Asian Resonance

Testing Lethal Concentration of Lead Acetate on *Clarias batrachus, Linn*

Prafulla Chandra Rout
Lecturer in Zoology
S.B.Women's College,
Cuttack, Odisha

Aimen Naaz
Research Scholar

Priyanka Sahoo
Lecturer in Zoology
Govt +2 SSD
MV-79, Kalimela
Malkangiri

Abstract

Indian cat fish Clarias batrachus, Linn. was treated with various concentrations of Lead acetate for 48 hrs . The LC50 determined by straight line graphical interpolation method was found to be 500ppm.The plot obtained is called as “Concentartion- response relationship” curve. Similarly, the LT50 of various concentrations were determined by “time-response relationship” curve and found to be 45 days for 25 ppm, 40 days for 50 ppm, 37 days for 75 ppm, 35 days for 100 ppm, 30 days for 125 ppm, 28 days for 150 ppm and 150 days for 15 ppm.. 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.

Keywords: Lead toxicity, Clarias batrachus, LC_{50}, LT50.,

Introduction

Life is diverse, and this diversity is now affected by pollutions. Pollution of air, water and soil has led to difficulties life activities so that many of the organisms have become extinct and many are in the verse of it. The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Canli 1998; Voegborlo *et al.,* 1999; Dirilgen, 2001; Vutukuru, 2005), and is getting extensively contaminate 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 Mc Bratney, 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, haematological, 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 Mirhashemi, *et al.,* 2010 Rout and Naik 2013a). 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 Rout and Naik 2013c). It has a great potential to serve as sensitive indicators, signaling exposure and understanding the toxic mechanisms of stressors in aquatic ecosystems (Vutukuru, 2005).

The impact of metals, are to be evaluated by toxicity tests, which are used to detect and evaluate the potential toxicological effects of chemicals on aquatic organisms. The 48hrs LC50 test paradigm is used to measure the susceptibility and survival potential of organisms exposed to particular toxic substances, such as heavy metals. Higher LC 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 a providing insights into individual 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 *al.,* 2008; Tawari-fufeyin *et al.,* 2008; Ramesh Khan *et al.,* 2011; Askari Hesni *et al.,* lethal effects of lead acetate on the freshwater fish are scanty. Hence, the present work is aimed to investigate the acute toxicity of lead acetate [($Pb(CHCOO)_2$] responses of the freshwater fish *Clarias batrachus, Linn.*

2.Materials and Methods:

2.1. Selection of *Clarias batrachus* as a model

According to WHO environmental health criteria No.180 (1996), because of their environmental conditions, fishes are excellent model for studying the effects of water and sediment-borne pollutants. There are several other good reasons for studying toxicity in fish:

- as many of the diseases are related to environmental quality.
- various pollutants have toxic potential, and
- Fishes are easy to obtain, there is an extensive body of knowledge, and their economic interest facilitates the finding of research resources.

Our model fish *Clarias batrachus*, *Linn.*, fulfils all the criteria as out lined above. It is easy to available and acclimatize due to its air breathing habit and hardly nature. The fish lives generally in oxygen-poor, polluted fresh water bodies and can be cultured for its economic and food value.

2.2. Procurement and Acclimatization:

Fishes of both sexes with varying weight, were collected from culture ponds of village MV 119 in Malkangiri district of Odisha. It is located between 21.1° N and 22.10° N latitude, 85.11° E and 86.22° E longitude. The village is located 500 meters mean sea level nearer to Bay of Bengal and comes under the '*Saptadhara River Valley system'*.

After collection, the fishes were maintained in the laboratory aquaria for about 10 days for acclimatization, following Dehadrai(1971). They were kept in large sized aquaria (size 2.5′ X 1′ X1′). Containing 80 litres of water each. Live earthworms and fish food were supplied daily with water was $1/10^{th}$ of their body weight. The water was changed daily with aeration.

The Physico-chemical profile of the aquaria water was monitored following APHA (2000) which was constant for control and experimental aquaria.

