The Journal of Animal & Plant Sciences, 21(2): 2011, Page: 176-181
ISSN: 1018-7081

ABSORPTION AND BIOACCUMULATION OF WATER-BORNE INORGANIC MERCURY IN THE FINGERLINGS OF GRASS CARP, *CTENOPHARYNGODON IDELLA*

M. S. Ahmed, Y. Aslam and W. A. Khan

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan
Corresponding author: drshafiq.ahmed@gmail.com

ABSTRACT

A study was conducted to evaluate the absorption and accumulation of total mercury (Hg) in various tissues of fingerlings of grass carp, *Ctenopharyngodon idella* when exposed to lower doses of water-borne Hg. Single breed fingerlings (10-12 cm) of grass carp were obtained from a commercial fish seed hatchery. Six groups (in triplicate) of fish (40 each) were maintained at 22° C, pH 7.0, hardness 140 mg/l and DO 7.0 mg/l in 90 liters of water in glass tanks. Each group was exposed to a sub-lethal dose of inorganic Hg as 0.0 µg/l (control), 0.1 µg/l, 1.0 µg/l, 2.0 µg/l, 2.5 µg/l and 3.0 µg/l for six weeks. Fish sampling was done on day zero and weekly thereafter. Five fish from each tank (15/treatment) were sacrificed; various tissues were collected and prepared for analysis of total mercury using cold-vapor atomic absorption spectrophotometry. Fish tissues that were directly exposed to Hg, the absorption and accumulation was significantly high ($P<0.05$) in skin (including mucus) (1.68 ± 0.012 µg/g dry wt.), gills (1.64 ± 0.013 µg/g dry wt.) and eyes (1.45 ± 0.011 µg/g dry wt.) as compared to the tissues from control group. While the tissues that were not directly in contact with water containing Hg had accumulated it significantly high ($P<0.05$) in muscles (2.03 ± 0.012µg/g dry wt.), intestine (1.39 ± 0.015 µg/g dry wt.) and liver (1.38 ± 0.013 µg/g dry wt.) as compared to tissues from zero treatment. This study clearly indicates that the fishes living in water bodies receiving industrial effluents and city waste water containing various toxicants particularly heavy metal ions, even at low concentration, absorb and accumulate these heavy metals in their various tissues like skin (including mucus), gills and intestine directly from water as well as alongwith the food during feeding. With the passage of time as the fishes grow, these metals accumulate in various internal organs like liver, muscles and intestine to a significantly high concentration not suitable for human consumption.

Key words: aquatic toxicants, Chinese carp, fish tissues, herbivore fish, sub-lethal dose, topical absorption, water pollution.

INTRODUCTION

The greatly increased circulation of toxic metals in soil, air and water resulted in the inevitable build up of each toxic material in the food through food chain available for human consumption (Peterson *et al.*, 1989). The industrial age has lead to more widespread occurrence of occupational diseases related to exposure to a variety of toxic metals including Hg (Forstner and Wittmann, 1998). Industrial development in Pakistan during past few decades has launched many industrial zones at major cities. These industries are producing tremendous amount of effluents that are drained into nearby rivers like Ravi. River Ravi, being seasonal receives enough rain water during monsoon season while in winter the amount of water decreases significantly. But the industrial effluents and city sewage are produced uninterrupted and enter continuously in the river untreated. A preliminary study was conducted and samples of water, aquatic plants, fishes and turtles were collected from river Ravi. The results revealed many fold (10-100) high level of various metals (Hg, Cd, Pb, Cu, Ni & Zn) in water, plant, fishes and turtle tissues (Ahmad, 2001).

Mercury being non-essential element is extremely toxic because of its high affinity to bind with thiol group (-SH) of the cell membrane even stronger than the affinity of cadmium to bind with sulphide (Nies, 1999). The uptake of inorganic waterborne Hg at gills prevents the active uptake of sodium, which resulted in the inability of freshwater fishes to maintain salt balance with their surroundings. Its uptake by the pseudobranch and choroids gland may result in additional disturbance of some regulatory mechanisms (Khuhawar and Languani, 2000). It is also documented that uptake of Hg from water in the gonadal tissue may be associated with the decreased egg production and hatching of young (Heath, 1995). Bioaccumulation of Hg is known to adversely affect liver, muscles, kidney and other tissues of the fish, disturb metabolism and hampers development and growth (Andre and Ribeyre, 1992).

