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Estimation of Acute Toxicity of Chromium to the Freshwater Catfish *Clarias batrachus* (Linn.)

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Abstract: *Anthropogenic activities has increased the discharge of various concentrations of both essential and nonessential metals into aquatic ecosystems. The present study has been conducted to determine the median lethal concentration of chromium to the freshwater fish Clarias batrachus following standard procedures and was found that 40.56ppm, 38.15ppm, 36.65 ppm and 35.50ppm for 24h, 48h, 72h and 96hours LC_{50} doses, whereas LC_0 as well as LC_{100} doses were 31 ppm and 40ppm respectively. The behavioural changes include abnormal coughing and jerky movements, erratic swimming, hyperactivity changes in opercular movement, copious amount of mucus secretion all over the body and loss of equilibrium were observed. All these observations can be considered to monitor the quality of water contaminated with heavy metal salts.*

Keywords: *Acute toxicity, Chromium, Clarias batrachus.*

1. Introduction

Metal contamination severely interferes with ecological balances of an ecosystem and anthropogenic inputs like waste disposal directly adds to the burden of environmental degradation (Farombi *et al.,* 2007), as metals cannot be destroyed through biological degradation and have the ability to accumulate the harmful chemicals in the aquatic ecosystem and consequently to humans (Di-Giulio and Hinton, 2008). Therefore, there is a need to develop rapid and sensitive screening methods as an aid for monitoring the presence of toxicants; besides water qualities, necessity for studying aquatic organisms as indicators of pollution was felt long back and quite a large number of organisms have been identified for this purpose (Vutukuru, 2005). Toxic heavy metals are increasingly being released into the environment with the advent of agricultural and industrial revolution in India. The heavy metals are reported to be ubiquitously present in the waste water which are generally released into aquatic ecosystems, because of water use in industrial processes as well as discharge of effluents from industry and urban development's (Mason, 1983; Demirak *et al.,* 2006; Fernandesa *et al.,* 2007) and toxic for aquatic organisms (Olsson, 1998; Kumar *et al.,* 2007; Kumar *et al.,* 2009) by changing genetic, physiological, biochemical and behavioural parameters (Scott and Sloman, 2004). Among the aquatic habitants, fish is the most susceptible to these elemental contaminants and more vulnerable to metal contamination than any other aquatic habitant (Alinnor, 2005). In recent years, much attention has been paid to the possible danger of metal poisoning in human as a result of consumption of contaminated fishes. Chromium, an important environmental pollutant, is present in the aquatic environment as a result of plating and electroplating factories, textile manufacturing facilities, steel producing factories, rinse waters, leather tanneries, dyeing, sanitary land fill leaching, the combustion of coal and oil, welding, bricks of furnaces, and wood-preserving industry (anthropogenic sources) (Palanippan and Karthikeyan 2009; Monterio *et al.,*. 2002; European Chemicals Bureau ECB 2005; ATSDR 2013). Chromium can exist in oxidation state ranging from −2 to + 6, but is most frequently found in the environment in the trivalent (Cr^{+3}) and hexavalent (Cr^{+6}) form (Eisler 1986). The hexavalent state is a major part of chromium in waters (Naja and Volesky, 2009). Hexavalent chromium easily penetrates biological membranes and for high oxidizing potential, is more toxic than the Cr^{+3} form (Eisler 1986; Meyers 1990; Jones 1990). Hexavalent chromium has been known to produce toxic, carcinogenic, teratogenic, and mutagenic effects in biological systems (Meyers 1990; Jones 1990; Patnaik 1992; Manahan 1992; Palmer and Plus 1994; Hodgson 2004; Naja and Volesky 2009; Velma *et al.,*. 2009). Hexavalent chromium exposure can induce adverse effects in different species of fish such as damage to DNA and tissue structures (Kuykendall *et al.,*. 2006; Irwin *et al.,*. 1997; Sesha Srinivas and Rao 1998), behavioral modifications (Mishra and Mohanty 2008), lifespan

(Perez-Benito 2006), metabolic changes (Vutukuru 2003), osmoregulatory upset (Van der Putte *et al.,*. 1982), survival and growth rate (Farag *et al.,*. 2006; Nguyen and Janssen 2002), and alternations at biochemical and haematological parameters (Sastry and Sunita 1983; Subashini *et al.,*. 2005; Velma *et al.,*. 2009). Hence, the present study has been proposed to determine the acute toxicity of chromium to the freshwater catfish *Clarias batrachus*(Linn.).

