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BIOACCUMULATION OF LEAD IN VARIOUS TISSUES OF THE FRESHWATER FISH *Catla catla* (HAMILTON, 1822)

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ABSTRACT

A study was conducted to evaluate the level of heavy metal lead (Pb) in various tissues of adult *Catla catla* when exposed to a sub-lethal concentration of 6.52 mgL^{-1} ($1/25^{th}$ of 96 hrs LC_{50} value) for a period of 120 days of exposure. The bioaccumulation of lead in the tissues studied was increased with duration of exposure periods. The pattern of lead accumulation was in the decreasing order of kidney > liver > gill > ovary > testis > brain at the end of 120 days of exposure. Preferential accumulation of lead was also observed in the tissues under experimentation. This may be due to the physiological differences and the position of each tissue in the fish. The maximum level of Pb was observed in kidney at the end of exposure period and which is related to the role of kidney as the excretory organ. The minimum concentration of Pb and the gender specific accumulation was also observed in gonads. Pb concentration was more in ovaries than the testis and this may be because of metabolic differences between the sexes. During the study period the level of Pb was minimum in brain. This may be due to the indirect contact of brain with the medium. Prolonged exposure of Pb may cause chronic nephropathy, hypertension, pathological changes and also reproductive impairments. Hence, the Pb level in the tissues of aquatic animals are occasionally monitored to ensure that the level do not constitute health hazards to consumers.

INTRODUCTION

In aquatic ecosystems, the heavy metals have received considerable attention due to their toxicity and accumulation in biota (Javed and Hayat, 1999). Metals have the tendency to accumulate in various organs of the aquatic organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards [Puel *et al.*, 1987; Godwin *et al.*, 2003; Rauf *et al.*, 2009]. Heavy metals like lead and other trace metals have high affininty for animal tissues where they are concentrated to varying levels (Rainbow and Dallinger, 1993; Huang, 2003; Martinez *et al.*, 2004). Lead is a microelement and has no physiological function in the organism (Neumann *et al.*, 1990; Cibulka, 1991). The divalent form of Pb(II) is the stable ionic form present in the environment and is thought to form, in which most lead is bio-accumulated by aquatic organisms. The main source of lead contamination are smelting works, transportation, rain, snow, soil, water, plants and animals, approximately 98% of lead in the atmosphere originates from the human activates (Fadrus, 1979; Strmiskova, 1992; D.W.A.F., 1996). When fishes are exposed to high level of metal ions in aquatic environment, their tissues tend to take up these metal ions through various routes from their surroundings. In natural Water, the total lead concentrations generally range between 0.05 and 10.00 mgL^{-1} (Galvin, 1996). Pb is also known to biologically accumulate in other tissues of fish, including skin and scales, gills, eyes, liver, kidneys and muscles (Rashed, 2001; Nussey *et al.*, 2000; Alves *et al.*, 2006, Spokas *et al.*, 2006; Javid et al ., 2007). The primary mode of uptake of aqueous Pb^{2+} in freshwater fishes is through their gills into the blood stream and is also ingested along with the food and water, finally absorbed in other tissues (Seymore, 1995; Ay *et al.*, 1999; Mac-donald *et al.*, 2002, Hansen *et al.*, 2007). Fishes exposed to high levels of lead exhibit a wide-range of effects including behavioral deficits, cancer, disease resistance, muscular and neurological degeneration and destruction, growth inhibition, mortality, reproductive problems, may cause structural lesions and functional disturbances and paralysis (U.S. EPA, 1976; Eisler, 1988; Jezierska and Witeska, 2001; Rademacher *et al.*, 2003). Little

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is known about the accumulation of lead (Pb) on fishes (Javid *et al.,* 2007; Korai *et al.,* 2008; Ahmed and Bibi, 2010; Nwani *et al.,* 2010; Kusemiju *et al.,* 2012). Hence, in the present investigation, an attempt has been made to estimate the accumulation of heavymetal lead in various tissues like gills, liver, kidney, testis, ovary and brain of the freshwater fish, *Catla catla*.

MATERIALS AND METHOD

Experimental design

This study was carried out in the laboratory of Zoology Department, Annamalai University during the month of January 2012 to April 2012. Adult *Catla catla* measuring 47.5 ± 2 cm in length and 890 ± 50 gm in weight were purchased from a commercial hatchery in Chidambaram. All fish were acclimatized to 12 hrs light/dark regimen in large cement tanks for two weeks prior to Pb exposure. Two large cement tanks, one treatment and a control each containing sixty adults were maintained at 22.01 ± 0.22^{o}C, pH 7.17 ± 0.14, hardness 160.01 ± 2.1 mgL^{-1} as $CaCo_3$ and DO 7.15 ± 0.24 mg/l. Lead nitrate salt (E. Merck, Analar grade) was dissolved in deionized water. The test fish were exposed to a sub lethal concentration of 6.52 mg PbL^{-1} (1 / 25th of 96 hrs LC_{50} value). All fish were fed with commercial fish feed, boiled egg white and rice bran cakes to an equivalent of 2 % body weight twice daily. Uneaten food and the feces were removed at 30 minutes after feeding from the tanks daily.

