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Heavy Metals in Mud, Water and Cultivated Grass Carp (*Ctenopharyngodon idella*) and Bighead Carp *(Hypophthalmichthys molitrix)* from Croatia

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Abstract: The fish meat is valuable foodstuff of animal source. Environment pollution also water pollution is dangerous for the health and life of organisms which live in it. Water among others can be polluted with heavy metals so, fish from that water can be used as the monitoring organisms for degree of environment pollution with heavy metals. The concentrations of heavy metals higher from allowed can make the fish as animal source, dangerous and harmful for human health. Concentrations of heavy metals (Pb, Cr, Mn, Zn, Cu and Fe) were measured in different organs (liver, kidney, intestine, spleen and skin + muscle) of grass carp (*Ctenopharyngodon idella*) and bighead carp (*Hypophthalmichthys molitrix*) and in mud and water from fish farm situated in the continental part of Republic of Croatia. All measurements were carried out by the fluorescence roentgen spectroscopy that uses energy dispersion (Energy Dispersive X-ray Fluorescence method (EDXRF). Significant difference was found between mud, water and fish organ composition ($p<0.05$) and those between fish tissue for lead ($p<0.01$) and manganese ($p<0.05$).

Key words: Grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypophthalmichthys molitrix*), mud, water, heavy metals, EDXRF

INTRODUCTION

Heavy metals enter aquatic systems by geological substance erosions from the atmosphere or by anthropological activity. Fish represents useful organisms for environmental pollution monitoring because they are able to accumulate chemical substances in the tissues where they are bioavailable (Odzak *et al*., 2000). The biological value and high digestibility of fish protein puts fish and fish meat in an increasingly significant place in human nutrition. Analysis of fish tissue for heavy metal content determines the health and hygienic safety of fish as an animal foodstuff and at the same time provides insight to pollution of water by heavy metals. Life in water depends on the correlation between fish and water. Considering the amount of harmful substances in water, they do not necessarily need to cause fish to die but they may make fish as an animal substance harmful to human health. Heavy metals are dangerous for living organisms due to their high toxicity, sustainability and tendency to accumulate in the ecosystem. They break down slowly in water and to not detoxify by metabolic processes. Heavy metals mainly enter fish organisms through the gills and skin and by food to a minor extent. Lead accumulates at the bottom of aquatic regions where the lead concentration is around four times greater than in water. It enters surface waters from mines and the lead, lead alloy and lead compound industries. The toxicity of lead to fish depends on the content of calcium and magnesium ions. The solubility of lead compounds is reversely proportional to the increase of water alkalinity (Novoselic, 2006).

The energy of X-rays enables identification of elements and the intensity of the spectrum lines enables determination of element concentrations in the sample (Valkovic, 1980, 1983, 1989; Jenkins *et al*., 1981) so, the heavy metal concentrations were determined by using the Energy Dispersive X-ray Fluorescence method (EDXRF). The objective of this research was to determine the heavy metal concentrations in samples of water, mud and organs of fish and to determine based on the results whether the heavy metal concentrations in edible parts of the analyzed are within the allowed limits as required by the standardization documents in the Republic of Croatia (RH) and the European Union (EU).

MATERIALS AND METHODS

Sampling and preparation for X-ray fluorescence spectroscopy: The samples of fish, grass carp (*Ctenopharyngodon idella*) and silver carp

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(*Hypophthalmichthys molitrix*) and the samples of water and mud were collected to determine the heavy metal concentrations by using the EDXRF method. Analyzed samples originated from a fish pond located in the continental part of Republic of Croatia where water flows supplying the pond run through an area without intensive agricultural production. The fish were previously caught for consumption purposes and prepared for transport to the marketplace. About 10 grass carps weighed 3.8 kg on the average and 10 silver carps weighed 4.10 kg on the average. Fish were 3 years old, approximately. From the pond where the fish were caught, 1 kg of mud and 1 L of water were taken.

