VOLUME 10 ISSUE 2 2024

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 10, No. 2, 991-1001 (2024)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Toxicity and Respiratory Effects of Heavy Metal Cadmium on the *Labeo rohita* Fingerlings

Vasanthi N. and Muthukumaravel K.*

Department of Zoology, Khadir Mohideen College (Affiliated to Bharathidasan University, Tiruchirappalli), Adirampattinam 614 701, Tamil Nadu, India

**Corresponding Author*

Received: 23rd April, 2024; **Accepted:** 6th June, 2024; **Published online:** 16th October, 2024

https://doi.org/10.33745/ijzi.2024.v10i02.098

Abstract: The present study evaluated the effect of sub lethal concentration of heavy metal, cadmium on oxygen consumption and gill histology of *Labeo rohita.* LC_{50} values for 96 h was determined for cadmium and was found to be 12.5 ppm. The fishes were exposed to 10% sub lethal concentrations of the 96 h LC_{50} value for 10, 20, and 30 days along with control. Decrease in O_2 consumption rate was observed in *L. rohita* exposed to 10% sub lethal concentration of heavy metal for a period of 10, 20 and 30 days. In this study, the oxygen consumption was gradually decreasing with increasing exposure periods. Maximum decline (-36.94%) over control in the rate of respiration was noticed in 10% sub lethal concentration after 30 days of exposure. Histopathological lesions in the gill of fresh water *L. rohita* were assessed following 10, 20 and 30 days of exposure to the cadmium. In the fish exposed to the cadmium, the most common lesions were fusion of gill lamellae, detachment of gill epithelium, hyperplasia and hypertrophy of respiratory epithelium in the gills. The lesions were comparatively most severe in 30 days exposed fish.

Keywords: Heavy metal, Cadmium, Toxicity, Oxygen consumption, Gill histology

Citation: Vasanthi N. and Muthukumaravel K.: Toxicity and respiratory effects of heavy metal cadmium on the *Labeo rohita* fingerlings. Intern. J. Zool. Invest. 10(2): 991-1001, 2024.

https://doi.org/10.33745/ijzi.2024.v10i02.098

Introduction

Fishes are valuable sources of high grade proteins, mineral salts including calcium, phosphorus and iodine, essential amino acids, omega 3 fatty acids and vitamins A, B, D and E (Tont, 1977). Fish proteins occupy an important place and it constitutes about 17-20%. Moreover, carbohydrate content of the fish flesh is very low and hence, fish can make valuable contribution to any diet (Holt, 1967). Besides providing food to man, fishes are sources of numerous by-products such as fish liver oil, fish flour, fish silage, fish glue, Isinglass etc. which have medical and economic importance. Considering their nutritional value, it is essential for any nation, which is desirous of

developing this rich protein source, to develop a efficient aquaculture system, for which maintenance of the environmental water quality is a prerequisite. However, increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment poses problems to humanity, particularly to the aquatic organisms (Mc Glashan *et al*., 2001).

Among the industrial wastes, heavy metals cause a hazard not only to mankind but also to other plants and animals. Heavy metals are non – degradable and are regarded as hazardous to the aquatic ecosystem for their environmental persistence and their ability for accumulation (Yigit and Altindag, 2006). Among the non – essential metals, cadmium is one of the most hazardous elements and is recognized carcinogen in mammals (Sunderman *et al*.,1991). It is also stable like other metal and does not degrade in the environment (Sax, 1974).

Cadmium (atomic weight 112.4 and atomic number 48), a naturally occurring non – essential element found in earth's crust associated with zinc and copper ores was discovered by Fredrich Stromesper in 1817, but was not used commercially until the end of the 19th century. This soft, silver – white metal was first used in paint pigments and as a substitute for tin in world war I. Today, about three – fourths of cadmium is used in battery, electroplating, mining, paints and dye industries (Forstner and Prosi, 1979). The environmental protection Agency (EPA) has found cadmium to potentially cause a variety of effects from acute exposures in human beings which include nausea, vomiting, abdominal pain and breathing difficulty. Chronic exposure to cadmium results in kidney dysfunction, lung cancer and prostate cancer (Roberts, 1999).

