom of maritime zones in sediments where the concentration of lead is of about four times higher than in water.

Lead toxicity for fish depends on water quality i.e., on the presence of calcium and magnesium ions. Liquefy ability of lead compounds decreases by the increase of alkalinity i.e., pH value of water. Lead is accumulated at

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the bottom of fish ponds from lead shotgun pellets remained after wildfowling. Iron in water appears most frequently in the form of colorless iron Hydroxycarbonate Fe $(HCO_3)_2$ which rapidly transforms to brown iron hydroxide Fe $(OH)_3$ by the activity of oxygen from the air and it makes water yellowish. Iron is melted similar to sugar and starch in water with pH value around 7. Such iron is harmful so there must n't be >0.1 mg L^{-1} of it in drinking water.

The same quantity applies to fish pond waters as well. If yellowish-brown deposits of iron oxide are accumulated on gills or fish roe, losses are possible.

Heavy metals enter a fish organism by food (zooplankton, phytoplankton and fauna of the bottom) or in a larger degree by the gills and skin so they can be bioaccumulated. Fish assimilate the metals by digestion, ion exchange of dissolved metals across lipophilic membranes and by adsorption on tissues and membrane surfaces. Bioaccumulation and pollution by metals causes irregularities in physiological processes in fish depending on concentration of certain metals in tissues (Alam *et al*., 2002).

Rashed (2001) determined that fish accumulate and concentrate heavy metals from sediments, water plants and water whereas Novoselic and Bogut and Pavlicevic determined that predator fish contain a larger quantity of contaminators in comparison to herbivores and herbivores in comparison to plants. Predator fish, pike (*Esox lucius*) and European catfish (*Silurus glanis*) are at the top of food chain in ecosystem of fish pond so concentrations of heavy metals in organs had to be determined, considering the possibility of their accumulation from the food.

Organs of predator fish which are fed on smaller water organisms, especially on juvenile fish species were examined. Since juvenile fish species couldn't accumulate heavy metals in a short period of time, it can be assumed that neither the researched fish could accumulate heavy metals from food in a larger quantity. Accumulation of heavy metals from water is possible.

A possibility of their accumulation from mud is especially important for European catfish (*Silurus glanis*) since it is a fish of a river bed which spends most of its life near to mud. By determining concentrations of heavy metals in certain fish tissues or organs and their analysis, health and hygiene safety of fish is determined. A degree of water pollution by heavy metals is noticed at the same time.

The data were examined to determine if the were significant differences in concentration of metals in fish organs compared to heavy metal concentrations determined in water and sediments.

MATERIALS AND METHODS

An approximate quantity of 1 kg of mud and 1 L of water were taken at regular fish catch for consumption needs in autumn, at carp fish pond located in the continental part of the Republic of Croatia. That fish pond supplies with water several currents which spring in forested area and flow through the area without intensive agricultural production. Per 10 pikes (*E. lucius*) and European catfish (*S. glanis*) were chosen among the fishes in transport pools which originated from the same fish pond as water and mud.

Fishes were of about 3 years of age and caught to be sold on the market for consumption needs. After determining their mass, soft tissue samples of particular organs (liver, kidney, intestine, spleen and skin+muscle) were dried at 80°C until their constant mass was achieved and then they were pulverized for the analysis as thick targets. About 2 g of sample were pushed into a disc of a 2 cm diameter. Thick targets prepared in the described way were analyzed by the EDXRF method (Jenkins *et al*., 1981; Orescanin *et al*., 2001).

The obtained results were recalculated to wet mass by calculating the difference of the dry matter mass and wet matter mass, in order to calculate the loss of wet mass (Orescanin *et al*., 2002). Sample of mud were prepared for EDXRF in the same way as the fish organs' samples. Water sample was filtrated through Millipore filter (0.45 μm pore size) in order to separate suspended particles. To analyze concentrations of lead, copper, zinc and iron in 100 mL of filtrate with the addition of hydrochloric acid and ammonium hydroxide, pH value was adjusted to pH = 3 (Orescanin *et al*., 2003).