Tissue sampling and Pb analysis

Fish tissue sampling were done on day zero and 15 days once upto 120 days of exposure. Six fish from control and treated were sacrificed and various tissues like gill, liver, kidney, gonads (testis and ovary) and brain were removed. Bioaccumulations of lead in the tissues were determined following the method of Kendall and Scanlon (1982). The tissues were dried separately in hot air oven at a temperature of 60°C for 24 hours. The dried materials were powdered using a mortar and pestle. 500 ml of powdered samples from each tissue were digested with a mixture of nitric acid and perchloric acid in the ratio 3:1 until it is almost dry and colourless. The final products were made upto 25 ml with double distilled water and the concentration of lead was analysed using an atomic absorption spectrophotometer (AAS) (Perkin-Elmer Model 2380). The results were represented in microgram per gram dry weight.

Statistical analysis

Statistical analysis of data was carried out with one – way analysis of variance (ANOVA) and ranked by using Duncan's multiple range test (Bruning and Kintz, 1968) to compare the data among various tissues study between days for analyzing the significance at 0.01, 1 and 5% levels.

RESULTS

The bioaccumulation of lead in the gill, liver, kidney, ovary, testis and brain increased with duration of exposure periods. The brain showed least amount of lead (Table 1 and Fig.1). An estimation of the levels of Pb in different tissues of the male and female fish of *Catla* exposed to sublethal concentration of lead (6.52 mg L^{-1}) for a period of 120 days exposure showed that maximum lead accumulation in kidney followed by liver, gill, ovary , testis and brain. Among the tissues studied the gill accumulated 1.44 µg/g after 15 days of exposure. The value showed an increasing trend and reached maximum after 120 days as 21.76 µg/g (Table 1 and Fig.1). Similarly in liver and kidney progressive accumulation of Pb occurred and reached maximum after 120 days of exposure. In liver, the value increased from 1.30 µg/g (15 days) to 28.63 µg/g after 120 days of exposure to sublethal concentration of lead. In kidney the rate of accumulation of lead varied from 1.10 µg/g to 31.20 µg/g after 120 days. The testis and ovary of the male and female fish accumulated low amount of lead as 8.42 µg/g and 9.68 µg/g respectively at the end of experimentation (120 days). The female fish accumulated more lead than male fish in the reproductive organs. Among the five tissues studied the brain showed least amount of lead accumulation. The value ranged from 0.50 µg/g after 15 days and 6.52 µg/g after 120 days of experimentation.

DISCUSSION

In this study, lead was accumulated in the decreasing order of kidney > liver > gill > ovary > testis > brain at the end of 120 days of exposure. The quantity of Pb accumulation has been reported to be directly related to the period of exposure and the pattern of accumulation was different in all the tissues studied (Table 1 and Fig. 1). At the beginning of exposure, Pb concentrations in the gills rapidly increase up to 90 days and then decline. The results of 90 days of exposure is in agreement with the report of Agrahari and Gopal (2007) who noted that the gills and liver showed highest accumulation of Pb and cadmium while the brain and kidney had the lowest in *Channa punctatus*. High concentration of metals in the gill upto 90 days of exposure has often been used as an indication of acute exposure, since the metals are fixed by absorption processes which occur very rapidly (Oladimeji and Offem, 1989; Noegrohati, 2006). The larger surface area of the gills in contact with the medium, the mucus layer present on general body surface as well as on the gills and its high affinity with ion transport activity of various mineral ions, then could probably account for the higher concentration of lead in the gill compared with the other organs studied (Roesijadi and Robinson, 1994; Allen, 1995a; Tao *et al*. 2000; Spokas, *et al.,* 2006).

This is more likely since the primary source of lead to the body is from the water via the gills. Seymore *et al.* (1995) also stated that the uptake of dissolved metals across the gills into the blood streams is the primary mode of uptake in freshwater fishes and as the pH of the water decreases the ionic state of the metal becomes more prevalent and toxicity increases acutely. Since, the iron oxides closely associated with the gills are involved in the carriage of respiratory gases. The presence of iron oxide in the gills is known to enhance lead disposition and Pb is known to strongly absorb onto iron oxides but not highly assimilated (Hare *et al.,* 1991). In this study, a distinction was not made between lead absorbed on the gill externally and those bound internally. At the end of 120 days of exposure a significant decrease ($P<0.01$) in Pb level was noticed in the gills. This result is in concurrent with the report of Olaifa *et al.* (2004) who recorded lower concentration of heavy metals in the gills and bones than muscles and intestines in *Clarias gariepinus* in lake and fish farm in Ibadan Nigeria. Though it is the route of entry of metal through active or

Table 1: Level of lead nitrate (µg/g dry wt.) in various tissue of *Catla catla* exposed to a sublethal concentration of 8.15 mgL^{-1} for a period of 120 days

