Research Journal of Fisheries and Hydrobiology, 6(4): 404-411, 2011
ISSN 1816-9112

ORIGINAL ARTICLES

Human Health Risk Assessment of Heavy metals in Fish from Freshwater

[1]Abolfazl Askary Sary; [2]Maryam Mohammadi

[1,2]Department of Fisheries, Ahvaz Branch, Islamic Azad University, Ahvaz, IRAN.

ABSTRACT

Contaminant levels in fish from particular regions of the world could allow people to make informed decisions about which fish to eat to reduce their risk from the contaminants for human. The high levels of heavy metals in freshwater of Khouzestan have toxic effects on fish metabolism and human, it is important to consider the biological effects of contamination on fish health in freshwater in Khouzestan. Fish are the major part of human diet and levels of contaminants in fish are of particular interest because of the potential risk to humans who consume them and in this research attention has focused on self-caught fish from downstream Karoon and Dez Rivers. This study was carried out to investigate contamination of Cd, Pb, Ni and Hg in gill, liver and muscle of fish in freshwater of Khouzestan, Iran. Heavy metals analysis: Cd, Pb and Ni were measured by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer, 4100 ZL). Hg concentration was determined with a Perkin–Elmer MHS-FIAS coupled to a Perkin–Elmer 4100 ZL spectrophotometer. The results indicated that the muscles of some fishes were highly contaminated by heavy metals and exceeded of WHO, FAO, MAFF, NHMRC and FDA guidelines.

***Key words*: Health Risk Assessment, Heavy metals, Fish, Fresh water**

Introduction

Fish is a healthy food because of its nutritional benefits related to its proteins of high biological quality, desirable lipid composition, valuable mineral compounds and vitamins. The particular composition of its lipid fraction, rich in essential ω-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and poor in cholesterol makes it as a primer food (Groth, 2010).

In fresh water systems, fish is one of aquatic products humans consume, and also provide a good indicator of trace element pollution (Rashed, 2001). In the last decades, industry and agriculture near the Karoon and Dez Rivers have been rapidly developed and human activities have increased. Under certain environmental conditions, heavy metals may be accumulated to a toxic concentration, and cause ecological damage (Freedman, 1989).

Heavy metals were of particular concern due to their toxicity and ability to be bio accumulated in aquatic ecosystems (Miller *et al*., 2002), as well as persistence in the natural environment. Heavy metals like cadmium, lead, mercury and nickel are among the most metallic pollutants. Nickel is essential metal since its play an important role in biological systems, whereas cadmium, lead and mercury are non-essential metals, as they are toxic, even in trace amounts (Fernandes *et al*., 2008).

For the normal metabolism of the fish, the essential metals must be taken up from water, food or sediment (Canli and Atli, 2003). These essential metals can also produce toxic effects when the metal intake is excessively elevated (Tuzen, 2003). Bioaccumulation of these metals is known to adversely affect liver, muscle, kidney, gill and other tissues of fish, disturb metabolism and hamper development and growth of fish (Dallinger *et al*., 1987).

Heavy metals tend to accumulate in advanced organisms through bio-magnification effects in the food chain. Thus they can enter into human body and accumulate in the human tissues to pose chronic toxicity. Chronic assimilation of heavy metals is a known cause of cancer (Nabawi *et al*., 1987).

Therefore, it is important to determine the concentrations of heavy metals in commercial fish in order to evaluate the possible risk of fish consumption for human health (Cid *et al*., 2001). Fish is the major part of human diet and it is not surprising that numerous studies have been carried out on metal accumulation in different species (Lewis *et al*., 2002).

Being the complexity of heavy metal bioaccumulation of fishes, it was important to study the heavy metal accumulation in different commercial fishes such as *Barbus grypus* and *Barbus xanthopterus* in freshwater for the food safely. The main objective of this study was to determinate the contents of heavy metals in the gill, liver and muscle of *Barbus grypus* and *Barbus xanthopterus* caught from downstream of Karoon and Dez Rivers in order to assess fish quality and to assess the health risk for humans. This could help us understand the

Corresponding Author: Maryam Mohammadi, Department of Fisheries, Ahvaz Branch, Islamic Azad University, Ahvaz, IRAN Postal cod: 618491-8411 P.O. Box1915
E-mail: M40_Mohammadi@yahoo.com

Res. J. Fish & Hydrobiol., 6(4): 404-411, 2011

enrichment behavior of heavy metals in downstream Karoon and Dez Rivers and emphasize the need to discard the most polluted tissues of the fish.

