Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 08, pp.2151-2155, August, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

A STUDY ON THE ACUTE TOXICITY OF LEAD NITRATE ($Pb(NO_3)_2$) ON THE FRESHWATER FISH *Catla Catla* (Hamilton, 1822)

Mohanambal, R. and *Puvaneswari, S.

Department of Zoology, Annamalai University, Annamalainagar – 608002. Tamil Nadu

ARTICLE INFO

Article History:
Received 26th May, 2013
Received in revised form
02th June, 2013
Accepted 05th July, 2013
Published online 23rd August, 2013

Key words:
Lead toxicity,
LC_{50},
Behaviour,
Catla Catla.

ABSTRACT

In this present study, the mortality rate of heavy metal lead nitrate ($Pb(NO_3)_2$) was determined in the freshwater fish *Catla Catla.* The median lethal concentration was determined using static and renewable method through Probit analysis. The median lethal concentration (LC_{50}) value of ($Pb(NO_3)_2$) was found to be 190.56, 181.81, 172.43 and 163.04 mgL^{-1} for 24, 48, 72 and 96 hrs respectively. The dose and time dependant responses (mortality rate and behavioural changes) in the test fish was observed. The behavioural changes observed in the experiment includes hyper activity, loss of balance, vertical and downward swimming pattern, frequent surfacing activity, convulsion, difficulty in breathing and mucus secretion over the body. No behavioural changes or death were observed in the control group during the experiment. The results of the study showed that, acute lead toxicity severely affects the normal behaviour and results in death.

INTRODUCTION

The contamination of freshwaters with a wide range of pollutants has become a matter of concern over the last few decades (Canli *et al.,* 1998; Voegborlo *et al.,* 1999; Dirilgen, 2001; Ohe *et al.,* 2004; Vutukuru, 2005), and is getting extensively contaminated with heavy metals released from domestic, industrial and other anthropogenic activities (Velez and Montoro, 1998; Conacher *et al.,* 1993). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Vosyliene and Jankaite, 2006; Farombi *et al.,* 2007). It poses serious risks to many aquatic organisms by changing genetic, physiological, biochemical and behavioural parameters (Scott and Sloman, 2004). Lead (Pb) is one among the heavy metal and its contamination in the water body has occurred on a global scale with adverse effects to human, environment health and damage caused to aquatic life especially fishes (Markus and McBratney, 2001). Lead found in the environment, urban, industrial and agricultural waste waters and its occurrence in the air, which is transported to the streams and rivers by runoffs where fish and other aquatic organisms take it up and incorporate it in their body (Weis and Weis, 1998; Chen and Folt, 2000). Several reports have indicated that Pb can cause neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, histopathological and histochemical changes all of them related to the dose and time of exposure to Pb (Reglero *et al.,* 2009; Abdallah *et al.,* 2010; Mirhashemi, *et al.,* 2010). Fish are largely used in evaluation of aquatic systems quality and some of their physiologic changes can be considered as biologic markers of environmental pollution (Dautremepuits *et al.,* 2004). It has a great potential to serve as sensitive indicators, signaling exposure and understanding the toxic mechanisms of stressors in aquatic ecosystems (Vutukuru, 2005).

***Corresponding author:** Puvaneswari, S. Department of Zoology, Annamalai University, Annamalainagar – 608002. Tamil Nadu.

