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Toxicity of Zinc to a Viviparous Fish, *Lebistes reticulatus* (Peters)

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Water pollution episodes have focused the attention of many scientists and also of public on the environmental problems. Water pollution not only affects the health and welfare of the people but also damages vegetation and properties. Heavy metals discharged by number of industries added into the aquatic ecosystem cause several irregularities in fish physiology (Doudoroff and Katz 1950; Sanglang and Halloran 1974; Sehgal et al. 1984). Information on the toxicity of heavy metals to fish has been summarised by some workers (Spehar et al. 1980; Saxena and Parashri 1983) Factors influencing the toxicity of zinc compounds and their mode of action on fish as well as other animals have been reviewed and discussed by Skidmore (1964). Still the toxic limits of heavy metals to fishes appear to be very limited. The purpose of this investigation was to examine the acute toxicity of zinc to L. reticulatus and sublethal toxicity to gonads of L. reticulatus after a long term exposure (20 days) to zinc.

MATERIALS AND METHODS

All bioassays were conducted with adult L. reticulatus (length 2.0 ± 0.51 cm; weight 70 ± 2.78 mg) obtained from Mahakal pond in the vicinity of Ujjain, India. The fishes were held in large aquaria for acclimatization to the laboratory conditions. The fish were fed with macerated prawn powder each day but not fed for two days prior to testing nor during the course of the toxicity tests. Acute toxicity tests have been done for the heavy metal, zinc sulphate. After 48-h acclimatization to the laboratory conditions static 96-h LC_{50} and LC_{100} bioassays were carried out on both male and female fish. Sdatic bioassays were performed by exposing various concentrations of zinc sulphate. The 96-h LC_{50} its 95% confidence limits and slope function of line were evaluated as per method recommended by Litchfield and Wilcoxan (1949). Log probability plot of per cent mortalities versus concentrations was analysed. The zinc from diluent water was removed prior to these tests. The specimens exposed to diluent water were taken as control.

The water was analysed at 24-h interval to determine the dissolved oxygen, biological oxygen demand, chemical oxygen demand, total hardness, alkalinity, free carbondioxide, chloride, pH and temperature as recommended in standard methods (APHA, AWWA and WPCF, 1975). The physicochemical analysis of control and test water was carried out as entered in Table 1.

Table 1. Physicochemical data of the water

Parameters	Control water Mean±SE	After treatment with zinc sulphate Mean±SE
Dissolved oxygen (mg/L	6.7±1.3	5.8±1.2
Biological oxygen demand (mg/L)	5.4±1.1	3.6±1.0
Chemical oxygen demand (mg/L)	15±1.9	26±1.5
Total hardness (mg/L)	134±2.6	118±2.3
Total alkalinity (mg/L)	90±1.4	85±1.2
Phenolphthalein alkalinity (mg/L)	9.1±1.5	5.5±1.1
Chloride (mg/L)	19.2±1.7	19.2±1.6
Free carbon dioxide(mg/L)	0	0
pH	8.4±1.2	8.3±1.1
Temperature °C	22±1.8	22±1.8

The long term response of zinc to gonads of *Lebistes reticulatus* were also carried out. Male and female fishes were exposed to 300 and 278 mg/L concentrations of zinc for twenty days respectively. Test solutions were prepared from stock solution. In each case static bioassays were employed. The fishes were sacrificed on 21st day. Gonads were dissected out, weighed and fixed in aquous Bouin's fluid. Serial paraffin sections were cut sagittaly and stained with Delafield's haematoxylin and eosin stains. The Gonosomatic index (GSI) were calculated (Pickford (1953). The number of cysts containing different stages of oogenesis and spermatogenesis were counted in sections of gonads in treated and control fishes. Statistical comparisons were done by Student 't' test.