2.3. The compound used:

The compound of lead used for present piece of work is lead Acetate $Pb(C_2H_3O_2)_2$, procured from Jhonson and Sons, London. It has a relative molecular mass of 328.28, it has a good solubility index i.e. 443gm/ltr of cold water.

Selection of lead acetate for experimental work was due to its following physical and chemical properties.

1. A white crystalline solid, soluble readily in cold water.
2. Called sugar of lead due to its sweet taste.
3. Anhydrous with relative density 3.5 and melting point 280°C.
4. It forms a variety of complex in solution and therefore reacts with different biomaterials.
5. The biological availability of lead acetate is highest in compare to other compounds (Dieter et.al.1993).

Hence for treatment, simply weighing it at desired dose and releasing to aquaria water is easy.

Calculations for lead content in Aquaria water.

Compound used: Lead Acetate $Pb(CHCOO)_2$: mol wt. 207 + (12 X 2 + 16X 2 + 1) X 2 $\Rightarrow$ 207 + (24 + 32 + 1) 2 $\Rightarrow$ 207 + 77 X 2 $\Rightarrow$ 321

1 mole of lead acetate has 321 gms of wt. or, 321 gm by part of lead acetate contain 207 gm of lead, Or, 321 mg by part of lead acetate contain 207 mg of Pb Or, 1 mg by part of lead acetate contain = 207/321= 0.65 Or, 1ppm of water 0.65 mg or 650 mg of lead. Hence,

Lead acetate in mg	**Contains lead in mg**
15	9.7
25	16.12
50	32.24
75	48.36
100	64.48
125	80.60
150	96.72

2.3.1. Determination of LC_{50} and LT_{50}

LC_{50} is that concentration of a toxicant at which 50 % of the test animal is killed in a specified time. For example, 48 - hours $LC_{50}$for a toxicant refers to that concentration of the toxicant at which 50 % of the test animals are killed. LC refers to lethal concentration and 50 to 50 % population. However, lethal concentration is used for aquatic media only. Another related term LT_{50} refers to lethal time required to kill 50 % population of the test animal.

The lethal concentration LC_{50} was determined following trial and error method of Omkar(1994), Verma and Srivastava (2007). At the beginning of the experimental work 10 large sized fishes were kept in each aquarium and different concentrations of lead acetate were applied. Taken 10 glass aquaria. Arranged them in a line on the laboratory table and then marked them number I, II, III, IV, V , VI , VII, VIII, IX and X. Filled up each aquaria with 40 liters of tap water. Then added toxicant at the rate of 17.5 mg/L, 15.5 mg/L, 31.5 mg/L, 62.5 mg/L,125 mg/L, 250mg/L, 500mg/L,1000mg/L and 2000mg/L in aquaria marked number II, III, IV, V , VI ,VII, VIII, IX and X respectively. Kept aquarium number 1 free from toxicant that works as **control,** while other aquaria are **experimental** ones. Then released 10 fishes in each aquarium including the control and observe mortality up to 48 hours The concentrations were increased logarithmic value and % mortality were calculated after 48 hrs for each concentration.

Table 1:
Determination of LC_{50} of Lead Acetate on *Clarias batrachus*, *Linn*. by Semi logarithmic interpolation method for 48 hours.

Sl.No. of Aquarium	No. of test Animals	Concentration of Lead Acetate in PPM	No of Death	% of death
I	10	Control – 0	0	0
II	10	7.5	0	0
III	10	15.5	1	10
IV	10	31.5	1	10
V	10	62.5	2	20
VI	10	125	3	30
VII	10	250	4	40
VIII	10	500	5	50
IX	10	1000	8	80
X	10	2000	10	100

Results and discussion: The graph was plotted between concentration of lead acetate and % mortality of *Clarias batrachus*. The plot thus obtained is called as "Concentration- response relationship" curve LC_{50} was found to be 500 ppm. Table. 1 outlines the results and figure 1 provides the concentration- response relationship.

