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Potential Use of the Freshwater Teleost, *Labeo rohita* (Hamilton, 1882) as a Bio-indicator of Zinc Toxicity

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ABSTRACT- Fish occupying the topmost niche in aquatic food chain has always been proved to be successful bio-indicators. The present study is focused on the effective use of *L. rohita*, an economically significant carp as a bio-indicator of zinc pollution through its several physiological, histopathological biomarkers. Primarily, acute toxicity test is performed in which the carp fingerlings are exposed to different concentrations (10, 20, 40, 80, 160, 320 ppm) of zinc sulphate. 96 hour LC_{50} value is determined to be 100 ppm. It is taken as lethal concentration and the fishes are exposed to it for a period of 96 hours during which wide range of behavioural abnormalities are evidenced like general hyperactivity, surfacing activity, hyper-opercular activity, and erratic swimming pattern. It is followed by loss of balance and convolutions. One fifth of the lethal concentration is taken (i.e., 10 ppm) as sub-lethal concentration and fishes are exposed to it for a period of 15 days during which growth, behaviour, oxygen consumption, histopathology, hematology and genotoxicity are studied. Negative growth performance is observed with insignificant length increment up to 0.24 % and significant weight reduction up to -2.38%. Wide range of behavioural abnormalities are evidenced which includes, erratic swimming, hyperactivity, surfacing activity and depression in appetite. Besides, general body discolouration and haemorrhage are observed as well. Rate of oxygen consumption showed a time dependant decrease which ranged up to -49.10%. Gills of the fishes are shown to have conspicuous histopathological alterations like lamellar necrosis, lamellar fusion, lamellar erosion, epithelial lifting and epithelial swelling.

Key-Words- Bioindicator, *L. rohita*, Zinc sulphate, Growth, Behaviour, Oxygen Consumption, Histopathology

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INTRODUCTION

Bioindicators are organisms that contain information on the quantitative aspects of quality of the environment. In the context of environmental monitoring studies, bioindicators reflect organisms that contain information on the quality of the environment. [1] considered the "bioindicative source of information" one of the pillars of modern environmental monitoring, since "bioindication is the breakdown of the information content of biosystems, making it possible to evaluate whole areas". Bioindication not only focus on the concentration and effects of contaminants in the environment and particularly in the organisms living in the environment [2].

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In the last 20 years, bioindicators have shown themselves to be particularly interesting and intelligent measuring systems.

An "ideal" indicator at least should have the characteristics as follows: (a) taxonomic soundness; (b) wide or cosmopolitan distribution; (c) low mobility (local indication); (d) well-known ecological characteristics; (e) Numerical abundance; (f) suitability for laboratory experiments; (g) high sensitivity to environmental stressor; (h) high ability for quantification and standardization [3].

The important characteristics of fish that makes it an ideal bioindicators can be summarized as follows:

1. Diverse class of vertebrates having around 28,000 species outnumbering other vertebrates [4].
2. Diverse body forms, lifestyles and habitat from freshwater to marine.
3. Bioaccumulate toxic substances and respond to low concentrations of environmental pollutants and mutagens.
4. Biochemical stress responses are quite similar to those found in mammals [5].

5. Located at the end of the aquatic food chain [6].

Fish around the world are found occupying almost any aquatic habitat. In particular, freshwater fish are severely threatened as the freshwater ecosystems are considered the most endangered of the world [7]. The importance of freshwater fish in ecotoxicology is a direct consequence of their importance in ecological and economic terms. Freshwater fish culture contributes the bulk of production derived from Indian aquaculture. The contribution of freshwater aquaculture to the total fish production in India has risen steadily from 17% a decade back to over 30% at present.

Freshwater fish culture is primarily comprised of Indian major carps (Catla, Rohu and Mrigal), with the secondary species including exotic Chinese carps. Among major carps, *Labeo rohita* (rohu) is the most popular freshwater fish species cultivated in Indian subcontinent. It occupies an outstanding position as the chief cultured species in aquaculture practices in India [8]. Also, it is the most important among the three Indian major carp species used in carp polyculture systems. It is highly delicious and prestigious fish species among other Indian major carps with good market demand [9]. Hence, it is chosen as the experimental fish. Numerous characteristics such as sensitivity to changes in any physico-chemical parameters of the water body, tendency to bioaccumulate xenobiotics discharged into water bodies etc. add to their value as a prime model in toxicological tests [10].

Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems, non-biodegradability, propensity of bio-magnification in food chain and their effects on the ecological equilibrium of the recipient aquatic body and diversity of aquatic organisms [11].

The world-wide emission of metals to the atmosphere (thousands of tons per year) by natural sources is estimated as: Ni: 26, Pb: 19, Cu: 19, As: 7.8, Zn: 4, Cd: 1.0, Se: 0.4 ($tx103.yr^{1}$). Whereas, from anthropogenic sources: Pb: 450, Zn: 320, Ni: 47, Cu: 56, As: 24, Cd: 7.5, Se: 1.1 (thousand t yr^{-1}). It is obvious from these numbers that Pb, Zn, Ni and Cu are the most important metal pollutants from human activities [12]. Currently, there are no guidelines on acceptable levels of Cu and Zn in the edible parts of fish suggested by EEC or FAO/ WHO [13].

Among heavy metals, zinc has an extensive industrial use in alloys, galvanizing, pigments and electrical equipments. Zinc is involved in animal growth and widely used metal cofactor of enzymes involved in protein, nucleic acid, carbohydrate and lipid metabolism that support life [14]. It is also added to the ponds as micronutrient for increasing in production of planktons and fish. Also, it is an essential trace element for organisms and plays a vital role in the physiology of living system but in higher concentrations can be toxic to organisms.

Its potential adverse effects in fishes ranges from interference with growth, reproduction, ATP production and mitochondrial electron-transport activity, osmoregulatory failure, pancreatic, gill or immunity damage, and/or behavior abnormalities .This is due to disturbance in acid-base balance, ion regulation, disruption of gill tissues and hypoxia in fish [15]. In the present study, zinc sulphate is used as the toxicant because, heavy metal salts contribute to a very serious type of pollution in fresh water because they are stable compounds and are not readily removed by oxidation, precipitation or other means and affect the activity of the animal .Also, its ionic form, Zn^{2+} is considered to be most toxic to organisms [16].

In the present work acute toxicity test will be carried out using various concentrations as LC_{50} is the biological index of 50% mortality in an exposed population. The 96-hour LC_{50} tests are conducted to measure the susceptibility and mortality potential of biota to particular toxic substances [17].

Studying fish growth under chronic exposure of toxicant is another yardstick to determining the stress caused by the water bone toxicant as it is considered as a reliable and sensitive indicator endpoint relating to chronic exposure of waterborne or dietary individual metals and their mixtures .The most effective indications of toxic pollution are the behavioral changes. It provides a unique perspective linking the physiology and ecology of an organism and its environment [18]. Oxygen consumption is widely considered to be a critical factor for evaluating the physiological response and useful variable for an early warning for monitoring aquatic organisms. Hence quantifying oxygen consumption in fishes under metallic stress can indicate the pollution status of the aquatic environment.

Histopathological alterations in the fish gills have been used in biomonitoring the effects of various pollutants in the aquatic environment [19]. The gills are used for histopathological studies as they are a multifunctional and complex organ with which fish make intimate contact with the surrounding water. It is well known that the gills contribute to the respiration, osmoregulation and excretion in fish. However, due to their close contact with the external environment, these are particularly sensitive to the changes in water quality. Thus histopathological studies of gills can be useful in indicating wide range of effects of pollutants on the organism.

MATERIALS AND METHODS

Experimental setup

The fingerlings of *Labeo rohita* are procured from Tamil Nadu fish farm, Thiruvallur district. They are treated with 0.1% $KMnO_4$ for dermal disinfection. The identification of the rohu fingerlings is re-confirmed following the diagnostic characters outlined by [9]. Only healthy fishes of uniform size (Length: 8.5 ± 0.5cms, Wt. 4.5± 1.50gms) are selected and acclimatized for 15 days in separate plastic troughs each containing definite number of fishes. The aquaria are thoroughly cleaned before filling with

dechlorinated water for keeping the experimental fish. During this period, fishes are fed with oil free groundnut cakes at 2 per cent of the body weight. The settled faecal matter and unutilized food particles are siphoned out from the aquaria each day using a plastic tube.

The toxicants used for this work is zinc sulphate ($ZnSO_4$, $7H_2O$). Acclimated fish are not fed 24-hr before the start of the tests. Care is taken to keep the mortality rate of fish not more than 5% in the last four days before the experiment was started. Water quality parameters (temperature, dissolved oxygen (DO), and pH) are periodically determined before the bioassay tests following A.P.H.A [20]. The water temperature is kept at 24 ± 10°C. Also the experimental medium is aerated in order to keep the amount of oxygen not less than 4 mg/l.