Organs	Control group	Exposure period (Days)							
		15	30	45	60	75	90	105	120
Gill	ND	1.44 ± 0.11 a^{*}	3.62 ± 0.27 a^{*} b^{NS}	5.12 ± 0.38 a^{*} b^{NS}	8.10 ± 0.61 a^{*} b^{*}	12.09 ± 0.92 a^{**} b^{**}	14.38 ± 1.09 a^{**} b^{NS}	16.03 ± 1.21 a^{**} b^{NS}	21.76 ± 1.65 a^{***} b^{**}
Liver	ND	1.30 ± 0.09 a^{*}	3.46 ± 0.26 a^{*} b^{*}	5.34 ± 0.40 a^{*} b^{*}	8.97 ± 0.68 a^{*} b^{*}	13.50 ± 1.02 a^{**} b^{*}	16.24 ± 1.23 a^{**} b^{*}	23.19 ± 1.76 a^{***} b^{**}	28.63 ± 2.18 a^{***} b^{**}
Kidney	ND	1.10 ± 0.08 a^{NS}	2.38 ± 0.17 a^{*} b^{*}	4.23 ± 0.32 a^{*} b^{*}	6.50 ± 0.49 a^{*} b^{*}	10.32 ± 0.78 a^{*} b^{*}	17.43 ± 1.32 a^{**} b^{*}	24.97 ± 1.90 a^{***} b^{**}	31.20 ± 2.37 a^{***} b^{**}
Testis	ND	0.87 ± 0.06 a^{*}	1.26 ± 0.09 a^{*} b^{NS}	2.39 ± 0.18 a^{*} b^{NS}	3.05 ± 0.22 a^{*} b^{NS}	3.57 ± 0.27 a^{*} b^{NS}	4.13 ± 0.31 a^{*} b^{NS}	6.05 ± 0.45 a^{**} b^{*}	8.42 ± 0.64 a^{**} b^{*}
Ovary	ND	0.97 ± 0.07 a^{NS}	1.58 ± 0.12 a^{*} b^{NS}	2.66 ± 0.20 a^{*} b^{NS}	3.27 ± 0.24 a^{*} b^{NS}	4.15 ± 0.31 a^{*} b^{NS}	4.98 ± 0.38 a^{*} b^{NS}	6.73 ± 0.51 a^{**} b^{*}	9.58 ± 0.73 a^{**} b^{*}
Brain	ND	0.50 ± 0.03 a^{NS} b^{NS}	0.92 ± 0.06 a^{NS} b^{NS}	1.47 ± 0.11 a^{*} b^{NS}	2.01 ± 0.15 a^{*} b^{NS}	2.87 ± 0.21 a^{*} b^{NS}	3.43 ± 0.26 a^{*} b^{NS}	4.70 ± 0.35 a^{*} b^{*}	6.52 ± 0.49 a^{*} b^{*}

Values are expressed as mean ± SD of six individuals
a – denotes the difference between control and treated groups
b – denotes the difference between respective experimental group and preceding experimental group
*** - (P < 0.001); ** - (P < 0.01); * - (P < 0.05); NS – Not significant; ND – Non deductable

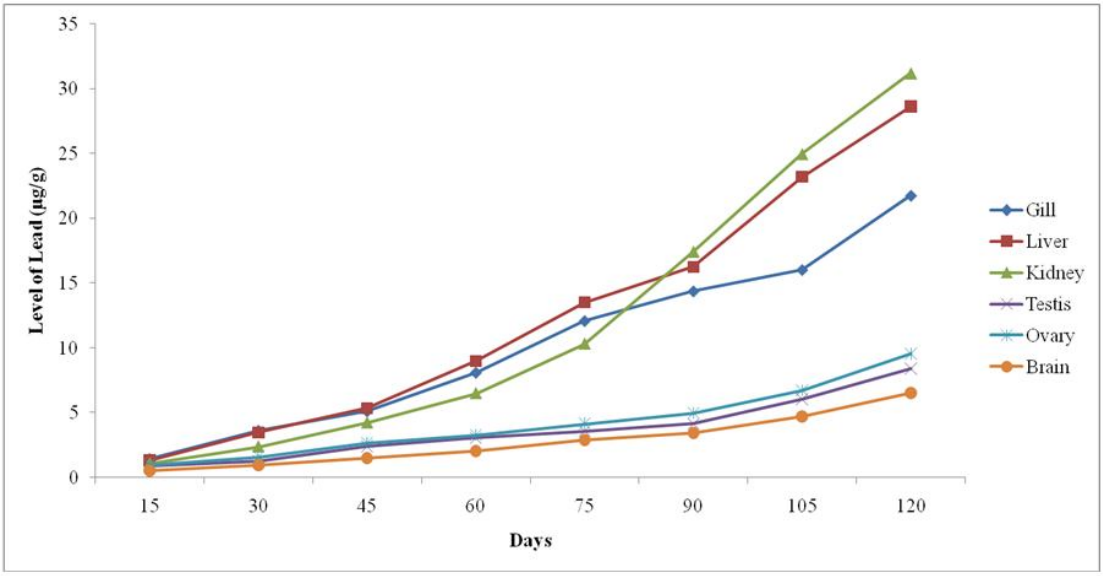

Figure 1: Level of lead (µg/g dry wt.) in various tissue of *Catla catla* exposed to a sublethal concentration of 6.52 mgL^{-1} for a period of 120 days

passive diffusion between the gill membrane and actual medium, as gills are the active sites of respiration and transport system associated with osmoregulation and excretion. Since, the layer is supposed to have carbohydrates and other sulphides with possible ion exchange properties and they facilitate a rapid exchange across the gill membranes to other tissues (Karthikeyan *et al.*, 2007). Thus, low Pb accumulation and a significant difference (P<0.01) was noted in the gills than other tissues (liver and kidney) of *Catla catla.*