The impact of metals, as well as other pollutants, on aquatic biota can be evaluated by toxicity tests, which are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms. The 96 hrs LC_{50} test paradigm is used to measure the susceptibility and survival potential of organisms exposed to particular toxic substances, such as heavy metals. Higher LC_{50} values are less toxic because greater concentrations are required to produce 50% mortality in organisms (Eaton *et al.,* 2005). The heavy metals that are toxic to many organisms at very low concentrations are mercury, cadmium and lead (Hilmy *et al.,* 1985). Behavior is a selective response that is constantly adapting through direct interaction with physical, chemical, social, and physiological aspects of the environment. Thus, the behavioral endpoints serve as valuable tools to discern and evaluate effects of exposure to environmental stressors. Behavioral endpoints that integrate endogenous and exogenous factors can link biochemical and physiological processes, thus providing insights into individual- and community-level effects of environmental contamination (Brewer *et al.,* 2001; Vogl *et al.,* 1999). Little is known about the lethal effects of lead (Pb) on fishes (Pickering and Henderson, 1966; Martinez *et al.,* 2004; Adeyemo *et al.,* 2008; Tawari-fufeyin *et al.,* 2008; Ramesh *et al.,* 2009; Ahmad Khan *et al.,* 2011; Askari Hesni *et al.,* 2011). Investigations on the lethal effects of lead nitrate on the freshwater fish *Catla catla* is scanty. Hence, the present work is aimed to investigate the acute toxicity of lead nitrate [$(Pb(NO_3)_2$] and its effect on behavioural responses of the freshwater fish *Catla catla.*

MATERIALS AND METHODS

LC_{50} determination

This study was carried out in the Laboratory of Zoology Department, Annamalai University, during the month of July 2011. Adult *Catla catla* (890 ± 50 gms and 47.7 ± 5 cms) were purchased from a local hatchery and were maintained in large cement tanks containing

aerated tap water. The other physico-chemical elements of the water and experimental condition were kept quite instant during the course of study by following the procedure of APHA (2000). Prior to the onset of treatment, all specimens were washed with 0.1% $KmnO_4$ solution to remove dermal infection and pollutants, if any. The fish were acclimatized to the laboratory conditions for 14 days. They were fed once daily with commercial feed, rice bran cake and boiled eggs white. Water was renewed every 24 hrs. The metal salt lead nitrate [$(Pb(NO_3)_2$], E-Merck) served as the test compound for determination of the median lethal concentration (LC_{50}) of lead. Stock solution of lead nitrate [Pb $(No_3)_2$] was made by dissolving 10 gms of lead nitrate in 1 liter of distilled water and the required concentrations were maintained in cement tanks with chlorine free tap water. A 96 hrs daily static renewal acute toxicity was conducted following the methods described by Sprague (1971). The fish were divided into several groups, each containing 6 individuals. Initial range finding experiments were conducted to drive the suitable concentrations for LC_{50} determinations. These groups were then exposed to different metal concentrations. The control group was not exposed to the test chemical and other groups were exposed. The concentrations used for acute toxicity estimation were 110.0, 120.0, 130.0, 140.0, 150.0, 160.0 170.0, 180.0, 190.0 and 200.0 mgL^{-1}. The experimental design included three replicates. The fish were not fed on the day before the beginning of the experiment or during the experiment. In the experimental tank, the water for the stressed groups and the water for the control group were renewed each day at the same time. The behavioral changes, morphological abnormalities and mortality of the fish were monitored during 96 hrs of exposure to each concentration of the toxicant. Each day, dead fish were counted and removed from the tank. The data from the experiment were used to estimate the LC_{50} value of lead nitrate. To calculate these values, the mortality observed in each treatment was determined and analyzed using the Finney probit analysis method (1971).

RESULTS AND DISCUSSION

Acute toxicity on mortalities

The pollution of aquatic environment by toxicants adversely affects the survival of aquatic organism including the commercially important fish species which form the dominant group of aquatic system (Somaraj *et al.,* 2005; Radhakrishnan Nair, 2006). The toxic effects of heavy metal on fish are multidirectional and manifested by numerous changes in the physiological and chemical processes of their body systems (Dimitrova *et al.,* 1994). The median lethal concentration (LC_{50}) of [$(Pb(NO_3)_2$] for *Catla catla* was derived for 24, 48, 72 and 96 hrs. The mortality data was subjected to probit analysis and plotted against log dose concentrations, resulting in a straight line [Fig. 1]. The LC_{50} values and 95% upper and lower confidence limits of Pb on *Catla catla* are given in Table - 1.

