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Original Article

Concentration of heavy metals (Pb, Cd) in muscle and liver of *Perca fluviatilis* and *Tinca tinca* in Anzali Wetland, southwest of the Caspian Sea

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Abstract: Anzali Wetland is one of the most important aquatic ecosystems of Iran located at southwest of the Caspian Sea. This wetland is a habitat for valuable fish with vital role in life cycle of this ecosystem. Assessment of pollutants concentration is rational due to complications of determining biological effects in a habitat. The present study examined the concentration of lead (Pb) and cadmium (Cd) in muscles and livers of two fish species i.e. *Perca fluviatilis* and *Tinca tinca* collected from Anzali Wetland, and their relationships with fish size (length and weight). The results showed the highest concentration of metals in liver, and the lowest in muscle tissues of both species. Highest concentrations of Cd (0.09) and Pb (3.66) were recorded in liver tissue of *T. tinca*. The results also showed significant negative correlation between metal concentrations and fish size. Highly significant (P<0.01) negative relationships were observed between fish length and Pb concentrations in liver of *P. fluviatilis*. Cd and Pb concentrations in liver of *P. fluviatilis* and Cd concentrations in the liver of *T. tinca* showed significant negative relationships (P<0.05) with size factors. The concentrations of Pb and Cd were lower than the maximum acceptable concentrations for fish proposed by MAFF thus safe for human utilization.

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Introduction

Awareness of heavy metal (HM) concentrations in fishes is essential in terms of management and human consumption (Rauf et al., 2009). Anthropogenic activities constantly enhance the amount of HMs in environment, especially in aquatic ecosystems. Pollution of HMs in aquatic ecosystems is increasing at an alarming rate worldwide due to human activities (Malik et al., 2010). HMs enter water reservoirs via atmosphere, drainage and soil erosion. Therefore, heavy metal pollutions are of great concern worldwide, and have a great ecological significance due to their toxicity and accumulative behavior. Hence, HMs can damage aquatic organisms (Matta et al., 1999). Researches have showed that fishes accumulate HMs in their tissues and their concentrations depend on many factors such as concentration and duration of exposure, salinity, temperature, hardness of water and metabolic rate of organisms (Pagenkopf, 1983; Allen, 1995). In polluted waters, HMs accumulate in organisms directly through skin and gill or indirectly via food chains (Sinha et al., 2002; Sure, 2003).

HMs cause mutation, disturb immune responses, change blood parameters, decrease organism's adaptation qualities and increase aquatics susceptibility to diseases. Also, HMs have toxic effects, altering physiological activities and biochemical parameters both in tissues and blood (Larsson et al., 1985; Nemesok and Huphes, 1988; Abel et al., 1986). Metals such as iron, copper, are essential due to their vital role in biological systems, whereas, lead and cadmium are non-essential and toxic. The essential metals can be toxic when their

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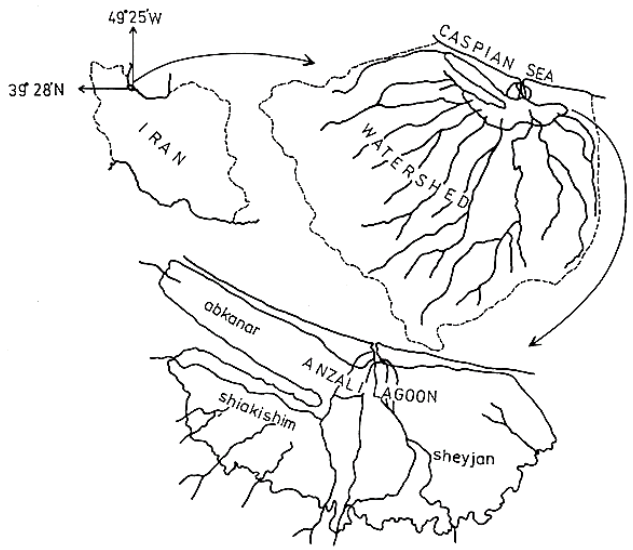

Figure 1. The map of Anzali Wetland at southern region of the Caspian Sea (North Iran).

concentration in aquatic environments increase beyond the tolerable level of organisms.

Since the toxic effects of metals have been recognized, HM levels in the tissues of aquatic animals are occasionally monitored. Because the HMs concentration in tissues reflects past exposure via water and/or food, hence it can demonstrate the current situation of animals before toxicity affects the ecological balance of populations in the aquatic environment and to assess their risk on human health (Canli et al., 1998). Therefore, this study was conducted to assess the concentrations of Pb and Cd, in muscle and liver of *Perca fluviatilis* and *Tinca tinca* collected from Anzali Wetland to estimate risk assessment of these fish species on consumer health.