In the other part of 100 mL for the analysis of chromium and manganese, pH value was adjusted to pH 11. Mettler Toledo digital pH meter was used for determining pH values. After adjusting pH value, each sample (in the bottle) was added 1% of pyrrolidine dithiocarbamate-APDC per 2 mL. Suspensions were filtrated through Millipore HAWP filter (0.45 μm pore size, diameter = 25 mm) after 20 min.

Millipore micro filtration system was used for filtration. A filter with sediment was air dried and protected with thin Mylar foil (2 μm), inserted in plastic housing and placed above the X-ray source in a spectrometer. Concentrations of metals (lead, chromium, manganese, iron, copper and zinc) in all the samples were analyzed by EDXRF method (Orescanin *et al*., 2004). Prepared targets were given impetus to emit a characteristic X-ray radiation by radioactive sources. We used ^{109}Cd as the source of X-rays. Prepared samples in

plastic housings were put directly on the source and after that we put them on a detector together with the source. A 2 μm thick Mylar foil was put between the sample and source and between the source and the detector. In order to notice X-rays which appear as the consequence of a characteristic X-radiation emission from the sample used semiconductor Si (Li) detector of Canberra type. To decrease the sound and improve energy resolution, the detector was cooled by liquid nitrogen. Active surface of the detector is 30 mm^2, its active diameter is 6.2 mm, thickness is 3 mm with 25 μm thick be window and a resolution of 165 eV on 5.9 keV (^{55}Fe).

A semiconductor Si (Li) detector was turned on 500 V. Electrical impulse appears as the interaction consequence of X-rays with the material of the detector. It is transmitted from a preamplifier to an amplifier which improves the proportion of characteristic lines and levels of forests. Genie Software-2000 (Canberra, Meriden, CT and USA) was used for spectrum collection. The time for counting was 40000 sec for samples of fish tissue, 7000 sec for samples of mud and 20000 sec for water samples. The data obtained by counting were analyzed by using WinAxil software, version 4.5.2. (Canberra, Eurisys Benelux and Belgium).

Calibration form for spectrum adjustment and quantitative and qualitative analysis was created based on the results of measuring standard reference material prepared of fish tissue (IAEA-MA-2-TM), lake sediment for a sample of mud (AEAI SL-1) and Merck standard solution for water sample. Standards were prepared and analyzed in the same way as unknown samples. Statistical processing of elements' results was performed by STATISTICA 8 program. Significance of differences between the mean element values was tested by using the One way ANOVA test.

RESULTS AND DISCUSSION

Concentrations of examined heavy metals were from 21 mg kg^{-1} for copper to 13220 mg kg^{-1} for iron in sample of mud then from 5 mg kg^{-1} for manganese to 116 mg kg^{-1} for iron in water sample (Table 1). Mean values of heavy metals concentrations (mg kg^{-1}) determined in samples of organs (liver, kidney, intestine, spleen, skin+muscle) of pike (*E. lucius*) are shown in Table 2 and shown in Fig. 1-5. It is obvious that mean values of all the researched elements were lowest in skin+muscle except for the concentration of manganese. Mean values of heavy metals concentrations in samples of organs (liver, kidney, intestine, spleen and skin+muscle) of European catfish (*S. glanis*) are shown in Table 3 and Fig. 1-5. The lowest concentrations in this case were determined in skin and muscle for zinc, iron and manganese.

Table 1: Mean value of heavy metal concentrations in samples of mud and water

	Concentrations of heavy metals (mg kg^{-1})	
Element (mg kg^{-1})	Mud	Water
Lead	22.0	9.5
Zinc	102.0	10.0
Copper	21.0	9.5
Iron	13220.0	116.0
Manganese	360.0	5.0
Chromium	48.5	13.0

Table 2: Mean values (±SD) of heavy metal concentrations determined in organs of pike (*E. lucius*)

	Concentrations of heavy metals (mg kg^{-1})					
Organs (n = 10)	Lead	Zinc	Copper	Iron	Manganese	Chromium
Liver	0.24	10.25	1.19	44.80	4.43	0.47
Kidney	0.12	33.65	0.52	22.62	2.72	0.38
Intestine	0.14	193.86	0.50	12.57	1.71	0.39
Spleen	0.10	19.14	0.35	73.71	0.77	0.29
Skin+muscle	0.09	5.24	0.30	4.98	0.92	0.18