In the lab, the samples of soft tissue of different organs (liver, kidney, intestine, spleen and skin + muscle) were dried at 80°C until they reached a constant mass. The pulverized solids were obtained to prepare thick targets and pressed around 2 g of the sample into a disc having a diameter of 2 cm for further analysis. The mud samples were dried at 80°C, pulverized and 2 g of the sample was pushed into a disc having a diameter of 2 cm to make a thick target for further analysis. The samples of water were filtered thought a Millipore filter to separate the deposit (Orescanin *et al*., 2001). The filtrate was split into two bottles. By adding HCl and aluminum hydroxide, the pH was adjusted to 3 in 1 part and to 11 in the other (Orescanin *et al*., 2003). For all measurements a digital Mettler Toledo pH was used. In each bottle 2 mL 1% (w/v) of Pyrrolidine Dithiocarbonate (APDC) was added and filtered through a Milipore HAWP filter after 20 min of complexation and dried the thick targets prepared in the air, protected by thin Mylar foil. The concentrations of metals (Pb, Cr, Mn, Zn, Cu and Fe) in all samples were analyzed by the EDXRF method (Orescanin *et al*., 2004). 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).

Excitation to emission of characteristics X-rays and recording: The targets prepared to emission of characteristic X-rays were excited by using a radioactive source. ^{109}Cd was used as the X-ray source. The samples were inserted directly onto the source in a polyethylene box and then all together on a detector. Between the samples, the source and the detector 2 μm Mylar foils were placed. A Canberra-type semiconductor Si (Li) detector was used to register the X-rays created as a result of the emission of characteristic X-rays from the sample. The detector was cooled by liquid nitrogen to reduce the noise and improve the energy resolution. The active surface of the detector is 30 mm^2, active diameter is 6.2 mm and it is 3 mm thick with a 25 μm thick Be-window and a resolution of 165 eV per 5.9 keV (^{55}Fe). The semiconductor Si (Li) detector was connected to 500 V.

The preamplifier conveys the current impulse occurring as a result of the interaction between the X-rays and the detector material to the amplifier improving the ratio between the characteristic lines and the noise level. We used Software Genie-2000 (Canberra, Meridon, CT, USA) to collect the spectrum. The counting time was 40,000 sec for the fish tissue samples, 7,000 sec for the mud samples and 20,000 sec for the water samples. The datas obtained by counting were analyzed by using the WinAxil software, version 4.5.2. (Canberra, Eurisys Benelux, Belgium). The calibration pattern for spectrum adjustment and quantitative and qualitative analyses was created on the basis of the result of measurement of standard reference material prepared from fish tissue (IAEA-MA-2-TM), sediment for the sludge samples (IAEA SL-1) and standard Merck solution for the water samples. The standards were prepared and analyzed in the same manner applied to the analyzed samples.

Statistical processing: We conducted the statistical processing of the obtained results on element concentrations by using the Statistica 8 software and we tested the statistical significance of the differences between the mean element values based on a $p<0.05$.

RESULTS AND DISCUSSION

The range values of the heavy metal concentrations expressed in mg kg^{-1} determined in soft tissue samples from organs (liver, kidney, intestine, spleen and skin + muscle) of grass carp and silver carp originating from a fish pond in the continental part of Croatia are provided in Table 1 and 2. A statistically significant difference was determined for concentrations of lead ($p<0.01$) and manganese ($p<0.05$) in soft tissues of grass carp and silver carp organs.

The heavy metal concentrations determined in mud and water samples originating from the same pond are shown in Table 3. A statistically significant difference between heavy metal concentrations was determined for mud, water and fish organs ($p<0.05$).

In this study, we analyzed the content and concentrations of heavy metals in soft tissues of two herbivorous cyprinid fish (grass carp and silver carp) and water and mud from a fish pond located in the continental part of Croatia. The fish were caught for consumption purposes. The meat of the analyzed fish species, grass carp and silver carp are particularly valued animal foodstuffs due to their easy digestibility and a large amount of Ω3 fatty acids.

Rashed (2001) determined that fish accumulate and concentrate heavy metals from mud, aquatic plants and water. Accumulated heavy metals, especially in amounts in excess of the allowed amounts, make parts of fish, i.e., an animal foodstuff hygienically unsafe and harmful to

Table 1: Heavy metal range concentration determined in organ samples of grass carp (*Ctenepharyngodon idella*)