Similarly, a plot between time period and % mortality for a particular concentration of lead acetate provided the LT_{50} value. This curve was called as "time-response relationship" curve. The LT_{50} was 45 days for 25 ppm, 40 days for 50 ppm, 37 days for 75 ppm, 35 days for 100 ppm, 30 days for 125 ppm, and 28 days for 150 ppm, the chronic 15 ppm treatment has a very long LT_{50} i.e. 150 days for the fishes weight for 200-250 gms.(Table 2 and Figure 2). The photographic plate shows differences between the behavioural patterns of the control and experimental fishes (Plate-1).

Table 2:
LT50 0f used concentrations of Lead Acetate on *Clarias batrachus*, *Linn*. during experimental Plumbism

Concentrations of Lead Acetate (ppm)	50% Lethal period of the chemical(days)
15	150
25	45
50	42
75	35
100	35
125	30
150	21

PLATE-1: Observations

i)Movement of the Control fish

ii) Magnified view of the gill movement of the fish just before death.

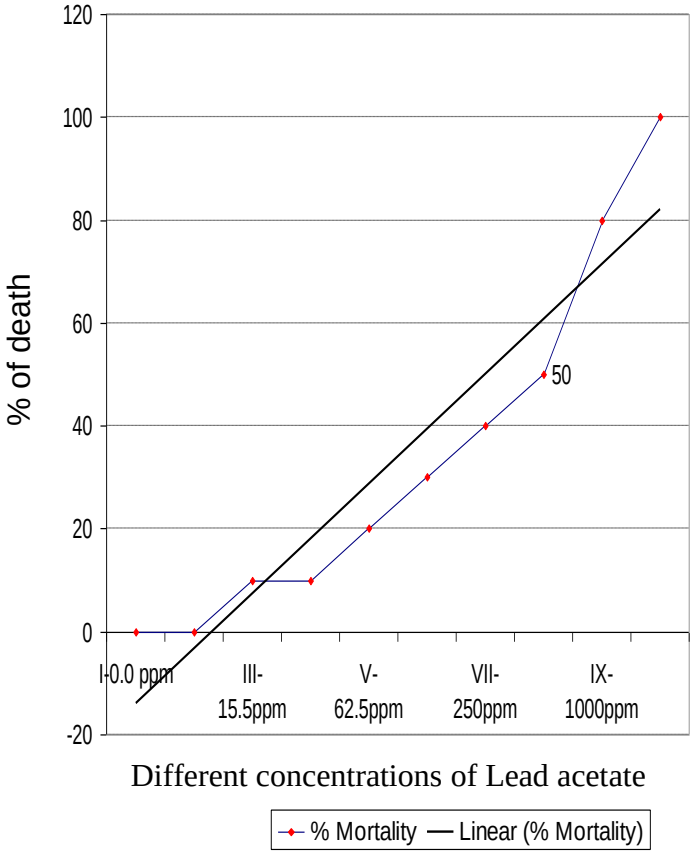

Fig.1: Determination of LC_{50} by semi logarithmic interpolation method.

