Int. J. Aquat. Biol. (2014) 2(3): 155-163
E-ISSN: 2322-5270; P-ISSN: 2383-0956
Journal homepage: www.ij-aquaticbiology.com

Original Article

Acute toxicity of alkali and alkaline earth metals on Rohu, *Labeo rohita* (Hamilton) egg and hatchlings

Anusaya Mallick*[1, 2], Bikash Chandra Mohapatra[2], Niranjan Sarangi[2]

[1]Department of Environmental Science, University of Kalyani, Kalyani, West Bengal -741235, India.
[2]Central Institute of Freshwater Aquaculture, (Indian Council of Agricultural Research), Bhubaneswar- 751 002, Orissa, India.

Abstract: The acute toxicity of salts of alkali and alkaline earth metals, such as sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) were studied on the egg and larval stages of Indian major carp *Labeo rohita* (Hamilton). The acute toxicity experiments were conducted followed by the range finding bioassay tests. The experiments were conducted in triplicates. The cumulative percentage of dead or damaged eggs at the end of 6, 12, 18, 24, 36, 48, 60, 72 and 96 hours was recorded for the calculation of LC_{50}. The increase in salt concentrations in water increased their toxicity and reduced the duration to damage 50% of the eggs. The eggs became smaller than their normal size and whitish before being damaged in the test solutions. Most of the exposed eggs and hatchlings tended to lay on the floor of the tank. The toxicity of the metals was in the order of K>Na>Mg>Ca. The 96 hours LC_{50} values were 3.25, 2.73, 28.9 and 20.52 ppm for sodium, potassium, calcium and magnesium, respectively.

Article history:
Received 14 November 2013
Accepted 18 April 2014
Available online 25 June 2014

Keywords:
Alkali and alkaline earth metals
Bioassay
LC_{50}
Rohu

Introduction

Indian major carps (IMC) are the most important groups of fishes cultured in Indian subcontinent. Rohu (*Labeo rohita*) belongs to the family cyprinidae and is found commonly in rivers and freshwater lakes, mainly in South-East Asia (Gupta et al., 1997). In order to bridge the gap between the ever-increasing demand and supply of the seed of the species, induced breeding plays a major role. Alkali and alkaline earth metals affect fish breeding, water hardening of eggs, growth and survival of hatchlings (Mallick et al., 2010).

The embryological stages of an organism have important considerations, when examining the effect of heavy metals and concentration of these in the body tissues. It can vary with the age or size of the organism (Bennett and Dooley, 1982; Newman and Mitz, 1988). The health of fish may be affected, either directly through uptake from the water or indirectly through their diet of vegetation, invertebrates or smaller fish (Kime et al., 1996). Metals released into aquatic ecosystems are responsible for several fish physiological irregularities (Sehgal and Saxena, 1986). These can also disturb the ion regulatory mechanism in aquatic organisms (Hansen et al., 1996). In an aquatic environment, metal toxicity can be influenced by various abiotic factors such as oxygen, calcium/water hardness (Skidmore, 1964; Cairns and Mount, 1990; Ghillebaert et al., 1995), pH and temperature (Cairns and Mount, 1990; Kotze et al., 1999). According to Rose et al. (1993) abiotic factors are defined as variables that exert a direct effect on individuals in the population.

The sodium level in water bodies is quite variable. Wide ranges of seasonal fluctuations of sodium in freshwater bodies have been reported by Khan and Siddqui (1974), Goel et al. (1986) and Khatavkar et al. (1990). High concentrations of sodium chloride have strong local effects in fish (Metelev et al.,

