Research Paper

Behavioural and Morphological Manifestations in *Labeo Rohita* (Hamilton-Buchanan) Under the Exposure of Lead Nitrate.

Zoology

KEYWORDS : Lead Nitrate, *Labeo rohita*, Behavioural Responses, Morphological Anomalies.

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ABSTRACT

This paper emphasises on the behavioural responses and morphological anomalies observed in the freshwater fish, Labeo rohita on being exposed to Lead Nitrate. The prominent changes in the behaviour observed were restlessness, swimming impairments, jerky movements, loss of equilibrium, drowning, hitting against the wall of test aquaria, increased and fast opercular movements, rapid surfacing and gulping of air and severe diarrhoea. Further, shedding of scales, fusion of fins, lesions on skin, eye deformities, muscular tetany, caudal bending/scoliosis, dullness in the body colour along with profuse mucus secretion were the morphological anomalies observed. Fishes in sub-lethal concentration were found to be under stress, no death was recorded. Control fishes were also monitored to record the normal behaviour. The study was for 60 days. The observations were recorded after 15, 30, 45 and 60 days of exposure.

Introduction

The pollution of rivers and streams with heavy metal contaminants has become one of the most critical environmental problems of the present day. Heavy metals add up into the aquatic ecosystems as the result of atmospheric deposition, leaching of minerals, soil erosion and anthropogenic activities and pose a high risk of life to aquatic organisms due to their persistent and toxic nature. These heavy metals can induce genetic disorders and physiological and behavioural alterations if not death to the exposed organisms. The detrimental effect of these pollutants on aquatic environments is incontestable (Livingstone *et al.*, 2001; Matsumoto *et al.*, 2006).

Behavioural changes are the physiological responses shown by the animal which are sensitive indicator of chemically induced stress in aquatic organisms (Remyla *et al.*, 2008). Since most fishes breathe in water in which they live, changes in the chemical properties of water are reflected in the behavioural activity of animals (Mushigeri, 2003). Any abnormal change in the behaviour, physiology and the morphology of fish indicates the deterioration of water quality, as the fishes are the biological indicators. Behaviour change is considered as a promising tool in ecotoxicology. The present study will help to investigate the behavioural and morphological changes in the freshwater fish, *Labeo rohita* on being exposed to different concentrations of the Lead Nitrate.

Materials and Methods

The healthy fish specimen of *Labeo rohita* with an average weight of 10±2 g and average length of 10±1 cm were collected from Nanoki fish farm located at Nanoki village of District Patiala, Punjab. They were acclimatized to laboratory conditions in dechlorinated tap water for 15 days in a plastic tank of 1000 litre capacity equipped with filters and aerators. During winters heaters are also used to protect the fingerlings from extreme cold. The water was changed after every 24 hours and fishes were given bath for 2-3 minutes in 0.1% KM_nO_4 solution for prevention of any disease. The fishes are fed with artificial feed equal to $1/10^{th}$ of the body weight of fish.

Toxicity tests have been performed in accordance with the standard methods given in APHA (2012). These were carried out in plastic tanks of 25 litre capacity. The 96hr LC_{50} of Lead Nitrate calculated by Probit analysis (Finney, 1971) was 34.20 mg/l. For chronic toxicity tests, $1/3^{rd}$, $1/5^{th}$, $1/7^{th}$ and $1/10^{th}$ dose of LC_{50} of Lead Nitrate were selected as sub-lethal concentrations which were 3.42mg/l, 4.84mg/l, 6.88mg/l and 11.4mg/l. Chronic bioassay experiments were performed using 10 fishes and exposing them to toxicant for 15, 30, 45 and 60 days along with the control. Toxicity tests had been aimed to observe various responses in behavioural and morphological changes in the fish organs.

Results

Control: The control fishes show compact schooling behaviour. They were found to scrap the bottom surface. When startled with the fishes they form a tight school. At the beginning of the experiment they remain close to each other but after a week when they get adapted, then cover whole area of the tank.

Treated:

Behavioural anomalies: During the toxicity tests the fishes were regularly examined and they showed the symptoms of restlessness, swimming impairments like irregular, erratic and darting swimming movements, uncontrolled and unsteady jerky movements, and loss of equilibrium followed by hanging vertically in water, drowning, hitting against the wall of test aquaria, trying to jump out of the test aquaria, rapid swimming movement around the test tank, increased and fast opercular movements, rapid surfacing and gulping of air, severe diarrhoea, hyper excitability, schooling disturbed, disrupted shoaling behavior, coughing, reduced feeding behaviour, after few hours became sluggish and lethargic.

These activities increase with the increase in the concentrations of Lead Nitrate. After few hours the fishes become lethargic and settle down at the bottom of the tank near the aeration rod. The discomfort seems to be higher at higher concentration. After every 24 hours similar observation have been found. With the increase in the number of days the fishes show less activity and after 30 days they seems to get adapted to the toxic environment upto some extent. On addition of the stock solution of Lead Nitrate the fishes showed increased activites for a few minutes to an hour and after that they become stable.