Material and methods

Sixteen specimens of *P. fluviatilis* and Twenty-three specimens of *T. tinca* were caught by local fishermen from Anzali Wetland, located at the southwest of the Caspian Sea, at 39°28'N, 49°25'W (Fig. 1). The specimens were transported to the laboratory on ice without delay and kept frozen at -25°C until further analyses. The age of specimens were determined using scales according to Bagenal and Tesch (1978). In addition, total length and weight of specimens were measured to nearest 1 mm and 1 gr, respectively. Following dissection, the liver and muscle tissues were removed, thawed (in an oven set at 90°C) and a weighted sample (0.5 g) of homogenized tissue was taken from each specimen. Each sample was placed in a Teflon digestion vessel with 12 ml mixture of nitric acid and perchloric acid (3:1 v/v) (Merck) (Canli and Atli, 2003). The mixture was heated to 120˚C for 45 minutes until the tissue was dissolved. The digests were diluted by adding distilled water prepared from stock standard solution (Merck). Metal concentrations were measured using an Inductive Coupled Plasma Mass Spectrometer (ICP-MS-300D) and metal concentration in a tissue was presented as µg metal/g dry weight.

Data were plotted on graphs to show their distributions. The linear regression were applied on data to analyze the relationships between size of specimens and HMs concentrations in the tissues. The concentration of HMs in tissues were also compared using One-way ANOVA test. All statistical analyses were carried out using SPSS statistical programs (Version 16).

Results

Table 1 shows numbers, length and weight ranges and length-weight relationships of examined specimens. Higher Cd concentrations were observed in liver tissues of both species. Pb concentrations in both tissues were higher than those of cadmium, especially in the liver (Table 2).

Table 3 shows the relationships between metal concentrations and fish length and weight. Significant negative relationship were found between length (and weight) of *T. tinca* and cadmium levels in its liver ($P<0.01$). Furthermore, in the liver of *P. fluviatilis* negative relationships were observed between length and HMs (Cd and Pb) levels ($P<0.05$) (Table 3).

Discussion

Heavy metals are considered as the most important pollutants of aquatic environments because of their toxicity and accumulation. The toxic effects of heavy metals, particularly cadmium and lead have been

Table 1. Size ranges and the relationships between weight and total length of *P. fluviatilis* and *T. tinca* from Anzali Wetland.

Species	**n**	**Age**	**Lenght rang (cm)**	**Weight rang (gr)**	**Equation[a]**	**r Value**
P. fluviatilis	16	1-4	17.6-23.4	87.5-156.3	Y=0.0793X+10.897	0.961
T. tinca	23	1-3	13-21	35 -144	Y=0.0708X+11.157	0.951

[a]Y is total length (cm) and X is total weight (g).

Table 2. The concentrations (µg /g d.w.) of heavy metals in the tissues of *P. fluviatilis*, and *T. tinca* collected from Anzali Wetland.

Fish	**Tissue**	**Cadmium**	**Lead**
		Mean ± SD	**Mean ± SD**
P. fluviatilis	Muscle	0.003 ± 0.001	0.62 ± 0.15
T. tinca		0.03 ± 0.02	1.15 ± 0.42
P. fluviatilis	Liver	0.08 ± 0.02	1.2 ± 0.28
T. tinca		0.09 ± 0.03	3.66 ± 0.5

Metal concentrations among the tissues from different fishes were compared statistically using one-way ANOVA. All comparisons were statistically significant ($P<0.05$).

Table 3. The relationships between heavy metal concentrations and total lengths and weights of fish (p Values are given in parenthesis) in the tissues of *P. fluviatilis* and *T. tinca*.a

Fish	Tissue	Data	Cadmium	Lead
P. fluviatilis	Muscle	Equation[a]	Y=18.47+(-422.44)X	Y=0.85+(-0.011)X
		p Value	NS[b]	NS
	Liver	Equation	Y=24.09+(-48.15)X	Y=3.73+(-0.012)X
		p Value	*(*)	*(*)
T. tinca	Muscle	Equation	Y=16.52+(-11.71)X	Y=0.75+0.02X
		p Value	NS (NS)	NS(NS)
	Liver	Equation	Y=0.23+(-0.008)X	Y=1.545+(-0.009)X
		p Value	**(*)	NS(NS)