Table 3: Mean values (±SD) of heavy metal concentrations determined in organ samples of European catfish (*S. glanis*)

	Concentrations of heavy metals (mg kg^{-1})					
Organs (n = 10)	Lead	Zinc	Copper	Iron	Manganese	Chromium
Liver	0.16	5.75	0.83	59.44	1.48	0.22
Kidney	0.12	6.33	0.39	33.00	1.06	0.16
Intestine	0.15	6.22	0.50	12.30	1.25	0.21
Spleen	0.10	5.17	0.31	63.20	1.09	0.27
Skin+muscle	0.19	4.50	0.50	6.77	0.89	0.23

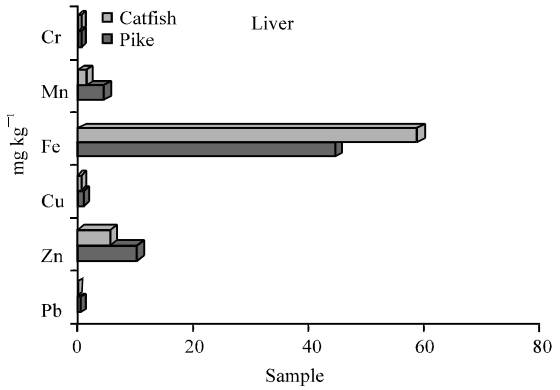

Fig. 1: Mean value of heavy metals concentrations (mg kg^{-1}) in liver samples of examined fish

Consistently with the results of the One way ANOVA test, a statistically significant difference was determined between concentrations of heavy metals in mud and water ($p<0.05$) and in fish organs ($p<0.05$) (mean value of each element in all the examined organs). Also, a statistically significant difference was determinated

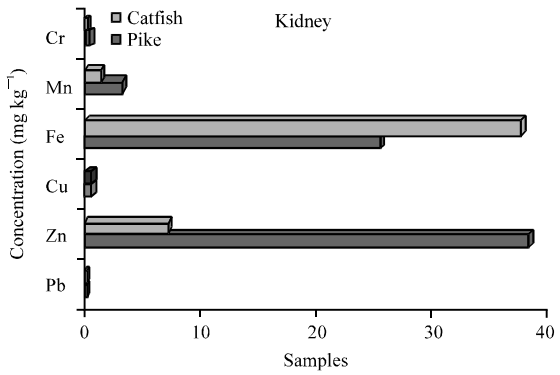

Fig. 2: Mean value of heavy metals concentrations (mg kg^{-1}) in kidney samples of examined fish

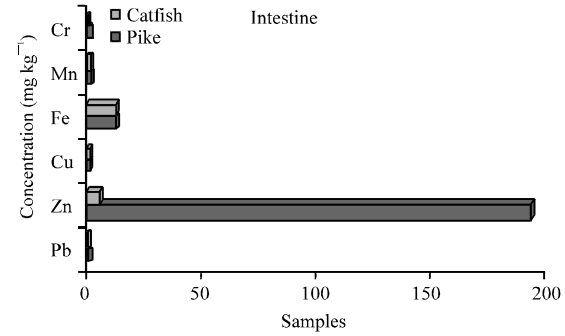

Fig. 3: Mean value of heavy metals concentrations (mg kg^{-1}) in intestine samples of examined fish

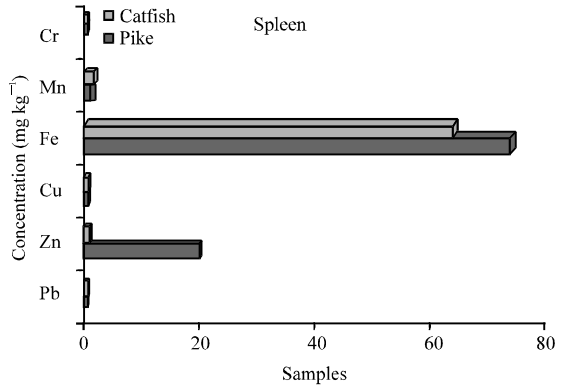

Fig. 4: Mean value of heavy metals concentrations (mg kg^{-1}) in spleen samples of examined fish