Fish liver exhibited highest tendency to accumulate lead. The higher levels of lead in liver relative to other tissues may be attributed to the affinity or strong coordination of metallothionein protein with these elements (Ikem *et al.*, 2003). In the present study after 90 days of exposure maximum quantity of Pb accumulation occurred in liver followed by gills, ovary, testis and brain. A significant increase (P<0.001) in the Pb level was observed at the end of 120 days of exposure. The general increase observed in the level of lead with increased exposure period might be due to increase in the level of low molecular weight metal-binding proteins such as hepatic metallothionein and have direct ratio with liver detoxification task, these results support with Henry *et al.* (2004) and Mansour and Sidky (2003) and report with Jalali (2003). Such an increase might be a response by the fish to remove lead from the circulation and hence reduce lethal effects. The increase in lead level might be due to a negative feedback mechanism whereby more lead enters the tissue and becomes bound to the metallothinnein. If this should happen, then the lead burden in the fish will increase with increased exposure time. In the present study, the accumulation of lead was maximum in kidney when compared to other tissues. The present findings are in concurrent with the findings of Ruangsomboon and Wongrat (2006) who observed maximum level of Cd in kidneys of the catfish *C. garipinus* and *Clarias macrocephalus*. A progressive increase in Pb accumulation was observed from 15 days and reached the maximum at 120 days of exposure. Similar results were obtained for various workers for various heavy metals (Zohouri *et al.*, 2001; Jayakumar and Paul, 2006; Puvaneswari and Karuppasamy, 2008; Vinodhini and Narayanan, 2008; Raikwar and Kumar, 2008). The concentrations of Pb in the kidneys rise slower than in liver, usually reaches maximum and a significant increase was noticed among the organs studied at the end of exposure period. Since, during depuration, the metal levels in kidney remain high or may even increase for some time, which is related to the role of

kidneys as excretory organs. Pollutants transformed in the liver may be stored there or excreted in bile or transported to other excretory organs such as gills or kidneys for elimination or stored in fat which is an extra hepatic tissue (Heath, 1991; Nussey *et al.*, 2000). The concentration of any pollutant in any given tissue therefore depends on its rate of absorption and the dynamic processes associated with its elimination by the fish. The increased level of lead in kidney, liver and gill in the present study was the capacity to accumulate Pb brought by blood from other parts of the body. Preferential accumulation of metals in the liver, kidney and gills has also been reported by Bilgrami *et al.,* 1996; Janlataeme *et al.,* 1999; Vinodhini and Narayanan, 2008; Rauf *et al.,* 2009;). Different organs in the body are known to accumulate a particular metal to a high level while others do not accumulate the metal though present in the medium (Javid *et al.,* 2007; Al-Kahtani, 2009). This may be primarily due to different metabolic activities (Allen, 1995a). It is well understood that 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 occurs. Therefore, the ability of each tissue to either regulate or accumulate metal ions can be directly related to the total amount of metal uptake in that specific tissue.

This metal regulation is due to the induction of low molecular weight metal-binding proteins, such as metallothionein which 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, tissue like liver, gills and kidney, which is a major producer of metal-binding proteins, show high concentrations of most heavy metals (Roesijadi and Robinson, 1994; Allen, 1995b; Heath, 1995). This may be yet another reason for the enhanced accumulation of Pb in the kidney, liver and gill. Additionally these tissues are rich in –SH groups with can bind with lead (Rema and Philip, 1997). 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). Studies have also indicated that fish are able to accumulate and retain heavy metals from their environment and that accumulation of metals in tissues of fish is dependent upon exposure concentration and duration as well as other factors such as salinity, temperature, hardness, metabolism of the animals, biological and genetic factors, species, age and tissues (Kotze *et al.,* 1999; Karthikeyan *et al.,* 2007; Otitoloju, 2001; Bu-Olayan and Thomas, 2008; Kamaruzzaman *et al.,* 2010). In gonads of both male and female fish of *Catla,* the rate of Pb accumulation was very slow at the initial periods of exposure and then it reached the maximum after 120 days of exposure.