[a]In the equations, Y is metal concentration (µg/g d.w.) and X is total length (cm) of fish. Asterisks indicate significant results.
[b]NS, not significant, $P>0.05$.
* $P<0.05$
** $P<0.01$

widely studied (Inskip and Piotrowsiki, 1985; Kurieshy and De siliva, 1993; Narvaes, 2002; Nishihara et al., 1985; Schoerder, 1965; Venugopal and Luckey, 1975). Cadmium and lead have no known biological functions in human physiology and might potentially be toxic even at trace concentrations (Robert, 1991). The symptoms of acute cadmium toxicity include high blood pressure, kidney damage, destruction of testicular tissue and destruction of red blood cells (Gupta and Mathur, 1983). Hence information regarding levels of heavy metals content in fish species may have some advantageous for reducing their risk in public health (Domingo et al., 2007).

In this study, metal concentrations varied in both examined fish species. These difference may be related to differences in ecological requirements, swimming behaviors, metabolic activities between fish species, feeding habits (Mormede and Davies, 2001; Romeoa et al., 1999; Watanabe et al., 2003), age and size of fish (Al-Yousuf et al., 2000; Linde et al., 1998) and their habitats (Canli and Atli, 2003).

In addition, the results showed that metal concentrations in the liver was higher than muscle tissue of both fish species. The dissimilarity in metal concentrations in various tissues is due to the induction of metal-binding proteins i.e. metallothioneins in liver. It is well-known that large quantity of metallothionein induction occurs in liver tissue of fishes (Heath, 1987; Canli and Furness, 1993a, b; Roesijadi and Robinson, 1994). Yilmaz et al. (2007) reported highest accumulation of cadmium, cobalt and copper in the liver of *Leuciscus cephalus* and *Lepornis gibbosus*, whereas the lowest accumulation was observed in muscle tissues of these species. It is commonly known that muscle is not a tissue in which HMs accumulate (Legorburu et al., 1988). Similar results in a number of fish species (Karadede and Unlo, 2000) show that muscle is not an active tissue in accumulating HMs. Canli and Atli (2003) recorded the highest concentration of cadmium in liver and the lowest in muscle tissues of the fish. Based on our results, the concentration of lead was higher than that of cadmium, particularly in the liver.

The results showed a negative relationships between fish sizes viz. length and weight and HM levels. Nussey et al. (2000) showed that accumulation of HMs (Cr, Mn, Ni, and Pb) were reduced with increasing the length of *Labeo umbratus*. Widianarko et al. (2000), showed the relationship between HMs (Pb, Zn, and Cu) concentration and size of *Poecilia reticulata* with a significant decline in lead concentrations with increasing fish size. Metabolic activities play an essential role in HM accumulation in aquatic organisms (Heath, 1987; Langston, 1990; Roesijadi and Robinson, 1994). Metabolic activity of a young individual is generally higher than that of older ones and therefore HM accumulation is higher in younger fish than elders (Elder and Collins, 1991; Douben, 1989; Canli and Furness, 1993b; Nussey et al., 2000; Widianarko et al., 2000). A probable reason for observed negative relationships between HM concentrations and size may be related to differences in metabolic activities between younger and older fish. Douben (1989) also showed that metal accumulation could reach a stable condition after a certain age. On the other hand, the dilution of tissue metal concentrations due to growth and/or lowered metabolic activity in old individuals may not be seen if metal concentration in water is higher than the capacity of these parameters. In this case, continuous accumulation of HMs may occur and positive relationships may be observed among animals in different sizes. High concentration of HMs in water can postpone fish growth causing variations in fish size (Heath, 1987; Weis and Weis, 1989; Friedmann et al., 1996).

The concentrations of Cd and Pb in examined *T. tinca* and *P. fluviatilis* specimens were below the guidelines for food summarized by MAFF (Cd, 0.2 μg/g wet wt.; Pb, 2.0 μg/g wet wt.) (Anan et al., 2005) and EEC (Cd, 0.05 μg/g wet wt.; Pb, 0.2 μg/g wet wt.) (Commission regulation, 2008) and therefore their consumption impose no risk for human health.

References

Abel P.D., Papoutsouglou S.E. (1986). Lethal toxicity of cadmium to *Cyprinus carpio* and *Tilapia aurea*. Bulletin of Environmental Contamination and Toxicology, 37: 382-386.