The present study was conducted to estimate the uptake and accumulation of total Hg in various tissues like skin, gills, eyes, liver, intestine and muscles of fingerlings of grass carp, *Ctenopharyngodon idella* when exposed to lower doses.

Fish were exposed only 6 weeks that will reveal bioaccumulation of total Hg (inorganic and methyl) in various tissues of fish then; it will be possible to predict dose-related accumulation in fishes already present (for quiet some time) in water bodies receiving various kinds of effluents carrying variable amount of heavy metals. *C. idella* is an herbivore fish mainly depends on aquatic vegetation (algae, phytoplankton and other plants) as food source. It was selected because of its continuously feeding habits; consuming large amount of vegetation daily, leading to very high uptake of toxicants and eventually accumulation, makes this fish pollution indicator of toxicants particularly heavy metals in any freshwater body.

MATERIALS AND METHODS

Experimental design: Single breed fingerlings of grass carp measuring 10-12 cm (±1.35cm) were purchased from a commercial fish seed hatchery. All fish were acclimatized to 12:12-hrs light: dark regimen in 90 L glass aquaria at 22.04 ± 0.19^{o} C, pH 7.24 ± 0.12, hardness 140.6 ± 1.6 mg/l and DO 7.21 ± 0.25 mg/l (table 1). Mercuric chloride (E. Merck, Darmstadt, Germany) was dissolved in deionized water and clear solution was obtained by adding few drops of acetic acid. The concentrations of Hg in water were adjusted and maintained to nominal values of 0.0 µg/l (control), 0.1 µg/l, 1.0 µg/l, 2.0 µg/l, 2.5 µg/l and 3.0 µg/l in the tanks for 6 weeks. All fish were fed with pelleted green leaf feed (Hg level 0.071 ± 0.03 µg/g dry weight) to an equivalent of 3% of wet body weight three times daily at equal intervals. Uneaten food and the feces were removed at 30 minutes after feeding from all tanks daily.

Tissue sampling and Hg analysis: Fish tissue sampling was done at one week interval from all treatments for 6 weeks. Five fish from each tank (15 fish / treatment) were sacrificed; their various tissues like skin (including mucus), gills, eyes (eye balls), muscles, liver and intestine were removed, wrapped in Teflon grade polythene bags and kept at –40^{o} C until analyzed. Fish tissue sample were pulverized in liquid nitrogen with glass mortar and a pestle (precleaned with 10% HNO_3) and allowed to air-dry overnight at room temperature to a constant weight. The dried samples were then acid-digested according to cold-vapor technique (Csuros and Csuros, 2002) and analyzed by continuous-flow, cold-vapor atomic absorption spectrophotometry (Scheuhammer and Bond, 1991). Standard curves were established by measuring different dilutions of Hg standards, the lowest dilution being 0.05 µg Hg/l (minimum detection limit = MDL). The accuracy and integrity of the sample analysis was monitored by regularly running check standards and deionized water blanks.

Statistical analysis: One-way nested ANOVA followed by the Tukey-Kramer multiple comparison test were used. An overall α value of 0.05 was used to assess significant differences. Data are presented as mean ± SEM. Most statistical analyses were conducted with STATISTICA data mining software, version 8 (StatSoft, Inc., Tulsa, OK). Bartlett's test was used to assess the homogeneity of variables.

RESULTS

The uptake and accumulation of total Hg (inorganic and methyl) in the skin (including mucus) at treatments 0.1 µg/l and 1.0 µg/l and 2.0 µg/l was high but not significantly different from control. At treatments 2.5 µg/l and 3.0 µg/l it was significantly high (P<0.01) from control as well as from 0.1 µg/l exposure (figure 1a). In the gills, total Hg uptake was high but not significantly different from control. At treatments 2.5 µg/l and 3.0 µg/l it was significantly high (P<0.01) from control as well as from 0.1 µg/l (figure 1b).