Materials And Methods

The concentration of heavy metals such as cadmium, lead, nickel and mercury were measured in the muscle, gills and liver of *B. xanthopterus* that collected by gillnet from down streams of Karoon and Dez Rivers in Khoozestan province during summer 2009. The number of samples was 48 fish in each river. After capture, fish were placed in plastic bags and transported to the laboratory in freezer bags with ice. The length and weight were measured which ranged from minimum and maximum value as 248.5 and 377.5mm for length and 173 and 561.3 g for weight of *B. Xanthopterus* in Karoon and Dez Rivers. After biometry, fish were immediately frozen at -20°C.

All samples were cut into pieces and labeled, and then all sampling procedures were carried out according to internationally recognized guidelines (UNEP,1991). Fish samples for heavy metals were put onto a dissection tray and thawed at room temperature. They were dissected using stainless steel scalpels and Teflon forceps using a laminar flow bench. In parallel gill, liver and a part of the muscle (dorsal muscle without skin) were removed and transferred in polypropylene vials. Subsequently, samples were put into an oven to dry at 90°C and reached constant weights in the oven. Before acid digestion, a porcelain mortar was employed to grind and homogenize the dry tissue samples. Aliquots of approximately 1g dried gill, liver and muscle were digested in Teflon beakers for 12 h at room temperature, and then for 4h at 100°C with 5 ml ultrapure nitric acid (65%, Merck).

Heavy metals analysis: Cd, Ni and Pb were measured by graphite furnace atomic absorption spectrophotometry (Perkin-Elmer, 4100 ZL). Hg concentration was determined with a Perkin–Elmer MHS-FIAS coupled to a Perkin–Elmer 4100 ZL spectrophotometer. Results are expressed as $mgkg^{-1}$ dry weight. The analytical procedure was checked using reference material [MESS-1, the National Center of Canada and CRM 277, the Community Bureau of Reference, Brussels, Belgium,and details were in (Robisch and Clark,1993; Meador *et al*.,1994)]. For each matrix, analyses of three blank samples were performed along with the samples. Quality control was assured by the analysis of reagent blank and procedural blanks.

Data statistics were performed using SPSS 17 software. Paired samples T-Test were used to compare differences between samples. A P-value ($p<0.05$) was considered statistically significant (Zhang *et al*., 2007).

Results:

Estimation Of Potential Public Health Risks:

The concentrations of heavy metals ($mgkg^{-1}$wet weight) in muscle of fish in Karoon and Dez Rivers were higher than WHO (1985) standard (Fig. 1,2).

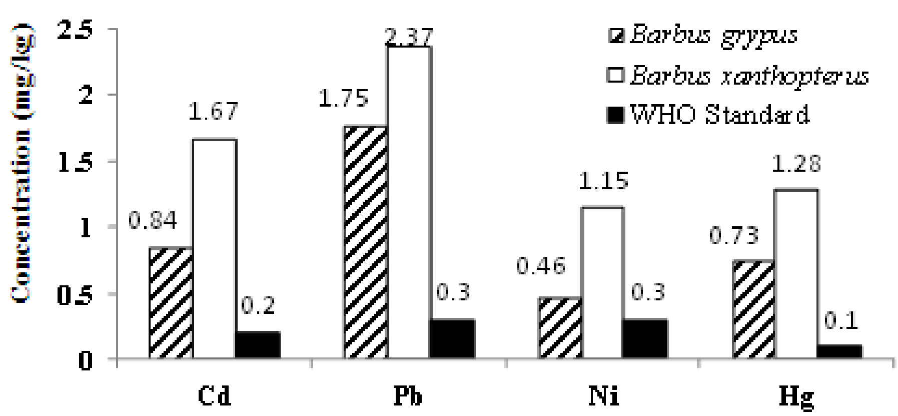

Fig. 1: Comparison of heavy metals in muscle of fish with WHO (1985) standard in Karoon River