Changes in oxygen consumption, as indices of energy expenditure, are a useful tool to assess the physiological stress on aquatic organisms (Lee, 1969). The oxygen consumption of the fish *Tilapia mossambica* has been studied when exposed to sublethal concentration of organochlorine pesticide DDT (Ravindran and Swami, 1987). *Labeo rohita* exposed to dimethyl parathion showed decline in the rate of oxygen consumption (Benggeri *et al*., 1984). Exposure of *Oreochromis mossambicus* to Quinalphos tends to depress the rate of oxygen uptake (Mathivanan, 2004). Stimulation of oxygen consumption by endrin has also been shown for mosquito fish, *Gambusia affinus* (Ferguson *et al*., 1966) and blue gills, *Lapomis machrochirus* (Huner *et al*., 1967). Saradhamani *et al.* (2009) reported the decreased rate of oxygen consumption in the fish *Catla catla* on exposure to herbicide, glyphosate.

Ramakrishnan and Sivakumar (1993) reported that the rate of oxygen consumption is decreased with increased concentrations of Quinalphos and increasing duration of exposure period. Toor and Kamal Deep (1974) observed a decrease in oxygen consumption of *Cyprinus carpio*, when exposed to lethal and sublethal concentrations of carbaryl diazinon, phospha-midon and fenitrothion. Rao *et al*. (1981) have reported a significant decrease in the oxygen consumption in the fish *Macrognathus aculeatum* when exposed to the sublethal concentrations of endosulphan.

Fish gills are regarded as a major site of respiration, osmoregulation and excretion and remain in close contact with the external environment and particularly sensitive to changes in the quality of water and considered the primary target of the contaminants. For this reason, they are considered excellent indicators of environmental quality (Wendelaar Bonga, 2008). Histopathological studies have been conducted to establish fundamental relationships between contaminant exposure and various biological responses. Histopathological investigation has been recognized as a valuable tool for assessment of the impact of environmental pollutants in fish (Heath, 1995; Teh *et al*., 1997; Schwaiger *et al*., 1996; Oliveira Ribeiro *et al*., 2002). Over the past few decades, the histopathological changes in gills under acute and chronic exposure to heavy metals have been studied in many fish species (Baker

1969; Matthiessen and Brafield, 1973; Gardner and Yevich, 1970; Wobeser, 1975; Crespo, 1982; Sriwastwa and Maurya, 1991; Gupta and Rajbanchi, 1995; Arellano *et al*., 2000; Rangsayatron *et al*., 2004; Gupta and Kumar, 2006; Hameed *et al*., 2005; Al-Attar, 2007; Muthukumaravel *et al*., 2008; Radhika and Krishnamoorthy, 2010; Georgieva *et al*., 2010; Patel and Bahadu, 2010). Hence, the present study was aimed to investigate the impact of cadmium on histological structure of gill of Indian major carp *Labeo rohita* in order to understand to mode of action, stress response and organ dysfunction.

Materials and Methods

Test chemicals:

The analytical grade cadmium sulphate ($3Cd.So_4).8H_2O$ was obtained from New India Chemical Enterprises, Cochin, India and used without further purification for the experiment.

Animal maintenance:

The freshwater healthy fingerlings of *Labeo rohita* (10±19 g b wt.; 8±0.5 cm) were selected for the experiment and were collected from Katherasan Aquafarm near Thanjavur, Tamil Nadu, India. The collected fish were acclimatized for one month in a large cement tank (1000 L capacity). During the acclimatization period, the fish were fed with rice bran and groundnut oil cake which had no detectable amount of cadmium. Food was provided once a day. The water was renewed daily to avoid accumulation and contamination of excretory materials and feeding was withheld 24 h before the commencement of the experiment.

Fish showing an abnormal behavior was removed as soon as possible. In the present study tap water free from chlorine was used which had the following physico-chemical characteristics (APHA, 1998)-- temperature 28±0.13°C, pH 7.6±0.04, salinity 1.2±0.13 ppt, D.O. 5.6±0.2 mg/l and total hardness 35±0.5 mg/l. Before the start of the experiment, suitable numbers of fish were transferred into two glass aquaria which were continuously aerated.

