International Journal of Academic Research and Development
ISSN: 2455-4197
Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 3; Special Issue 1; February 2018; Page No. 04-08

Effects of some heavy metals on toxicity of the fresh water fish: *Rasbora daniconius*

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Abstract
Heavy metals pose a great threat to aquatic organism not only because they are highly toxic in low quantity but also because of their potential characteristic of combining with biological moleculs. Metals like mercury, cadmium and lead all show a great affinity for sulfhydryl (-SH) groups and exert toxic effects largely by combining with such groups on proteins. One of the most significant effect of metallic pollution is that aquatic organisms can adsorb and accumulate concentrations in their tissues. For example, there may be up to 15 times as much mercury present in fish. The LC10 and LC50 values for heavy metals were summarized in (Table 1 & 2).The LC10 of lead acetate for 24, 48, 72, and 96 hours were 3.34,3.80, 1.99 and 1.90 ppm respectively. The LC10 of zinc sulphate for 24, 48, 72 and 96 hours were 6.64, 5.71, 2.99 and 1.99 ppm respectively. The LC10 of nickel chloride for 24, 48, 72 and 96 hours were61.07, 18.63, 23.09 and 9.29 ppm respectively. The LC50 of zinc sulphate for 24, 48, 72 and 96 hrs were 14.6, 12.65, 9.38 and 6.26 ppm respectively and LC50 of nickel chloride for 24, 48, 72 and 96 hrs were 82.78, 69.15, 44.86 and 29.22 ppm respectively. Where as in preset study no such behavioral changes were noticed in the control fish, which remained active and healthy throughout the experimental period.

Keywords: heavy metals, *Rasbora daniconius*, toxicity

Introduction

Water pollution has focused the attention of both the scientific community and the public on environmental problems. not only does water pollution affect the health and welfare of people and organisms, but it also damage vegetation and properties Sehgal and Saxena (1986) [25]. Metals with an atomic number greater than 20 are considered as heavy metals. Introduction of metals into aquatic system are due to both natural causes ans human activities.

Heavy metals pose a great threat to aquatic organism ont only because they are highly toxic in low quantity but also because of their potential characteristic of combining with biological moleculs. Metals like mercury, cadmium and lead all show a great affinity for sulfhydryl (-SH) groups and exert toxic effects largely by combining with such groups on proteins. This result in disruption of enzymes mediated processes in the cells Laws, (1981) [19]. Another quality of these metals is that they rapidly adsorb to particular materials such as detritus and suspended, sediments ans particle like organism such as plankton.

When these particles are ingested by other organisms, metals get transferred and in the process the whole food chain may get the metals transferred in a series. Benthic organism are the most likely candidates to be affected by metals in sediments because the benthic environment is the ultimate respiratory of particulate materials that wash into aquatic system. Invariable sediments near open drain out falls contain high metals concentration because of discharge from municipal and industrial wastes. One of the most significant effect of metallic pollution is that aquatic organisms can adsorb ans accumulate concentrations in their tissues. For example, there may be up to 15 times as much mercury present in fish as in algae. (Ajmal *et al.*; 1983) [3] have reported elevated level of Cd, Co, Cu, Cr, Fe, Mn, Ni, P bans Zn in the fish ans submerged plants from the Ganges river. There are many reports on toxic effects of certain metallic compounds. (Banerjee and Banerjee 1988, kohli *et al*. 1988, joseph 1989; and Sinha *et al*. 1992) [8, 18, 27]. Increasing urbanization, rapid industrialization and development of agrarian technology has led to the use of innumerable synthetic chemicals and productions of hazardous waste. Although the green revolution through out the world has successfully met the challenge of hunger, the use of thousands of chemicals as fertilizers, insecticides, fungicides, herbicides, rhodenticides and other biocides has posed serious problems of environmental pollution leading threat to the human beings, wildlife and important biotic components of the food chain. The modern style of living and ever-increasing needs of mankind has led to rapid industrial development; as a result thousands of water-based industries have come up during last three-four decades. The industries like pulp and paper, textile, tanneries, distilleries, electroplating, fertilizer, food and dairy products, thermal and nuclear power plants and other plastic and allied products has been another serious problem of environmental pollution.

Water bodies serves as the ultimate sink for all sorts of chemicals used for any purpose and all sorts of hazardous wastes produced by industries. Some of these directly find their way to aquatic ecosystems, while other reaching indirectly. Some of the toxicants are biodegradable and being converted into nontoxic substances over a period of time depending on self-purification capacity of water body, while some of like heavy metals are either non-degradable or slowly degradable resulting in accumulation and bio-concentration within the ecosystem and its biotic components.