Determination of LC_{50}

Stock solution of zinc sulphate is prepared by dissolving appropriate amount $ZnSO_4$ as Zn salt in distilled water. The working concentrations are prepared from this standard stock. The fish are exposed to Zn ($ZnSO_4$) to know the acute toxicity at 24, 48, 72 and 96 hrs. During acute toxicity tests, fishes are exposed to wide range of toxicant concentrations such as 10, 20, 40, 80, 160 and 320 ppm in a static water system for 96-hr. All experiments are carried out for a period of 96 hours. No food is given to the fish during the experimental period. Ten fingerlings are introduced in each trough containing 10 liters of water with required amount of toxicant. In order to avoid the sudden stress to fish, the concentrations of metals in aquariums are increased gradually, 50% test concentration being reached in three and half hours and full toxicant concentration in seven hours. The number of dead fish are counted every 12 hours and removed from the aquaria as soon as possible. The screening test is continued to assess the concentration at which all the fingerlings survived for 96 hrs and likewise the concentration at which most of the fishes died simultaneously. The mortality rate is determined at the end of the 96^{th} hour.

The 96^{th} hour LC_{50} value was determined by adopting the straight line graphical interpolation method [21]. During the experimental period the control and toxicant exposed fishes are kept under constant observation to study behavioral abnormalities. The behavioral changes of the fish exposed to the toxicant are photographed and evaluated as regard to behavioral anomalies. The behavioural changes in each fishes are calculated as frequency and the decrease or increase in frequency is evaluated and recorded.

Chronic Toxicity Test

One tenth of the LC_{50} (10 ppm) is selected as sublethal concentration and ten fishes are introduced in each test group. The control and zinc sulphate exposed fishes are kept under continuous observation for 20 days and during this period various parameters such as length/weight differences, behavioural changes, oxygen consumption, histopathological changes in gill, and qualitative haematological and micronucleus test are carried out at the interval of 10 days (i.e., 1^{st} day, 10^{th} day & 20^{th} day). This is done by sacrificing the fishes taken from control and test group at the end of each day and experimentations are carried out.

Fish Growth

The standard length and total weight of the surviving organisms in the test and the control are evaluated with a high precision scale and gage, respectively. Growth parameters including percentage increase or decrease in standard length and total weight are studied during chronic exposure of fishes to the sublethal concentration of the toxicant at 1^{st} day, 10^{th} day and 20^{th} day respectively.

Percentage increase in length and weight is calculated using the formula:

$$\frac{B-A}{A} \times 100$$

Where, A= Initial Mean Length/Weight, B= Final Mean Length/Weight

The percentage changes in length and weight are evaluated and recorded.

Behavioural Studies

Behavioural parameters are observed in the control group and the test group exposed to sublethal concentrations of the toxicant during the experimental period (1^{st} day, 10^{th} day and 20^{th} day). The behavioural changes in each fishes are calculated as frequency and the decrease or increase in frequency is evaluated and recorded.

Oxygen consumption

Rate of oxygen consumption is measured for sublethal concentrations by following the method of [22]. For determining oxygen consumption, dissolved oxygen is first measured following Winklers Iodometric method. Each experiment is done in triplicate and the mean value is calculated and recorded.

Histopathology

Histopathological studies of gills of the control and toxicant exposed fishes are carried out during experimental period. The fishes from both the control and experimental group are dissected. After dissecting the fish, gills are removed and fixed in 4% formalin solution for 24 hr. The tissue are routinely dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax. Sections of 4-6 µm thick are cut, processed and stained with heamatoxylin and eosin (H&E) following [23]. They are examined under compound light microscope, Dewinter to discern their general architecture and histological details. Photomicrographs are taken using Image analysis software, Capture Pro.

RESULTS

Acute toxicity & 96 hours LC_{50}

The mortality rate of *L. rohita* fingerlings exposed to different concentrations of $ZnSO_4$ is shown in Table 1. It is evident that the mortality rate showed a gradual increase with the increase in the concentration of $ZnSO_4$ and duration of exposure. No death of fingerlings is observed in the control group. The percentage mortality of *L. rohita* is 10 %, 20 %, 30 %, 40 %, 80 % and 100 % at the end of 96 hours exposure to $ZnSO_4$ concentrations of 10 ppm, 20 ppm, 40 ppm, 80 ppm, 160 ppm and 320 ppm respectively (Table 1).