Preparation of stock solution and determination of 96 h LC50 value of cadmium:

Stock solution of cadmium was prepared by dissolving 1 g of cadmium in an appropriate amount of water. For the determination of median tolerance limits or LC_{50}, different concentrations of cadmium (5, 10, 15, 20, 25 and 30 mg/l) were prepared from the stock and added in separate glass aquaria containing 50 L of water. Three replicates were run for each concentration and 10 fishes of equal size and weight were introduced. The test water was renewed at 24 h interval and freshly prepared cadmium was added to maintain the concentration of cadmium at a constant level. A concurrent control of 30 fish in three different glass aquaria was maintained under identical conditions. The mortality/survival of fish was recorded after 96 h and median lethal concentration (LC_{50}) was calculated (Finney, 1978). $1/10^{th}$ value (1.25 mg/l) of the LC_{50} value for 96 h (12.5 mg/l) was taken as the sublethal concentration for experiment (Sprague, 1971).

Sub lethal studies:

For sub lethal toxicity tests 200 fingerlings were selected and divided into four groups (one control and three experimental) with 50 fish in each aquarium filled with water. The desired concentration (1.25 mg/l cadmium solution which is $1/10^{th}$ of 96 h LC_{50}) of the toxicant was added directly in order to maintain constant concentration of the toxicant. The experiment was conducted for 30 days and sampled at 10 days interval and no mortality was observed during the above treatment period. At the end of the stipulated periods (10^{th}, 20^{th} and 30^{th} day) of exposures fish were randomly selected and sacrificed for histological studies.

Estimation of Oxygen Consumption:

A series of rectangular glass jars, each with one liter capacity were used as aquarium. They were filled with water. Care was taken to avoid trapping of air bubbles. Only one fish was introduced into each aquarium and a thick layer of coconut oil was spread on the surface of the medium to prevent

the contact of the medium to the atmosphere and to prevent the fish from reaching the atmospheric air.

Before starting the experiment, the initial oxygen content of water used for the preparation of animal chambers was estimated by collecting a sample into a narrow mouth, glass stoppered sample bottle of known volume following the Winkler's method (Annon, 1984). A healthy fish was allowed to respire for one hour in animal chambers. After one hour, samples from respiratory chamber were taken into the sample bottle of known volume through siphon system and the dissolved oxygen was estimated.

The initial oxygen content of water was determined by collecting the sample in a narrow mouthed glass stoppered sample bottle of known volume. To this 1 ml of manganous sulphate solution was added followed by addition of 1 ml of alkaline iodide solution. The bottle was stoppered and shaken vigorously and kept in a dark place to prevent any photochemical reaction for about 15 min. A few drops of conc. sulphuric acid were added into the sample bottle in order to dissolve the precipitate. The precipitate was completely dissolved by shaking vigorously. 20 ml of the sample was taken in a clean conical flask and the liberated iodine was titrated against sodium thiosulphate using four to five drops of starch as indicator. The disappearance of blue colour was taken as end point. The burette values were tabulated. The final oxygen content of the respiratory chamber was also determined in the same manner. Oxygen consumed by the fish was calculated by finding out the difference between the initial and final oxygen content in the animal chambers. Also the rate of oxygen consumption per gram weight of the fish per hour was calculated and the values were expressed as ml O_2/g/h.

The dissolved oxygen content in the water was calculated using the following formula:

$$O_2 \text{ content ml/l} = \frac{K \times 200 \times \text{volume of } Na_2S_2O_3 \text{ consumed} \times 0.698}{\text{Volume of the sample titrated}} \times 100$$

$$\text{Where } K = \frac{\text{Volume of sample bottle}}{\text{Vol. of sample bottle} - \text{Vol. of reagents added}}$$

0.698 is the conversion factor to convert parts per million to ml/l; 200 is the constant which is obtained by multiplying the equivalent weight of oxygen and normality of $Na_2S_2O_3$ and 100 ml.

Histological investigations:

On 10th, 20th and 30th day, fish were taken out, sacrificed and the gill was excised out. The gill tissue was fixed in Bouin's fluid and then they were processed (Gurr, 1959) and embedded in paraffin wax (58-60 °C). Serial sections of 8 µm thickness were cut and deparafinised sections were stained in haematoxylin and counterstained with aqueous eosin.

Results

LC_{50} value – 96 h:

In the present study the 96h LC_{50} value of cadmium for *Labeo rohita* was estimated and found to be 12.5 mg/l.