* Corresponding author: Anusaya Mallick
E-mail address: anusaya.cifa@gmail.com

1983). Potassium is a naturally occurring element, which remains in lesser concentration than calcium, magnesium and sodium in freshwater. The potassium level in aquatic habitats is quite variable from 0.2-35.0 ppm. Mohan and Zafar (1986) demonstrated that the potassium is fast accumulated with the passage of time and doubling up in every fifteen years in the aquatic habitat. According to Metelev et al. (1983) and Mohapatra (1999) the symptoms of poisoning due to potassium compounds are analogous to those of poisoning with sodium compounds. Calcium is a very important cation for the animal body because not only takes part in the structural formation, but also in the various metabolic and physiological activities in the body. The increase in environmental calcium rises the tolerance of aquatic animals to ammonia toxicity (Tomasso et al., 1980). Calcium is a primary structural component of hard tissues such as bones, exoskeleton, scales and teeth of aquatic organisms. Freshwater fish obtain magnesium ions through active uptake from the environment or from dietary sources. Fish eggs contain a significant amount of magnesium associating with the yolk (Hayes et al., 1946). Therefore, the yolk may serve as a magnesium source for the developing embryo during the early developmental stage. Magnesium is essential for cellular respiration and neuromuscular transmission (Lall, 1989).

Egg hatchability and hatching time are more sensitive indicators of toxicity than “standard” end point, like mortality and growth (Pyle et al., 2002). Except one report of Mohapatra (1999) on the toxicity studies on fingerlings of *Catla catla*, no work has been done on the determination of tolerance limits of alkali and alkaline earth metals on egg and larvae of Indian major carps viz., *Catla catla*, *Labeo rohita* and *Cirrihinus mrigala*. Therefore, the present study was planned to investigate the comparative sensitivity of rohu egg and hatchlings to alkali and alkaline earth metals through toxicity tests.

Materials and methods

Test containers: The glass jar tanks with 20 L capacity were used as test containers after with laboratory detergents, then with 100% acetone and tap water. After each test, the containers were washed appropriately with acid to remove metals, bases and organic compounds. Each of the test containers was provided with facilities of continuous aeration.

Test solutions: The test solutions were prepared by dissolving the calculated amount of salts, such as sodium chloride, potassium chloride, magnesium chloride and calcium chloride as per the experiments in the dilution water of the jars. The solutions were prepared immediately prior to the initiation of the experiments. De-chlorinated tap water was used as dilution water for control tests and for making concentrations of the test substances. The dilution water was clean, uncontaminated and of constant quality.

Test concentrations: Selection of test concentrations was made as per the protocol of APHA (1998). The concentrations of the metals ware expressed as parts per million (ppm). The test organisms were exposed to a range of salt concentrations in logarithmic scale, such as 0.0001, 0.001, 0.01, 0.1, 1.0, 10, 100, 1000 and 10,000 ppm. Based on the results of the range finding bioassay, the concentrations 0.5-10,000; 0.05-1000; 1-10,000 and 1-5000 ppm for the salts of sodium, potassium, magnesium and calcium, respectively were selected as the test concentrations for definite bioassay experiment. To avoid contamination, the controls were maintained little away from bioassay tanks.

Bioassay of egg: The rohu eggs of appropriate quality were selected for the experiment. Eggs were received from CIFA carp hatchery at Bhubaneswar and were transferred to the experimental tanks with test media on arrival at laboratory, and the containers were calibrated to determine the approximate number of eggs for experimentation. The eggs were measured volumetrically before release into the test containers including control tanks. The percentage of dead/ damaged eggs at the end of every 6, 12, 18, 24, 48, 72 and 96 hours were recorded and tabulated. To avoid contamination, the dead eggs were removed

Table 1. Percentage death of eggs or hatchlings of rohu exposed to different concentrations of sodium chloride (NaCl) salt solution

Conc. (ppm)	% death of eggs and hatchlings of rohu in different exposure time (hours)								
	6	12	18	24	36	48	60	72	96
0.5	30	35	40	40	40	40	40	40	40
1	45	50	50	50	50	50	50	50	50
10	50	50	50	50	55	55	55	55	55
100	50	50	50	50	55	55	60	70	70
1000	60	75	80	90	100	100	100	100	100
10000	65	85	100	100	100	100	100	100	100
Control	10	15	15	20	20	25	30	35	40

Table 2. Percentage death of eggs or hatchlings of rohu exposed to different concentrations of potassium chloride (KCl) salt solution

Conc. (ppm)	% death of eggs and hatchlings of rohu in different exposure time (hours)								
	6	12	18	24	36	48	60	72	96
0.05	20	25	30	30	45	45	45	45	50
0.1	20	30	35	45	45	45	45	45	45
1	30	30	40	50	50	50	50	50	50
10	35	45	50	50	60	60	65	65	75
100	40	55	70	90	100	100	100	100	100
1000	45	60	80	100	100	100	100	100	100
Control	10	20	20	30	30	30	30	30	35

