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Determination of Heavy Metal (Cu, Pb and Zn) Concentrations in Muscle Tissue of *Hypophthalmichthys molitrix*, *Cyprinus carpio* and *Ctenopharyngodon idella* Caught from Zarivar Wetland, western Iran

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ABSTRACT

The present research report the heavy metal (Cu, Pb and Zn) contamination in muscle tissue of three species of fish (silver carp, common carp and grass carp) caught from Zarivar international wetland. Heavy metal concentrations (ìg g^{-1}, wet weight; means±S.D.) in silver carp, common carp and grass carp muscle tissue were: Cu- 0.006 ±0.005, 0.01±0.008 and 0.013.91±0.011, Pb- 0.008±0.006, 0.006±0.003 and 0.011±0.007, Zn- 0.013±0.009, 0.017±0.014 and 0.009±0.008, respectively. Comparative evaluation of these metals in different fish species showed that the average concentrations of Cu, Pb and Zn of all species is significantly lower than adverse level for the species themselves and for human consumption when compared with FAO/WHO permissible limits. Therefore, their contribution to the total body burden of these heavy metals can be considered as negligibly small.

Key words: Heavy metal, Food safety, Maximum contamination limit, *Hypophthalmichthys molitrix*, *Cyprinus carpio*, *Ctenopharyngodon idella.*

INTRODUCTION

The presence of metals in aquatic ecosystems originates from the natural interactions between the water, sediments and atmosphere (Kalay and Canil, 2000; Sankar *et al*. 2006). Heavy metals may enter an aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills, shipping and harbor activities and atmospheric deposits (Nair *et al*. 2006). The contamination of heavy metals in the various parts of organisms are determined primarily indicative of the level of the pollution in the environment (Canbek *et al*. 2007). Aquatic organisms are widely used to monitor environmental health due to anthropogenic impacts (Hellawell, 1986; Evans *et al*. 1993; Rashed, 2001; Rajeshkumar and Munuswamy, 2011).

Of many aquatic organisms, fish is a valuable biomonitor of environmental pollution (Padmini and Usha, 2008). Chronic contamination by heavy metals and organic pollutants in the marine environment is a severe problem particularly in estuaries. This has prompted numerous investigations on the effects of these pollutants on the biological functions of aquatic organisms and in particular defense mechanisms in fish (Wood, 1991). Its complexity and constant contact with the external environment make the gill the first target to waterborne pollutants (Mallatt, 1985; Perry and Laurent, 1993; Fracacio *et al*. 2003). The effect of different contaminants on gill and liver

causing biochemical and morphological changes have been analyzed in several studies (Monterio *et al*. 2005; Garcia-Santos *et al*. 2006; Romao *et al*. 2006; Fernandes *et al*. 2007). Several studies were done to measure and determine the effects of heavy metals and trace elements on ecosystem and human. The studies showed that decreased content of antioxidative elements, such as Zn, Se and Mn and increased content of some elements including Cu, Co and As, which probably elevate the oxidative stress, can cause some inflammatory diseases and cardiac functional disorders (Barandier *et al*. 1999; De-lorgeril *et al*. 2001; Salehifar *et al*. 2008; Shokrzadeh *et al*. 2009; Topuzoglu *et al*. 2003). On the other hand, Pb toxicity can lead to growth retardation, neuronal defects and anemia in children. Also, hepatotoxicity, nephrotoxicity and neurotoxicity can be occurred following Pb chronic toxicity (Tabari *et al*. 2010).

Cu under ionic forms Cu_2^+, Cu_2OH^+ and $CuOH^+$ is toxic to fish (Ashraf *et al*. 2006). Zn is present in many enzymes involved in important physiological functions like protein synthesis and constitutes about 33 ppm of adult body weight (Ashraf *et al*. 2006). Pb poisoning is generally ranked as the most common environmental health hazard (Goyer, 1994). Pb absorption may constitute a serious risk to public health. Pb may induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular diseases in adults. Over the past decade the levels in food have decreased significantly owing to the awareness of lead as a health problem and source related efforts to reduce the emission of Pb (Suppin *et al*. 2005).