2. MATERIALS AND METHODS

Healthy specimens of *Clarias batrachus*(length 10 ± 0.5 cm and 12 ±1 g of body weight) were collected locally at Chidambaram, Tamilnadu, India, and were confined to large plastic aquaria bearing tap water for a period of two weeks in the laboratory for acclimation. They were kept in batches (10 each) in 50L glass tanks filled with dechlorinated tap water under constant filtration. The fish were fed with minced goat liver on every day for a period of three hours and the water was renewed on every day by routine cleaning of aquaria to remove unconsumed food, faecal matter or death fish (if any). Feeding was allowed 3hours before the renewal of the medium. Prior to the commencement of the experiment feeding was stopped. The physico–chemical characteristics of the normal water and water dissolved with chromium for calculating LC_{50} dose was analysed as per standard procedures (APHA,2008) (Table 1).

The analytical grade potassium dichromate ($K_2Cr_2O_7$) (CAS no: 7778-50-9) with 99.7 % purity was obtained from Merck (Darmstadt, Germany) and used without further purification for the laboratory experiment. Test solution was prepared by dissolving 2.828 g of potassium dichromate with double distilled water to make 1000 mg/L chromium (Palanippan and Karthikeyan2009) and then diluted to desired chromium with double distilled water. For the analysis of acute toxicity, fourteen groups of 10 fish each were exposed separately to 100 1 of 31 to 44 ppm of Potassium dichromate solution prepared in well water. Parallel control groups were also kept without the addition of Potassium dichromate. Media were renewed after every 24 h. Following the completion of each experiment, the test containers were cleaned with detergent soap and rinsed with 5 per cent nitric acid and subsequently dried. This procedure is very helpful to remove the metal content from adhering to the wall of the trough. The trough was covered with nylon net to prevent the escape of fish. Preliminary range finding tests were conducted to avoid delay and to save time and manual effort. These exploratory bioassays help to determine the approximate range of concentrations of the toxicant. A wide range of concentrations *viz.,* 10, 20, 30, 40, 50, 60, 70 and 80 ppm of Potassium dichromate solutions were prepared. Then the test were conducted in the rectangular plastic troughs by allowing ten fish of *Clarias batrachus*in each plastic trough containing 10 litres of water with required concentration of the Chromium. The test water was renewed daily according to the acute static toxicity test (U. S. E. P. A., 1975). The median lethal concentration (LC_{50}) for 24, 48, 72 and 96h were determined using trimmed spearman Karber method (Hamilton *et al.,* 1977).

3. RESULT

Table 1 shows the Physico-chemical characteristics of the normal water and water dissolved with chromium. The analysed parameters were within the permissible limit. When the toxicant was added the odour of water became unpleasant.

Table1.*Physico-chemical characteristics of the normal water and water dissolved with Potassium dichromate*

Parameters	Normal water	Water dissolved with Chromium
Odour	Odourless	Unpleasant
Temperature (˚C)	28	29
pH	7.3	6.2
Dissolved Oxygen (mg/L)	7.6	6.9
Total Hardness (mg/L)	225	210

Table 2 shows the data on preliminary test, to assess the concentration at which all fishes survived for 24 hours and likewise the concentration at which most of the fishes died simultaneously. The result indicates that toxicity effect of Chromium on *C. batrachus*fell within the range of concentration of the metal from 35ppm to 40 ppm and beyond 40 ppm of Chromium, all the fishes died.