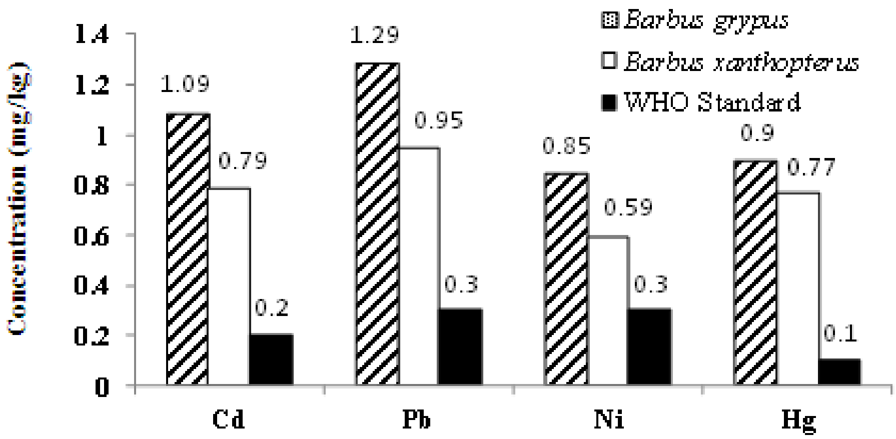

Fig. 2: Comparison of heavy metals in muscle of fish with WHO (1985) standard in Dez River

Cadmium:

The maximum cadmium level permitted in fish is 0.2 mg/kg for WHO (1985), 0.5 mg/kg for FAO (1983), 0.2 for MAFF (1995), 0.05 mg/kg for NHMRC (1990) and 2 mg/kg for FDA (1996) (Table 1). Generally, in this research cadmium levels in fish from freshwater were found to be higher than legal limits and have significant differences ($P<0.05$) in fish samples with WHO, FAO, MAFF and NHMRC legal limits, except for FDA which cadmium levels in fish samples were lower than FDA legal limit.

Lead:

The maximum lead level permitted is 0.3 mg/kg for WHO (Czarnezki, 1985), 0.5 mg/kg for FAO (1983), 2.0 mg/kg for MAFF (1995) and 1.5 mg/kg for NHMRC (1990). Generally, lead levels in analyzed fish samples were found to be higher than legal limits, except for lead levels in fish in comparison with MAFF and NHMRC which were lower than MAFF and NHMRC legal limits and only lead level in *B. xanthopterus* in Karoon River was higher than MAFF legal limit and lead levels in fish from Karoon River were higher than NHMRC legal limit. Lead levels have significant differences ($P<0.05$) in fish samples with WHO and FAO legal limits (Table 1).

Nickel:

The maximum nickel level permitted for fishes is 0.3 mg/kg according to WHO (1994) standard. Generally, nickel levels in analyzed fish samples were found to be higher than legal limit and have significant differences ($P<0.05$) in fish samples with WHO legal limit (Table 1).

Mercury:

The maximum mercury level permitted is 0.1 mg/kg for WHO (1985), 1 mg/kg for NHMRC (1990) and 0.1-0.5 mg/kg for FDA (1996) standards. Generally, mercury levels in analyzed fish samples were found to be higher than legal limits except for mercury levels in *B. grypus* from Karoon River and in *B. grypus* and *B. xanthopterus* from Dez River in comparison with NHMRC which were lower than NHMRC legal limit and mercury levels have significant differences ($P<0.05$) in fish samples with WHO and FDA legal limits (Table 1).

Table 1: Standard of heavy metals in muscle of fish

Standard	Cd	Pb	Ni	Hg
WHO	0.2	0.3	0.3[c]	0.1
FAO	0.5	0.5	-	-
MAFF	0.2	2	-	-
NHMRC	0.05	1.5	-	1
FDA	2	-	-	0.1-0.5

References: WHO, 1985, 1994; Czarnezki, 1985; FAO, 1983; MAFF, 1995; Colling *et al*., 1996; Darmono and Denton, 1990.

The concentrations of heavy metals (mgkg^{-1}wet weight) were detected in gill, liver and muscle of *Barbus grypus* and *Barbus xanthopterus* in Karoon and Dez Rivers. Pb level was higher than the other metals (P<0.05). Distribution patterns of metal concentrations in the gill, liver and muscle of *Barbus grypus* in Karoon River follows the sequence: Pb>Cd>Hg>Ni, in the muscle and liver of *Barbus grypus* in Dez River and in the *Barbus xanthopterus* in Karoon and Dez Rivers follows the sequence: Pb>Cd>Hg>Ni and in the gill of *Barbus grypus* in Dez River and in the *Barbus xanthopterus* in Karoon and Dez Rivers follows the sequence: Pb>Cd>Ni>Hg respectively (Fig. 3,4). The distribution patterns of Cd, Pb, Ni and Hg in tissues of *B. grypus* in Karoon River follows the order: gill> liver> muscle. The distribution patterns of Cd, Pb, Ni in tissues of *B. grypus* in Dez River and in the *B. xanthopterus* in Karoon and Dez Rivers follows the order: gill> liver> muscle. Hg level in the *B. grypus* in Dez River and *B. xanthopterus* in Karoon River follow the order: liver> gill> muscle and Hg level in the tissues of *B. xanthopterus* in Dez River follow the order: liver> muscle> gill. Heavy metal concentrations in the gill, liver and muscle of *B. grypus* and *B. xanthopterus* in Karoon and Dez Rivers were higher in the gill and liver, when compared with muscle except for Hg level that in the gill, liver and muscle of *B. xanthopterus* in Dez River follow the order: liver> muscle> gill.