dangerous level that lead to fish death. Shah and Altindu (2005), who have also suggested that the accumulation of a heavy metal has a direct effect on the LC_{50} values of the respective metal in fish. The results of the present work strongly concurrent with the findings of Guven *et al.* (1999), Shyang and Chen (2000), Karuppasamy (2001), Kanabur and Sannadurgappa (2001), Subathra and Karuppasamy (2003), Martinez *et al.* (2004), Puvaneswari and Karuppasamy (2007), Askari Hesni *et al.* (2011) and Nekoubin *et al.* (2012). Askari Hesni *et al.* (2011) reported a 96 hrs LC_{50} value of [$(Pb(NO_3)_2$] as 426.49 mgL^{-1} to the milk fish *Chanos chanos.*At the same time, Martinez *et al.* (2004) found out the 96 hrs LC_{50} value of the same metal salt as 95 $mgPbL^{-1}$ to the neotropical fish *Prochilodus lineatus*, 300.45 mgL^{-1} in *Clarias batrachus* (Ahmad khan *et al.,* 2011), 378 mgL^{-1} to the cat fish *C. batrachus* (Shamshun Nehar *et al.,* 2010), 268.065 mgL^{-1} to the Sea kutum *Rutilus frisii kutum* (Gharedaashi *et al.,* 2013) and 2.624 mgL^{-1} to the juvenile common carp (Nekoubin *et al.,* 2012). Srivastava and Mishra (1979) recorded the 96 hrs LC_{50} of Pb as 19 ppm to the test fish *Colisa fasciatus*. However, Hodson *et al.* (1978) found 2.4 ppm Pb for the 21-day LC_{50} of *Salmo gairdneri*. Shah and Altindu (2005) recorded the 96 hrs LC_{50} for *Tinca tinca* as 6.5 ppm for Cd and 300.0 ppm for Pb. In contrast with these results, the present study determined a 96 hrs LC_{50} of 163.04 $mgPbL^{-1}$ for *Catla catla*. The above mentioned 96 hrs LC_{50} values are disagreed with the present investigations, this may be due to the differences in the test species, age and also the difference in the abiotic factors.

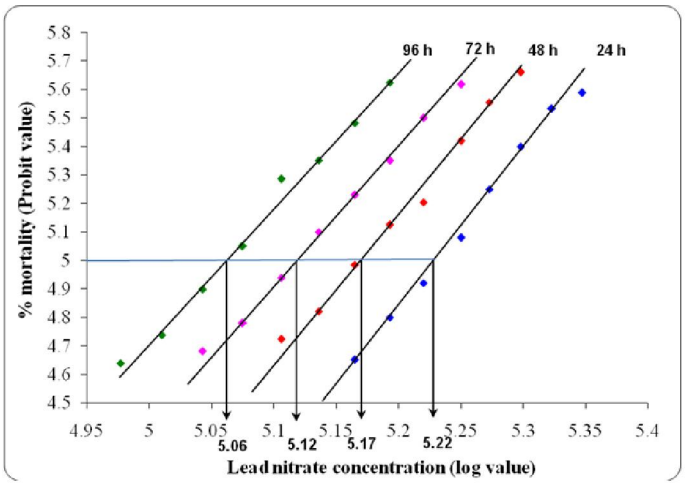

Fig. 1. Relation between concentration of lead nitrate and probit mortality of *Catla catla*

The values obtained by toxicity testing (LC_{50} value) are vary and dependent on the conditions under which tests were performed, so that interpretation of LC_{50} values needs to be done with caution

Table 1. Median lethal concentration of heavy metal lead nitrate to *Catla catla*

Exposure period (hrs)	LC_{50} (mgL^{-1})	95% confidence limit (mgL^{-1})		Regression Co-efficient (Y)	Slope function (SF)
		Lower	Upper		
24	190.56	189.61	191.516	Y = 48.26 + (2.15)X	1.41
48	181.81	180.86	182.77	Y = 44.71 + (2.19)X	1.48
72	172.43	171.50	173.38	Y = 41.28 + (2.16)X	1.45
96	163.04	162.09	164.73	Y = 38.32 + (2.13)X	1.43