Table2. *Mortality percentage of exposed to different concentrations of Chromium*

Concentration (ppm)	No. of Specimens	Mortality Percentage				Remarks
		24h	48h	72h	96h	
31	10*	0	0	0	0	LC_0
32	10*	0	0	0	10	
33	10*	0	0	10	20	
34	10*	0	0	20	40	
35	10*	0	10	40	50	LC_{50**}
36	10*	0	20	50	60	
37	10*	10	30	60	70	
38	10*	20	50	70	80	
39	10*	40	60	80	90	
40	10*	50	70	90	100	LC_{100***}
41	10*	60	80	100	100	
42	10*	70	90	100	100	
43	10*	90	100	100	100	
44	10*	100	100	100	100	

**Total number of specimens tested in 14 sets of experiments; LC_{50} 96 h dose.*

***Incipient LC_{50} 96 h dose; *** Incipient LC_{100} 96 h dose.*

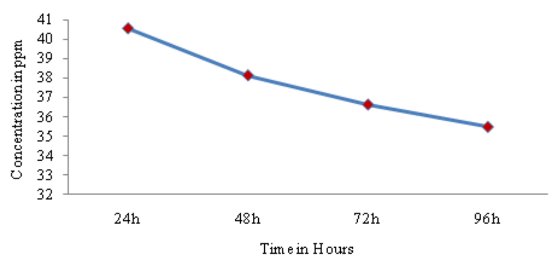
The preliminary test showed that beyond 40 ppm of chromium, all the test fishes died. Therefore, the concentration of chromium falling the range between 35 and 40 ppm were prepared and ten number of test fish were introduced to a confined narrow range of concentration viz. 35, 36, 37, 38, 39, 40 and 41ppm of chromium solutions for a period of 24, 48, 72 and 96h of intervals (Table 3).

Table3. *LC_{50} for Chromium (with 95% confidence limit) estimated by Trimmed Spearman – Karber method.*

Test Duration	**LC_{50} (ppm)**	**95% lower confidence limit**	**95% upper confidence limit**
24h	40.56	40.55	40.57
48h	38.15	38.14	38.16
72h	36.65	36.58	36.72
96h	35.50	35.42	35.58

Linear regression curve was constructed taking test concentrations and corresponding percent mortalities on log and probit scales respectively which was utilized to estimate the LC_{50} values at different time intervals (Fig. 1) and the LC_{50} was calculated as 35. 50 ppm at 95% confidence limit.

Fig. 1 Showing the Toxicity Curve of Chromium



The behavioural responses of the fish at various concentrations of chromium were observed at regular intervals to ascertain the impact of the toxicant on the organism. When released in the toxic media, the fish showed abnormal behaviour as hyper-excitability, increased opercular movement as well as fish were often seen swimming with jerky movements on the surface of water and tried to jump out of the container. Further, the fish tried to remain almost in vertical position perpendicular to the base of the container with the mouth facing upward, the fish lost balance, settled to bottom and died.

4. Discussion

A toxicity test using aquatic organisms plays an important role in the development of proposals for environmental management and protection (Hoi, 2004). Lethal Concentration of 50% (LC50) tests can measure the susceptibility and survival potential of animals to particular toxic substances such as heavy metals. Higher LC50 values are less toxic because greater concentrations are required to produce 50% mortality in animals (Hedayati *et al.*, 2010). Heavy metals such as mercury and chromium are toxic to aquatic animals at very low concentrations and are never beneficial to living beings (Gooley *et al.*, 2000; Shuhaimi-Othman *et al.*, 2010). The acute toxicity test is used to determine the concentration of a test material or the level of an agent that produces a deleterious effect on a group of test organisms during a short-term exposure under controlled conditions (Rani *et al.*, 2011). All toxicants are capable of severally interfering with the biological systems that producing damage to the structure and function of a particular organism and ultimately to its survival. Acute toxicity test constitutes 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 (Spacie and Hamelink, 1985). Toxicity is a characteristic feature of an individual organism's response to a chemical at a particular concentration or dosage for a specific period. Acute toxicity tests are conducted to measure the impact of toxicant on aquatic animals within a short period of four days. During acute toxicity test, organisms are transferred to freshly prepared, desired toxicant medium at periodic intervals usually 24 hours. In the present investigation the LC_{50} value is estimated and it is concerned with the determination of the median lethal response or median tolerance limit, which is no more than a condition which produces a 50 per cent mortality or permits a 50 per cent survival of the test organisms under the experimental conditions during a specific time interval (Sprague, 1973). Moreover, the study on toxicity impact deals, in general, with the reaction of a living organism in an aquatic environment and there is a generalized view that the toxicity of a chemical of the individual species.