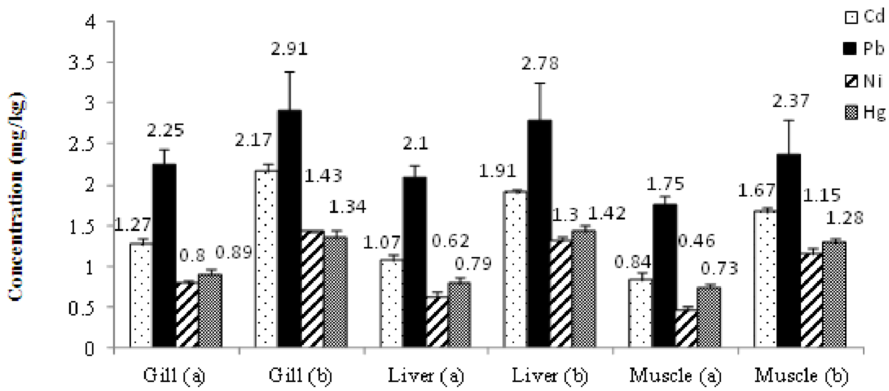

Fig3: Concentrations of heavy metals (mgkg^{-1}) in tissues of fish in Karoon River
a: *Barbus grypus*; b: *Barbus xanthopterus*

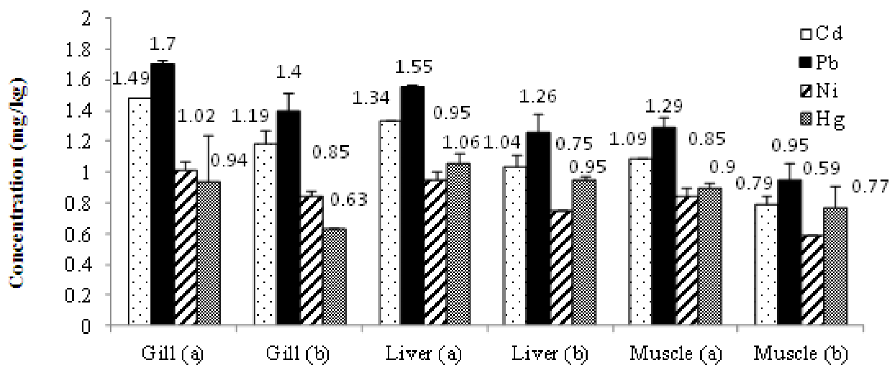

Fig4: Concentrations of heavy metals (mgkg^{-1}) in tissues of fish in Dez River
a: *Barbus grypus*; b: *Barbus xanthopterus*

Discussion:

The results showed that the muscle was not an active tissue regarding the accumulation of metals. The metal concentration in muscle is important for the edible parts of the fish. Fish generally accumulate contaminants from aquatic environments, have been largely used in food safety studies. The maximum cadmium level permitted in fish is 0.2 mg/kg for WHO (1985), 0.5 mg/kg for FAO (1983), 0.2 for MAFF (1995), 0.05 mg/kg for NHMRC (1990) and 2 mg/kg for FDA (1996) (Table 1). Generally, in this research cadmium levels in fish from freshwater were found to be higher than legal limits and have significant differences (P<0.05) in fish samples with WHO, FAO, MAFF and NHMRC legal limits, except for FDA which cadmium levels in fish samples were lower than FDA legal limit. In the literature cadmium levels in the muscle of fish samples have been reported as 0.42 mg/kg in *Labeo rohita* and 0.41 mg/kg in *Ctenopharyngodon idella* in lake of Bhopal, India (Malik *et al*., 2010), 0.01 µg/g in *Elops lacerta* and 0.03 µg/g in *Psettias sebae* in Calabar River of Nigeria

(Ekpo and Ibok, 1999). Adverse effects from cadmium can occur in fish with dietary levels of 0.1 ppm (Eisler, 1985). Cadmium may accumulate in the human body and may induce kidney dysfunction, skeletal damage and reproductive deficiencies (Commission of the European Communities, 2001).