Our findings are in close agreement with the results of James *et al.* (2003) who have reported that the accumulation of metal in the test fish has linearly increased with the increasing of exposure period under long-term experimentation. Further, the concentration of Pb was minimum in gonads when compared with kidney, liver and gill. The minimum concentration of Pb in gonads of this present observation is concurrent with Dural *et al.* (2006) and Puvaneswari and Karuppasamy (2008) and these results shows that gonads as target organs and can also accumulate heavy metals. Bioaccumulation of chemicals and gender susceptibility can depend on several factors such as changes in metabolic rate (Metcalfe-Smith, 1994; Karadede and Unlu, 2000; Canli and Atli, 2003; Yilmaz and Yilmaz, 2007), physiological differences in sex among fish (Van den Broek, *et al.,* 2002), hepatic metabolism (Gunderson *et al.,* (2001), reproductive state and size variations (Burger *et al.,* 2006),variations in the hormones and the available number of active sites in the proteins and cytochrome – 450 for the binding of metals in male and female fish (Jargensen and Pedersen, 1994). This might be one of the reasons for high concentration of Pb in the ovary of female fish. On the contrary, Yilmaz and Yilmaz (2007) found that the level of heavy metals in male shrimps was higher than that of females. They suggested that the faster-growing sex (usually the female) can be expected to contain lower concentrations of metals, but not necessarily a smaller total body burden (Pourang *et al.,* 2004). From other researches, it is clear that the gender-related effects occur in various fishes exposed to contaminants (Burger *et al.,* 2004; Pyle *et al.,* 2005). This increased level of Pb in gonads, attributed to the pathological changes of gonads, in turn affected the reproduction of the test fish *Catla catla* (unpublished data). This is in close agreement with the findings of Puvaneswari (2008). She stated that the accumulation of the heavymetal Cd caused pathological changes in gonads which inturn affected the reproductive capacity of the test fish Heteropneustes *fossilis*. The brain of *Catla* showed least accumulation of Pb among the tissues under investigation.

This may be due to the indirect contact with the medium i.e. the transport of Pb through blood to the brain. Lead exposure has been associated with behavioural anomalies, learning impairment, memory loss, damaged cognitive functions in humans and experimental animals. Lead intoxication has been shown to produce anemia by inhibiting activity of one of the principal enzymes δ-ALAD (delta-aminolevulinic acid dehydratase) of heme synthesis (Hodson *et al.,* 1978; Arnvin *et al.,* 1980; Lockitch, 1993; Ruff *et al.,* 1996; Burden *et al.,* 1999). The aquatic toxicity of lead has shown to alter a number of haematologic events and δ-ALAD activity in fish. Deformability of erythrocytes and increase lipid peroxidation has been implicated in rat model of lead toxicosis. Lead is highly neurotoxic agent known to chemically interact with the system at cellular and molecular levels. This is also evident in our present findings (unpublished data). Severe or prolonged exposure to Pb may also cause chronic nephropathy, hypertension and reproductive impairment. Pb inhibits enzymes, alters cellular calcium metabolism and slows nerve conduction (Lockitch, 1993).

It is important to know that fishes containing variable concentration of toxicants including heavy metals from various sources like industries, agriculture runoff or domestic wastewater, may have accumulated heavy metals in their tissues as they grow and these toxicants and metals will be transferred to humans (being at the end of food chain) when consumed and may impair body metabolism (WHO, 1980; Ceirwyn, 1995; Pourang *et al*., 2005). *Catla catla*, is a preferred freshwater fish for human consumption in Indian sub-continental region. It is found naturally in all freshwater bodies including rivers and lakes which are receiving untreated industrial effluents and city wastewater containing various toxicants and heavy metals that may lead to the accumulation of toxicants and heavy metal including Pb in their tissues. In conclusion, bio monitoring of trace metal

pollution in a aquatic system is necessary. Since, the toxic effects of metals have been recognized, heavy metal levels in the tissues of aquatic animals are occasionally monitored to ensure that the level do not constitute health hazards to consumers. Periodic monitoring of lead and other heavy metals in both the fishes and aquatic system to ensure continuous safety of people in the area is recommended.

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REFERENCES

Agrahari, S., and Gopal, K. 2007. Fate and toxicity of cadmium and toxicity of Cadmium and lead accumulation in different tissues (gills, liver, kidney and brain) of a fresh water fish *Channa punctatus*. J. Ecophysiol. Occup. Hlth. 7: 151-155.

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

Al-kahtani, M.A. 2009. Accumulation of heavy metals in Tilapia fish *(Oreochromis niloticus*) from Al-Khadoud Spring, Al-Hassa, Saudi Arabia. Am. J. Appl. Sci. 6(12): 2024-2029.

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

Allen, P. 1995b. Chronic Accumulation of Cadmium in the Edible Tissues of *Oreochromis aureus* (Steindachner): Modification by Mercury and Lead. Arch. Environ. Contam. Toxicol., 29: 8-14.

Alves, L. C., Glover and Wood, C. M. 2006. Dietary Pb accumulation in juvenile freshwater rainbow trout (*Oncorhynchus mykiss*). Archiv. Environ. Contam. Toxicol., 51(4): 615-625.

Arnving, E., Grandjean, P., Beckman, J. 1980. Neurotoxic effects of heavy lead exposure determined with psychological test. *Toxicol. Lett.*, 5: 399-404.

Ay, O, Kalay., M. Tamer., L., and Canli, M., 1999. Copper and lead accumulation in tissues of freshwater fish, *Tilapia zilli* and its effects on the branchial Na^+ K^+ ATPase activity. Bull. Environ. Contam. Toxicol., 62: 160-168.

Bilgrami, K. S., Sanjib-Kumar, S.S., and Kumar, S. 1996. Rivers biota as indicators and scavengers of heavy metal pollution. Nat. Acad. Sci. Lett. 19(11-12):205-207.

Bruning, J. L., and Kintz, B. L. 1968. Computational Hand Book of Statistics. Foresman and Company, Dallas, USA. pp. 1-308.