Table 3. Percentage death of eggs or hatchlings of rohu exposed to different concentrations of magnesium chloride ($MgCl_2$) salt solution

Conc. (ppm)	% death of eggs and hatchlings of rohu in different exposure time (hours)								
	6	12	18	24	36	48	60	72	96
1	30	35	40	40	45	45	45	45	45
10	40	40	45	50	50	50	50	50	50
100	45	45	50	50	50	60	60	60	65
1000	50	50	55	70	90	100	100	100	100
10000	50	55	65	100	100	100	100	100	100
Control	20	20	20	30	30	30	40	40	40

Table 4. Percentage death of eggs or hatchlings of rohu exposed to different concentrations of calcium chloride ($CaCl_2$) salt solution

Conc. (ppm)	% death of eggs and hatchlings of rohu in different exposure time (hours)								
	6	12	18	24	36	48	60	72	96
1	30	35	45	40	40	40	40	40	40
10	40	45	50	50	50	50	50	50	50
100	40	45	50	50	60	60	65	65	70
1000	50	60	80	90	100	100	100	100	100
5000	55	70	80	100	100	100	100	100	100
Control	20	20	20	20	20	30	40	40	40

from the experimental tanks immediately.

Data analysis: The data obtained from the experiments were processed by probit analysis (Finney, 1971; Reish and Oshida, 1987; Mohapatra and Rengarajan, 1995) for determination of LC_{50} values in computer using SPSS software. The lethal concentrations were plotted against time in hours to get "Toxicity curve"(Seegertet al., 1979).

Determination of toxicity of elementary form of metals: Grams of compound containing 1.0 g element= Molecular weight of compound/Molecular weight of element i.e., 1.0 g of NaCl, KCl, $CaCl_2$ $2H_2O$ and $MgCl_2$ $6H_2O$ contain 0.39, 0.524, 0.273 and 0.12 g of Na^+, K^+, Ca^{++} and Mg^{++}, respectively.

Results

Range finding bioassay: In range finding bioassay a wide range of concentrations i.e. 0.0001, 0.001, 0.01, 0.1, 1.0, 10, 100, 1000 and 10,000 ppm were selected for each salt solution. No mortality was observed in

Table 5. LC_{50} values of salts of alkali and alkaline earth metals on eggs and hatchlings of rohu, *Labeo rohita*

Exposure Period (hours)	**LC_{50} (ppm)[a]**			
	NaCl	**KCl**	**$MgCl_2$**	**$CaCl_2$**
6	5561	1170	9122.5	3743.8
12	2471.1	560.6	6890.1	2326.4
18	345.7	188.8	4413.8	1075.1
24	353.1	17	387.8	256.1
36	93.2	7.2	193.4	64.5
48	21.6	6	58.8	59.1
60	13.9	6	38.8	45.4
72	4.1	4.6	36.2	42.7
96	3.3	2.7	20.5	28.9

[a] After 18 hours of exposure the eggs hatched to hatchlings

Table 6. LC_{50} values of elementary forms of alkali and alkaline earth metals on eggs and hatchlings of rohu, *Labeo rohita*

Exposure Period (hours)	**LC_{50} (ppm)**			
	Na^+	K^+	Mg^{++}	Ca^{++}
6	2168.8	613.1	1094.7	1022.1
12	963.7	293.7	826.8	635.1
18	134.8	98.9	529.6	293.5
24	137.7	8.9	46.5	69.9
36	36.3	3.8	23.2	17.6
48	8.4	3.2	7.1	16.13
60	5.4	3.2	4.6	12.4
72	1.6	2.4	4.3	11.6
96	1.3	1.4	2.5	7.9

0.0001-0.01 ppm for all the tested salts i.e. sodium chloride, potassium chloride, calcium chloride and magnesium chloride, but 75% mortality occurred at 10,000 ppm in case of salts of sodium and magnesium, 1000 ppm for potassium and 5000 ppm for calcium in 96 hour of exposure. Hence, the concentrations of 0.5, 1, 10, 100, 1000 and 10,000; 0.05, 0.1, 1, 10, 100 and 1000; 1, 10, 100, 1000 and 10,000; and 1, 10, 100, 1000 and 5000 ppm were selected for the salts of sodium, potassium, magnesium and calcium, respectively for conducting definitive bioassay.