Zarivar Lake (ZL) is fresh water body with an area of about 750 ha and average water depth of 4-5 meters in far west of Iran located in 35°-30' to 35°-35' North and 46°-06' to 46°-09' East in the North of Kurdistan province, Iran (Figure 1). Zarivar Lake is a typical ecosystem of great importance in regard to biodiversity and to aesthetic value. The fish species found most commonly in the lake are Cyprinus carpio, Ctenopharyngodon idella, Hypophthalmichthys molitrix, Capoeta damascina, Pseudorasbora parva, Chalcalburnus sp, Carassius auratus, Gambusia affinis and Mastacembelus mastacembelus. Previous research showed that the pollutants transferred to ZL and regarding the intensity of pollution production, the non point source pollution related to agricultural activities was first rank among other pollutant as community wastewater, solid waste, grassland pollution and forest. These pollutions are transferred directly to wetland and threaten the biological systems of ZL (Ghaderi and Ghafouri, 2006).

The aim of this study was to provide baseline information on heavy metal (Cu, Pb and Zn) contamination in muscle tissue of three fish species from ZL, to determine whether these metals are within permissible limits for human consumption.

MATERIALS AND METHODS

Chemical and reagents

All chemical reagents were of analytical reagent grade, purchased from Merck (Germany). All solutions were prepared with doubly distilled water. Stock standard solution of Cu(II), Pb(II), and Zn(II) (1,000 mg L^{-1}) were prepared by dissolving the appropriate amount of metal salts in doubly distilled water and diluting to 1,000 ml in the volumetric flask. As supporting electrolyte, 0.1 M acetate–acetic acid buffer (pH= 4.5) was used.

Apparatus

All voltammetric measurements were carried out using a polarographic processor, model 746 VA (Metrohm), in combination with a polarographic stand model 747 VA (Metrohm). The electrode stand consists of a hanging mercury drop electrode (HMDE) as working electrode, a double junction Ag/AgCl (3 M KCl, saturated AgCl, and 3 M KCl in the bridge) as reference electrode, and platinum wire, with considerably larger surface area than that of HMDE, as auxiliary electrode. All potentials quoted are relative to the Ag/AgCl reference electrode. Stirring was carried out by a large Teflon road with 2,000 rpm speed. A 780 pH Meter (Metrohm), equipped with a combined Ag/AgCl glass electrode was used for pH measurements. Eppendorf reference variable micropipettes were used to pipette microliter volumes of solutions. All glasswares were soaked overnight in 10% (v/v) nitric acid, followed by washing with 10% (v/v) hydrochloric acid, and rinsed with doubly distilled water and dried before using.

Sample preparation

Although voltammetric techniques are inherently precise and accurate, the results obtained using these techniques may be invalidated due to contamination caused by poor sample handling and preparation. Therefore, stringent conditions should be routinely used for trace analysis. In this study fish samples were cleaned with distilled water and then dissected. 2 g of muscle tissue of each fish sample was removed and weighed for the analysis. For estimation of heavy metal content, 2 g of each tissue was taken in a 100-ml Borosil beaker. To this, 2 ml of HNO_3 and 1 ml of $HClO_4$ were added and kept for digestion on a hot plate at 100°C till complete digestion was achieved. It was ensured that the residue obtained after digestion was free from organic matter which acts as impurities in metal analysis (Sobhanardakani *et al*. 2011a).

Sample analysis

To analysis of Cu(II), Pb(II) and Zn(II) concentrations in the muscle of fish species (Hypophthalmichthys molitrix, Cyprinus carpio and Ctenopharyngodon idella), 5 ml of each sample solution and 1 ml acetate–acetic acid buffer solution were transferred into the electrochemical cell and diluted to 10 ml by doubly distilled water. The solution was deaerated by passing pure nitrogen for 5 min. The deposition potential was controlled at (-0.25, -0.75 and -1.0 for Cu, Pb and Zn respectively) and applied to a fresh mercury drop while the solution was stirred. After the deposition step and further 10 s (equilibrium time), the voltammograms were recorded. Different concentrations of the standard metal ions were added to the cell. The solution was stirred and purged with nitrogen for 1 min after each spike. Finally, the concentrations of Cu(II), Pb(II) and Zn(II,) were calculated in the sample solutions by using the standard addition method (Sobhanardakani *et al*. 2011a; Sobhanardakani *et al*. 2011b).