Toxicity testing is essential component of water pollution evaluation and the study of changes in physico-chemical parameters only does not help much in the assessment of effect of pollution in aquatic biota. The history of toxicity testing dates back to the time of Aristotle (over 2000 years ago) when he observed the effect of seawater on freshwater animals. Toxicology arose as the formal discipline in early 1980's and since then man has been serious of knowing the adverse effects of chemicals and drugs on human beings. After 1940's interest was generated towards the effects of chemical and wastes to non-human organisms like fish. The toxicologists have demonstrated and advocated the utility of experimental toxicity testing of industrial waste and other toxicants to fish or predicting potential damage to the aquatic fauna of water bodies.

Heavy metal contamination of the environment resulting from anthropogenic activities such as mining operations and industrial activities is of concern because they exhibit behavior consistent with those of persistent chemicals. Heavy metals are also known to have toxic effects at very low concentrations, as well as high concentration,. Unlike many organic contaminants that lose toxicity with biodegradation, metals can not be degraded further and that their toxic effects can be very long lasting. Heavy metals have a tendency to bioaccumulate and end up as permanent addition to the environment.

The toxicity of heavy metals lies in the capacity of their selective effect on enzymes of nerve tissue – cholinesterase (ChE), which lid to excessive accumulation of acetylcholine in the organism. (Other enzymes like esterase, protease and peroxides are also inhibited by organophosphate compounds and slightly enhance the activity of catalyze.

The aquatic environment in recent times is witnessing an unprecedented in pour of various kinds of biocides in alarming quantities and sources of such release are too numerous to be mentioned. Rapid industrialization and consequent discharge of effluents into water system made heavy metals a major pollutants of aquatic ecosystem. The common toxic metals found in industrial effluents are cadmium, chromium, nickel, lead, copper, zinc etc. are non essential elements and do not require by the animal. Therefore any accumulation of these metals is a burden on the organism and is likely to prove as a source of toxicity. Gupta and Chakrabarti, (1994) [27] reported toxicity of zinc to Notopterus notopterus and Punctius japanicus, Zyadan and Abdel, (2000) studied the toxicity and bioaccumulation of copper, zinc and cadmium in fishes. Villar *et al.*, (2000) [21] determined lethal concentration of copper in the neurotropical fish, Cnesterodon decemmaculatus.

Material and Method

Fishes of average size *Rasbora daniconius* were regularly collected from 'Van' river 10 km away from Parli-vaijnath, Dist Beed (Marathwada region) Maharastra state. Experiments related to toxicity evaluation was carried during June 2016 to September 2016. All physicochemical parameter of the water used to perform toxicity evaluation were maintained as per the record available for the 'Van' river from the municipal council Parli-vaijnath *Rasbora daniconius* is sensitive to aquatic disorders and easily maintainable in the laboratory. *Rasbora daniconius* mainly feeds on insects, crustaceans and filamentous algae and other suitable organisms found very close to the surface of water. The fishes have oblique cleft of mouth, which helps to capture various insects, larvae and other floating organisms.

These fishes were brought to the laboratory without any mechanical injury. The fishes were acclimatized in well-aerated water, the physiochemical characters of the aged test water were (average values) temperature: 27°C-28°C: conductivity:0.72 m MHO; dissolved oxygen 6.2mg/1);total hardness (as CaCo3);72 ppm (70-90ppm);total alkalinity:26.4 ppm(22.6-28.2ppm): total acidity:6.4 ppm (6.0-7.0ppm):Ph7.8 (7.6-7.9) for two weeks before being used for tests.

Acute toxicity tests were conducted over 96 hrs.The experimental thought caontaining 5 liters dechlorinated water were used to keep the animals. Stock solution of the toxivants wrer prepared in double –distilled water and added to the test medium to get desired concentration of heavy metals. After every 12 hours the polluted water was changed by the fresh solution of the same concentreation of heavy metals. The resulting mortality was noted in the range of 10 to 90% for each concentration for the duration of 24,48,72 and 96 hrs. Each experimental was repeated to obtain constant results.

The data collected was analyzed statically by means of probit method on transforming toxicity curve (% mortality Vs. concentration), which allows the average median lethal concentration of LC_{50} to be calculated for 24,48,72 and 96 hrs. Dead fishes were counted individually. The criterion for death was the failure of the animal to respond to the pricking of its foot with a needle.