The LC_{50} values for 24, 48, 72 and 96 hrs of exposures were estimated as 190.56, 181.81, 172.43 and 163.04 $mgPbL^{-1}$ respectively. This present results are clearly indicated that, the mortality increased with an increase in concentration and required the decreases of exposure time to bring about 50 percent mortality of fish. At the same time no mortality and behavioural changes were observed in the control groups. Further, the present findings indicate that, the mortality of the test fish to [$(Pb(NO_3)_2$] was dose and time dependant and this reflects the regular mode of action which may due to accumulation and subsequent magnification of [Pb $(No_3)_2$] up to (Walker *et al.,* 1996). Amongst fish species, considerable differences in sensitivity to lead have been reported (Salmerón-Flores *et al.,* 1990). According to Demayo *et al.* (1981), lead toxicity is a function of water hardness, species tested, and fish age. Increased water hardness reduces lead toxicity to fish due to a significant inorganic complexation process that controls Pb availability to fish (Hodson *et al.,* 1984). Pickering and Henderson (1966) showed that in soft water (20 mg $CaCO_3L^{-1}$) the 96 hrs LC_{50} for *Pimephales promelas* and *Lepomis macrochirus* was 5.6 and 23.8 mg Pb L^{-1}, whereas in hard water (360 mg $CaCO_3L^{-1}$) 96 hrs LC_{50} was 482 and

442 mg PbL^{-1}, respectively. Darmayati and Hindarti (1994) found that young juvenile milkfish are more sensitive to hexavalent Cr than to Cd and they obtained 96 hrs LC_{50}values for Cr and Cd of 22.45 mgL^{-1} and 38.9 mgL^{-1} respectively. Diaz (1994) reported that the approximate 96 hrs LC_{50} for Cd in juvenile *Chanos chanos* is 27.3 mgL^{-1}. From the previous report of Pb toxicity on various fish species indicate that the toxicity of Pb to aquatic organisms varies with life stages of organism, test water criteria and duration of exposure. The wide difference in LC_{50} values of Pb to various species might be due to the mode of toxic potentiality and responses of animals under static conditions. Thus, the test employing the single species may provide information about the environmental risks of a toxicant (Taylor *et al.,* 1991).

Behavioral abnormalities

The test fish showed various behavioral changes at different lead concentrations. The type, rate and duration of the behavioral changes increased with increased in concentrations. In all of the treatments, fish were hyperactive and attempted to escape from the tank during the first hours at which movement occurred. No behavioral changes or death occurred in the control group at any time during the experiment. All control fish were active and swam normally. Abnormal behavior was not expected to occur spontaneously in the control group. At the same time, the treated fish tried to escape from the tank and increased mucus secretion was also observed. The behavioural disorders included loss of balance, respiratory difficulty, slowness of motion, frequent surfacing activity and increased mucus secretion were observed after 48 hrs of exposure. These toxic effects increased as the dose increased. After 72 hrs of exposure in higher concentration the secretion of mucus increased and the fish turns upside down in the water and became motionless, sideways swimming and loss of balance were observed and the anal fin, the anus and the area around the eyes were bloody. The present observations were concurrent with the reports of Puvaneswari and Karuppasamy (2007). They observed these abnormal behaviours in Indian catfish *Heteropneustes fossilis* exposed to cadmium toxicity. Relatively increased breathing rate at the beginning and reduced rate as later revealed by opercular movements. Finally after prolonged period of exposure, the decrease in opercular movement and corresponding increase in frequency of surfacing of test fish clearly indicates the adaptively shifts towards aerial respiration and the fish tries to avoid contact with the metal through gill chamber (Karuppasamy, 2001; Gharedaashi *et al.,* 2013).The hyper activities in the test fish, which have higher metabolic activity could require higher levels of oxygen and thus could have a higher respiration or breathing rate (Canli and Kargin, 1995). Heavy extrudation of mucus over the body and dispigmentation are attributed to the endocrine/pituitary gland under toxic stress, causing changes in the number and area of mucus glands and chromatophores (Pandey *et al.,* 1990). The accumulation and increased secretion of mucus in the fish exposed to lead nitrate may be an adoptive response perhaps providing additional protection against corrosive nature of the metal and to avoid the absorption of the toxicant by the general body surface. This is in agreement with the earlier findings of Das and Mukherjee, (2003), Yilmaz *et al.* (2004), Prashanth *et al.* (2005) and Subathra and Karuppasamy (2003).