Rate of oxygen consumption:

Oxygen consumption of fish, *L. rohita* exposed to sublethal concentration of Cadmium is presented in Table 1. Decrease in O_2 consumption rate was observed in *L. rohita* exposed to 10% sub lethal concentration of heavy metal for a period of 10, 20 and 30 days.

The rate of oxygen consumption in control *L. rohita* were 0.579, 0.584 and 0.582 ml/O_2/g/h at 10,20 and 30 days, respectively (Table 1; Fig. 1). The fish exposed to sub lethal concentrations of cadmium showed the oxygen consumption at the rate of 0.518, 0.439 and 0.367 ml/O_2/g/h at 10% sub lethal concentrations after10, 20 and 30 days, respectively (Table 1, Fig. 1). In this study, the oxygen consumption was gradually decreasing with increasing exposure periods. Maximum decline (-36.94%) over control in the rate of respiration was noticed in 10% sub lethal concentration on 30 days of exposure (Table 1).

Table 1: Changes in the oxygen uptake of *Labeo rohita* at sublethal concentration of cadmium (O_2ml/g/h)

S. No.	Experimental group	Exposure periods days		
		10	20	30
1.	Control	0.579±0.070	0.584±0.065	0.582±0.068
2.	10% SLC	0.518±0.028	0.439±0.036	0.367±0.046
3.	%Variation	-10.54	-24.83	-36.94

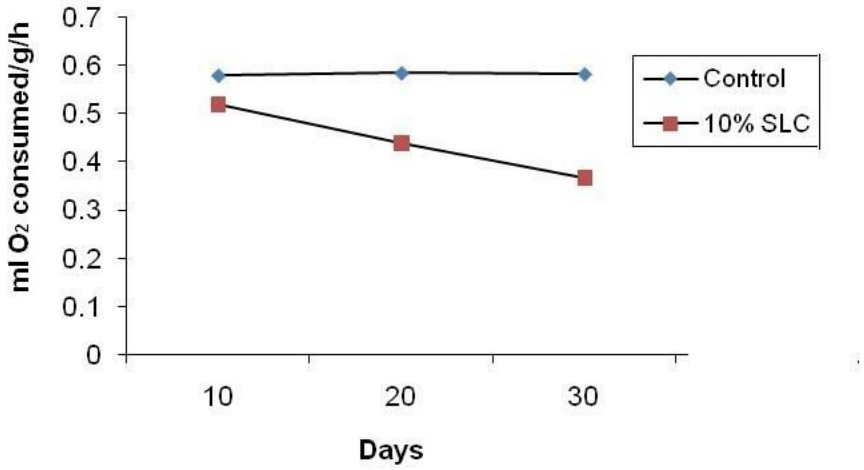

Fig. 1: Changes in oxygen uptake of *Labeo rohita* fingerlings at sub lethal concentration of cadmium.

Histological investigations:

The structure of gill of *Labeo rohita* consists of highly vascular plate like process called primary and secondary lamellae. The secondary lamellae of the gill appeared as finger- like structure and are covered with thin layer of epithelial cells. They are very thin, slender and attached on either side of the primary lamellae (Fig. 2a).

After sublethal exposure of Cd, the gill of *Labeo rohita* showed marked histological changes. Appreciable changes were noticed in the histology of gill after 10 days treatment including fusion of secondary lamellae, degeneration of epithelium and vaculation (Fig. 2b)

The damage was more severe and progressive after 20 days of exposure. The primary and secondary gill lamellae were damaged to a great extent. Hypertrophy, hyperplasia, necrosis and lamellar fusion were observed (Fig. 2c). However, such changes were drastic to the extent that disintegration of lamellar epithelium, fusion of secondary lamellae and degenerated secondary lamellae were found in the 30 days treated fish (Fig. 2d).

Discussion

The rate of oxygen consumption is a true index of the metabolic state, particularly in aquatic animals whose body is continuously bathed by the surrounding water and is a valuable indication of sub lethal stress. When the pesticides enter the living system, they primarily affect the metabolism, which is concerned with oxygen consumption. Respiration is one of the most important processes as oxygen taken up during this process is essential to provide energy for life activities in the living organisms. The O_2 consumption is a very sensitive physiological process and the change in respiratory activity has been used as an indicator of stress in animals exposed to toxicants (Sarkar, 1999).