Bioassay results: The percentage of damaged or dead eggs was not similar in all experimental concentrations. In higher concentrations, the percentage of damaged eggs was high. Egg became smaller, whitish just before death and coelomic content turned opaque or white. Most of the exposed eggs and hatchlings after death were settled on the aquarium floor. The cumulative percentage of dead or damaged eggs; or hatchlings after hatching out from eggs at 6, 12, 18, 24, 36, 48, 60, 72 and 96 hours of exposure was recorded for the calculation of LC_{50} (Tables 1-4). The results of toxicity studies expressed in terms of LC_{50} values obtained from probit analysis for different salts are given in Table 5. The median lethal concentration (LC_{50}) decreased gradually with the increase in exposure time from 6 to 96 hours. The rank order of toxicity of metal salts for rohu egg and hatchlings was found to be potassium chloride > sodium chloride > magnesium chloride > calcium chloride.

The toxicity of inorganic solids such as sodium, potassium, calcium and magnesium were calculated from the results of bioassay studies with their salts and presented in table 6. Based on 96 h LC_{50} values, potassium was found to be more toxic to rohu larval stages and the least was the calcium. The toxicity order was K>Na>Mg>Ca (Table 6).

Toxicity curve: The toxicity curves were obtained for different salts by plotting the log time against the log LC_{50} values (Fig. 1A-D). The toxicity curve may not pass through all the LC_{50} points on the graph at all times. It gives the overall picture of the progress

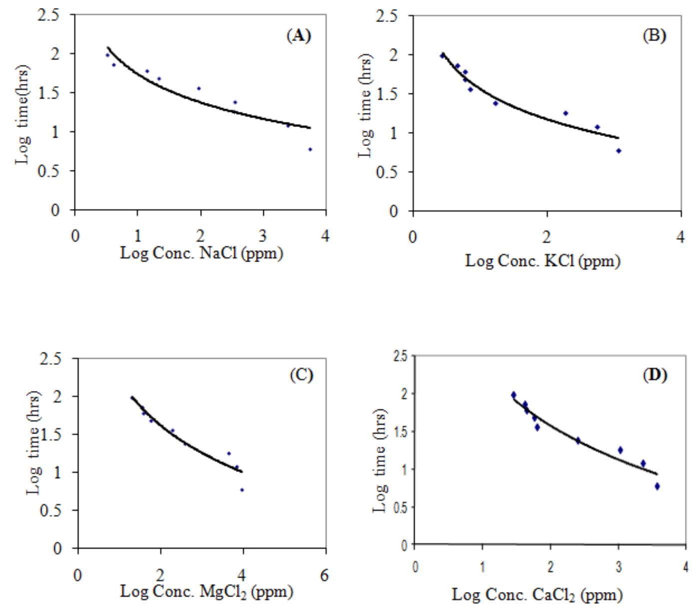

Figure 1. Toxicity curve for rohu eggs hatchlings exposed to different lethal concentrations of NaCl, KCl, $MgCl_2$, and $CaCl_2$ up to 96 hours.

Table 7. LC_{50} values of elementary forms of alkali and alkaline earth metals on eggs and hatchlings of rohu, *Labeo rohita*