Further, the test chemical produces effects on the skin at the site of absorption and is then transported systemically to produce its typical effects on the central nervous system and other organs (Askari hesni *et al.,* 2011). The site of the highest concentration of the chemical is not often, the target organ of toxicity. Lead is concentrated in bone, but its toxicity is due to its effects in soft tissues, particularly the brain. The target organ most frequently involved in systemic toxicity is the CNS (brain and spinal cord) (Klaassen, 2008), resulting in loss of coordination and locomotion, instability followed by hyper excitability, tremors and convulsions (Wouters and Vanden Brecken, 1978). For this reason, exposure to lead can affect the normal behavior of the test fish. Thus, the results of this study clearly illustrate that the toxic effects (mortality and behavioral changes) of lead nitrate on *Catla catla* varied with increasing heavy metal concentrations and in response to such water conditions as temperature, pH and dissolved O_2. The study demonstrated that the test fish *C.catla* can be used as an effective bioindicator for acute pollutants such as lead nitrate. Finally, death resulting from acute lead nitrate, in the test fish might be due to increased gastric haemorrage, convulsion and suffocation (Valee and Ulmer, 1972). In conclusion, acute toxicity test constitute only one of the many tools available to the aquatic toxicologists but they are the basic means of provoking a quick, relatively inexpensive and reproducible estimate of the toxic effects of a test material. The assessment of toxicity on fish exposed to a particular toxicant will reveal facts regarding the health of given ecosystem and would eventually help us to propose policies to protect the ecosystem. It will also reveal the organisms sensitivity to a particular toxicant that would help us to determine the permissible limit of a toxicant in an ecosystem.

Acknowledgments

The authors are grateful to the Professor and Wing Head, Department of Zoology, Annamalai University for providing necessary facilities.

REFERENCES

Abdallah, G.M., El-Sayed,S.M.and Abo-Salem,O.M. 2010. Effect of lead toxicity on coenzyme Q levels in rat tissues. Food Chem. Toxicol., 48: 1753-1756.

Adeyemo, O.K., Ajani,F., Adedeji, O. B., Ajiboye O.O. 2008. Acute Toxicity and Blood Profile of Adult *Clarias gariepinus* Exposed to Lead Nitrate. The Internatinal Journal of Hematology. Volume 4 Number 2.

Ahmad Khan, H., Malabika Sikdar-Bar, Kamlesh, B., Abdullah Wani, A., Ahmed Pervaiz, P. 2011. Lead Nitrate Induced Histopathological Changes in the Gills of the African *Clarias batrachus.* Journal of Applied Sciences Research, 7(7): 1081-1086.

APHA. 2000. Standard methods for examination of water and wastewater. 20th Edn. American Public Health Association Washington D C. p.1976.

Ashraj, W. 2005. Accumulation of heavy metals in kidneyand heart tissues of *Epinephelus microdon* fish from the Arabian Gulf. Environ. Monit. Assess. 101(1-3): 311-316.

Askari Hesni, M. A., Dadolahi-Sohrab,A., Savari and Mortazavi M.S. 2011.Study the Acute Toxicity of Lead Nitrate Metal Salt on Behavioral Changes of the Milkfish (*Chanos chanos*) World Journal of Fish and Marine Sciences 3 (6): 496-501.

Brewer, S.K., Little, E.E., DeLonay, A.J., Beauvais, S.L., Jones, S.B., Ellersieck, M.R. 2001. Behavioral dysfunctions correlate to altered physiology in rainbow trout (*Oncorynchus mykiss)* exposed to cholinesterase-inhibiting chemicals. Arch. Environ. Contam. Toxicol. 40: 70-76.