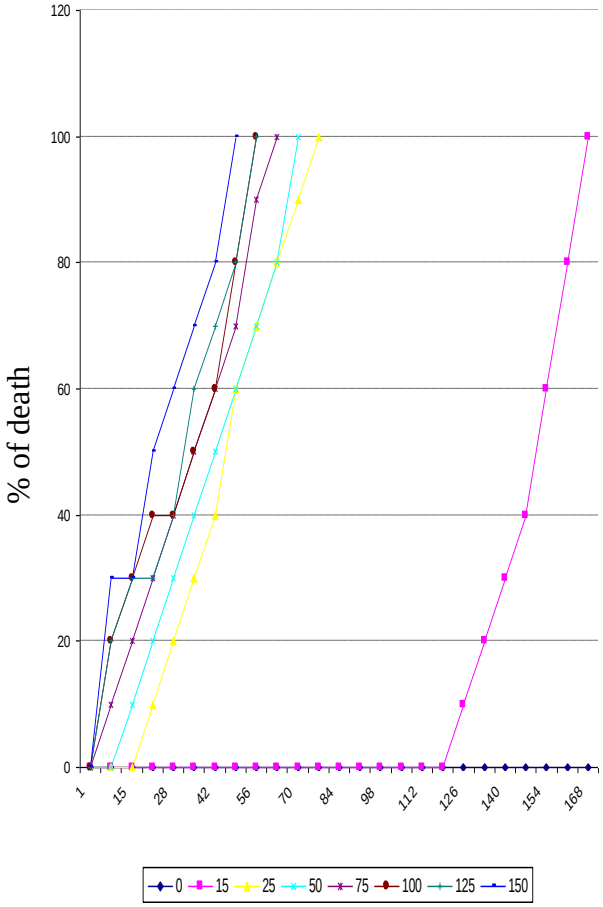

Fig.2 : Determination of LT_{50} lead Acetate on *Clarias batrachus* by semi logarithmic interpolation method.

3.1 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 [$(Pb(CHCOO)_2$] for *Clarias batrachus* was derived for 48 hrs. The mortality data was subjected to semi-logarithmic interpolation method and plotted against log dose concentrations, resulting in almost a straight line [Fig. 1]. The LC50 values and 95% upper and lower confidence limits of Pb on *Clarias batrachus* are given in Table - 1. The LC_{50} values for 48 hrs of exposures was estimated as 500- mgPbL-l (PPM) . This present result is 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 [$(Pb(CHCOO)_2$] was dose and time dependant and this reflects the regular mode of action which may due to accumulation and subsequent magnification of [$(Pb(CHCOO)_2$] up to 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 $Pb(CHCOO)_2$] 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 48 hrs LC_{50} of 500 mgPbL-1 for *Clariac batrachus*. 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. 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 (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 CaCO3L–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 CaCO3L–1) 96 hrs LC_{50} was 482442 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).

3.2 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. . 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 acetate 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, (Rout and Naik 2013b) 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 acetate on *Clarias batrachus* varied with increasing heavy metal concentrations and in response to such water conditions as temperature, pH and dissolved O2. The study demonstrated that the test fish *C. batrachus* can be used as an effective bio-indicator for acute pollutants such as lead nitrate. Finally, death resulting from acute lead acetate, in the test fish might be due to increased gastric hemorrhage, convulsion and suffocation (Valee and Ulmer, 1972) as it can be observed in the Plate 1.

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.

Acknowledgement:

The Authors are grateful to Dr P. S. Roy, Program Co-coordinator of the Refresher Course on Experimental Biology, Sponsored by Indian National Science Academy at IISER kolkata , Dr Sumana Annagiri HOD behavioural Studies in IISER Kolata, who inspired to conduct such experiments in this remote Maoist area. Also thanks are due to the Principal Govt. Sc. College, Malkangiri and DWO,ITDA Malkangiri who provided funds to establish the laboratory allowed to carry such experiments.

References:

1. 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.
2. 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.
3. 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.
4. APHA. (2000). Standard methods for examination of water and wastewater. 20th Edn. American Public Health Association Washington D C. p.1976.