Statistical analyses

Statistical analysis was performed using SPSS 15.0 version (SPSS Inc., Chicago, IL, USA) statistical package. Data were grouped according to species. One-way analysis of variance was used to test for differences in tissue metal concentrations. Data were log-transformed to improve normality before analysis to meet the underlying assumptions of the analysis of variance; the values given are therefore geometric means. The differences between the metal concentrations in different species were analyzed using the t-test. Possibilities less than 0.05 were considered statistically significant ($p < 0.05$).

RESULTS AND DISCUSSIONS

Because of high sensitivity of anodic stripping voltammetry, this method is applied to

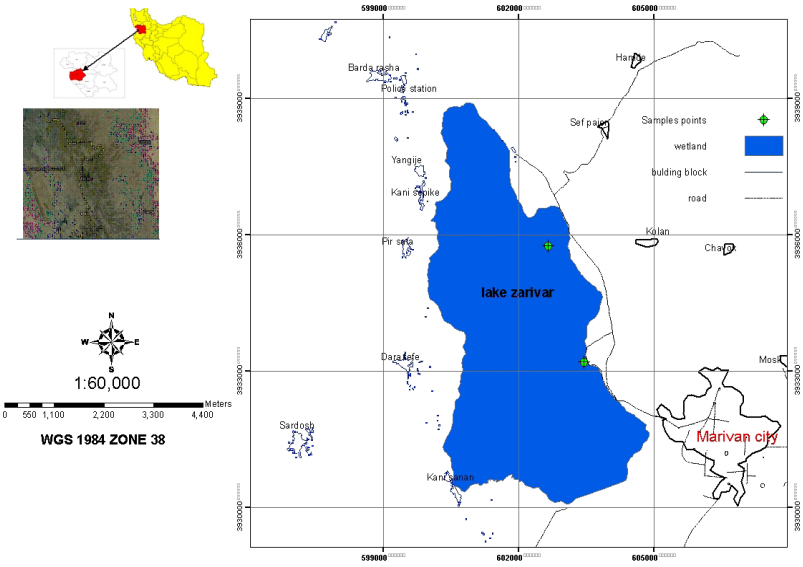

Fig. 1: Location of Zarivar Lake in Marivan city, Western Iran

the determination of Cu, Pb and Zn in the muscle tissue of fish species (Hypophthalmichthys molitrix, Cyprinus carpio and Ctenopharyngodon idella).

The concentrations of Cu, Pb and Zn in fish species are presented in Table 1 along with the statistical parameters. Statistical analysis of the data for all fish species showed significant differences among all of the samples. Figure 2 shows the comparative levels of Cu, Pb and Zn in the various species of fish. It can be seen that the average concentrations Cu in grass carp is more than 2 times higher than common carp. Similarly the Pb for grass carp is much higher than common carp as well. On the other hand, the average Zn content in grass carp was much lower than in common carp. Statistical grouping of the concentrations of each element in the different species of fish by ANOVA and the Tukey test are shown in table 1. The results indicated that there were significant differences within and between all of the evaluated brands ($p<0.05$).

The levels of heavy metals in fish depend upon many factors like the duration of exposure of fish to contaminants in the water, the feeding habits of each fish species, the, concentrations of contaminants in the water column, water chemistry, any contamination of fish during handling and processing, and fish sex, weight, season (Kagi and Schaffer, 1998; Boadi *et al*. 2011).

It is known that seafood is a good source of dietary Cu, which is an essential element for humans but where a very high intake (>120 ìg g^{-1}) can again cause adverse health problems, such as liver and kidney damage (WHO, 1996; Aucoin *et al*. 1999; Ikem and Egeibor, 2005). Whereas the maximum permitted Cu level established by the FAO (1983) and WHO (1996) is 30 ìg g^{-1} and 20 ìg g^{-1} by MAFF (1993), the, literature values ranged from 0.23 to 9.49 ìg g^{-1} for muscles of fish from the Marmara Sea (Keskin *et al*. 2007), 0.7-27 ìg g^{-1} for muscles of fish from Lake Budi (Tapia *et al*. 2006), 0.74-2.24 ìg g^{-1} for muscles of fish from Iskenderun Bay and 0.32-6.48 ìg g^{-1} for muscles of fish from the Marmara, Aegean and Mediterranean Seas (Turkmen *et al*. 2006; Turkmen *et al*. 2008), 0.15-5.06 ìg g^{-1} for muscles and whole fish from Turkish Seas (Tepe *et al*. 2007), 0.001-6.29, 0.001-57.3, 0.001-15.9 and 0.08-32.9 ìg g^{-1} for muscle tissues of anchovy, red mullet, mackerel and picarel respectively from the Croatian waters of the Adriatic Sea (Bilandz¡ic´ *et al*. 2011), 0.157 and 1.206 ìg g^{-1} for muscle tissue of milk fish from less polluted and polluted sites of Kaattuppalli Island, India respectively (Rajeshkumar and Munuswamy, 2011), Sobhanardakani *et al*. reported that Cu in the muscle tissue of five fish species (Otolithes ruber, Pampus argenteus, Parastromateus niger, Scomberomorus commerson, Onchorynchus mykiss) ranged from 0.007-0.23 ìg g^{-1} (Sobhanardakani *et al*. 2011b).