Canli, M., and Kargin, F.1995. A Comparative study on heavy metal (Cd, Cr, Pb and Ni) accumulation in the tissue of carp, *Cyprinus carpio* and Nile fish, *Tilapia nilotica. Tur. Jour. Zool*., 19: 165-171.

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

Chen, C.Y. and Folt, C.L. 2000. Bioaccumulation and diminution of arsenic and lead in a freshwater food web. Environ. Sci. & Technol., 34: 3878-3884.

Conacher, H. B., Page, B. D. and Ryan, J. J. 1993. Industrial chemical contamination of foods [Review]. Food Addit.Contam. 10(1): 129-143.

Darmayati, Y. and Hindarti, D.1994. Acute toxicity of cadmium and hexavalent chromium to tropical fish milkfish (*Chanos chanos*). In the proceedings IOC-WESTRAC Third International Scientific Symposium, pp: 245-250.

Das, B.K. and Mukerjee, S.C. 2003. Effect of pyrethroid on the behaviour of *Heteropneusta fossilis.* Comp. Biochem. Physiol. C. Toxicol.Pharmacol. 134(1): 109-112.

Dautremepuits, C., Betoulle, S., Paris-Palacios S. and Vernet, G. 2004. Immunol-related perturbations induced by copper and chiston in carp *Cyprinus carpio*). Arch Environ. Contam. Toxicol., 47 : 370 – 378.

Demayo, A., Taylor, M. C., Taylor, K. W. and Hodson, P. V. 1981. Toxic effects of lead and lead compounds on human health, aquatic life, wildlife plants, and livestock. *Critical Reviews in Environmental Control, 12(4)*: 257-305.

Diaz-Ravina M., Baath E., Frostegard A. 1994. Multiple heavy metal tolerance of soil bacterial communities and its measurement by a thymidine incorporation technique. Applied Environmental Microbiology 60: 2238–2247.

Dimitrova, M. S., Tishinova, T., and Velcheva, V. 1994. Combined effects of zinc and lead on the hepatic superoxide dismutase-catalase system in carp. *Comp. Biochem. Physiol., 108C*: 43-46.

Dirilgen, N., 2001. Accumulation of heavy metals in fresh water organisms: Assessment of toxic interactions. Turk. J. Chem. 25(3): 173-179.

Eaton, A.D., Clesceri,L.S., Greenberg A.E. and Franson, M.A.H. 2005. Standard Methods for the Examination of Water and Waste Water. 19 ed. The American Public Health Association. Washington.

Farombi, E. O., Adelowo, O. A. and Ajimoko, Y. R. 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias gariepinus*) from Nigeria ogun river. Int.J. Environ. Res. Public Health. 4(2): 158-165.

Finney, D.J. 1971. Probit Analysis, Cambridge University Press, London/New York, pp: 20-47.

Gharedaashi, E., Imanpour, M.R. and Taghizadeh,V. 2013. Determination of median lethal concentration (LC_{50}) of copper sulfate and lead nitrate and effects on behavior in Caspian sea kutum (*Rutilus frisii kutum*)**.** Journal of Toxicol. and Environ. Health Sci., Vol. 5(1) pp. 12-16.

Guven, K., Ozbay,C., Unlu E. and Satar,A. 1999. Acute lethal toxicity and accumulation of copper in *Gammarus pulex* (L.) (Amphipoda). *Tur. Jour. Biol.,* **23**: 513-521.

Hilmy, A.M., Shabana M.B. and Dabees, A.Y. 1985. Bioaccumulation of cadmium: Toxicity in *Mugil cephalus*. Comparative Biochemistry and Physiol., 81: 139-143.

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

Hodson, P. V., Whittle, D. M., Wong, P. T. S., Borgmann, U., Thomas, R. L., Chau, Y. K., Nriagu, J. O. and Hallet, D. J. 1984. Lead contamination of the Great Lakes and its potential effects on aquatic biota. *In*: J. O. Nriagu and M. S. Simmons (eds.), *Toxic contaminants in the Great Lakes*. John Wiley and Sons, Indianapolis, In.