96 hours LC_{50} is calculated to be 100 ppm following the graphical method. 10 ppm i.e., $1/10^{th}$ of the 96 hour LC_{50} is taken as the sublethal concentration for further studies.

Table 1: Mortality record of *Labeo rohita* fingerlings exposed to $ZnSO_4$ for 96 hours

$ZnSO_4$ conc. (ppm)	No. of fingerlings	No. of dead at the end of 96 hours	% Mortality
10	10	1	10
20	10	2	20
40	10	3	30
80	10	4	40
160	10	8	80
320	10	10	100

Fish growth

Chronic exposure of sublethal concentration (10 ppm) of Zinc sulphate to *L. rohita* affecting fish growth parameters such as mean length, mean weight and their percentage changes are recorded (Table 2 & 3).

Fishes in the control showed significant increase in length from 2.42% to 6.77% during experimental period i.e. 1^{st} day to 20^{th} day. Their weight increased from 1.10% to 2.21%. The experimental fishes showed significant variations in length and weight growth in comparison to that of control. In experimental fishes, there is an insignificant increase in length upto 0.23% and their weight lost exponentially from -0.47% to -2.38%.

Fishes in the control group showed normal feeding behaviour in contrast to the toxicant exposed fishes where there is reduced food intake. Hence, weight increment is observed in the control fishes and weight reduction is evidenced in the experimental fishes. The stress created in the fish by the heavy metal is believed to have influenced its feeding behaviour resulting in weight reduction. Thus in present study it is observed that under chronic exposure to sub-lethal concentration of the toxicant, there is a stunted growth in length and drastic fall in weight, which can be attributed to the toxic effect of zinc sulphate.

Table 2: Growth Performance of *L. rohita* during chronic exposure to sublethal concentration of $ZnSO_4$

Estimation	Control			10 ppm		
Average Mean Length (cm)	8.26	8.46	8.82	8.64	8.64	8.66
±SD	0.2408	0.1816	0.1303	0.5727	0.5727	0.6066
±SE	0.1077	0.0812	0.0583	0.2561	0.2561	0.2712
Change (%)	-	2.42	6.77	-	0	0.23

Table 3: Growth Performance of *L. rohita* during chronic exposure to sublethal concentration of $ZnSO_4$

Estimations	Control			10 ppm		
Average Mean Weight (gm)	4.52	4.57	4.62	4.19	4.17	4.09
±SD	0.8435	0.8672	0.8698	0.5345	0.5430	0.5793
±SE	0.3772	0.3878	0.3889	0.2390	0.2428	0.2591
% Change	-	1.10	2.21	-	-0.47	-2.38

Behavioural Changes

Behavioural changes of the fishes are monitored every 24 hours up to 96 hours during acute exposure to lethal concentration i.e., 100 ppm. Similarly the behavioural changes are monitored at 1st, 10th and 20th day during chronic exposure to the sublethal concentration i.e., 10 ppm. Its behavioural parameters are quantified by numbering the occurrence of such behaviours in different fishes and recorded as increase or decrease (Table 4 & 5). The control fishes in both the acute and chronic toxicity test behaved normally without significant disturbances. But, experimental fishes have exhibited wide range of behavioural abnormalities which reflects the animal's defensive response to the toxic environment.

Table 4: Behavioural Responses of *L. rohita* during acute exposure to lethal concentration (LC_{50} -100 ppm) of $ZnSO_4$

Behavioural abnormalities	Control	24 hrs	48 hrs	72 hrs	96 hrs
Hyperactivity	-	++	++	+++	-
Erratic swimming	-	+	++	+++	-
Opercular beat frequency	-	++	++	+++	++++
Surfacing activity	-	+++	++	+	-
Loss of balance	-	-	-	+	++
Convolutions	-	-	-	-	+

The increase or decrease in the frequency of behavioural parameters is shown by numbers of (+) sign. And the (-) sign indicate normal behavioural conditions

Table 5: Behavioural Responses of *L. rohita* during chronic exposure to sublethal concentration of $ZnSO_4$

Behavioural abnormalities	Control	10 ppm		
		I day	X day	XX day
Hyperactivity	-	++++	+++	-
Erratic swimming	-	++++	++	-
Opercular beat frequency	-	+	+++	++++
Surfacing activity	-	++	++++	++
Lethargy	-	-	++	++++
Depression in appetite	-	-	++	+++

The increase or decrease in the level of behavioural parameters is shown by numbers of (+) sign.
The (-) sign indicate normal behavioural conditions.