Fishes are considered to be most significant biomonitors in aquatic systems for the estimation of metal pollution level (Rashed, 2001; Authman, 2008), they offer several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats (Lamas *et al.*, 2007). In addition, fish are located at the end of the aquatic food chain and may accumulate metals and pass them to human beings through food causing chronic or acute diseases (Al-Yousuf *et al.*, 2000). Studies from the field and laboratory works showed that accumulation of heavy metals in a tissue is mainly dependent on water concentrations of metals and exposure period; although some other environmental factors such as water temperature, oxygen concentration, pH, hardness, salinity, alkalinity and dissolved organic carbon may affect and play significant roles in metal's accumulation and toxicity to fish (Benaduce *et al.*,2008; Has-Schön *et al.*,2008; Linbo *et al.*, 2009; Sassi *et al.*, 2010; Ebrahimi and Taherianfard, 2011; Jitar *et al.*, 2014).

In the present study, susceptibility of the fish *C. batrachus*to the lethal effect of chromium was duration and concentration dependant as mortality increased with an increase in its concentration. The behavioural response of the fish towards the toxicant was also investigated in the present study. Behavior provides a unique perspective linking the physiology and ecology of an organism and its environment: (Little and Brewer, 2001). Any change in the behavior of fish indicates the deterioration of water quality, as fish are the biological indicator and hence index of environmental suitability and the cost of survival (Halappa and David, 2009). Chemicals in sublethal concentrations present in aquatic environment are too low to cause rapid death directly but may affect the functioning of the organisms, disrupt normal behavior and reduce the fitness of natural population (Susan *et al.*, 2010). The impact of chemicals on the life of aquatic organisms is often acute resulting in mass mortality or chronic changes in behaviour. Such behavioural alterations are very sensitive indicators of stress imposed on fish by the environment (Cooke *et al.*, 2000). Fish behavior under stress conditions provides important information for aqua culturists (Christiansen *et al.*, 2004). Methods of monitoring and quantifying the behavioural response have become potential alternatives for assessing stress, disease, water pollution and toxic material in water (Kane *et al.*, 2004).

Fishes exposed to chromium showed speedy movements as compared to control. The increase in swimming activity may be due to disruption of shoaling behavior which occurs because of the stress of the toxicant (Venkata *et al.*, 2008). Fast swimming was also observed by Yaji *et al.*, (2011) in *Orechromisniloticus* treated with Cypermethrin. Similar observation found by Ramesh and Saravanan (2008) in *Cyprinuscarpo* exposed to Chlorpyrifos. Increased ventilator movement was also noted in the present study. Under toxic condition the oxygen supply becomes deficient and so the fish breathe rapidly (Susan *et al.*, 2010). Movements like S jerking, threat and burst swimming were increased in the experimental fishes when exposed to chromium. Similar observations were reported by Nimila and Nandan (2010) in *Etroplus maculatus* when it is treated with lindane and by AL-Akel (2000) in carbaryl treated fishes. S jerk and burst swimming were also observed by Marigoudar *et al.*, (2009) *in*

Labeo rohita exposed to cypermethrin. In present study, copious secretion of mucus all over the body and increased gulping of air was also noted. The results of these studies may provide guidance to select acute toxicity to be considered in field bio monitoring efforts designed to detect the bioavailability of chromium and early warning indicators of chromium toxicity in Clarias batrachus.