The maximum lead level permitted is 0.3 mg/kg for WHO (Czarnezki, 1985), 0.5 mg/kg for FAO (1983), 2.0 mg/kg for MAFF (1995) and 1.5 mg/kg for NHMRC (1990). Generally, lead levels in analyzed fish samples were found to be higher than legal limits, except for lead levels in fish in comparison with MAFF and NHMRC which were lower than MAFF and NHMRC legal limits and only lead level in *B. xanthopterus* in Karoon River was higher than MAFF legal limit and lead levels in fish from Karoon River were higher than NHMRC legal limit. Lead levels have significant differences (P<0.05) in fish samples with WHO and FAO legal limits (Table 1). In the literature lead contents in the muscle of fish have been reported as 0.68 mg/kg in *Barbus xanthopterus* and 0.66 mg/kg in *Barbus rajanorum* in Atatürk Dam Lake, Turkey (Alhas *et al*., 2009), 1.23 mg/kg in *Tor grypus* in Atatürk Dam Lake, Turkey (Oymak *et al*., 2009), 0.39 mg/kg in *Labeo rohita* and 1.32 mg/kg in *Ctenopharyngodon idella* in lake of Bhopal, India (Malik *et al*., 2010), 0.02 mg/kg in *Elops lacerta* and 0.01 mg/kg in *Psettias sebae* in Calabar River of Nigeria (Ekpo and Ibok, 1999). Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adult (Commission of the European Communities, 2001).

The maximum nickel level permitted for fishes is 0.3 mg/kg according to WHO (1994) standard. Generally, nickel levels in analyzed fish samples were found to be higher than legal limit and have significant differences (P<0.05) in fish samples with WHO legal limit (Table 1). In the literature nickel content in the muscle of fish have been reported as 0.08 mg/kg in *Barbus xanthopterus* and 0.04 mg/kg in *Barbus rajanorum mystaceus* in Atatürk Dam Lake, Turkey (Alhas *et al*., 2009), 0.16 mg/kg in *Tor grypus* in Atatürk Dam Lake, Turkey (Oymak *et al*., 2009), 0.20 mg/kg in *Labeo rohita* and 0.67 mg/kg in *Ctenopharyngodon idella* in lake of Bhopal, India (Malik *et al*., 2010). Excessive amount of nickel are hazardous for humans, producing disturbances in magnesium, zinc and manganese, metabolism (Anke and Seifert, 2005). Nickel is also considered to be carcinogenic (Salnikow and Kasprzak, 2005).

The maximum mercury level permitted is 0.1 mg/kg for WHO (1985), 1 mg/kg for NHMRC (1990) and 0.1-0.5 mg/kg for FDA (1996) standards. Generally, mercury levels in analyzed fish samples were found to be higher than legal limits except for mercury levels in *B. grypus* from Karoon River and in *B. grypus* and *B. xanthopterus* from Dez River in comparison with NHMRC which were lower than NHMRC legal limit and mercury levels have significant differences (P<0.05) in fish samples with WHO and FDA legal limits (Table 1). In the literature mercury levels in the muscle of fish samples have been reported as 0.77 mg/kg in *Labeo rohita* and 0.14 mg/kg in *Ctenopharyngodon idella* in lake of Bhopal, India (Malik *et al*., 2010), 0.12 mg/kg in *Liza parsia* in Sunderban mangrove wetland of northeast India (Saha *et al*., 2006). Mercury toxicity cause growth deficits and affects fish organs. In humans, mercury is toxic to the developing fetus and considered a possible carcinogen (Ikem and Egilla, 2008). Mercury is a known human toxicant and the primary sources of mercury contamination in man are through eating fish (Emami Khansari *et al*., 2005).

In this study the highest levels of Cd, Pb and Ni were found in the gill of fish in two rivers and Hg level in the liver was higher than other tissues except for *B. grypus* in Karoon River. The highest level of Hg in the liver has been corroborated by Kennedy (Kennedy, 2003). Havelkova *et al*., (2008) reported that the target organ for Hg accumulation in fish from heavily contaminated localities was the liver. The results of our study were similar to the above study. The liver has the ability to accumulate large quantities of pollutants from the external environment and also plays an important role in storage, redistribution, detoxification and transformation of pollutants (Evans *et al*., 1993). Studies carried out with various fish species have shown that heavy metals accumulate mainly in metabolic organs such as liver that stores metals to detoxificate by producing metallothioneins (Hogstrand and Haux, 1991).