The absorption and accumulation of total Hg in the eyes at treatments 2.5 µg/l and 3.0 µg/l was significantly high (P<0.01) from control as well as from 0.1 µg/l exposure but no significant difference was observed among other treatments (figure 1c). The accumulation of total Hg in the muscle and intestine at treatment 3.0 µg/l was significantly high (P<0.05) from control as well as 0.1 and 1.0 µg/l (figure 1d-e). The accumulation in liver was high but non-significant (P>0.05) from other treatments but significant (P<0.05) from control group throughout the exposure time (figure 1f).

The maximum values of total Hg accumulation in each tissue (Hg µg/g dry wt.) were calculated for the highest treatment. The data indicated the following rank order of total Hg absorbed (from highest to lowest mean total Hg in each tissue) in the muscles (2.03 ±0.012 µg/g), skin (including mucus) (1.68 0.012 µg/g dry wt.) > gills (1.64 ±0.013 µg/g dry wt.) > eyes (1.45 ±0.011 µg/g) > intestine (1.39 ±0.015 µg/g) > liver (1.38 ±0.013 µg/g dry wt.), (figure 2).

DISCUSSION

Fishes are ideal indicator of heavy metal contamination in aquatic ecosystem because they occupy different trophic levels and are of different sizes and ages (Eromosele *et al*., 1995, Gupta and Dua, 2002). Since fishes are located at the end of food chain in the aquatic ecosystem, they reflect the status of water quality and are indicator of water pollution particularly heavy metals. Grass carp is an herbivore fish, consumes large amount of vegetations (phytoplankton, algae, grasses and other aquatic plant), mostly contaminated with heavy metals including Hg and accumulates metal ions gradually in its body (various tissue and organs) as it grows.

Metabolically fish can regulate metal concentration to certain extent where after bioaccumulation will occur in various tissues (Heath, 1995). The ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal that can be taken up by that specific tissue. Furthermore, physiological differences and the position of each tissue in fish can also influence the bioaccumulation of a particular metal (Kotze, 1997). The uptake of metal ions (including Hg) was higher in tissues which are directly exposed to the water containing metal ions and lower in tissues which were not or less exposed to metal present in contaminated water (Zhou and Wong, 2000; Javid *et al*., 2007; Korai1 *et al*., 2008; Rauf *et al*. 2009; Ahmed and Bibi, 2010). In the present study, the tissues like skin and gills were directly exposed to inorganic mercury and the accumulation was observed during start of exposure which increased significantly high ($P<0.05$) with the passage of time; at the end of exposure. This happened because of exposure to high concentration and for longer time when compared to other groups. Intestine also showed significant accumulation because of continuous ingestion of Hg ions from exposure water as well as alongwith the food during feeding. This accumulation gradually increased in the intestinal tissues with the passage of time. In the liver, the accumulation of mercury was very low at the start of exposure but it increased with the passage of time because liver is more prone to accumulation because of its metabolic activities. But it is noted that the induction of low molecular weight metal-binding proteins, such as metallothionein are closely related to heavy metal exposure and metals taken up from the environment can be detoxificated by binding on these proteins (Roesijadi and Robinson, 1994; Canli *et al.*, 1997; Kotze, 1997; Khuhawar and Languani, 2000). Therefore, tissue like liver which is a major producer of metal-binding proteins shows high concentrations of most heavy metals detoxification (Thomas *et al*., 1983; Amiard *et al*., 1987; Donald and Ostrander, 1993; Roesijadi and Robinson, 1994; Allen, 1995; Heath, 1995) which eventually result in the clearance of heavy metal ions from the body. Furthermore, the physiological differences and the position of each tissue in the fish can also influence the accumulation of a particular metal (Donald and Ostrander, 1993; Heath, 1995; Kotze, 1997). In other words, the amount of a metal accumulated is influenced by various environmental, biological and genetic factors, leading to the differences in metal accumulation between different individuals, species, age, tissues, seasons and sites (Kotze *et al*., 1999).