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REFERENCES

Al-Aker A.S. and Shamsi M.J.K. (2000) Comparitive study of toxicity of carbaryl and its impact on the behaviour and carbohydrate metabolism of cichlid fish, *Oreochro misniloticus* (Linnaeus, 1758) and catfish *Clarias gariepinus* (Burchell,1822) from Saudi Arabia. *Egypt. J. Aquat Biol and Fish* 4(2), 211-227.

Alinnor I(2005). Assessment of elemental contaminants in water and fish samples from Aba River. *Environ. Monit. Assess.* 102: 15-25.

Al-Yousuf MH, El-Shahawi MS, Al-Ghais SM (2000) Trace metals in liver, skin and muscle of Lethrinuslentjan fish species in relation to body length and sex.Sci Total Environ 256: 87-94.

APHA (2008): Standard methods for examination of water and waste water.19th ed. American Public Health Association, Washington DC.

ATSDR (2013) Toxicological profile for chromium. U.S. Department of Health and Human services, Agency for Toxic Substances and Disease Registry, Atlanta, Georgia.

Authman MMN (2008) Oreochromisniloticus as a biomonitor of heavy metal pollution with emphasis on potential risk and relation to some biological aspects. Global Vet., 2(3): 104-109.

Benaduce APS, Kochhann D, Flores ÉMM, Dressler VL, Baldisserotto B (2008) Toxicity of cadmium for silver catfish Rhamdiaquelen (Heptapteridae) embryos and larvae at different alkalinities. Arch Environ ContamToxicol 54: 274-282.

Christiansen T.S., Ferno A., Holm J.C., Privitera L., Bakke S., and Fosseidengen J.E. (2004) Swimming behavior as an indicator of low growth rate and impaired welfare in Atlantic halibut (*Hippoglossus hippoglossus*L.) reared at three stocking densities. *Aquaculture,* 230 (1-4), 137-151.

Cooke S.J., Chandroo K.P., Beddow T.A., Moccia R.D. and McKinley R. S. (2000) Swimming activity and energetic expenditure of captive rainbow trout. *J.Appl.Ecol*13,12-23.

Demirak, A.; Yilmaz, F.; Levent Tuna, A. and Ozdemir, N. (2006): Heavy metals in water, sediment and tissues of *Leuciscus cephalus*from a stream in southwestern Turkey. Chemosphere, 63: 1451-1458.

Di-Giulio RT, Hinton DE.The Toxicology of Fishes. Taylor and Francis 2008; 319-884.

Domingo JL (1996) Chromium: a review of the reproductive and developmental toxicity. ReprodToxicol 10: 175-182.

Ebrahimi M, Taherianfard M (2011) The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River. Iran J Fish Sci 10: 13-24.

Eisler R (1986) Chromium hazards to fish, wildlife, and invertebrates: a synoptic review. U.S. Fish and Wildlife. Service Biol Rep 85(1.6): 60 pp.

European Chemicals Bureau (ECB) (2005) European Union Risk Assessment Report. Chromium trioxide, sodium chromate, sodium dichromate, ammonium dichromate and potassium dichromate risk assessment. EUR Report No. 201508 EN. ECB.

FaragAM, May T, Marty GD, Easton M, Harper DD, Little EE, Cleveland L (2006) The effect of chronic chromium exposure on the health of Chinook salmon (Oncorhynchus tshawytscha). AquatToxicol 76(3–4):246–257.

Farombi EO, Adelowo OA, Ajimoko YR. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish *Clariasgariepinus* from Nigeria Ogun river. International Journal of Environmental. Research Public Health 2007; 4(2):158-165.

Fernandesa, C.; Fontainhas-Fernandesb, A.; Peixotoc, F. and Salgadod, M.A. (2007): Bioaccumulation of heavy metals in *Liza saliens* from the Esmoriz –Paramos coastal lagoon, Portugal. Ecotoxicology and Environmental Safety, 66: 426-431.