In *B. grypus* in Karoon River, the highest levels of Cd, Pb, Ni and Hg were found in the gill. The gills are the first organs to be exposed to resuspended sediment particles, so they can be significant sites of interaction with metal ions (Pawert *et al*., 1998). Gills are the uptake site of waterborne ions, where metal concentrations increase especially at the beginning of exposure, before the metal enters other parts of organism (Kamaruzzaman *et al*., 2010). Metal concentration in the gills could be due to the element complexing with the mucus, which is impossible to remove completely from between the lamellae, before tissue is prepared for analysis. The absorption of metals on to the gill surface, as the first target for pollutants in water, could also be an important influence in the total metal levels of the gill (Heath, 1987). Different heavy metal levels were observed in the gill, liver and muscle of *B. grypus* and *B. xanthopterus* in Karoon and Dez Rivers (P<0.05) (Fig3, 4).

Heavy metal concentrations varied significantly depending upon the type of fish tissues and locations. The comparison of mean concentrations of metals in all tissues of *B. grypus* and *B. xanthopterus* in Karoon and Dez Rivers shows that the metal levels of tissues are very variable in fishes (Canli and Atli, 2003; Fernandes *et al*., 2007). The observed variability of heavy metal levels in different species depends on feeding habits (Romeoa *et al*., 1999), ecological needs, metabolism (Canli and Furness, 1993), age, size and length of the fish (Linde *et* *al*., 1998) and their habitats (Canli and Atli, 2003). Concentrations of heavy metals detected in the muscle, gill and liver samples showed different capacities for accumulating.

In general, the highest metal concentrations were found in the liver and gill. Thus, the liver and gill in fish are more often recommended as environmental indicator organs of water pollution than other fish organs (Karadede *et al*., 2004). The relatively high concentrations of heavy metals in liver and gill were also found in different species of fish in Tigris River and Atatürk Dam Lake (Karadede and Ünlü, 2000). Alhas *et al*., (2009) reported in *Barbus xanthopterus* and *Barbus rajanorum mystaceus* in Atatürk Dam Lake, Turkey, heavy metal concentrations in gill and liver were maximum, while these concentrations were least in muscle. So, the results of our study were similar to the above studies.

The results from this research indicated that metal accumulation depended on the tissues probably as a consequence of metabolic needs, physiochemical properties, and detoxification processes specific for each element. The results of this study showed that Pb was the highest accumulating metal compared with other metals ($P<0.05$), as Pb is a microelement naturally present in trace amount in all biological materials in soil, water, plants and animals, it has no physiological function in the organism (Cibulka, 1991).

The high levels of Pb in the Karoon and Dez Rivers have toxic effects on fish metabolism, it is important to consider the biological effects of contamination on fish health and human health who consumption fish. Fish samples should be analyzed more often in freshwater in Khouzestan with respect to toxic elements.

Conclusion:

Consumption of fish contaminated with the heavy metals cadmium, lead, nickel and mercury is the most likely route for human exposure in Khouzestan, Iran. This study was carried out to provide information on contaminants in fish which can pose a health risk to the fish themselves and to humans who consume them. The high levels of heavy metals in freshwater of Khouzestan have toxic effects on fish metabolism and human, it is important to consider the biological effects of contamination on fish health. In this research we demonstrate that *B. grypus* and *Barbus xanthopterus* are good bioindicators of environmental exposure to heavy metals in freshwater and it is important to consider the biological effects of contamination on fish health and human who consumption them. The results indicated that the muscles of some fishes were exceeded of WHO, FAO, MAFF, NHMRC and FDA guidelines.

References

Alhas, E., S.A. Oymak and H.K. Akin, 2009. Heavy metal concentrations in two barb, *Barbus xanthopterus* and *Barbus rajanorum mystaceus* from Ataturk Dam Lake, Turkey. Environmental Monitoring and Assessment, 148: 11-18.

Anke, M and M. Seifert, 2005. Nickel-ein fuer die Flora, Fauna and den Menschen essentielles und toxisches Spurenelement. In Experimentelle Modelle der Spurenelementforschung, ed. W. Windisch and C. Plitzner, pp:133-78. Muenchen: Herbert Utz Verlag.

Canli, M and G. Atli, 2003.The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environmental Pollution, 121(1): 129-36.

Canli, M and R.W. Furness, 1993.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.

Carpene, E and M. Vasak, 1989. Hepatic metallothionein from goldfish (*Carassius auratus*). Comparative Biochemistry and Physiology part B: Biochemistry and Molecular Biology, 92: 463-468.

Cibulka, J., 1991. Academia Praha, p: 426.

Cid, B.P., C. Boia, L. Pombo and E. Rebelo, 2001. Determination of trace metals in fish species of the Ria de Aveiro (Portugal) by electrothermal atomic absorption spectrometry. Food Chemistry, 75: 93-100.