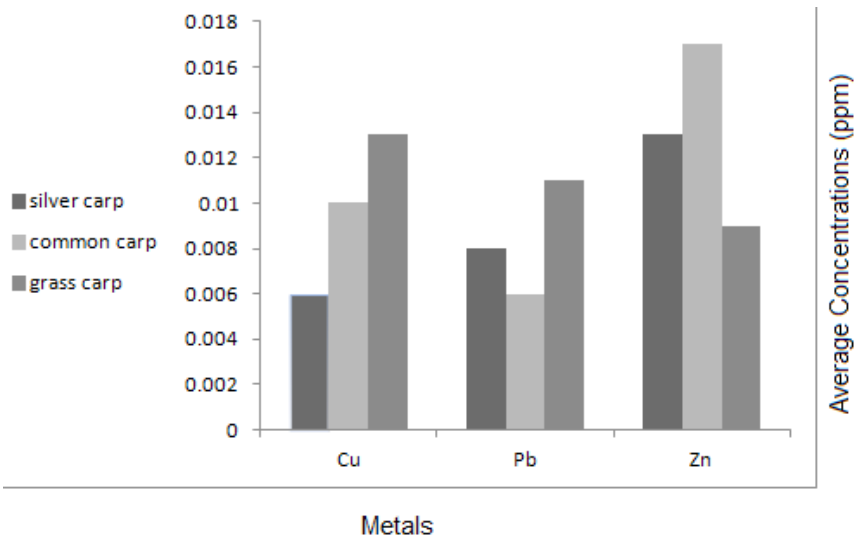

Fig. 2: Comparative levels of selected heavy metals in fish species

The maximum Pb level permitted reported by the WHO (1996) is 2.0 ìg g¯¹, 0.5 ìg g¯¹ by the FAO (1983) and 2.0 ìg g¯¹ by the MAFF (1995). Eboh *et al*. (2006) reported that Pb in the muscle, gills and liver tissue of five common commercially available fish species in Nigeria (catfish, tilapia, ilisha, bonga and mudskipper) were found in the range of 0.001-0.002 ìg g¯¹ but did not find any heavy metal residues in salmon and mackerel species. On the other hand, the mean concentration of Pb (4.27-6.12 ìg g¯¹) reported by Canli and Atli (2005) in muscle tissues of six different fish species (Sparus auratus, Atherina hepsetus, Mugil cephalus, Trigla cuculus, Sardina pilchardus and Scomberesox saurus), Rajeshkumar and Munuswamy (2011) reported that Pb in the muscle tissue of milk fish from less polluted and polluted sites of Kaattuppalli Island, India were found in the range of 0.035 and 0.058 ìg g¯¹ respectively, Bilandz¡ic´ *et al*. (2011) reported that Pb in the muscle tissues of anchovy, red mullet, mackerel and picarel from the Croatian waters of the Adriatic Sea in the range of 0.001-0.34, 0.001-0.27, 0.002-0.24 and 0.001-0.46 ìg g¯¹, respectively. Tabari *et al*. (2010) reported that the concentration of Pb in the muscle tissues of three species (Cyprinus carpio, Mugila auratus and Rutilus frisikutum) at 12 fishing site form Southern Caspian Sea, Iran in the range of 53.7 to 168.9 ìg g¯¹, Sobhanardakani *et al*. (2011a) reported that Pb in the muscle tissue of five fish species (Otolithes ruber, Pampus argenteus, Parastromateus niger, Scomberomorus commerson, Onchorynchus mykiss) ranged from 0.007-0.09 ìg g¯¹.