Gooley GJ, Gavine FM, Dalton W, De Silva SS, Bretherton M, Samblebe M. Feasibility of aquaculture in dairy manufacturing wastewater to enhance environmental performance and offset costs. Final Report DRDC Project No. MAF001. Marine and Freshwater Resources Institute Snobs Creek 2000.

Halappa R. and David M. (2009) Behavioural responses of the freshwater fish, *Cyprinus carpio*(Linnaeus) following sublethal exposure to chloropyrifos. *Turk. J. Fish. Aquat. Sci.* 9, 233-238.

Hamilton, M.A.; Russo, R.C.; Thurston, R.V., (1977). Trimmed Spearman Karber method for estimating median lethal concentrations in toxicity bioassays.Environ. Sci. Tech., 11: 714-719.

Has-Schön E, Bogut I, Kralik G, Bogut S, Horvatić J, *et al.*,. (2008) Heavy metal concentration in fish tissues inhabiting waters of "BuškoBlato" reservoir (Bosnia and Herzegovina). Environ Monit Assess 144: 15-22.

Hedayati A, Safahieh A, Savar A, Ghofleh MJ. Detection of mercury chloride acute toxicity in Yellowfin sea bream (*Acanthopagrus latus*). World Journal of Fish and Marine Science 2010; 2: 86-92.

Hodgson E (2004)A textbook ofmodern toxicology. JohnWiley&Sons, Inc., Publication, Hoboken.

Hoi ND. Toxicology of water environment fora.Research institute of aquaculture, Ministry of Aquaculture, Vietnam 2004; 43.

Irwin RJ, Van Mouwerik M, Stevens L, Seese MD, Basham W (1997) Environmental contaminants encyclopedia. National Park Service, Water Resources Division, Fort Collins, Colorado.Distributed within the Federal Government as an Electronic Document (Projected public availability on the internet or NTIS: 1998).

Jitar O, Teodosiu C, Oros A, Plavan G, Nicoara M (2014) Bioaccumulation of heavy metals in marine organisms from the Romanian sector of the Black Sea. N. Biotechnol. 2014 Dec 9.pii: S1871-6784(14)02197-9.

Jones RE (1990) Hexavalent chrome: threshold concept for carcinogenicity. Biomed Environ Sci 3:20–34.

Kane A.S., Salierno J.D., Gipson G.T., Molteno T.C.A. and Hunter C.A.(2004) Video based movement analysis system to quantify behavioural stress response of fish. *Water Res.* 38(18), 3993-4001.

Kumar P, Prasad Y, Patra AK and Swarup D (2007).Levels of Cadmium and Lead in Tissues of Freshwater Fish (*Clarias batrachus*L.) and Chicken in Western UP (India).*Bull. Environ. Contam.Toxicol.*79: 396-400.

Kumar P, Prasad Y, Patra AK, Ranjan R, Patra RC, Swarup D and Singh SP (2009). Ascorbic acid, garlic extract and taurine alleviate cadmium induced oxidativee stress in freshwater catfish (*Clarias batrachus*). *Sci. Total Environ.* 407: 5024–5030.

Kuykendall JR, Miller KL, Mellinger KN, Cain AV (2006) Waterborne and dietary hexavalent chromium exposure causes DNA-protein crosslink (DPX) formation in erythrocytes of largemouth bass (Micropterus salmoides). AquatToxicol 78:27–31.

Lamas S, Fernández JA, Aboal JR, Carballeira A (2007) Testing the use of juvenile Salmotrutta L. as biomonitors of heavy metal pollution in freshwater. Chemosphere 67: 221-228.

Linbo TL, Baldwin DH, McIntyre J, Scholz NL (2009) Effects of water hardness, alkalinity, and dissolved organic carbon on the toxicity of copper to the lateral line of developing fish. Environ ToxicolChem 28: 1455-1461.