Collings, S.E., M.S. Johnson, R.T. Leach, 1996. Metal contamination of Angler-caugh fish from the Mersey estuary. Marine environmental research, 41(3): 281-297.

Commission of the European Communities, 2001. Commission Regulation (EC) No. 221/2002 of 6 February 2002 amending regulation (EC) No. 466/2002 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities, Brussels, 6 February 2002.

Czarnezki, J.M., 1985. Accumulation of lead in fish from Missouri streams impacted by lead mining. Bulletin of Environmental Contamination and Toxicology, 34: 736-745.

Dallinger, R., F. Prosi, H. Senger and H. Back, 1987. Contaminated food and uptake of heavy metals by fish: A review and proposal for further research. Oecologia, 73: 91-98.

Darmono, D., G.R.W. Denton, 1990. Heavy metal concentration in the banana prawn (*Penaeus merguiensis*) and leader prawn(*P.monodon*)in the Townsvile region of Australia. Bulletin of Environmental Contamination and Toxicology, 44: 479-486.

Eisler, R., 1985. Cadmium-hazards to fish, wildlife and invertebrates: a synoptic review. US Fish and Wildlife Service Reports, 85(1-2): Washington, DC.

Ekpo, B.O and U.J. Ibok, 1999.Temporal variation and distribution of trace metals in freshwater and fish from Calabar River, S.E. Nigeria, Environmental Geochemistry and Health, 21: 51-66.

Emami Khansari, F., M. Ghazi-Khansari and M. Abdollahi, 2005. Heavy metal content of canned tuna fish. Food Chemistry, 93: 293-296.

Evans, D.W., D.K. Dodoo and P.J. Hanson, 1993.Trace-element concentrations in fish livers: implications of variations with fish size in pollution monitoring. Marine Pollution Bulletin, 26: 329-334.

FAO (Food and Agriculture Organization), 1983. Compilation of legal limits for hazardous substances in fish and fishery products, FAO Fishery Circular, Food and Agriculture Organization of the United Nations, Rome., 464: 5-10.

Fernandes, C., A. Fontainhas-Fernandes, D. Cabral and M.A. Salgado, 2008. Heavy metals in water, sediment and tissues of Liza saliens from Esmoriz-Paramos lagoon, Portugal. Environmental Monitoring and Assessment, 136: 267-275.

Fernandes, C., A. Fontainhas-Fernandes, F. Peixoto and M.A. Salgado, 2007. Bioaccumulation of heavy metals in Liza saliens from the Esmoriz-Paramos coastal lagoon, Portugal. Ecotoxicology and Environmental Safety, 66 (3): 426-431.

Freedman, B., 1989. Environmental Ecology. The Impacts of Pollution and other Stresses on Ecosystem Structure and Function.; Academic Press: London.

Groth III, E., 2010. Ranking the contributions of commercial fish and shellfish varieties to mercury exposure in the United States: implication for risk communication. Environmental Research, 110: 226-236.

Havelkova, M., J. Blahova, H. Kroupova, T. Randak, I. Slatinska, D. Leontovycova, R. Grabic, R. Pospisil and Z. Svobodova, 2008. Biomarkers of contaminant exposure in Chub (*Leuciscus cephalus*) – a biomonitoring of major rivers in the Czech Republic. Sensors, 8: 2589- 2603.

Heath, A.G., 1987. In: Water Pollution and Fish Physiology, CRP press, Florida, p: 245.

Hogstrand, C and C. Haux, 1991. Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionein. Comparative Biochemistry and Physiology part C: Comparative Pharmacology, 100: 137-141.

Ikem, A and J. Egilla, 2008. Trace element content of fish feed and bluegill sunfish (*Lepomis macrochirus*) from aquaculture and wild source in Missouri. Food Chemistry, 110: 301-309.

Kamaruzzaman, B.Y., M.C. Ong and S.Z. Rina, 2010. Concentration of Zn, Cu and Pb in some selected marine fishes of the Pahang Coastal Waters, Malaysia. American Journal of Applied Sciences, 7(3): 309- 314.

Karadede, H and E. Ünlü, 2000. Concentrations of some heavy metals in water, sediment and fish species from The Atatürk Dam Lake (Euphrates), Turkey. Chemosphere, 41: 1371-1376.

Karadede, H., S.A. Oymak and E. Unlu, 2004. Heavy metals in mullet, Liza abu, and catfish, Silurus triostegus, fromthe Ataturk Dam Lake (Euphrates), Turkey. Environment International, 30: 183-188.