RESULTS AND DISCUSSION

The result of water analysis are given in Table 1. Total hardness of the water was high and the range was 118-134 mg/L. The high alkalinity was also observed with zinc. Das (1978) opines that high alkalinity indicates pollution. Mount (1966) reported the effect of total hardness and pH on the acute toxicity of zinc to selected fresh water fishes. They agreed that pH 7.5 and high value of total hardness, reduce the mortality rate in the solution of zinc sulphate. But the investigations made by Holcombe et al. (1978) with acute toxicity of zinc to rainbow and brook trout were due to increase in the pH with increasing hardness and alkalinity. Similar observations were also made in respect of zinc sulphate.

The 96-h LC 25, 50, 75 bioassays, slope function and confidence limits for female and male fishes are shown in Table 2.

Table 2. Bioassay LC 25,50,75 value slope functions and 95% confidence limits of fish *Lebistes reticulatus* (Peters) for zinc sulphate

Fish *Labistes reticulatus* male/female	No. of fishes	96-h LC mg/L			Slope function, 95% confidence limits (mg/L)
		LC 25	LC 50	LC 75	
Male	20	240	300	375	1.37(272.1-332.4)
Female	20	212	278	325	1.30(251-30-326.4)

No mortality was recorded in control fish for 96-h.

The male and female fishes exposed to the sublethal concentration of zinc sulphate for 20 days exhibited profound changes in gonads. In the control ovaries the oocytes were found dispersed along the wall of cavity and were comparatively small in size. Younger germ cells such as oogonia and oocytes were small with little cytoplasm, large nucleus and nucleoli. The immature oocytes were larger than oogonia. The maturing oocytes exhibit yolk vesicles while the mature oocytes were in yolk accumulation phase. The female fish exposed to sublethal concentration (278 mg/L) of zinc displayed the typical histological characters of increased percentage of atretic oocytes, reduction in percentage of mature oocytes, pyknotic nuclei, numerous small vacuoles in cytoplasm. No apparent alteration was indicated in control fish during the experimental periods. The GSI after twenty days treatment decreased significantly (Table 3). The percentage of different oogenetic states in control and treated fishes are given in Table 4.

Table 3. Gonosomatic indices of female and male *Labistes reticulatus* (Peters) after 20 days treatment of zinc sulphate

Groups	No.of fish	Body weight (mg) Before zinc exposure Mean±SE	After zinc exposure Mean±SE	Weight of gonads (mg) Mean±SE	Gonosomatic indices (GSI) Mean±SE
Females (control group)	10	68.1±0.95	68.5±0.95	6.2±1.31	9.19±7.12
Females (Sublethal zinc treated)	10	68.0±0.12	60.1±0.13	4.6±1.23	7.62±0.01*
Males (Control group)	10	68.5±1.22	61.3±1.21	6.3±1.25	4.21±1.40
Males (Sub-lethal zinc treated)	10	68.0±1.22	61.2±1.02	4.2±1.20	6.81±1.02*

* $p \angle 0.001$

The testis of control fish looked almost spherical in structure. It was packed with numerous acini bounded by peritonial membrane. The seminiferous tubules were absent. Each cyst represented a particular stage of spermatogenesis viz. spermatogonia, spermatocytes, spermatids, spermcysts and spermatophores.

Table 4. Percentage of different oogenetic stages in *Lebistes reticulatus* (Peters) after twenty days treatment of zinc sulphate

Oogenetic stages	Control fishes Mean±SE	Sublethal zinc treated fishes Mean±SE
Oogonia	8.1±1.23	12.0±0.32*
Immature oocytes	24.0±1.70	45.0±1.37*
Maturing oocytes	30.0±0.52	11.0±0.34*
Mature oocytes	33.0±0.92	11.0±0.20*
Atretic oocytes	5.0±1.12	21.0±0.40*

* $p < 0.001$

The fish treated with sublethal concentration (300 mg/L) of zinc sulphate for 20 days exhibited drastic changes in the testis. The elaborate vacuolization was observed in spermatocytes. The cysts of spermatogonia, spermatocytes, spermatids and sperms exhibited significant reduction in their counts (percentage). A remarkable increase in the percentage of atretic spermatophores population was observed in all exposed fishes (Table 5)