Result

Acute toxicity tests were carried out in the laboratory condition for 24,28, 72 and 96 hours duration for three metal and its combination like lead acetate, zinc sulphate, nickel chloride, acute toxicity tests were conducted by the method described by Finney (1951) [15] and simplified by Busvine (1971) [11]. The regression equation wre obtained by heavy metals. The result obtained after toxicity evaluation of *Rasbora daniconius* are cited in table.

Table 1: Comparison of safe concentration, Lc_{10} and Lc_{50} values of heavy metals

Sr. No.	Heavy metals	Time Of Exposure	Regression Equation $Y=\bar{Y}+B(X-X)$	Lc_{50} Val-ue (ppm)	Varia-Nce 'V'	X^2	Fiducial Limit (PPM)		Lethal Dose (PPM)	Safe Conc-Etration
							M_1	M_2		
	Lead acetate	24 HRS.	4.9054804 x(+4.7602726)	10.52	0.001662	0.156999	0.90141	1.06121	252.48	1.247
		48 HRS.	4.9054804 x (+3.7162278)	8.43	0.002732	0.084951	0.770041	0.974952	404.64	
		72 HRS.	4.9054804 x (+2.5855242)	6.26	0.005669	0.019805	0.569297	0.864436	450.72	

		96 HRS	4.9054804 x (+3.762278)	4.22	0.002732	0.084951	0.469011	0.673922	405.12	
	Zinc sulphate	24 HRS.	4.7460526 x (+2.5767973)	14.6	0.005972	0.453196	0.866422	1.169365	350.40	1.154
		48 HRS.	4.9054804 x (+3.7162278)	12.65	0.002732	0.084951	0.946132	1.151043	607.20	
		72 HRS.	4.9054804 x (+2.5855242)	9.38	0.005669	0.019805	0.745388	1.040527	675.36	
		96 HRS	4.9054804 x (+2.5855242)	6.26	0.005669	0.019805	0.569297	0.864436	600.96	
	Nickel chloride	24 HRS.	4.9054804 x (+9.7036221)	82.78	0.000398	0.034408	1.859548	1.937795	1986.72	1.197
		48 HRS.	4.7460526 x (+2.2507655)	69.15	0.007843	0.539762	1.497435	1.844587	3319.20	
		72 HRS.	4.7897398 x (+4.4462689)	44.86	0.001914	0.027927	1.497623	1.669117	3239.92	
		96 HRS	4.7460526 x (+2.5767973)	29.22	0.005972	0.453196	1.167457	1.470395	2805.12	

Table 2: Relative toxicity of heavy metals to *Rasbora daniconius*

Sr. No.	Name of heavy metal	Safe concen-tration	24 HRS.		48 HRS.		72 HRS.		96 HRS.	
			LC_{10}	LC_{50}	LC_{10}	LC_{50}	LC_{10}	LC_{50}	LC_{10}	LC_{50}
1.	Lead acetate	1.247	3.34	9.72	3.80	8.43	1.99	6.26	1.90	4.22
2.	Zinc sulphate	1.154	4.64	14.60	5.71	12.65	2.99	9.38	1.99	6.26
3.	Nickel chloride	1.197	61.07	82.78	18.63	69.15	23.09	44.86	9.29	29.22

The LC10 and LC50 values for heavy metals were summarized in table. The LC10 of lead acetate for 24,48, 72 and 96 hours were 3.34, 3.80, 1.99 and 1.90 ppm respectively. The LC10 of Zincsulphate for 24, 48, 72 and 96 hours were 6.64, 5.71, 2.99 and 1.99 respectively. The LC10 of nickel chloride for 24, 48, 72 and 96 hours were 61.07, 18.63, 23.09 and 9.29 ppm respectively.

Among all the LC10 values of lead acetate compared to zinc sulphate and nickel chlorie. The lead acetate is more toxic dividedly zinc sulphate and nickel chloride. *Rasbora daniconius* is more sensitive to these metals. Approximately the LC10 values for zinc sulphate are two times greater than lead acetate and 30 times greater than nickel chloride.