Al-Yousuf M.H., El-Sahahawi M.S., Al-Ghasis S.M. (2000). Trace metals in liver, skin and muscle of *Lethrinus lentjan* fish species in relation to body length and sex. The Science of the Total Environment, 256: 87-94.

Allen P. (1995). Chronic accumulation of cadmium in the edible tissues of *Oreochromis aureus* (Steindachner): Modification by mercury and lead. Archives of Environmental Contamination and Toxicology, 29: 8-14.

Anan Y., Kunito T., Tanabe S., Mitrofanov I.G., Aubrey D. (2005). Trace element accumulation in fishes collected from coastal waters of the Caspian Sea. Marine Pollution Bulletin, 51: 882-888.

Bagenal T.B., Tesch F.W. (1978). Age and growth. In: T.B. Bagenal (ed). Methods for assessment of fish production in fresh waters, IBP Handbook, Oxford, London, Edinburgh, and Melbourne: Blackwell

Scientific Publications, pp: 101-36

Canli M., Atli G. (2003). The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) Levels and the size of six Mediterranean fish species. Environmental Pollution, 121: 129-136.

Canli M., Ay O., Kalay M. (1998). Levels of heavy metals (Cd, Pb, Cu, and Ni) in tissue of *Cyprinus Carpio*, *Barbus Capito* and *Chondrostoma regium* from the Seyhan River. Turkish Journal of Zoology, 22 (3): 149-157.

Canli M., Furness R.W. (1993a). Heavy metals in tissues of the Norway lobster *Nephrops norvegicu,* effects of sex, size and season. Chemical Ecology, 8: 19-32.

Canli M., Furness R.W. (1993b). Toxicity of heavy metals dissolved in sea water and influences of sex and size on metal accumulation and tissue distribution in the Norway lobster *Nephrops norvegicus*. Marine Environmental Research, 36: 217-236.

Commission regulation. (2008). Amending regulation the European Union (EEC), setting maximum level for certain concentration in food stuff. Official Journal of the European Union, 629: 6-9.

Domingo J.L., Bocio A., Flaco G., Llobet J.M. (2007). Benefits and risks of fish consumption. Part I. A quantitative analysis of the intake of omega-3 fatty acids and chemical contaminants. Toxicology, 230: 219-226.

Douben P.E. (1989). Lead and cadmium in stone loach (*Noemacheilus barbatulus* L.) from three rivers in Derbyshire. Ecotoxicology and Environmental Safety, 18: 35-58.

Elder J.F., Collins J.J. (1991). Freshwater molluscs as indicators of bioavailability and toxicity of metals in surface systems. Reviews of Environmental Contamination and Toxicology, 122: 37-79.

Friedmann A.S., Watzin M.C., Brinck-Johnsen T., Leiter J.C., Medina J., Hernandez F., Pastor A., Beferfull J.B., Barbera J.C. (1996). Low levels of dietary methylmercury inhibit growth and gonadal development in juvenile walleye (*Stizostedion vitreum*). Aquatic Toxicology, 35: 265-278.

Gupta B.N., Mathur A.K. (1983). Toxicity of heavy metals. Indian Journal of Medical Sciences, 37: 236-240.

Gupta A., Rai D.K., Pandey R.S., Sharma B. (2009). Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. Environmental Monitoring Assessment, 157: 449-458.

Heath A.G. (1987). Water pollution and fish physiology. CRC press, Florida, USA.

Inskip M.J., Piotrowsiki J.K. (1985). Review of the health effects of methyl mercury. Journal of Applied Toxicology, 5: 113-133.

Kalay M., Ay O., Canli M. (1999). Heavy metal concentrations in fish tissues from the Northeast Mediterranean Sea. Bull. Environ. Contamination and Toxicology, 63: 673-681.

Karadede H., Unlu E. (2000). Concentrations of some heavy metals in water, sediment and fish species from the Ataturk dam lake (Euphrates), Turkey. Chemosphere, 41: 1371-1376.

Kurieshy T.W., De siliva C. (1993). Uptake and loss of mercury, cadmium and lead in marine organisms. Indian Journal of Experimental Biology, 31: 373-379.

Larsson A., Haux C., Sjöbeck M. (1985). Fish physiology and metal pollution: Results and experiences from laboratory and field studies. Ecotoxicology and Environmental Safety, 9: 250-281.

Legorburu I., Canton L., Millan E., Casado A. (1988). Trace metal levels in fish from Unda River (Spain) Anguillidae, Mugillidae and Salmonidae. Environmental Technology Letters, 9: 1373-1378.