During acute exposure to the toxicant, hyperactivity and erratic swimming behaviour steadily increased from 24 to 72 hours. Also, frequency of opercular beat steadily increased from 24 to 96 hours. Surfacing activity decreased exponentially from 24 to 72 hours. Remarkably at 96 hours, no sort of abnormalities like hyperactivity, erratic swimming or surfacing activity are observed. Loss of balance is noticed from 72 hours and finally convolution is observed with decreased frequency at 96 hours which is a sign of imminent death (Fig. 1).

Fig 1: *L. rohita* exhibiting loss of balance as an ethological response after 72 hrs exposure to Lethal concentration of Zinc Sulphate

During chronic exposure to the toxicant, hyperactivity and erratic swimming are observed with highest frequency at the 1st day of exposure which later declined during 10th day. Opercular beat frequency, Lethargy and depression in appetite increased considerably during later period i.e., 10th and 20th day. Surfacing initially increased at 1st and 10th day but, later declined at 20th day. Hyperactivity and erratic swimming are not at all observed at 20th day. Food intake reduced remarkably resulting in considerable loss of weight affecting the normal growth of the fish. This can be attributed to the depression in appetite evidenced in the fishes. Besides, reduced body pigmentation and hemorrhage near gills are observed as well (Fig. 2).

Fig. 2: *L. rohita* showing hemorrhage around the gills and general dis-colouration of the body during chronic exposure to sublethal concentration of $ZnSO_4$

Oxygen Consumption

The rate of oxygen consumption by the fingerlings of *L. rohita* in relation to the chronic exposure to sublethal concentration i.e., 10 ppm with their percentage changes is shown in Table 6. In the control group of fishes, constant rate of oxygen consumption is maintained throughout the experimental period. But the test fishes showed wide range of differences in rate of oxygen consumption from that of control. A steady decline in the rate of oxygen consumption is evidenced in test fishes compared to that of control. During 1st day of exposure, the rate of oxygen consumption declined by -18.94%. During 10thday of exposure, it is further decreased by -28.18%. The maximum decline in rate of oxygen consumption by -49.10 % is observed during 20th day of exposure (Table 6).

Table 6: Oxygen consumption (ml/g/L/hr) of *L. rohita* following exposure to sublethal concentration (5 ppm) of $ZnSO_4$

Estimations	Control	10 ppm		
		Exposure period in days		
		I	X	XX
Oxygen consumption	0.3875	0.3141	0.2783	0.1972
± SD	0.0127	0.0411	0.0151	0.0151
±SE	0.0073	0.0237	0.0087	0.0087
% Change	-	-18.94	-28.18	-49.10

Values are the mean of triplicate observations

Histopathology

Histopathological alterations are seen in the experimental fishes when compared to the control. It is evidenced that in the control, gills are in normal architecture with the secondary gill lamella appearing as finger-like structures which is thin, slender and attached on either side of the primary gill lamellae.

The gills usually possess double rows of filaments or primary lamellae from which arise perpendicularly the secondary lamellae.The primary gill lamella is lined by a thick stratified epithelium that contains numerous mucous and chloride cells responsible for excessive mucus secretion. Chloride and mucus cells are present between secondary lamellae. The secondary gill lamellae are highly vascularised and surrounded by a thin layer of epithelial cell. It consists of respiratory epithelial cells, pillar cells situated between blood capillaries. Chloride cells are located at the base of two adjacent lamellae (Fig. 3 & 4).