The LC50 of lead acetate for 24, 48, 72 and 96 hours were 9.72, 8.43, 6.26 ppm respectively. The LC50 of zinc sulphate for 24, 48, 72 and 96 hrs were 14.6, 12.65, 9.38 and 6.26 ppm respectively and LC50 of nickel chloride for 24,48, 72 and 96 hrs were 82.78, 69.15, 44.86 and 29.22 ppm respectively. The accuracy calculated for log LC50 value are cited in table. The head various ‘V’ of log LC50 for lead acetate 234, 48, 72 and 96 hrs 0.001662, 0.002732, 0.005669 and 0.002732 respectively. The variance value of log LC50 for zinc sulphate for 24,48, 72 and 96 hrs were 0.005972, 0.002732, 0.005669 and 0.005669 respectively. The variance value for log LC50 nickel chloride for 24, 48, 72 and 96 hrs were 0.00398, 0.00183, 0.001914 and 0.005972 respectively. Lethal doses for heavy metals are shoed in table. The order of toxicity in decreasing manner is lead acetate and zinc sulphate, lead acetate, nickel chloride.

Fiducial limit are summarized in table for lead acetate for 24, 48, 72 and 96 hrs. were 0.90141 to 1.06121, 0.770041 to 0.974952, 0.509297 to 0.864436 and 0.469011 to 0.673955 ppm respectively. For zinc sulphate for 24, 48, 72 and 96 hrs. where 0.866427 to 1.169365, 0.946132 to 1.151043, 0.745388 to 1.040527 and 0.569297 to 0.864436 ppm respectively. Fiducial limits for nickel chloride for 24, 48, 72 and 96 hrs. were 1.859548 to 1.937795, 1.497435 to 1.844587, 1.497623 to 1.66117 and 1.167452 to 1.470395 ppm respectively.

Safe concentration of heavy metals are 1.247, 1.147 and 1.197ppm respectively.

Lethal doses for heavy metals are in decreasing manner is lead acetate < zinc sulphate > nickel chloride.

Discussion

The quantitative study of pollutants in aquatic organisms offers an interesting challenge to the researchers. Heavy metals are well known environmental pollutants, they often persist, circulate and eventually accumulate throughout the food chain, thus cause a serious threat to non-target organisms. (Aktar and Mohun; 1995) [4]. Radhakrishnaiah (1988) [24] observed copper concentration in freshwater fish Labeo rohita. Legorburus *et al*., (1988) observed that fish from the more polluted places show higher metal levels. Jaffar *et al*., (1988) observed that, there is a positive correlation between the concentrations of zinc and arsenic in the fish muscle and in water. Tulsi *et al*., (1992) [29] showed significant accumulation of Lead in blood and tissue in the freshwater fish, Anabas testudineus. Lead bio-accumulation showed organ-specific distribution with high levels in blood followed by kidney, gill, liver and brain and comparatively lesser amounts in the ovary and muscle. Cuvin, (1994) [14] observed that, concentration of cadmium and mercury in Oreochromis niloticus increased with exposure period. Seymore *et al*., (1996) [26] studied bioaccumulation of chromium and nickel in selected tissues of freshwater fish Barbus marquensis. They observed that chromium and nickel accumulated highest in blood, followed by the bile and vertebrae, while skin accumulated the lowest amount. Have shown that the heavy metal concentrations were usually lowest in muscle and highest in the liver or the gills. Heavy metals entering aquatic systems are generally sequestered in bottom sediments. Mason *et al*., (2000) [9] observed that, concentration of heavy metals is more in detoxifying organs in fresh water in vertebrates and fish.

The recent decade has been applied to investigation on acute and sublethal toxicity of heavy metal interaction and their biochemical and physiological impact on indicator species such as fishes. The model fish species used for this type of investigation are rainbow trout, Onchorhynchus mykiss and freshwater fish Tilapia nilotica**.** It has been demonstrated that heavy metal get accumulated in various tissues of the T. nilotica observed that, freshwater fish C. mrigala and C. carpio when exposed to acute concentrations of cadmium and lead, there was more accumulation of heavy metals in liver followed by gill and muscle.

The metal interactions not only influence the acute toxicity but also alter the bioaccumulation patterns of these metals in the exposed fishes.

The effects of model combination of seven heavy metals (Cu, Zn, Ni, Cr, Pb, Cd and Mn) on the rainbow trout Onchorhynchus mykiss at all stages of development were investigated by Vosyliene *et al*., (2003). According to parameters the fish larvae show higher sensitivity to the model metal combination compared to embryos and adult fish. The maximum toxic effect of model combination was observed during hatching period.