Salts	Species	Value (ppm)	Toxicity	Source
NaCl	Roach and Tench	10,000-11,000	Not toxic within 24 hrs	Metelev et al.,1983
		13,000	Mortality after 1 day	
	Carp (100-150g)	15,000	Toxic	
	Trout (fry and fingerlings)	10,000	Non-toxic for several hrs	
	Catla catla	15,600	6 hr LC_{50}	Mohapatra, 1999
		13,700	12 hr LC_{50}	
		10,900	24 hr LC_{50}	
		10,800	48 hr LC_{50}	
		10,100	72 hr LC_{50}	
		9,000	96 hr LC_{50}	
	Sarotherodon mossambicus	31,300	24 hr LC_{50}	
		30,300	48 hr LC_{50}	
		29,700	72 hr LC_{50}	
		27,600	96 hr LC_{50}	
	Labeo rohita (Egg)	5561	6 hr LC_{50}	Present study
		2472.1	12 hr LC_{50}	
	Labeo rohita (Hatchling)	345.7	18 hr LC_{50}	
		353.1	24 hr LC_{50}	
		93.2	36 hr LC_{50}	
		21.6	48 hr LC_{50}	
		13.9	60 hr LC_{50}	
		4.1	72 hr LC_{50}	
		3.3	96 hr LC_{50}	
KCl	Perch and white salmon	10,000	Toxic after 18 hrs	Metelev et al.,1983
	Fish	1000	Toxic	
		500	Non toxic	
	Emerald shiner, Feathead minnow, Golden shiner, White bass, Carp	10,000	No mortality up to 24 hrs	Snyder et al., 1991
	Catla catla	3350	12 hr LC_{50}	Mohapatra, 1999
		1830	24 hr LC_{50}	
		1270	48 hr LC_{50}	
		800	72 hr LC_{50}	
		3400	96 hr LC_{50}	

Table 7. Continued.

	Sarotherodon mossambicus	3,400	12 hr LC_{50}	
		1,750	24 hr LC_{50}	
		920	48 hr LC_{50}	
		900	72 hr LC_{50}	
		810	96 hr LC_{50}	
	Labeo rohita (Egg)	1170	6 hr LC_{50}	Present study
		560.6	12 hr LC_{50}	
	Labeo rohita (Hatchling)	188.8	18 hr LC_{50}	
		17	24 hr LC_{50}	
		7.2	36 hr LC_{50}	
		6	48 hr LC_{50}	
		6	60 hr LC_{50}	
		4.6	72 hr LC_{50}	
		2.7	96 hr LC_{50}	
$MgCl_2$ $6H_2O$	Three-spined stickle back	2,300	Can withstand up to 6 months without damage	Metelev et al.,1983
	Perch and White salmon	10,000	Can tolerate up to 24 hours	
	Carp (100g)	6000	Can tolerate for 3-4 days without damage	
	Brown trout fry	4900	Can live up to 8 days	
	Barbel	10,000	Can withstand for 4 weeks	
		15,000	A percentage die after 5 days	
		20,000	Die after 1hrs	
	Carp	1000-15,000	Can tolerate for 4 weeks	
		20,000	A percentage die after 5 days	
		30,000	Die after 4 hours	
	Eel	10,000	Live for 4 weeks	
		15-20,000	Die after 14 days	
	Catla catla	18,500	12 hr LC_{50}	Mohapatra, 1999
		18,300	24 hr LC_{50}	
		18,200	48 hr LC_{50}	
		18,000	72 hr LC_{50}	
		17,900	96 hr LC_{50}	
	Sarotherodon mossambicus	38,000	12 hr LC_{50}	
		36,500	24 hr LC_{50}	
		36,100	48 hr LC_{50}	
		35,600	72 hr LC_{50}	
		35,100	96 hr LC_{50}	
	Labeo rohita (Egg)	9122.5	6 hr LC_{50}	Present study
		6890.1	12 hr LC_{50}	
	Labeo rohita (Hatchling)	4413.8	18 hr LC_{50}	
		387.8	24 hr LC_{50}	
		193.4	36 hr LC_{50}	
		58.8	48 hr LC_{50}	
		38.8	60 hr LC_{50}	
		36.2	72 hr LC_{50}	
		20.5	96 hr LC_{50}	
$CaCl_2$ $6H_2O$	White Salmon, carp and perch	10,000	Toxic after 16-29 hours	Metelev et al.,1983
	Juvenile brown trout	13,900	Toxic after 10 days	
	Carp, rainbow trout and barbel	5,000	Not toxic in 4 weeks	
	Fishes	15,000	Toxic in 1 hr to several days	
	Eel	27,000	Can tolerate	
	Catla catla	7,500	12 hr LC_{50}	Mohapatra, 1999
		6,350	24 hr LC_{50}	
		4,950	48 hr LC_{50}	
		4,100	72 hr LC_{50}	
		3,950	96 hr LC_{50}	

Table 7. Continued.