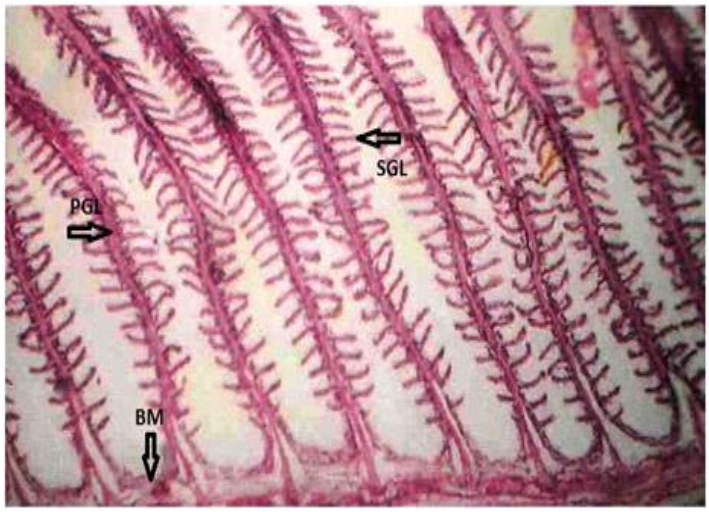

Fig.3: Photomicrograph of L.S of gills in control fish showing: B- Basement Membrane; P-Primary Gill Lamella; S-Secondary Gill

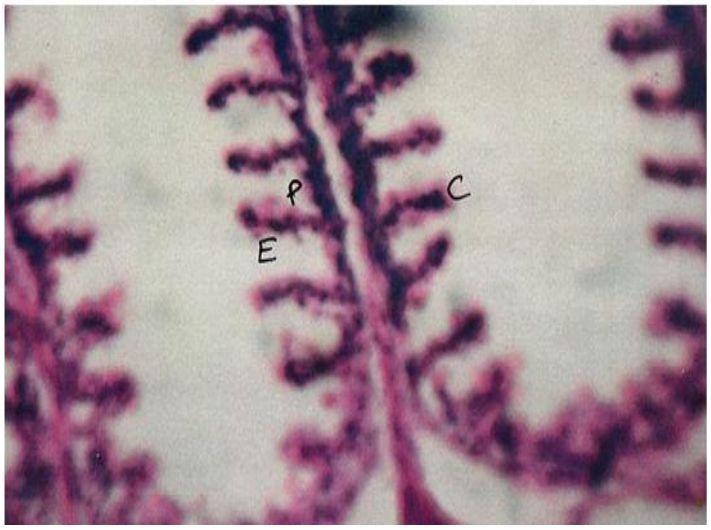

Fig. 4: Photomicrograph of L.S of gills in control fish showing various cells. E-Epithelial cell; C-Chloride cell, and P-Pillar cell

Histological observation of gills of treated fishes showed degenerative changes when compared with that of control. A number of histopathological alterations indicate the toxic potential of zinc sulphate to the tissues of gills. At initial period of exposure, i.e., 10th day, the experimental fishes exposed to 10 ppm showed slight degenerations like, fusion of secondary gill lamellae and erosion of secondary gill lamellae. During later period i.e., they showed remarkable changes like epithelial swelling, lamellar necrosis and epithelial lifting (Fig. 5 & 6).

Fig. 5: Photomicrograph of L.S of gills in $ZnSO_4$ exposed fish showing various abnormalities. N-Necrosis in Lamellae; EL-Epithelial Lifting; SGE-Swelling of Gill epithelium (H & E, x400)

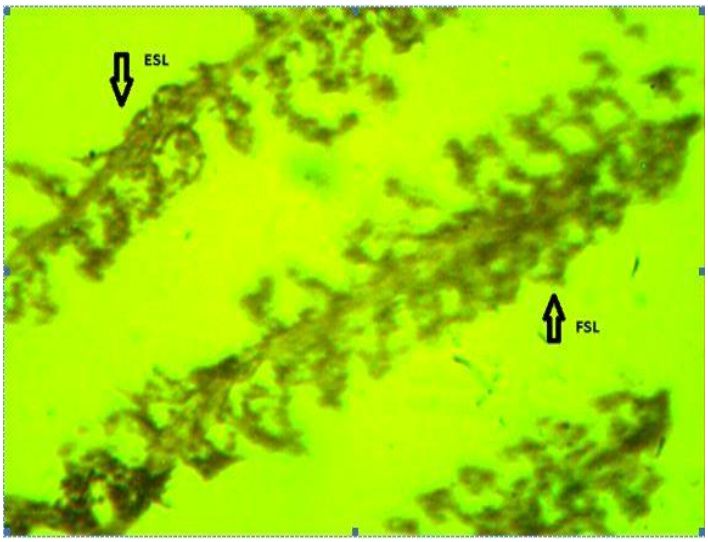

Fig. 6: Photomicrograph of L.S of gills in $ZnSO_4$ exposed fish showing various abnormalities. F-Fusion of Secondary Gill Lamellae; ESL-Erosion of Secondary gill lamellae (H & E, x400)

DISCUSSION

Fishes are the successful bioindicators that can be used to monitor the health of an aquatic ecosystem. They have proved to be of significance as bioindicators of the so-called ecological integrity [24]. Fishes are known to exhibit such wide range of such biological responses which can be quantified through various approaches by following studies.