Kanabur, V. and Sannadurgappa, D. 2001. Acute toxicity of phenol and cresol to a freshwater fish *Oreochromis mossambicus*. Environ. Ecol., 19(4): 756-758.

Karuppasamy, R. 2001a. Evaluation of acute toxicity levels and behavioral responses of *Channa punctatus* (Bloch) to phenyl mercuric acetate. *Ecol. Env. Cons*., 7(1): 75-78.

Klaassen, C.D. 2008. Casarett and Doull's Toxicology The Basic Science of Poisons, Seventh Edition.McGraw-Hill, Medical publishing division, New York, pp: 35-40.

Markus, J. and McBratney, A.B. 2001. A review of the contamination of soil with lead II. Spatial distribution and risk assess/smelt of soil lead. Environ. Intl., 27: 399-411.

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

Mirhashemi, S.M., Moshtaghie,A.A., Ani, M. and Aarabi, M.H. 2010. Lead toxicity on kinetic behaviors of high and low molecular weight alkaline phosphatase isoenzymes of rat, *in vivo* and *in vitro* studies. J. Biol.Sci., 10: 341-347.

Nekoubin,H., Gharedaashi.E., Hosseinzadeh M. and Imanpour,M.R. 2012. Determination of LC_{50} of Lead Nitrate and Copper Sulphate in Common Carp (*Ciprinus carpio*). American-Eurasian Journal of Toxicological Sciences 4 (2): 60-63.

Ohe, T., Watanabe, T. and Wakabayashil, K. 2004. Mutagens in surface waters: A review. Mutat. Res., 567: 109-149.

Pandey, A., Kunwar, O.K. and Munshi, J.S.D. 1990. Integumentary Chromatophores and mucus glands of fish as indicator of heavy metal pollution. *Jour. Freshwater Biol.,* 117-121.

Pickering, Q. H. and Henderson, P. 1966, The acute toxicity of some heavy metals to different species of warmwater fishes. *Int. J. Air Water Pollut., 10*: 453-463.

Prashanth, M.S., David, M and Mathed, S.G. 2005. Acute toxicity of Paper mill effluents on the behaviour of fresh water Indian Cat fish *wallagonia attu*. J. Environ. Biol. 26(1): 73-82.

Puvaneswari, S. and Karuppasamy, R. 2007. Acute toxicity bioassays of cadmium chloride on Indian catfish, *Heteropneustes fossilis*. *Aquacult.,* 8(2): 155-163.

Radhakrishnan Nair, 2006. Changes is acid and alkaline Phosphate activity during sub-lethal exposure of *Cyprinus carpio* and *Oreochromis mossambicus* to copper and chromium. Asian J. Microbial Biotech. Environ. Sci., 8(4):817-827.

Ramesh, M., Saravanan, M and Kavitha, C. 2009. Hormonal responses of the fish, *Cyprinus carpio*, to environmental lead exposure. African Journal of Biotechnology. Vol. 8 (17) : pp. 4154-4158.

Reglero, M.M., Taggart,M.A., Monsalve-Gonzalez,L.and Mateo, R. 2009. Heavy metal exposure in large game from a lead mining area: effects on oxidative stress and fatty acid composition in liver. Environ. Pollut., 157: 1388-1

Salmerón-Flores, P., Melendez-Camargo, M. E. and Martinez-Tabche, L. 1990, Efecto hepatotóxico y nefrotóxico del plomo sobre la tilapia (Sarotherodon aureus). An. Esc. Nac. Cienc. Biol. Mex., 33 : 147-156.

Scott, G.R. and Sloman, K.A. 2004. The effects of environmental pollutants on complex fish behaviour: integrative behavioural and physiological indicators of toxicity. Aquat. Toxicol. 68: 369-392.

Shah, S.L. and Altindu, A. 2005. Effects of Heavy Metal Accumulation on the 96-h LC Values in 50 Tench, *Tinca tinca L*., 1758. Turkish J. Vet. And Animal Sci., 29: 139-144.