Bu-olayan, and A.H., Thomas, B.V. 2008. Trace metals toxicity bioaccumulation in Mudskipper *Periophthalmus waltoni* Koumas 1941 (Gobiidae: Perciformes). Turk. J. Fish. Aquat. Sci. 8: 215-218.

Burden, V.M., Sandheinrich, M.B. and Caldwell, C.A. 1999. Effects of lead on the growth and S-amino-levulinic-acid-dehydratase activity of Juvenile Rainbow Trout. *Oncorhynchus mykiss*. *Environ. Poll.*, 101: 285-290.

Burger, J., Fossi, C., McClellan–Green, P., and Orlando, E. F. 2006. Methodologies, bioindicators and biomarkers for assessing gender – related differences in wildlife exposed to Environmental chemicals. Environ. Res. 1-18.

Burger, J., Orlando, E.F., Gochfeld, M., Binczik, G.A., and Jr. Guillette, L.J. 2004. Metal levels in Florida Gar (*Lepisosteus platyhincus*) from Lake Okeechobee. Environ. Monit. Assess., 90: 187-201.

Canli, M., and Atli, G. 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ. Pollut., 121(1): 129-136.

Canli, M., Stagg, R. M., and Rodger, G. 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.

Cibulka, J. 1991. Academia Praha, 426.

D.W.A.F., 1996. Department of Water Affairs and Forestry. *South African Water Quality Guidelines*, (2nd edition). *Aquat. Ecosys.*, 7: 159–67

Dural, M., Lugal Goksu, M. Z., Ozak, A. A., and Derici, B. 2006. Bioaccumulation of some heavy metals in different tissues of *Dicentrarchus labrax* L. 1758, *Sparus aurata* L. 1758 and *Mugil cephalus* L. 1758. From the Camlik Lagoon of the Eastern Cost of Mediterranean (Turkey). Environ. Monit. Assess. 118 (1-3): 65-74.

Eisler, R. 1988. Lead hazards to fish, wildlife, and invertebrates: A synoptic review; biological Report 85 (1.14); Contaminant Hazard Reviews 14; U.S. Fish and Wildlife Service: Laurel, MD.

Fadrus, H., Maly, J., and Sedlack, M. 1979. Management and control of heavy metals in the environment. 493.

Galvin, R.M. 1996. Occurrence of metals in water. An Overview. Water S.A. 22: 7–18

Godwin, T.H., Young, A. R., Holmes, M.G.R., Old, G. H. and Hewitt, N. 2003. The temporal and spatial variability of sediment transport and yields within the Bradford Beck Catchment, West Yorkshire. Sci. Total Environ. 314(a) 316: 475-494.

Gunderson, M. P., LeBlanc G. A., and Jr. Guillette, L. J. 2001. Alterations in sexually dimorphic biotransformation of testosterone in American alligators (*Alligator mississippiensis*) from Contaminated lakes. Environ. Heal. Perspect. 109: 1257-1264.

Hansen, B. H., Garmo, O. A., Olsvik, P. A. and Andersen, R. A. 2007. Gill metal binding and stress gene transcription in brown trout (*Salmo truta*) exposed to metal environment: The effect of preexposure in natural populations. Environ. Toxicol. Chem. 26: 944-953.

Hare, L, Saouter, E., Campbell, P.G.C., Tessier, A., Ribeyre, F., and Boudou, A. 1991. Dynamic of cadmium, lead, and zinc exchange between nymph of the burrowing malefly *Hexagenia rigidia* (Ephemeroptera) and the environment. C. J. Fish: Aquat. Sci. 48: 39.

Heath, A. G. 1995. Water pollution and fish physiology.Lewis Publishers, Boca Raton, Florida, USA.

Heath, A.G. 1991. Water Pollution and Fish Physiology. Lewis Publishers, Boca Raton, Florida, USA., ISBN: 0873716329, pp: 359.

Henry, F., Amara, R., Courcot, L., Lacouture, D. and Bertho, M.L. 2004. Heavy metals in four fish species from the French coast of Estern English Channel and Southern Bight of the North Sea. Environ. Int., 30: 675-683.

Hodson, P. V., Blunt B.R., and. Spry, D.J. 1978. Chronic toxicity of water borne and dietary lead to rainbow trout (*Salmo gairdneri*) in lake Ontario water. Water Res., 12: 869-878.

Huang, W. 2003. Heavy metal concentrations in the common benthic fishes caught from the coastal waters of Eastern Taiwan. J. Food Drug Anal. 2(4): 324-330.

Ikem, A., Egiebor, N. O. and Nyavor, K. 2003. Trace elements in water, fish and sediments from Tuskegee lake, Southeastern USA. Water Air Soil. Pollut. 149: 51-75.

Jalali, B. 2003. Pusy Disease of Growing Fish. Manne Ketab, Tehran, ISBN: 964-96764-8-1.

James, R., Sampath, K., and Edward, D. S. 2003. Copper toxicity on growth and reproductive potential in an Ornamental fish, *Xiphophorus helleri*. Asian Fish Sci. 16: 317-326.

Janlataeme, S., Kruatrachus, M., Kaewsawangcap, S., Chitramvong, Y., Sretarugsa, P., and Upatham, E. 1999. Acute toxicity and bioaccumulation of lead in the snail *Filopaludina* (Siamopaludin*) martensi Martensil martensi* (Frauenfeldt). J. Sci. Soc. Thailand.2:237-247.