- ✓ Toxicology
- ✓ Physiology
- ✓ Histopathology

In the present work, acute toxicity tests are conducted. Also, numerous studies are undertaken such as growth performance, ethology, oxygen consumption, histopathology, hematology and micronucleus assay during chronic exposure to sublethal concentration of the toxicant to establish the potentiality of the fish species in indicating the metallic pollution of the aquatic ecosystem.

The LC_{50} value of 100 ppm found in the current study is significantly lower than the values reported by several authors [25-27]. These varying interpretations can be attributed to physico-chemical parameters of water such as pH, temperature, water hardness, dissolved oxygen and alkalinity [28]. Stunted growth evidenced in this study is in agreement with earlier works. [29-30] observed similar growth reduction in guppies under chronic exposure of zinc. [31] also reported decrease in growth performance of *Cirrhinus mrigala* under chronic exposure of zinc. Under optimum conditions, at appropriate temperature and at sufficient quantities of food, the fish increase in both body length and mass.

Behavioural anomalies like erratic swimming, loss of balance and hyperactivity observed in present study are similar to those reported by [32,33] during acute exposure of zinc cyanide and sodium cyanide to *C. mrigala* and *L.*

rohita respectively. Similar behavioural changes are manifested in *Clarias batrachus* exposed to zinc sulphate in another relevant study [34].

The treated fishes exhibited wide behavioural anomalies under sublethal concentration of zinc. The control fishes exhibited normal swimming pattern but the toxicant exposed fishes exhibited irregular, erratic and darting swimming movements and loss of equilibrium which is due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyperstimulation [35]. The increased opercular movements in the initial period of exposure may be to support enhanced physiological activities in stressful habitat and later decreased, possibly due to accumulation of mucus over the gill filaments.

Surfacing phenomenon i.e., significant preference of upper layers in exposed group may be a demand of higher oxygen level during the exposure period [36]. Surfacing activity is seen decreasing in toxicant exposed fishes indicating physiological incapability in procuring definite proportion of its oxygen requirement from the atmosphere [37]. Increased gill opercular movements observed initially may possibly compensate the increased physiological activities under stressful conditions [38].

Acute exposure of zinc cyanide and copper cyanide to *C. mrigala* and *C. catla* respectively exhibited gradual decline in oxygen consumption as reported in the present work [32,33]. [41] reported initial decrease and subsequent increase in oxygen consumption in *L. rohita* exposed to zinc sulphate which is in contrast with the present observations. The altered rate of oxygen consumption observed in the present study may be due to the disruption of respiratory process caused by damage of gill epithelium [40].

The histopathological changes observed in this work are similar to those reported in earlier works. Swelling of gill epithelium, fusion of secondary gill lamellae and lamellar necrosis observed in the present work are also evidenced in gills of *L. rohita* exposed to sublethal concentration of endosulfan [41]. Lamellar fusion and lamellar erosion observed in the present investigation are similarly reported in another work carried out by [42]. Epithelial lifting seen in the present work is observed as well in *L. rohita* exposed to textile mill effluent [43]. The results showed that the response to stress induced by zinc caused considerable histological alterations in the gills of *L. rohita*. Therefore, the evidence of pathological alterations in the gills of *L. rohita* appears to be a useful bio-marker of pollutant exposure and its effects on freshwater fish.

CONCLUSIONS

The present study confirms that zinc sulphate in high concentrations has manifold effects on *L. rohita* fingerlings affecting growth parameters, respiratory physiology and gill structure, ethology, haematological parameters and it is observed to possess genotoxic potential which thereby could cause deleterious effects on its survival. Changes in the behavior, physiology, hematology, growth parameters and gentoxic response of *L. rohita* hence, could be taken as biological indicators of water quality and thereby a tool for bio-monitoring. Zinc is an essential trace element required for different physiological functions and plays an important role in cellular metabolism. However, it becomes toxic when elevated concentrations are introduced into the aquatic environment due to anthropogenic factors. It is hence essential that sustainable, eco-friendly aquacultural practices along with abatement of aquatic pollution would help in conservation of fish diversity and maintain of water quality.

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