Linde A.R., Sanchez-Galan S., Izquierdo J.I., Arribas P., Maranon E., Garcya-Vazquez E. (1998). Brown trout as biomonitor of heavy metal pollution: effect of age on the reliability of the assessment. Ecotoxicology and Environmental Safety, 40: 120-125.

MAFF. (2000) Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1997. Aquatic Environment Monitoring Report No. 52. Center for Environment, Fisheries and Aquaculture Science, Lowestoft, UK.

Malik N., Biswas A.K., Qureshi T.A., Borana K., Virha R. (2010). Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. Environmental Monitoring and Assessment, 160: 267-267.

Mormede S., Davies, I.M. (2001). Heavy metal concentrations in commercial deep-sea fish from the Rockall Trough. Continental Shelf Research, 21: 899-916.

Matta J., Milad M., Manger R., Tosteson T. (1999). Heavy metals, lipid peroxidation, and cigatera toxicity in the liver of the Caribben barracuda (*Sphyraena barracuda*). Biological Trace Element Research, 70:

69-79.

Narvaes D.M. (2002). Human exposure to mercury in fish in mining areas in the Philippines. FAO/WHO Global forum of food safety regulation. Morocco: Marrakec.

Nemesok J.G., Huphes Z.G.M. (1988). The effects of copper sulphate on some biochemical parameters of rainbow trout. Environmental Pollution, 49: 77-85.

Nishihara T., Shimamato T., Wen K.C., Kondo M. (1985). Accumulation of lead, cadmium and chromium in several organs and tissues of carp. Journal Hygienic Chemistry, 31: 119-123.

Nussey G., Van Vuren J.H.J., du Preez H.H. (2000). Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *Labeo umbratus* (Cyprinidae), from Witbank dam, Mpumalanga. Water Sa-Pretoria 26: 269-284.

Pagenkopf G.K. (1983). Gill surface interaction model for trace metal toxicity to fish. Role of complexation, pH, water hardness. Environmental Science and Technology, 17(6): 342-347.

Rauf A., Javed M., Ubaidulla M. (2009). Heavy metal levels in three major carps (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) from the river Ravi, Pakistan. Pakistan Veterinary Journal, 29(1): 24-26.

Robert G. (1991). Toxic effects of metals. In: Casarett and Doull's toxicology. Pergamon Press, pp: 662-672.

Roesijadi G., Robinson W.E. (1994). Metal regulation in aquatic animals: mechanism of uptake, accumulation and release. In: D.C. Malins, G.K. Ostrander (Eds.). Aquatic Toxicology (Molecular, Biochemical and Cellular Perspectives. Lewis Publishers, London. p 539.

Romeoa M., Siaub Y., Sidoumou Z., Gnassia-Barelli M. (1999). Heavy metals distribution in different fish species from the Mauritania coast. The Science of the Total Environment, 232: 169-175.

Schoerder H.A. (1965). Cadmium as a factor in hypertension. Journal of Chronic Diseases, 18: 647.

Sinha A.K., Dasgupta P., Chakrabarty S., Bhattacharyya G., BhattacharJee S. (2002). Bio- accumulation of heavy metals in different organs of some of the common edible fishes of Kharkai River, Jamshed pur. Indian Journal of Environmental Science Health, 46: 102-107.

Sure C.B. (2003). Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. Parasitology, 126: 53-60.

Venugopal B., Luckey T. (1975). Toxicity of non-radioactive heavy metals and their salts. In: F. Coulston (Ed.). Heavy metal toxicity, safety and hormology. Academic press, George Thieme Stuttagart, New York.

Watanabe K. H., Desimone F.W., Thiyagarajah A., Hartley W. R., Hindrichs A.E. (2003). Fish tissue quality in the lower Mississippi River and health risks from fish consumption. The Science of the Total Environment, 302(1-3): 109-126.

Widianarko B., Van Gestel C.A.M., Verweij R.A., Van Straalen N.M. (2000). Associations between trace metals in sediment, water, and guppy, *Poecilia reticulata* (Peters), from urban streams of Semarang, Indonesia. Ecotoxicology and Environmental Safety, 46: 101-107.

Yilmaz F., Ozdemir N., Demirak A., Tuna A.L. (2007). Heavy metal levels in two fish species *Leuciscus cephalus* and *Lepomis gibbosus*. Food Chemistry, 100: 830-835.