Table 1. Physico-chemical parameters maintained in the water for fingerlings of *Ctenopharyngodon idella* during Hg exposure. Data is presented as ±SEM, n=21

Weeks	Temperature (C°)	pH	Hardness (mg/l)	Dissolved oxygen (mg/l)
0	22.0	7.1	141	7.5
1	21.8	7.1	142	6.8
2	21.9	7.4	141	7.4
3	22.4	7.2	139	7.2
4	22.2	7.3	143	7.4
5	22.1	7.2	138	7.3
6	21.9	7.4	140	6.9
Mean	22.04 ±0.19	7.24 ±0.12	140.6 ±1.6	7.21 ±0.25

When fishes are exposed to low concentrations of various metal ions in an aquatic environment they uptake the biologically available metal ions directly from the surrounding water as topical absorption through gills and skin or through the ingestion of contaminated food. Metals when enter the digestive tract alongwith ingestion of food or water reach at intestinal level, are absorbed by the blood and reach at the organ of metabolic activity like liver where they get accumulated. Their depositions also occur later in the muscles, skeleton and other internal organs (Fergusson, 1990; Eromosele *et al.*, 1995; Heath, 1995; Simon and Boudou, 2001; Javid *et al*., 2007; Korai1 *et al*. 2008; Rauf *et al*., 2009). But, it is well known that all metal ions taken up by a fish through any route are not totally accumulated because fish can regulate metal concentrations to a certain extent, after which accumulation will occur. Therefore, the ability of each tissue to either regulate or accumulate metal ions can be directly related to the total amount of metal accumulated in that specific tissue. This metal regulation is due to induction of low molecular weight metal-binding proteins, such as metallothionein are closely related to heavy metal exposure and metals taken up from the environment can be detoxified by binding on these proteins (Roesijadi and Robinson, 1994; Canli *et al.,* 1997; Kotze, 1997). Therefore, tissues like liver, which is a major producer of metal-binding proteins, show high concentrations of most heavy metals detoxification (Thomas *et al*., 1983; Amiard *et al*., 1987; Roesijadi and Robinson, 1994; Allen, 1995; Heath, 1995) which eventually result in clearance of heavy metal ions from the body. Furthermore, the physiological differences and the position of each tissue in the fish can also influence the accumulation of a particular metal (Heath, 1995; Kotze, 1997). In other words, the amount of a metal accumulated is influenced by various environmental, biological and genetic factors, leading to the differences in metal accumulation between different individuals, species, tissues, seasons and sites (Kotze *et al*. 1999).