Jargensen, L. A., and Pedersen, S. 1994. Trace metals in fish used for time trend analysis and as Environmental indicators. Mar. Pollut. Bull. 28: 24-32.

Javed, M., and Hayat, S. 1999. Heavy metal toxicity of river Ravi aquatic ecosystem. Pakistan J. Agric. Sci., 36: 1–9.

Javid, A., Javed, M., Abdullah, S., and Ali, Z. 2007. Bioaccumulation of lead in the bodies of major carps (*Catla catla, Labeo rohita* and *Cirrhinus mrigala*) during 96h LC_{50} exposures. Int. J. Agri. Biol. 9(6): 909-912.

Jayakumar, P., and Paul, V. I. 2006. Patterns of cadmium accumulation in selected tissues of the catfish *Clarias batrachus* (Linn) exposed to sublethal concentration of cadmium chloride. Vet. Arch., 76: 167-177.

Jezierska, B., and Witeska, M. 2001. Metal Toxicity to Fish, Wydawnictwo Akademii Podlaskiej, Siedlce, 318.

Kamaruzzaman, B.Y., Akbar, B., Jalal, K.C.A., and Shahbudin, S. 2010. Accumulation of metals in the gills of Tilapia fingerlings (*Oreochromis niloticus*) from in vitro toxicology study. J. Fish. Aquat. Sci. 5(6): 503-509.

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

Karthikeyan, S., Palaniappan, PL.RM., and Sabhanayakan, S. 2007. Influence of pH and water hardness upon Nickel accumulation in edible fish *Cirrhinus mrigala*. J. Environ. Biol. 28: 484-492.

Kendall, R.J. and Scanlon, P.F. 1982. A rapid method for analysis of tissues for heavy metals using atomic absorption spectrophotometer. *Northwest Sci*., 56: 265-267.

Korai, L., Sahato, G. A., Kazi, T. G., and Lashari, K. H. 2008. Lead Concentrations in Fresh Water, Muscle, Gill and Liver of *Catla Catla* (Hamilton) from Keenjhar Lake. Pak. J. Anal. Environ. Chem. 9(1): 11 – 19.

Kotze, P. J. 1997. Aspects of water quality, metal contamination of sediments and fish in the Olifants River, Mpumalangi, Rand Afrikaans University, South Africa, 157 pp.

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

Kusemiju, Victor, Amoruwa, Patience and Aderinola J. Oluwatoyin, 2012. Accumulation of lead in the tissues of freshwater catfish *Clarias gariepinus* exposed to static nominal concentrations of lead nitrate Agric. Biol. J. N. Am. 3(12): 510-515.

Lockitch, G. 1993. Perspectives on lead toxicity. Clin. Biochem., 26: 371-381. PMID: 8299207.

Mac-Donald, A., Silk, L., Schwartz, M. and Playle, R. C. A. 2002. A lead-gill binding model to predict acute lead toxicity to rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. physiol. (Part C) 133: 227-242

Mansour, S.A., and Sidky, M.M. 2003. Ecotoxicological studies. 6. The first comparative study between lake Qarun and Wadi E1-Rayan wetland (Egypt). With respect to contamination of their major components. Food Chem. 82: 181-189.

Martinez, C.B.R., Nagae, M.Y., Zaia, C.T.U., and Zaia, D.A.M. 2004. Acute morphological and physiological effects of lead in the neotropical fish, *Prochilodus lineatus*. Braz. J. Biol. 64(4):797-807.

Metcalfe-Smith, J. L. 1994. Influence of species and sex on metal residues in freshwater mussels (family Unionidae) from the St. Lawrence River, with implications for bio monitoring programs. Environ. Toxicol. Chem. 13: 1433-1443.

Neumann, J. Lopuchovsky, J. and Zapletal, O. 1990. *Chemisation, Agriculture, Pharmacology and toxicology* (In Czech) *SZN Praha*, 1st Ed., 304.

Noegrohati, S. 2006. Bioaccumulation dynamic of heavy metals in *Oreochromis niloticus Berkala MIPA* 16(2):29-40.

Nussey, G., VanVuren, J. H. J. and Du Preez, H. H. 2000. Bioaccumulation of Chromium, Manganese, Nickel and Lead in the Tissues of the Moggel, *Labeo umbrata* (*Cypr inidae*) from Witbank Dam, Mpumalanga. Water S.A., 26: 269-284.

Nwani, C.D., Nwachi, D.A., Okogwu, O.I., Ude, E.F., and Odoh, G.E. 2010. Heavy metals in fish species from lotic freshwater ecosystem at Afikpo, Nigeria J. Environ. Biol. 31(5): 595-601 .

Oladimeji, A.A., and Offem, B.O. 1989. Toxicity of lead to *Clarias lazera, Oreochromis niloticus, Chironomus tentuns* and *Benacus sp. Water, Air and Soil Pollution* 44:191-201.

Olaifa, F. G., Olaifa, A. K., and Onwude, T. E. 2004. Lethal and sublethal effects of copper to the African Cat fish (*Clarias gariepinus*). Afr. J. Biomed. Res. 7: 65-70.