Morphological anomalies: The morphological changes in the body of fish fingerlings on being exposed to different concentrations of Lead Nitrate for different number of days include shedding of scales, fusion and hyper extension of fins, split and necrosis of fins, lesion on skin, eye deformities, muscular tetany, caudal bending, decalcified skull, lower lip extension, clumping of gills, dullness in the body colour along with profuse mucus secretion and its coagulation. These anomalies seem to appear after 30 days of exposure and are clearly visible in 45 and 60 days of exposure and seem to be more prominent at higher concentrations of the toxicant.

Discussion

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Behavioural

During the present study the behaviour and the condition of fish specimens in both the control and the experimental tanks were noted for 24 hours. The test fish when exposed to different sublethal concentrations of Lead Nitrate exhibited a number of abnormal behavioural responses like faster opercular activity, surfacing and gulping of air, erratic swimming with jerky movements, hyper-excitability, schooling disturbed, tendency of escaping from aquaria, loss of balance, etc. These activities increased initially for few hours but subsequently get reduced when the fish becomes lethargic. These activities goes on increasing with the increase in the concentrations of Lead Nitrate and increase with the number of days of exposure but decrease and become normal after 15 to 20 days of exposure as the fish gets adapted. The normal behaviour of fish at 15, 30, 45 and 60 days of exposure indicates its adaptability to the sub-lethal concentration due to long term exposure. These results are in agreement with Drummond (1986), Bhoraskar and Kothari (1997), P. S. Joshi (2011).

When the solution is added a rapid opercular movements, gulping of air, increase in surfacing, followed by excited swimming and coughing was observed. This may be because of improper ventilation or inconvenience in breathing (Katja *et al.,* 2005; Ural and Simsek, 2006; Shwetha *et al.*, 2009). The substantial reduction in the growth of fish was observed in the sub-lethal concentrations as compared to the control group. This may be due to reduced amount of diet consumed by the fish due to toxicant stress, which was immediately utilized and was not stored in the body weight. This is in agreement with the earlier studies (Sancho *et al.,* 1998; Dembele *et al.*, 2000; Kalavathy *et al.*, 2001). Lateral swimming and loss of equilibrium were probably due to the impairment of nervous and muscular system which may be due accumulation of acetylcholine in synaptic and neuromuscular junctions (Sinha and Kumar, 1992; Rao *et al.*, 2005). Defecation was increased and more fecal matter was found at the bottom of the aquarium than control.

Behavioural changes are the most sensitive indication of potential toxic effects. Impact of different toxicants on the behavior of *Labeo rohita* have been studied by various workers (Marigoudar *et al.*, 2009; Anita *et al.*, 2010; Nagaraju *et al.*, 2011) and the results are in conformity with present observations**.** Similar results were also observed in different fishes treated with different heavy metals by many workers (Santha *et al.,* 2000; Subathra and Karuppasamy, 2003; Sivakumar *et al.,* 2006).

Morphological

The morphological deformities such as shedding of scales, discolouration, lesions on skin, split and necrosis of fin, eye deformities, scoliosis, damaged skull, lower lip extension, copious amount of mucus secretion all over the body were observed. The percentage of all these deformities was increased with the increasing concentrations of lead nitrate and duration of exposure. Fish body becomes lean towards the abdominal position. Similar observations were reported by Helappa *et al.* (2009) and Anita *et al.* (2010).

Increase in the secretion of mucus all over the body in the fish exposed to Lead Nitrate may be an adaptive response providing additional protection against corrosive nature of the metal and to avoid the absorption of the toxicant by the general body surface. Similar observations were made by many workers (Santhakumar, 2000; Sivakumar *et al.,* 2006 and Shallangwa, 2011).

The pale red colour of the gills and their clumping increases with the increasing of concentration of toxicant. The fishes lost their natural coloration and become almost pale yellow in colour. Eye deformities such as micropthalmia, anapthalmia and exopthalmia were also observed which may be due to the apoptosis of cells in the eye or reduction in the diameter of eye socket as reported by Tayor *et al.*, 2012. The extent of caudal bending (scoliosis) was pronounced at higher concentrations after 30 to 40 days of exposure which may be a sort of paralysis caused due to the inhibition of muscular AChE activity resulting in blockage of neural transmission.

Conclusions

The behavioural and morphological changes show direct response of the animals to the pollutants. This type of study can be useful to compare the sensitivity of various species of aquatic animals and to derive safe environmental concentration of the toxicants by which there is no lethality and stress to the animals. These studies can be used as a sensitive model to monitor the aquatic pollution. The current study evidenced that lead is highly toxic and had a detrimental impact on the behavioural responses and morphology of the fish, *Labeo rohita* at sublethal concentrations. It affects the ability of animals to adapt to its environment and affect the central and peripheral nervous system. These responses can be used as a tool in biomonitoring programme to monitor ecotoxicity risk of lead to the fish.

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