Turkmen *et al*. (2009) determined the metal levels in the muscle of 12 fish species from the Aegean and Mediterranean Seas and reported that the level of Zn in muscle of fish was 3.51-53.5 ìg g¯¹. Sivaperumal *et al*. (2007) reported that Zn in the muscle tissue of 23 fish species were obtained from internal markets of India ranged from 0.66-39.2 ìg g¯¹. Yilmaz *et al*. (2007) analyzed Zn in the muscle of two fish species (Leuciscus cephalus and Lepomis gibbosus) caught from Saricay, South-West Anatolia in the range of 6.35-28.55 ìg g¯¹. Yilmaz (2009) analyzed Zn in the muscle of 127 fish samples of three fish species (Anguilla anguilla, Mugil cephalus, Oreochromis niloticus) caught from Köyceðiz Lake-Mugla in Turkey. In their study the lowest metal contents were found in the edible parts (muscle) of all species. However, Zn for O. niloticus; Zn for A. anguilla; and Zn for M. cephalus were higher than those established by Turkish Food Codex and WHO limits for human consumption in the edible parts of fish samples and posed a risk for human health, Rajeshkumar and Munuswamy (2011) reported that Zn in the muscle tissue of milk fish from less polluted and polluted sites of Kaattuppalli Island, India were found in the range of 0.233 and 0.324 ìg g¯¹ respectively, Sobhanardakani *et al*. (2011b) reported that Zn in the muscle tissue of five fish species (Otolithes ruber, Pampus argenteus, Parastromateus niger, Scomberomorus commerson, Onchorynchus mykiss) ranged from 0.005-0.04 ìg g¯¹. Dural *et al*. (2007) analyzed Zn concentration in the muscle tissue of three fish species (Dicentrarchus labrax, Sparus aurata and Mugil cephalus) from the Tuzla lagoon, Turkey and reported that Zn contents ranged from 8.27±41.50, 8.82±99.8 and 12.2±76.98 ìg g¯¹ in autumn, winter and spring 2001 respectively. Agusa *et al*. (2005) reported that Zn in the muscle of 12 species of marine fish collected from coastal areas in Malaysia ranged from 16.4-1730.0 ìg g¯¹. Cohen *et al*. (2001) reported that Zn in the muscle of six fish species caught from Mugu Lagoon, Malibu Lagoon and Ballona Wetlands in shoutern California (F. parvipinnis, A. affinis, G. mirabilis, L. armatus, M. galloprovincialis and T. californianus) ranged from

Table. 1: Heavy metal contents μg g^{-1}) for various species of fish

Metal	silver carp		common carp		grass carp	
	Range	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD
Cu	0.002-0.009	0.006 ± 0.005^{a}	0.009-0.013	0.01 ± 0.008^{c}	0.008-0.017	0.013 ± 0.011^{b}
Pb	0.003-0.011	0.008 ± 0.006^{a}	0.004-0.009	0.006 ± 0.003^{d}	0.008-0.016	0.011 ± 0.007^{b}
Zn	0.007-0.014	0.013 ± 0.009^{b}	0.01-0.021	0.017 ± 0.014^{d}	0.004-0.015	0.009 ± 0.008^{c}

Vertically, letters a, b and c show statistically significant differences ($p<0.05$).

12.0-650.0 ìg g¯¹. Mormede and Davies (2001) reported that Zn in the muscle tissue of monkfish (Lophius piscatorius), black scabbard (Aphanopus carbo), blue ling (Molva dyp terygia), blue whiting (Micromesistius poutassou) and hake (Merluccius merluccius) were obtained from the continental slope of Rockall Trough, west of Scotland ranged from 0.37-3.90 ìg g¯¹.

CONCLUSION

The results from this study suggested that significant differences existed in the metal concentrations across three different fish species. Also, analytical data obtained from this study shows that the metal concentrations for the fishes were generally within the FAO/WHO, U.S. FDA and U.S. EPA recommended limits for fish (table 1). There is therefore there is no serious health risk associated with the consumption of the three studied metals in the fishes analyzed. Both low-risk groups (adolescents and adults) and high-risk groups (pregnant mothers and children) must, based on the results obtained, reduce their consumption of fish. Therefore more research and assessments of seafood quality is needed in many countries to provide more data and help safeguard the health of humans.

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