LEGEND TO THE FIGURES

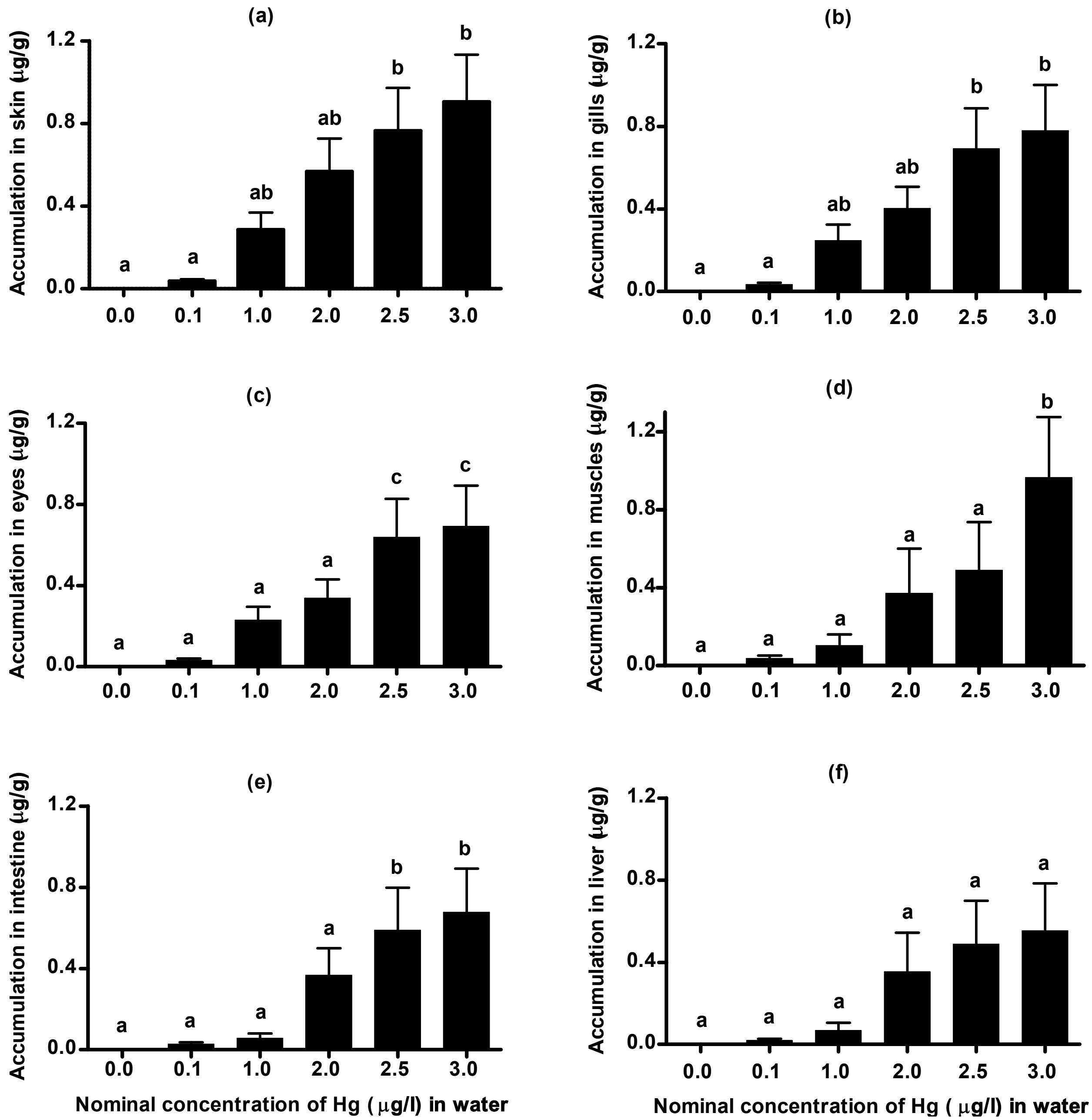
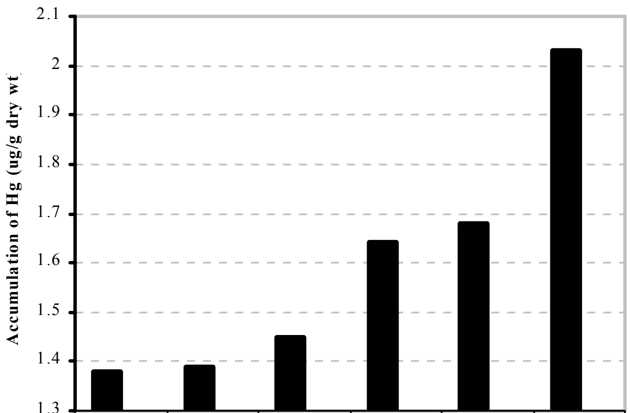


Figure 1

Figure 1 (a-f): Fingerlings of an herbivore fish, *Ctenopharyngodon idella* were exposed to various nominal concentrations of water-borne mercury (Hg) for 42 days. Fish tissue samples were taken on day 0 and weekly thereafter and analyzed by continuous-flow cold-vapor atomic absorption spectrophotometry for uptake and accumulation of total Hg in various tissues. The level of uptake and accumulation of total Hg (µg/g dry weight) in skin (a), gills (b), eyes (c), liver (d), muscles (e) and intestine (f) is presented. Bars represent the mean values (± SEM). Bars with common letters are not significantly different, $P<0.05$), n=15.