Sarotherodon mossambicus	24,400	12 hr LC_{50}	
	23,000	24 hr LC_{50}	
	21,800	48 hr LC_{50}	
	21,700	72 hr LC_{50}	
	21,400	96 hr LC_{50}	
Labeo rohita (Egg)	3743.8	6 hr LC_{50}	Present study
	2326.4	12 hr LC_{50}	
Labeo rohita (Hatchling)	1075.1	18 hr LC_{50}	
	256.1	24 hr LC_{50}	
	64.5	36 hr LC_{50}	
	59.1	48 hr LC_{50}	
	45.4	60 hr LC_{50}	
	42.7	72 hr LC_{50}	
	28.9	96 hr LC_{50}	

of the test and also indicates when acute lethality has stopped. With longer exposure times, the curve tends to be parallel to the time axis.

Discussion

Acute toxicity test is necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals. Bioassay is necessary to determine the concentration of a toxicant, which may be allowed in receiving waters without adverse effects on the living resources (Standing Committee of Analysts, 1981; Ward and Parrish, 1982; Reish and Oshida, 1987). Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety (Ward and Parrish, 1982; Mohapatra and Saha, 2000).

The median lethal concentration (LC_{50}) of the metals sodium, potassium, calcium and magnesium on rohu eggs and hatchlings decreased gradually with the increase in exposure time from 6 to 96 h. The toxicities of the salts were seen in the order of potassium chloride > sodium chloride > magnesium chloride > calcium chloride. Potassium was found more toxic and calcium was the least. These toxicity values cannot be are compared directly with the available results of other workers, but, a comparative statement is given in Table 7.

Because of eggs and larval stages, the toxicity values in the present study for rohu were found less than the values reported by Mohapatra (1999). Metelev et al. (1983) reviewed that symptoms of magnesium poisoning in fish are similar to those with sodium salts. In fish (Crusian carp) the first indication is sluggish eye movement and subsequently they turn on their sides. In the present experiment the damaged eggs and hatchlings before death remained on the floor of the tanks.

Acknowledgements

We are thankful to the Director, Central Institute of Freshwater Aquaculture, Bhubaneswar, Odisha, India for providing facilities for research work.

Reference

APHA. (1998). Standard methods for the examination of water and wastewater, 16th ed., American Public Health Association, Water Pollution Central Federation and American Water Works Association, New York, USA.

Bennett R.O., Dooley J.K. (1982). Copper uptake by two sympatric species of killifish *Fundulus heteroclitus* (L.) and *L. majalis* (Walkbaum). Journal of Fish Biology, 21: 381-398.

Cairns J. (Jr)., Mount D.I. (1990). Aquatic toxicology. Environmental Science and Technology, 24 (2): 154-160.

Finney D.J. (1971). Probit Analysis. Univ. Press, Cambridge, 333 p.

Ghillebaert F., Chaillou C., Deschamps F., Roubaud P. (1995). Toxic effects at three pH levels of two reference molecules on common carp embryo. Ecotoxicology and Environmental Safety, 32: 19-28.

Goel H., Kohli G.S., Lai H. (1986). Serum phosphohexose isomerase levels in patients with head and neck cancer. Journal of Laryngology and Otology, 100: 581-585.

Gupta M.V., Dey M. M., Dunham R., Bimbao G. (1997). Proceedings of the collaborative research and training on genetic improvement of carp species in Asia. Bhubaneswar, India. ICLARM Work, Doc. No. 1.

Hayes F.R., Darcy D.A., Sullivan C.M. (1946). Changes in the inorganic constituents of developing salmon eggs. Journal of Biological Chemistry, 163: 621-631.

Hansen H.J.M., Olsen A.G., Rosenkilde P. (1996). The effect of Cu on gill and esophagus lipid metabolism in the rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology, 113 (C) 1: 23-29.

Khan A.A., Siddqui A.Q. (1974). Seasonal change in the limnology of a perennial fish pond at Aligarh. Indian Journal of Fisheries, 21: 463-478

Khatavkar S.D., Kulkarni A.Y., Goel P.K. (1990). Phytoplankton flora of some freshwater bodies in South-western Maharashtra. Environment and Ecology, 8: 267-275.