In this study, the rates of O_2 uptake of whole organism were altered by cadmium. The

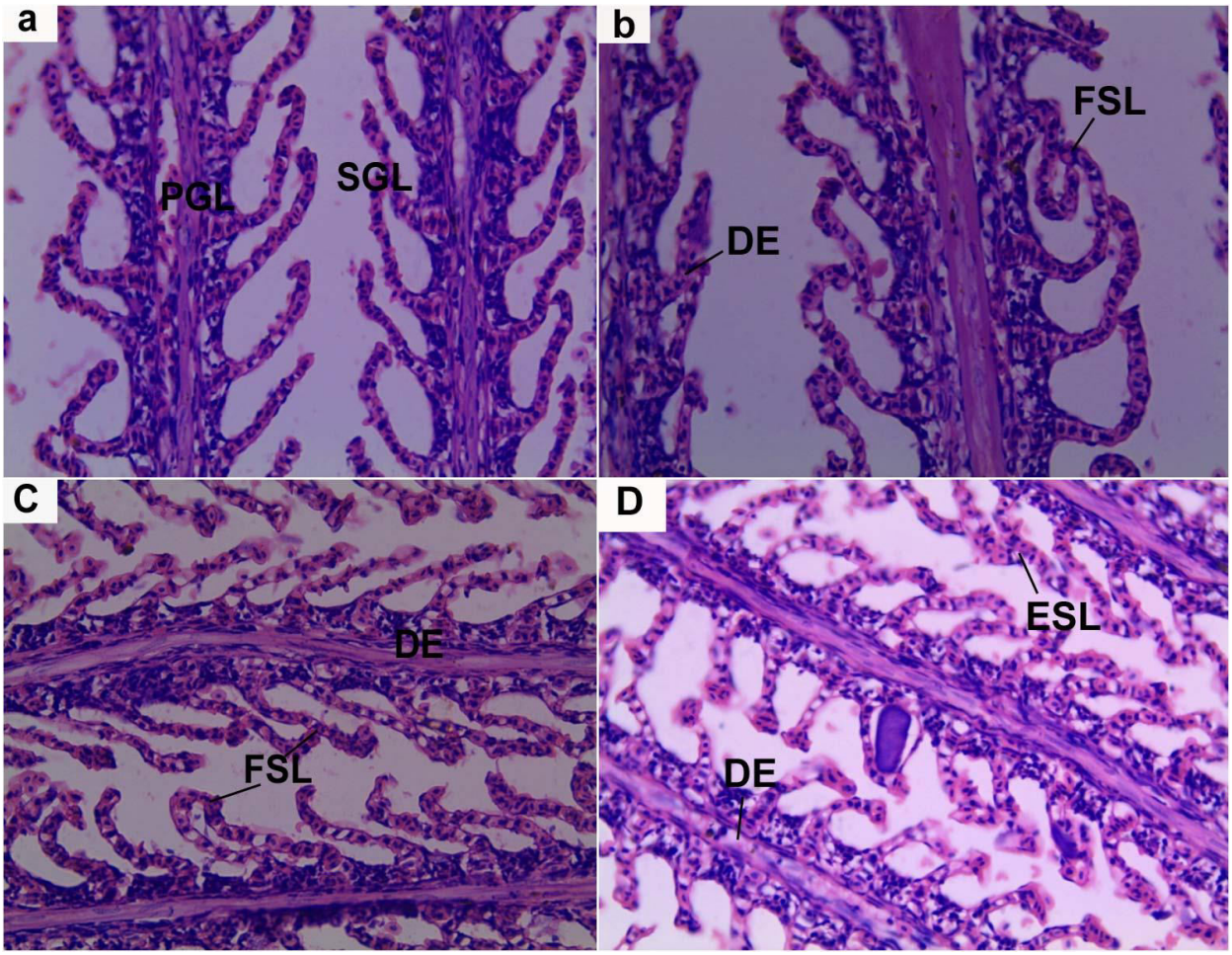

Fig. 2: Histopathological lesions in the gill of *Labeo rohita* exposed to 10% sub lethal concentration of cadmium at different durations. (a) Control, PGL-Primary Gill Lamellae, SGL- Secondary Gill Lamellae; (b) 10 days treated, FSL-Fusion of secondary lamellae, DE- Degeneration of epithelium; (c) 20 days treated, FSL - Fusion of secondary lamellae, DE- Degeneration of epithelium; (d) 30 days treated,DE- Degeneration of epithelium, ESL- Erosion of secondary lamellae.