It is worth documenting that fishes from water reservoirs receiving effluents even in less volume or low concentration of toxicants containing heavy metals from



	Liver	Intestine	Eyes	Gills	Skin	Muscles
Mean	1.38	1.39	1.45	1.64	1.68	2.029
± SEM	0.013	0.015	0.011	0.013	0.012	0.012

Figure 2. The rank order of uptake and bioaccumulation (from highest to lowest) of total mercury (Hg µg/g dry weight) in various tissues of fingerlings of *Ctenopharyngodon idella* at the highest concentration and maximum exposure time. Data is presented as ±SEM.

industries, agriculture runoff and domestic wastewater may have accumulated heavy metals in their fillet as theygrow, that will be transferred to humans when consumed and may impair body metabolism (WHO, 1980; Donald and Ostrander, 1993; Ceirwyn, 1995; Pourang *et al*. 2005; Palaniappan *et al.* 2009). *C. idella* is a preferred fish for human consumption in Pakistan as well as in Indian subcontinent, found in various water bodies like rivers and lakes receiving toxic effluents, is accumulating metals including mercury in the fillet. River Ravi is located in the vicinity of Lahore, Pakistan which is continuously receiving untreated industrial effluents and city wastewater containing high contents of various heavy metals (Ahmad, 2001). *C. idella* is an equally important fish among other commercial fishes. While being in river Ravi until they reach the consumable size (1-2 kg) would have already accumulated very high contents of Hg in its various tissues including muscles (consumable part). Our earlier studies (Ahmad, 2001) have revealed that Hg level in the water of river Ravi is 1.25-1.7 ppm (more than 2000 times higher than WHO standards for drinking water and number of fish species (*Cirrhina mrigala*, *Catla catla*, *Oreochromis niloticus*, *Notopterus notopterus*, *Laboe calbasu*) about 1 year old had already accumulated very high level of Hg (1.03 – 1.21 ppm) in their muscles which is more than 2000 times higher than the permissible level of Hg in food recommended for human consumption (US.EPA, 1976; WHO, 1980). It is therefore, recommended that the consumption of *C. idella* and similar other freshwater fishes obtained from contaminated water bodies like river Ravi should be prohibited.

Acknowledgments: Present research grant was awarded to the first author that was funded by Faculty Development Program, 2005-06, University of the Punjab, Lahore, Pakistan.

REFERENCES

Ahmad, F. (2001). Some heavy metal (Hg, Cd, Pb, Cu, Ni and Zn) concentrations in water, sediment and aquatic organisms from Nala Deg and river Ravi at Baloki headwork. M. Phil thesis, G C University, Lahore, Pakistan. pp 96

Ahmed, M. S. and S. Bibi, (2010). Uptake and bioaccumulation of waterborne lead (Pb) in the fingerlings of a freshwater cyprinid, *Catla catla* L. The J. Anim. and Plant Sci. 20 (3): 201-207

Allen, P. (1995). Accumulation profiles of lead and cadmium in the edible tissues of *Oreochromis aureus* during acute exposure. J. Fish. Biol., 47(4): 559-568.

Amiard, J. C., C. Amiard-Triquet and C. Metayer, (1987). Comparative studies of patterns of bioaccumulation, toxicity and regulation of some metals in various estuarine and coastal organisms. J. Exp. Mar. Ecol., 106: 73-89.

Andre, J. M. and F. Ribeyre (1992). Mercury contamination levels and distribution in tissues and organs of delphinids from eastern tropical pacific, in relation to biological and ecological factors. URA- CNRS- UNI-BORDEAUX-FRANCE. Environ. Res., 30: 42-72.

Canli, M., R. M. Stagg and G. Rodger (1997). The induction of metallothionein in tissues of Norway lobster, *Nephrops norvegicus* following exposure to cadmium, copper and zinc: the relationship between metallothionein and the metals. Environ. Pollut., 96: 343-350.

Ceirwyn. S. J. (1995). Analytical Chemistry of Foods. Blackwell Academic Press. pp: 24-79.

Csuros, M and C. Csuros, (2002). Environmental Sampling and Analysis for Metals. Lewis Publishers, CRC Press New York, pp 237-238.

Donald, C. M and G. K. Ostrander (1993). Aquatic Toxicology: Molecular, Biochemical and Cellular Perspectives. Lewis Publishers, Boca Raton, Florida, USA.

Eromosele, C. O., I. C. Eromosele, S. L. M. Muktar and S. A. Birdling (1995). Metals in fish from the upper Benue river and lakes Geriyo and Njuwa in northeastern Nigeria. Bull. Environ. Contam. Toxicol., 54: 8-14.