Kime D.E., Ebrahimi M., Nysten K., Roelants I., Rurangwa E., Moore H.D.M., Ollevier F. (1996). Use of computer assisted sperm analysis (CASA) for monitoring the effects of pollution on sperm quality of fish, application to the effects of heavy metals. Aquatic Toxicology, 36: 223-237.

Kotze P.J., Dupreez H.H., Van Vuren J.H.J. (1999). Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus* from the Olifants River, Mpumalanga, South Africa. Water SA, 25 (1): 99-110.

Lall S.P. (1989). The minerals. In: Fish Nutrition (Ed.) J. H., Halver, Academic Press, London-San Diego-California, pp: 220-252.

Mallick A., Mohapatra B.C., Sarangi N. (2010). Effect of alkali and alkaline earth metals on embryonic stages of rohu, *Labeo rohita* (Ham.) In: A. Sinha, S. Datta & B.K. Mahapatra (Eds.). Diversification of Aquaculture, Narendra Publishers, New Delhi, pp: 229-240.

Metelev V.V., Kanaev A.I., Dzasokhova N.G. (1983). Water Toxicology. Amerind Publishing Co. pvt. Ltd., New Delhi, pp: 216.

Mohan K.S., Zafar A.R. (1986). Water quality of two drinking water reservoirs of Hyderabad (India). Pollution Research, 5: 111-122.

Mohapatra B.C., Rengarajan K. (1995). A manual on bioassays in the laboratory and their techniques. CMFRI, 64:1-75.

Mohapatra B.C. (1999). Purna saline tract, Maharastra state: Assessment of ground water quality through fish bioassays. D.Sc. Thesis, Berhampur Univ., Berhampur, Orissa. 101 p.

Mohapatra B.C., Saha C. (2000). Aquatic pollution and management. Central Institute of Freshwater Aquaculture, Bhubaneswar, pp: 1-363.

Newman M.C., Mitz S.V. (1988). Size dependence of zinc elimination and uptake from water by mosquito fish, *Gambusia affinis* (Baird and Girard). Aquatic Toxicology, 12: 17-32.

Pyle G.B., Wanson S.M., Lehmkuhl D. M. (2002). Toxicity of uranium mine receiving waters to early life stage fathead minnows (*Pimaphales promelas*) in the laboratory. Environmental Pollution, 16: 243-255.

Reish D.L., Oshida P.S. (1987). Manual of methods in aquatic environment research. Part 10. Short-term Static Bioassay. FAO Fish. Technical paper, 247: 1-62.

Rose K.A., Cowan J.H., (Jr)., Houde E.D., Coutant C.C. (1993). Individual based modeling of environmental quality effects on early life stages of fishes: A case study using striped bass. American Fisheries Society Symposium, 14: 125-145.

Seegert G.L., Brooks A.S., Castle I.R.V., Gradall K. (1979). The effects of monochloramine on selected riverine fishes. Transactions of the American Fisheries Society, 108: 88-96.

Sehgal R., Saxena A.B. (1986). Toxicity of zinc to a viviparous fish *Lebistes reticulatus* (Peters). Environmental Contamination and Toxicology, 36: 888-894.

Snyder F.L., Fisher S.W., Schneider B. (1991). Evaluation of potassium chloride for removal of zebra mussel veligers from commercial fish shipments. Journal of shellfish research, 11 (1): 238-239.

Standing Committee of Analysts (1981). Acute toxicity testing with aquatic orgamisms. Methods for the Examination of Waters and Associated Materials. Her majesty's Stationery Office, London.

Skidmore J.F. (1964). Toxicity of zinc compounds to aquatic animals, with special reference to fish. The Quarterly Review of Biology, 39 (3): 227-247.

Tomasso J.R., Goudie C.A., Simco B.A., Davis K.B. (1980). Effects of environmental pH and calcium on ammonia toxicity in channel catfish. Transactions of the American Fisheries Society, 109: 229-234.

Vander Merwe M., Van Vuren J.H.J., Du Preez H.H. (1993). Lethal copper concentration levels for *Clarias gariepinus* (Burchell, 1822) - a preliminary Study. Koedoe, 36 (2): 77-86.

Ward G.S., Parrish P.R. (1982). Manual of methods in aquatic environmental research. Part 6. Toxicity Tests. FAO Fisheries Technical Paper, No 185 FIRI/T185.