Otitoloju, A.A. 2001. Joint action toxicity and bioaccumulation of heavy metals. Ph.D. Thesis. University of Lagos. 225pp.

Pourang, N., Dennis, J. H. and Ghourchain, H. 2004. Tissue distribution and redistribution of trace elements in shrimp species with the emphasis on the roles of metallothionein. Ecotoxicol., 13: 519-533.

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

Puel, D., Zsuerger, N., and Breittmayer, J. P. 1987. Bull. Environ. Contam. Toxicol., 38: 700.

Puvaneswari, S. 2008. Effects of cadmium on the reproductive biology, Embryonic and early development and growth in

Heteropneustes fossilis (Bloch) Ph.D., Thesis submitted to the Annamalai University, Annamalainagar, Tamilnadu.

Puvaneswari, S., and Karuppasamy, R. 2008. Bioaccumulation pattern of Cadmium in *Heteropneustes fossilis* (Bloch), related to sex groups after chronic exposure Annamalai University Science Journal 44 pp: 65-74.

Pyle, G. G., Rajotte, J. W. and Couture, P. 2005. Effects of industrial metals on wild fish populations along a metal contaminant gradient. Ecotoxicol. Environ. Saf., 61: 287-312.

Rademacher, D. J., Steinpreis, R. E. and Weber, D. N. 2003. Effects of dietary lead or dimercapto sussinic acid exposure on regional serotonin and serotonin metabolic content in rainbow trout (*Oncorhynchus mykiss*). Neurosci. Lett. 339: 156-160.

Raikwar, M. K., and Kumar P. 2008. Toxic effect of heavy metals in livestock health. Veter. World, 1(1): 28-29.

Rainbow, P.S. and Dallinger, R. 1993. Metal uptake regulation and excretion in freshwater invertebrate. In: Ecotoxicology of Metals in Invertebrates (ed.) P.S. Rainbow and R. Dallinger Lenis publishers: 119 – 131.

Rashed, M. N. 2001. Cadmium and lead levels in fish (*Tilapia nilotica*) tissues as biological indicator for lake water pollution. Environ. Mont. Asses. 68: 75-89.

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

Ruff, H.A., Markowitz, M.E., Bijur, B.E. and Rosen, J.F. 1996. Relationship among blood lead levels, iron deficiency and cognitive development in two years old children. *Environ. Hlth. Perspect.*, 104: 180-185.

Rema, L. P., and Philip, B. 1997. Accumulation of an essential metal (Zinc) and a non-essential metal (Mercury) in different tissues of *Oreochromis mossambicus* (Peters). Ind. Jour. Exp. Biol. 35: 67-69.

Roesijadi, G. and Robinson, W. E. 1994. Metal regulation in aquatic animals: Mechanisms of uptake, accumulation and release. In: Aquatic Toxicology. Molecular, Biochemical and Cellular Perspectives

Ruangsomboon, S., and Wongrat, L. 2006. Bioaccumulation of cadmium in an experimental Aquatic food chain involving phytoplankton *(Chlorella vulgaris),* zooplankton *(Moina macrocopa),* and the predatory catfish *Clarias macrocephalus* and *C. gariepinus*. Aquat. Toxicol., 78: 15-20.

Seymore, T. 1995. Manganese, lead and strontium bioaccumulation in the tissues of the yellow fish, *Barbus marequensis* from the lower Olifants River, Eastern Transvaal Water, South Africa, pp241-244.

Spokas, E. G., Spur, W. B., Smith, H., Kemp, F. W., and Bogden, J.D. 2006. Tissue lead concentration during chronic exposure of *Pimephales promelas* (fathead minnow) to lead nitrate in aquarium water. Environ. Sci. Technol. 40: 6852-6858.

Strmiskova, G. 1992. Lead in the environment and food (In Slovak). Nut. Hlth., 37: 19.

Tao, S., Li, H., Lui, C., and Lam, K. C. 2000. Fish uptake of inorganic and mucus complexes of lead. Ecotoxicol. Environ. Saf. 46: 174-180.

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.

Van den Broek, J. L., Gledhill K. S., and Morgan, D. G. 2002. Heavy Metal Concentrations in the Mosquito Fish, *Gambusia holbrooki,* in the Manly Lagoon Catchment. In: UTS Freshwater Ecology Report 2002, Dept. Environ. Sci. Univ. Technol., Sydney.

Vinodhini, R., and Narayanan, M. 2008. Bioaccumulation of heavy metals in organs of freshwater fish *Cyprinus carpio* (Common carp). Int. J. Environ. Sci. Tech. 5(2):179-182.

WHO.1980. International Standards for drinking water. World Health Organization. pp. 96-145.

Yilmaz, A. B. and Yilmaz, L. 2007. Influences of sex and seasons on levels of heavy metals in tissues of green tiger shrimp (*Penaeus semisulcatus* de Hann, 1844). Food Chem. 101: 1664-1669.

Zohouri, M. A., Pyle, G. G., and Wood, C. M. 2001. Dietary Ca inhibits waterborne Cd uptake in Cd exposed rainbow trout, *Oncorhynchus mykiss*. Comp. Biochem. Physiol. Toxicol. Pharmacol. 130: 347-356.

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