	Heavy metal range concentration values (mg kg^{-1}) (*Ctenepharyngodon idella*)					
Organs	Pb	Cr	Mn	Zn	Cu	Fe
Liver	0.24-0.68	0.36-0.760	9.49-8.320	174.51-31.24	2.54-4.61	54.10-73.18
Kidney	0.11-0.29	0.19-0.620	3.61-3.710	4.58-17.92	0.39-0.98	24.31-80.12
Intestine	0.32-0.52	0.34-25.48	6.67-6.990	12.23-16.98	1.03-1.27	24.33-27.21
Spleen	0.21-0.49	0.37-64.09	10.26-10.19	6.18-9.340	0.56-0.61	76.15-82.13
Skin + muscle	0.20-0.29	0.13-0.190	2.41-3.170	2.97-3.790	0.34-0.51	4.93-6.110

Table 2: Heavy metal range concentration determined in organ samples of silver carp (*Hypophthalmichthys molitrix*)

	Heavy metal range concentration values (mg kg^{-1}) (*Hypophthalmichthys molitrix*)					
Organs	Pb	Cr	Mn	Zn	Cu	Fe
Liver	0.14-0.27	0.35-0.740	3.46-5.49	11.51-8.86	0.47-0.92	61.42-63.76
Kidney	0.12-0.16	0.32-0.420	1.49-4.36	4.90-7.34	0.52-0.56	46.59-55.22
Intestine	0.14-0.18	0.47-0.580	1.17-1.02	7.02-7.12	0.68-0.72	23.13-27.92
Spleen	0.11-0.14	0.36-0.680	1.49-1.57	5.15-6.21	0.52-0.57	64.09-67.34
Skin + muscle	0.19-0.20	0.30-10.14	1.51-1.67	3.02-3.11	0.54-0.56	11.14-11.27

Table 3: Mean values of heavy mental concentrations in mud and water samples from a fish pond located inthe continental part of Croatia

	Concentration (mg kg^{-1})	
Elements	Mud	Water
Pb	29.60	12.66
Cr	56.00	13.66
Mn	463.33	6.66
Zn	79.00	17.00
Cu	28.00	13.30
Fe	17613.33	150.00

human health. As grass carp feeds on higher aquatic plants and silver carp feeds on phytoplankton according to Rashed (2001) and Alam *et al.* (2002), we wanted to study whether there are any heavy metals in the muscles, i.e., edible parts of the analyzed fish and if so whether the determined concentrations are within the limits allowed for consumption. We determined the concentrations of six metals which are the most significant ones considering the maximum allowed concentrations in fish meat for human consumption according to the standardization documents of Croatia and the EU (Anonymous, 1997, 2001a, b).

The results obtained show that the concentrations of all analyzed heavy metals in all investigated organs, other than chromium, copper and iron in the skin and muscles are higher in grass carp than in silver carp. These concentrations are also higher than the concentrations in carp organs (Matasin *et al.*, 2008). Although, Rashed (2001) determined that fish accumulate and concentrate heavy metals from mud, aquatic plants and water. These findings and the findings of Matasin *et al.* (2008) show that grass carp which feeds on higher aquatic plants, accumulates and concentrates the analyzed heavy metals in concentrations higher than those in carp and silver carp. The results of this research indicate that grass carp should be used as the indicating fish species (cyprinid) for determination of pollution with respect to heavy metals. The highest heavy metal concentrations in liver samples confirm the data provided by Goldfisher *et al.* (1970) which determine that metabolically active organs such as the liver accumulate heavy metals the most while the high tolerance threshold of the liver is ascribed to the location of metals in the liver lysosomes where they become harmless. The heavy metal concentrations in skin and muscle samples collected from the analyzed fish show that the tissues of these organs are not active heavy metal accumulators.

Having analyzed the heavy metal concentrations by using the EDXRF method in soft tissues of organs in consumption fish, grass carp and silver carp and in mud and water, we determined a statistically significant difference between heavy metal concentrations ($p<0.05$). In addition, we determined a statistically significant difference between lead ($p<0.01$) and manganese ($p<0.05$) concentrations in soft tissues of grass carp and silver carp which confirms that fish bioaccumulate heavy metals. Higher heavy metal concentrations in grass carp than in silver carp indicate that grass carp should be selected to determine environmental pollution with respect to heavy metals. The heavy metal concentrations in the edible parts of fish (skin and muscles) are lower than the maximum allowed concentrations in accordance with the standardization documents of Croatia and the EU (Anonymous, 2001a, b).

CONCLUSION

The study shows that grass carp is fish of choice for determinations of environment pollutions with heavy metals. Determinate values do not over cross maximal allowed concentrations of heavy metals in edible fish parts (skin + muscle).

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