Kennedy, C.J., 2003. Uptake and accumulation of mercury from dental amalgam in the common goldfish, *Carassius auratus*. Environmental Pollution, 121: 321-326.

Lewis, M.A., G.I. Scott, D.W. Bearden, R.L. Quarles and M. James, 2002. Fish tissue quality in near- coastal areas of the Gulf of Mexico receiving point source discharges. Science of the Total Environment, 284: 249-261.

Linde, A.R., S. Sanchez-Galan, J.I. Izquierdo and P. Arribas, 1998. Maranon E, Garcy´' a- Vazquez E. Brown Trout as biomonitor of heavy metal pollution: effect of age on the reliability of the assessment. Ecotoxicology and Environmental Safety, 40: 120-5.

MAFF, 1995. Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1993. *Aquatic Environment Monitoring Report* No. 44. Direcorate of Fisheries Research, Lowestoft.

Malik, N., A.K. Biswas, T.A. Qureshi, K. Borana and R. Virha, 2010. Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. Journal of Environmental Monitoring and Assessment, 160(4): 267-276.

Meador, J.P., R.C. Clark Jr, P.A. Robisch, D.W. Ernest, J.T. Landahl and U. Varanasi, 1994. *National Benthic Surveillance Project: Pacific Coast*. Trace element analyses for cycles I to V (1984–1988).National Oceanic and Atmospheric Administration, National Marine Fisheries Service Technical Memorandum NMFSNW FSC-16, 206.

Miller, J.R., P.J. Lechler, K.A. Hudson-Edwards and M.G. Macklin, 2002. Lead isotopic fingerprinting of heavy metal contamination, Rio Pilcomayo basin, Bolivia. Geochemistry: Exploration. Environmental Analytical, 2: 225.

Nabawi, A., B. Heinzow and H. Kruse, 1987. As, Cd, Cu, Pb, Hg and Zn in fish from Alexandria Region, Egyption [J]. Bulletin of Environmental Contamination and Toxicology, 39: 889-897.

Oymak, S.A., H. Karadede-Akin and N. Dogan, 2009. Heavy metal in tissues of *Tor grypus* from Atatürk Dam Lake, Euphrates River-Turkey. Biologia, 64(1): 151-155.

Pawert, M., E. Muller and R. Triebskorn, 1998. Ultra structural changes in fish gills as biomarker to assess small stream pollution. Tissue and Cell, 30: 617- 626.

Rashed, M.N., 2001. Monitoring of environmental heavy metals in fish from Nasser lake. Environment International, 27: 27-33.

Robisch, P.A and R.C. Clark, 1993. Sample preparation and analyses of trace metals by atomic absorption spectroscopy. In: Lauenstein GG, Cantillo AY, editors. Sampling and Analytical Methods of the National Status and Trends Program, National Benthic Surveillance and Mussel Watch Project 1984–1992, vol. III. Comprehensive Descriptions of Elemental Analytical Methods. US Department of Commerce, National Oceanic and Atmospheric Administration. Technical Memorandum NOS ORCA, 71: 111-150.

Romeo, M., Y. Siau, Z. Sidoumou and M. Gnassia- Barelli, 1999. Heavy metal distribution in different fish species from the Mauritania coast. Science of the Total Environment, 232: 169-175.

Saha, M., S.K. Sarkar and B. Bhattacharya, 2006. Interspecific variation in heavy metal body concentrations in biota of Sunderban mangrove wetland, northeast India. Environment International, 32: 203-207.

Salnikow, K and K.S. Kasprzak, 2005. Ascorbate depletion: A critical step in nickel carcinogenesis Environmental Health Perspectives, 113: 577-84.

Tuzen, M., 2009. Toxic and essential trace element contents in fish species from the Black Sea, Turkey. Food and Chemical Toxicology, 47: 1785-1790.

UNEP.Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons, 1991. Reference Methods for Marine Pollution Studies No.12, Rev. 2.UNEP. Nairobi. 17.

WHO, 1985. Review of potentially harmful substances - cadmium, lead and tin. WHO, Geneva. (Reports and Studies No. 22. MO /FAO /UNESCO /WMO /WHO /IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution).

World Health Organization, 1994. Quality Directive of Potable Water, 2nd ed, WHO, 197.

Zhang, Z., L. He, J. Li and Z.B. Wu, 2007. Analysis of Heavy Metals of Muscle and Intestine Tissue in Fish – in Banan Section of Chongqing from Three Gorges Reservoir, China. Polish Journal of Environmental, 16(6): 949- 958.