5. 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.
6. 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.
7. 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.
8. 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.
9. 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.
10. 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.
11. Conacher, H. B., Page, B. D. and Ryan, J. J. (1993). Industrial chemical contamination of foods [Review]. Food Addit.Contam. 10(1): 129-143.
12. 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.
13. Das, B.K. and Mukerjee, S.C. (2003). Effect of pyrethroid on the behaviour of *Heteropneusta fossilis.* Comp. Biochem. Physiol. C. .Pharmacol. 134(1): 109-112.
14. 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.
15. Dehadrai, P.V. (1971): Physiological adaptation of air breathing fishes in relation to adverse environmental features. First workshop of the All India Coordinated Research Project on culture of Airbreathing fishes, Cuttack September. P.11.
16. 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.
17. 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.
18. Dimitrova, M. S., Tishinova, T., and Velcheva, V. (1994). Combined effects of zinc and lead on the hepatic superoxide dismutasecatalase system in carp. *Comp. Biochem. Physiol., 108C*: 43-46.
19. Dirilgen, N., (2001). Accumulation of heavy metals in fresh water organisms: Assessment of toxic interactions. Turk. J. Chem. 25(3): 173-179.
20. 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.
21. 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.
22. 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 (LC50) 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.
23. 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.
24. 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.
25. 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.
26. 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.
27. Kanabur, V. and Sannadurgappa, D. (2001). Acute toxicity of phenol and cresol to a freshwater fish *Oreochromis mossambicus*. Environ. Ecol., 19(4): 756-758.
28. 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.
29. 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.
30. 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.
31. 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
32. Mirhashemi, S.M., Moshtaghie,A.A., Ani, M. and Aarabi, M.H. (2010). Lead toxicity on kinetic behaviors of high and low molecularweight alkaline phosphatase isoenzymes of rat, *in vivo* and *in vitro* studies. J. Biol.Sci., 10: 341-347.
33. Nekoubin,H., Gharedaashi.E., Hosseinzadeh M. and Imanpour,M.R.(2012). Determination of LC50 of Lead Nitrate and Copper Sulphate in Common Carp (*Ciprinus carpio*). American-Eurasian Journal of Toxicological Sciences 4 (2): 60-63.
34. Ohe, T., Watanabe, T. and Wakabayashil, K. (2004). Mutagens in surface waters: A review. Mutat. Res., 567: 109-149.
35. Omkar, P.A.(1994): Toxicology. S. Chand and Co, New Delhi.P.494.

36. 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.
37. 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.
38. 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.
39. Puvaneswari, S. and Karuppasamy, R. (2007). Acute toxicity bioassays of cadmium chloride on Indian catfish, *Heteropneustes fossilis*. *Aquacult.,* 8(2): 155-163.
40. 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.
41. Ramesh, M., Saravanan, M and Kavitha, C. (2009). Hormonal responses of the fish, *Cyprinus carpio*, to environmental lead . African Journal of Biotechnology. Vol. 8 (17) : pp. 4154-4158.
42. Rout P. C. and Niak B. N. (2013a). Quantitative Precipitation tests for anti-avidin during experimental plumbism in Clarias batrachus, Linn. Asian resonance vol II(III) July issue.
43. Rout P. C. and Niak B. N. (2013b). Kinetics of Lead and bio-concentration factor (BCF) in different tissues of *Clarias batrachus* during experimental Plumbism. IJSRP.Vol.3(8) pp 325-340.August issue.
44. Rout Prafulla Chandra, Naik BN (2013 c). Tracing out Correlation between Blood Lead and Heamatological Parameters in *Clarias batrachus, Linn*. During Experimental Plumbism. IJCR Vol. 5 (9).In press.
45. 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
46. 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.
47. 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.
48. 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.
49. 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.
50. 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.
51. 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
52. Sprague, J.B., (1971). Measurement of pollutant toxicity to fish. III. Sublethal effects and 'safe' concentrations. Water Res.,5:245–266.
53. 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.
54. 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.
55. 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.
56. 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.
57. Valee, B. L. and Ulmer, D. D. (1972). Biochemical effects of mercury, cadmium, and lead. Annu. Rev. Biochemical 41(10): 91 -128.
58. Velez, D. and Montoro, R. (1998). Arsenic speciation in manufactured seafood products: a review. J. Food Protect. 61(9): 1240-1245.
59. Verma P.S. and Srivastava P.C. (2007). Advanced Practical Zoology. S. Chand Publication New Delhi p 256.
60. 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.
61. 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.
62. Vosyliene, M. Z. and Jankaite, A. (2006). Effect of heavy metal model mixture on rainbow trout biological parameters.Ekologia.4:12-17.
63. 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.
64. Walker, C. H., Hopkin, S. P., Sibly, R. M. and Peakall, D. B. (1996), Principles of ecotoxicology. Taylor and Francis, London.
65. 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.
66. 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.
67. WHO. (1996): Principles and methods of assessing direct immunotoxicity associated with exposure to chemicals. Environmental health criteria 180. Published by WHO, Geneva.
68. 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.