Fergusson, J. E. (1990). The Heavy Elements: Environmental Impact and Health Effects. Pergamon Press, Oxford. U. K.

Forstner, U. and G. T. M. Wittmann, (1998). Pollution in the Aquatic Environment. Springer Verlag, Berlin.

Gupta, N. and A. Dua, (2002). Mercury induced architectural alterations in the gills surface of a freshwater fish, *Channa punctatus.* J. Environ. Biol., 23: 383-386.

Heath, A. G. (1995). Water Pollution and Fish Physiology. Lewis Publishers, Boca Raton, Florida, U.S.A.

Javid, A., M. Javed, S. Abdullah and Z. Ali (2007). Bio-accumulation of lead in the bodies of major carps (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) during 96-h LC50 exposures. Int. J. Agri. Biol., 9: 909-912.

Khuhawar, M. R. and S. N. Languani (2000). Determination of mercury by liquid chromatography in freshwater fishes using 2 – thiophenaldehyde – 4 – phenyl – 3-thiosemicarbazone. Pak. J. Sci. Res., 44: 253 – 256.

Korai1, A. L., G. A. Sahato1, T. G. Kazi and K. H. Lashari (2008). Lead concentrations in fresh water, muscle, gill and liver of *Catla catla* (Hamilton) from Keenjhar Lake. Pak. J. Anal. Environ. Chem., 9: 11-19.

Kotze, P. J. (1997). Aspects of water quality, metal contamination of sediment and fish in the Olifants Rivers, Mpumalanga. M.Sc. Thesis, Rand Afr. Univ., South Africa.

Kotze, P. J., H. H. DuPreez and J. H. J. Van Vuren, (1999). Bioaccumulation of Cu and Zn in *Oreochromis mossambicus* and *Clarias gariepinus* from the Olifants River Mpumalanga, Rand Afrikaans University, South Africa 12 pp

Nies, D. H. (1999). Microbial heavy metal resistance. Appl. Microbial. Biotechnol., 51: 730-750.

Palaniappan, P. L. R. M., N. Krishnakumar and M. Vadivelu (2009). Bioaccumulation of lead and the influence of chelating agents in *Catla catla* fingerlings. Environ. Chem. Letters, 7: 51-54.

Peterson, R. H., A. Sreedharan and S. Ray (1989). Accumulation of trace metals in three species of fish from lakes in New Brunswick and Nova Scotia (Canada): Influence of pH and chemical parameters. Water Pollut. Res. J. Can., 24: 101-117.

Pourang, N., S. Tanabe, S. Rezvani, and H. Dennis (2005). Trace element accumulation in edible tissues of five sturgeon species from the Caspian Sea. Eviron. Mont. Asses., 100: 89-108.

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

Roesijadi, G. and W. E. Robinson (1994). Metal regulation in aquatic animals: Mechanisms of uptake, accumulation and release. (In: Malins and Ostrander (Eds) Aquatic Toxicology, Lewis Publishers, CRC Press, Florida, USA).

Scheuhammer. A. M. and D. Bond, (1991). Factors affecting the determination of total mercury in biological samples by continuous-flow cold vapor atomic absorption spectrophotometry. Biol. Trace Elem Res., 31: 119-129.

Simon, O. and A. Boudou, (2001). Direct and trophic contamination of the herbivorous carp, *Ctenopharyngodon idella* by inorganic mercury and methyl mercury. Ecotoxicol. Environ. Saf., 50: 48-59.

Thomas, D. G., A. Cryer, F. D. L. G. Solbe and J. Kay, (1983). A comparison of the accumulation and protein binding of environmental cadmium in the gills, kidneys and liver of rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol., 76c: 241-246.

U.S. EPA. (1976). Effects of exposure to heavy metals on selected fresh water fish: toxicity of copper, cadmium, chromium, and lead to eggs and fry of seven fish species. Environmental Research Laboratory, Office of Research and Development, Duluth, MN. 600/3-76-105.

WHO (WORLD HEALTH ORGANIZATION). 1980. International Standards for Drinking Water. pp. 96 – 145.

Zhou, H. Y. and M. H. Wong, (2000). Mercury accumulation in freshwater fish with emphasis on the dietary influence. Water Res., 34: 4234-4242.