Shamshun Nehar, Chandan Kumar, Kavita Mishra and Mamta Kumari, 2010. Lead induced alteration in blood profile of air breathing catfish, *Clarias batrachus* linn. The Bioscan:Special issue, Vol. 2.

Shyong, W. J. and Chen, H. C. 2000. Acute toxicity of copper, cadmium and mercury to the freshwater fish *Varicorbinus barbatus* and *Zacco barbata*. Acta. Zool. Taiwanica, 11(1):33-45.

Somaraj, R., Ranjith Singh, A. and Pushparah, A. 2005. Pesticide induced impairment on the carbohydrate metabolism is the fish *Mystus vittatus*. J. Curr. Sci., 7(2): 551-576

Sprague, J.B., 1971. Measurement of pollutant toxicity to fish. III. Sublethal effects and 'safe' concentrations. Water Res.,5:245–266.

Srivastava, A.K. and Mishra, S.1979. Blood dyscrasia in a teleost, *Colisa fasciatus* after acute exposure to sublethal concentrations of lead. J. Fish Biol., 24: 199-203.

Subathra, S. and Karuppasamy, R. 2003. Bioassay evaluation of acute toxicity levels of cadmium on mortality and behavioral responses of an air-breathing fish, *Channa punctatus* (Bloch.). *Jour. Experi. Zool. India,* 62(2): 245-250.

Tawari-Fufeyin, P., Igetei J.and Okoidigun M.E. 2008. Changes in the catfish (*Clarias gariepinus*) exposed to acute cadmium and lead poisoning Bioscience Research Communications. Vol. 20, No. 5.

Taylor, E. J., Maund S.J. and Pascoe,D. 1991. Toxicity of four common pollutants to the fresh water macro vertebrates *Chironomus riparius* Meigen (Insecta: Diptera) and Gammarus pulex (L.) (Crustacea: Amphipoda). Arch. Environ. Contam. Toxicol., 21: 371-76.

Valee, B. L. and Ulmer, D. D. 1972. Biochemical effects of mercury, cadmium, and lead. Annu. Rev. Biochemical 41(10): 91 -128.

Velez, D. and Montoro, R. 1998. Arsenic speciation in manufactured seafood products: a review. J. Food Protect. 61(9): 1240-1245

Voegborlo, R. B., Methnani, A. M. E. and Abedin, M. Z. 1999. Mercury, cadmium and lead content of canned Tuna fish. Food Chem. 67(4): 341 - 345.

Vogl, C., Grillitsch, B., Wytek, R., Hunrich Spieser, O., Scholz, W. 1999. Qualification of spontaneous undirected locomotor behavior of fish for sublethal toxicity testing. Part I. Variability of measurement parameters under general test conditions. Environ. Toxicol. Chem. 18 (12): 2736-2742.

Vosyliene, M. Z. and Jankaite, A. 2006. Effect of heavy metal model mixture on rainbow trout biological parameters. Ekologia.4:12-17.

Vutukuru, 2005. Acute effects of Hexavalent chromiumon survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian Major carp, *Labeo rohita*. Int. J. Environ. Res. Public Health. 2(3): 456- 462.

Walker, C. H., Hopkin, S. P., Sibly, R. M. and Peakall, D. B. 1996, Principles of ecotoxicology. Taylor and Francis, London.

Weis, J.S. and P. Weis P. 1998. Effects of exposure to lead on behavior of mummichog (*Fundulus heteroclitus* L.) larvae. J. Experimental Marine Biology and Ecol., 222: 1-10.

Wouters, W. and Van den Brecken. 1978. A comparative study on mercury, Zinc and copper, accumulation and recovery in the tissues of fishes. Gen. Pharmacol. 9: 387-392.

Yilmaz, M., Gul, A. and Erbasli, K. 2004. Influence of water hardness on cadmium toxicity to *Salmo gairdneri* (Rush). Bull. Environ. Contam. Toxicol. 56(4): 575-582.

****