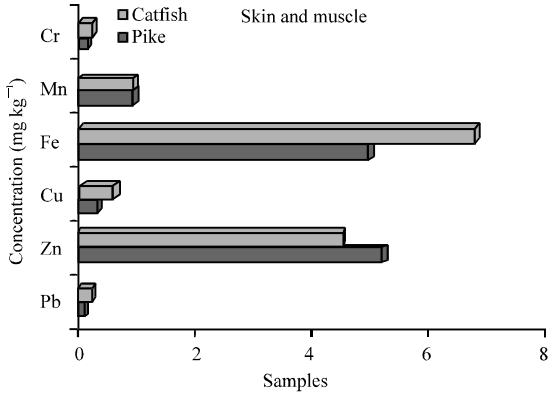

Fig. 5: Mean value of heavy metals concentrations (mg kg^{-1}) in skin and muscle of examined fish samples

between fish organs of pike and European catfish ($p<0.05$) for chromium. While the highest concentrations of several elements were determined in the liver of both pike (*E. lucius*) and European catfish (*S. glanis*) (copper and manganese), the lowest values were determined in skin and muscle i.e., edible fish parts are especially valuable (except for manganese in pike (*E. lucius*) and lead, copper and chromium in European catfish (*S. glanis*)).

It was proved in previous study that heavy metals enter water systems (Alam *et al*., 2002) and fish accumulate and concentrate heavy metals thus showing environmental pollution. At the same time, they are especially valuable foodstuff of animal origin in human nutrition. Therefore, it was important to determine concentrations of heavy metals in environment, water and mud and in fish. In this study were determinated concentrations of 6 elements and some of them are important considering their maximally allowed concentrations in fish meat for human nutrition. Statistically significant difference in conducted analyses was determined between the concentrations of heavy metals in mud and water ($p<0.05$) and in fish organs ($p<0.05$). Since concentrations of researched heavy metals are significantly higher in water and mud from those in fish organs, the findings confirm the findings of Rashed (2001) that fish accumulate and concentrate heavy metals from mud (water plants) and water.

All the obtained results show that the lowest concentrations of heavy metals were determined in skin and muscle of fish (except for the concentration of lead and chromium in European catfish (*S. glanis*) which confirms the finding of Karadede *et al*. (1997) that these organs are not active in heavy metals accumulation. Heavy metals are mostly accumulated by metabolically active organs like liver which stores metals for detoxification by producing metalloids (Carpene and Vasak, 1989) and high tolerance threshold is attributed to storing metals in liver lysosomes where they become harmless (Goldfischer *et al*., 1970). The results of the researches also show that the highest concentrations of the most researched heavy metals in liver samples. All the obtained results are shown in tables to make an easy reference and comparison. Maximally allowed value for

lead in fish muscle is 0.2 mg kg^{-1} of wet mass by an international regulation (European Commission, 2006). In the Republic of Croatia, maximally allowed value is also 0.2 mg kg^{-1}, consistently with the Regulation on revisions on quantities of pesticides, toxins, mycotoxins, metals and histamines and similar substances which can be found in foodstuffs and on other conditions regarding health safety of foodstuffs and items of general use.

The findings of the lowest concentrations of heavy metals and by that concentration of lead in muscle and skin, the edible parts of both pike (*E. lucius*) and European catfish (*S. glanis*) show that the determined concentrations are lower than the maximally allowed. In regards to the contamination by heavy metals, fish is a safe foodstuff in health and hygiene terms.

CONCLUSION

By determining concentrations of heavy metals by EDXRF method in water, mud and organs of pike (*E. lucius*) and European catfish (*S. glanis*) from a carp fish-pond located in the continental part of the Republic of Croatia, a statistically significant relation was determined between concentrations of heavy metals in mud and water ($p<0.01$) and in fish organs ($p<0.05$). The obtained results show that the highest concentrations of the most of the researched heavy metals were determined in liver and the lowest in skin and muscle of fish. The determined values for lead in skin and muscle are lower than the maximally allowed concentration of lead in fish muscle (European Commission, 2006).

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