Little E.E. and Brewer S.K. (2001) Neurobehavioral toxicity in fish. In *Target Organ Toxicity in Marine and Freshwater Teleosts New Perspectives: Toxicology and the Environment.* Vol. 2.Systems.Schlenk, D. and W. H. Benson (eds). Pp 139-174. Taylor and Francis, London andNew York.

Manahan SE (1992) Toxicological chemistry. Lewis Publishers, an Imprint of CRC Press, Boca Raton.

Marigoudar S.R., Ahmed R.N. and David M. (2009) Impact of Cypermethrin on behavioural responses in the freshwater teleost, *Labeo rohita*(Hamilton).*World J. Zool.* 4(1),19-23.

Mason, C.F. (1983): Biology of freshwater pollution. Longman Inc., NY, 250 pp.

Meyers E (1990) Chemistry of hazardous materials.Prentice Hall Career and Technology. Prentice-Hall Inc, Engle wood Cliffs.

Minelli L, Veschetti E, Giammanco S, Mancini G, Ottaviani M (2000) Chromium in Italian waters: monitoring and speciation of V(IV) and V(V). Microchem J 67: 83-90.

Mishra AK, Mohanty B (2008) Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch). Environ Toxicol Phar 26:136–141.

Monterio MIC, Fraga ICS, Yallouz AV, de Oliveria NMM, Riberio SH (2002) Determination of total chromium traces in tannery effluents by electrothermal atomic absorption spectrometry, flame atomic absorption spectrometry and UV-visible spectrophotometric methods. Talanta 58:629–633.

Naja and Volesky, 2009

Naja GM, Volesky B (2009) Toxicity and Sources of Pb, Cd, Hg, Cr, As, and radionuclides in the environment.In:Wang LK, Chen JP, Hung YT, Shammas NK (eds) Heavy metals in the environment. CRC Press LCL, Boca Raton, pp 13–61.

Nguyen LTH, Janssen CR (2002) Embryo-Larval toxicity tests with the African catfish (*Clarias gariepinus*): comparative sensitivity of endpoints. Arch Environ ContamToxicol 42:256–262.

Nimila P.J and Nandan B. (2010) Effect of organochlorine pesticide lindane (**γ**-HCH) on behavior of *Etroplus maculates* (Bloch, 1795). *Poll Res*. 29(4), 577-582.

Olsson PE (1998). Disorders associated with heavy metal pollution. In: *Fish Diseases and Disorders Volume 2 (Non-infectious Disorders).* (Eds. Leatherland JE and Woo PTK) CABI International, U.K. pp 105-131.

Palanippan PLRM, Karthikeyan S (2009) Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish Cirrhinusmrigala individually and in binary solutions with nickel. J Environ Sci 21:229–236.

Palmer CD, Plus RW (1994) Natural attenuation of hexavalent chromium in ground water and soils, USEPA Ground Water Issue, EPA/540/5-94/505. U.S Govt Print Office, Washington, D. C.

Patnaik P (1992) A comprehensive guide to the hazardous properties of chemical substances. Van Nostrand Reinhold Publishers, New York.

Perez-Benito JF (2006) Effects of chromium (VI) and vanadium (V) on the lifespan of fish. J Trace Elem Med Biol 20(3):161–70.

Ramesh M. and Saravanan M. (2008) Haematological and biochemical responses in a fresh water fish *Cyprinus carpio* exposed to Chlorpyrifos. *Int. J. Integrative Biol*. 3(1**)**, 80-83.

Rani MJ, John MMC, Uthiralingam M, Azhaguraj R. Acute Toxicity of Mercury and Chromium to *Clarias batrachus*(Linn) Bioresearch Bulletin 2011; 5: 368-372.

Rashed MN (2001) Monitoring of environmental heavy metals in fish from Nasser Lake. Environ Int 27: 27-33.