Table 5. Percentage of different spermatogenetic cysts in *Lebistes reticulatus* (Peters) after twenty days treatment of zinc sulphate

Spermatogenetic stages	Control fish Mean ± SE	Sublethal zinc treated fishes Mean ± SE
Spermatogonias	10.2 ± 2.13	8 ± 2.01
Spermatocytes	43.0 ± 1.21	37 ± 1.50
Spermatids	15.0 ± 1.60	11 ± 1.40*
Spermatophores	25.0 ± 1.50	16 ± 1.25*
Atretic spermatophores	7.0 ± 1.24	31 ± 3.1*

* $p < 0.001$

Sublethal exposure of zinc to *Lebistes reticulatus* inhibited the gonadal response in both female and male fishes. Some scientists reported that the pollutants disturb the normal histology and function of reproductive organs in fishes (Sehgal *et al.* 1984;

Saxena and Garg 1978). The results of the present investigations on the treatment with zinc were found to be the same as reported by Saxena and Garg, 1978. The histological abnormalities, low percentage of mature oocytes and high percentage of atretic oocytes were found in treated group. Sehgal et al. (1984) reported the comparative effects of two heavy metallic salts on the testis of *Lebistes reticulatus* and noted that both pollutants produced deleterious effects on the spermatogenesis. The present investigations are in good agreement with the above referred report. The GSI decreased significantly in both female and male fishes which reflect the lowered gonadal activity in zinc exposed fishes. The effect of heavy metal pollution on fish testis was further determined (Ahsan and Ahsan 1974; Sangalang and O Halloran 1974). With this knowledge we can say that pollutants like heavy metals cause deleterious effects on fish.

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REFERENCES

WPCF (1975) Standard methods for the analysis of water, sewage and industrial wastes. American Public Health Association Inc, Newyork

Ahsan SN, Ahsan J (1974) Degenerative changes in the testis of *Clarias batrachus* (Linn) caused by cadmium chloride. Indian J Zool 15 39-43

Das SM (1978) High pollution in lake Nainital UP as evidenced by biological indicators Sci & Cult 44 236-237

Doudoroff P, Katz M (1950) critical review of literature on the toxicity of industrial wastes and their component to fish I Alkalies Acids and Inorganic Gases Sewage. Industr Wastes 22 1432-58

Holcombe Carry W, Rober W Andrew (1978) The acute toxicity of zinc to rainbow trout and brook trout comparisons in hard and soft water US environment protection agency DuluthMN Publication EPA-600/3-25 78-98

Litchfield JT, Wilcoxan F (1949) A simple method of evaluating dose effect experiments. J Pharm exp Ther 96 99-113

Mount DI (1966) The effect of total hardness and pH on acute toxicity of zinc to fish. Int J Air Wat Poll 10 49-56

Pickford GE (1953) A study of hypophysectomized males Kill Fish F. heteroclitus L.Bull Binham Ocenog Collect 14 5-41
Sangalang GB, Halloran MJO (1974) Adverse effects of cadmium on brook trout testis and on in vitro testicular endrogen biosynthesis. Biol Report 9 374-403
Saxena PK, Garg M (1978) Effect of insecticidal Pollution on ovarian recrudesence in the fresh water teleosts C. punctatus (Bloch). Indian J exp Biol 16 690-691
Saxena OP, Parashari A (1983) Comparative study of the toxicity of six heavy metals to Channa punctatus. J Environ Biol 4 91-94
Sehgal R, Tomar V, Pandey AK (1984) Comparative effects of two heavy metallic salts on the testis of viviparous teleost Lebistes reticulatus (Peters). J Environ Biol 5 195-192
Skidmore JF (1964) Toxicity of zinc compounds to aquatic animals with special reference to fish. Quart Rev Biol 39 227-247
Spehar RL, Carlson RW, Lemke AE, Mount DI, Pickering QH, Snarski VM (1980) Effect of pollution on fresh water fish. J Wat Poll Cont Fede 52 1703-1768

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