The ecotoxicological effect expressed as mortality of four metal ions (Cd, Cu, Zn, Al) and their associations on the star larvae of C.plumosus was determined by Fargasova (2001). The effect of individual metals was introduced as acute toxicological effect and expressed as LC_{50} values. On the basis of LC_{50} values the toxicity of metals after 96 hrs treatments was ranked as Cu > Cd > Zn > Al. Similar trend of toxicity was also observed in the present investigation. Among these three metal, Lead proved to be more toxic then zinc and nickel. The toxicity in combination was either increased for decreased because of antagonism or synergisms. Further the study reveled that when in metal pairs in which the original metal is at low concentration shows increased toxicity in combination is the effect of synergism for example in the present investigation the combination of zinc and nickel increased toxicity due to synergism. Similarly, lead showed synergism with zinc and antagonism with nickel. The present investigation reports unique kind of effect such as antagonism and synergism of metal toxicity for the freshwater fish species R. daniconius. On the other hand, in the present investigation toxicity evaluation of heavy metals lead acetate, zinc sulphate, nickel chloride, lead acetate and zinc sulphate, lead acetate and nickel chloride, zinc sulphate and nickel chloride, and lead acetate and zinc sulphate and nickel chloride was conducted on the freshwater fish R. daniconius and LC50 values were calculated. The LC50 values for 96 hrs exposures were 4.22, 6.26, 29.22, 3.68, 16.20, 30.13 and 25.21 ppm respectively R. daniconius to metal combination clearly exhibited, antagonism and synergism. lead acetate and zinc sulphate show synergism, as toxicity of combination was more where as the model combination of lead, zinc and nickel showed antagonism, as toxicity was decreased. The nickel had antagonism for both that is lead and zinc. This type of data is very scanty in case of the freshwater fishes where as the toxicity studies performed on non-fish species are in agreement with our findings. Similarly in the pattern of sublethal toxicity due to metal combination has been reported incase of juvenile rainbow trout. Onchorhynchus mykiss after single and combined exposure to metal and other pollutants by Ait -Aissas al., (2003) [1]. Impact of metal interaction on accumulation and elimination of heavy metals have also been documented (Allen, 1995; Kargin and Cogun 1999; Cicik *et al*., 2004) [5, 7]. These showed either antagonistic or synergistic effects of metal combination on the freshwater fishes.

Use of freshwater fishes as bioindicator of metal pollution have already been proposed. Rashed (2001a, 2001b) studied (Co, Cu, Cr, Ca, Fe, Mn, Ni, Sr, Pb, Cd and Zn) in different tissues of fish, T. nitotica from Nassar Lake to assess both the water pollution with the metals and lethal levels of these metals in the fish. The author has demonstrated that the metal levels in the fish increased with the increased levels of metals in the lake water. Other studies for using fish as biomarker for water pollution was conducted that metal concentrates in the fish tissue increased with increased metal concentration in the aquatic ecosystems. Kalfakakon and Akrida reported that Ca, Mg, Fe, Cu, Zn, and Pb exhibited bioaccumulation from water to fish. They demonstrated that metal concentration in fish are higher than in water which indicates the bioaccumulation of metals in the fishes and therefore freshwater fishes can be developed as the bioindicators of metal pollutions of aquatic ecosystems. The present investigation also proposes use of freshwater fish R. daniconius species as a model indicator to assess the heavy metal pollution status of the aquatic ecosystems.

The safe concentration for R. daniconius to the heavy metals were lead acetate, zinc sulphate, nickel chloride, lead acetate and zinc sulphate, lead acetate and nickel chloride, zinc sulphate and nickel chloride, and lead acetate and zinc sulphate and nickel chloride were 1.247, 1.154, 1.197, 1.14, 1.306, 1.182 and 1.165, ppm. From these results is clear that lead acetate more toxic either individual or combined form for R. daniconius in the present study.

In the present study the fish showed characteristic change in behavior when transferred to experimental chambers having different metal. The fishes survived rapidly in the experimental media and were trying to jump out of water at short intervals. Later the fishes exhibited restlessness by erratic opercular movement, difficulty in respiration, convulsions and short erratic jerky movements, which is in apparent with the studies of Lohar *et al*., (2000) [21] and Mubarak Begam, (1998) [22]. The fishes in experimental chambers showed mucous secretion to avoid toxic environment. Where as in preset study no such behavioral changes were noticed in the control fish, which remained active and healthy throughout the experimental period. The fast swimming activity may be due to the irritating effect of the exposed heavy metal where as the excessive secretion is a kind of avoidance by the fish. The safe concentration for *Rasbora daniconius* to the heavy metals were lead acetate, zinc sulphate, nickel chloride were 1.247, 1.154 and 1.197 ppm. From these results is clear that lead acetate more toxic for *Rasbora daniconius* in the present study.

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