Sassi A, Annabi A, Kessabi K, Kerkeni A, Saïd K, *et al.*,. (2010) Influence of high temperature on cadmium-induced skeletal deformities in juvenile mosquitofish Gambusia affinis). Fish PhysiolBiochem 36: 403-409.

Sastry KV, Sunita KM (1983) Enzymological and biochemical changes produced by chronic chromium exposure in a Teleost fish, Channa punctatus. Toxicol Lett 16:9–15.

Scott GR, Sloman KA (2004). The effects of environmental pollutants on complex fish behaviour: integrative behavioural and physiological indicators of toxicity. *Aquat.Toxicol.* 68: 369-392.

Sepe A, Ciaralli L, Ciprotti M, Giordano R, Fumari E, Costantini S (2003) Determination of cadmium, chromium, lead and chromium in six fish species from the Adriatic Sea. Food Addit Contam 20: 543-552.

Sesha Srinivas V, Rao BM (1998) Chromium induced alterations in the gill of the freshwater teleost fish, Labeo rohita. Indian J Compar Anim Physiol 17(1):31–33.

Shuhaimi-Othman MY, Nadzifah AK, Ahmad. Toxicity of Copper and Cadmium to Freshwater Fishes. World Academic of Science Engine and Technology 2010; 65:869-871.

Soares SS, Martins H, Gutiérrez-Merino C, Aureliano M (2008) Chromium and cadmium in vivo effects in teleost cardiac muscle: Metal accumulation and oxidative stress markers. Comp Biochem Physiol C 147: 168-178.

Spacie A, Hamelink JL. Bioaccumulation, In: Fundamentals and aquatic toxicology methods and applications (Eds) Rand, GM. and Petrocelli, SR. Hemisphere Publishing Corporation, New York 1985; 495-525.

Sprague, J.B. (1973): The ABC pollutant bioassays using fish. In: Biological methods for the Assessment of water quality. ASTM STP 528, American society for testing and materials, USA, 6-30.

Subashini P, Manavalaramanujam R, Ramesh M, Geetha N (2005) Changes in selected biomarkers in freshwater teleost fish, Cyprinuscarpio var. communis exposed to sublethal concentrations of chromium sulphate toxicity. J Environ SciEng 47: 65–68.

Susan A.T., Sobha K. and Tilak K.S. (2010) A study on acute toxicity, oxygen consumption and behavioural changes in the three major carps, *Labeo rohita*(ham), *Catla catla*(ham) and *Cirrhinus mrigala*(ham) exposed to Fenvalerate. *Bioresearch Bulletin* 1, 33-40.

USEPA (United States Environmental protection Agency). (1975): Method for acute toxicity tests with fish, macro invertebrates, and amphibians. Guidelines EPA – 600/3 – 75.

Van der Putte I, Laurier MBHM, Van Eijk GJM (1982) Respiration and osmoregulation in rainbow trout (Salmo gairdneri) exposed to hexavalent chromium at different pH values. AquatToxicol 2(2): 99–112.

Velma V, Vutukuru SS, Tchounwou PB (2009) Ecotoxicology of hexavalent chromium in freshwater fish: a critical review. Rev Environ Health 24(2):129–145.

Venkata Rathnamma V., Vijayakumar, M. and Philip, G.H. (2008) Acute toxicity and behavioural changes in freshwater fish *Labeo rohita* exposed to Deltamethrin. *J.Aqua Biol*. 23(2), 165-170.

Vutukuru SS (2003) Chromium induced alterations in some biochemical profiles of the Indian major carp, Labeorohita (Hamilton). Bull Environ ContamToxicol 70:118–123.

Vutukuru SS (2005): Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major Carp, *Labeo rohita*. International Journal of Environmental. Research Public Health 2005; 2: 456-462.

Yaji A.J., Auta J., Oniye S.J., Adakole J.A. and Usman J.I. (2011) Effects of Cypermethrin on behavior and biochemical indices of freshwater fish *Oreochro misniloticus*. *EJEAFChe.*10(2), 1927-1934.