decreased oxygen uptake could be suggested as a equal to gill damage or due to hypochronic microcytic anaemia under pesticide stress (Koundinya and Ramamurthi, 1980), Heavy metal interfered with respiration by damage of gill and inhibition of enzyme system at mitochondrial levels resulting in reduced oxygen uptake. The drop in oxygen uptake of cadmium treated *L. rohita* indicates the onset of severe hypoxia under toxicant stress, which has triggered the metabolic pathways of fish. Similar observations have been reported in *L. rohita* after chlordane, and metasystox (Bansal *et al*., 1979).

A similar decrease in oxygen uptake has been reported in *Mystus vittatus* exposed to thiodon (Reddy and Gomathy, 1977), in *Sarotherodon mossambicus* exposed to thiodon (Vasanthi and Ramasamy, 1987) and in *C. carpio* due to methyl parathion exposure(Nagarathinama and Ramamurthi, 1982). Das *et al*. (1999) observed decline in the rate of oxygen consumption when *Channa punctata* was exposed to synthetic pyrethroid, cypermethrin. Sublethal concentrations of deltamethrin, a pyrethroid, have decreased oxygen consumption in *O. mossambicus* (Nazeemual Khane *et al*., 1992). Similar result has been observed when the same species of fish was exposed to metasystox as reported by Natarajan (1984) and this has been explained as due to the injury caused to the red blood corpuscles and

reduction in the RBC count. A reduction in haemoglobin content and erythrocyte population resulting in hypochromic microcytic anaemia have also been suggested as reasons for drop in oxygen uptake in *S. mossambicus* exposed to lethal concentration of Sumithion and Sevin (Ranganatha and Ramamurthi, 1979). When *O. mossambicus* was exposed to quinalphos, decreased consumption of oxygen has been noted (Mathivanan, 2004). The increasing concentrations of the textile dye effluent decreased the oxygen consumption in *O. massambicus* (Baskaran *et al*., 1989). Similar result has been obtained by Hingorani *et al*. (1979) in the fish *L. rohita* when exposed to different concentrations of textile dye effluent. Exposure of the cat fish *Macrons keletius* to dimethoate has declined the rate of oxygen consumption (Shahul Hameed and Vadamalai,

1986).

Fish gills are considered as most vulnerable organ for the toxicants (Srivastava *et al*., 1989; Dutta *et al*., 1996; Kumar *et al.*, 2010) because they are in direct contact with the surrounding water. Alterations in gill structure affect the normal functioning of vital physiological processes such as gas and ion exchanges, osmoregulation, excretion of nitrogenous wastes and acid – base equilibrium (Maina, 1998; Wendelaear Bonga and Lock, 2008).

Pathological lesions induced by cadmium were characterized by hypertrophy, hyperplasia, fusion of secondary lamellae and necrosis. Several authors have reported histopathological anomalies in gills of other species exposed to heavy metals. Gupta and Rajbanshi (1995) observed fusion and clumping of gill lamellae in mercury treated *Rasbora daniconius*. Mazon *et al*. (2002), Venkatesan and Subramanian (2007) and Campagna *et al*. (2008) noted similar types of gill lesions in copper treated fish. Gupta and Kumar (2006) also noted serve gill lesions in mercury treated *Cirrhinus mrigala*. In the present study, degeneration of gill lamellae, necrosis and edema of secondary lamellae were apparent in *Labeo rohita* exposed to cadmium. These observations are quite comparable to pathological lesions induced in gills by nickel in *Hypophthalmichthys molitrix* (Athikesavan *et al*., 2006), by inorganic mercury treatment in *Salvelinus alpines* (Oliveira Ribeiro *et al*., 2002) and by treatment with cadmium in grass carp *Ctenophanyngodon idella* (Espina *et al*., 2000). Radhika and Krishnamoorthy (2010) also noted alterations in gill tissues of cadmium exposed *Oreochromis mossambicus*. The present study showed that the gills of *Labeo rohita* exposed to Cd during 30 days presented a higher occurrence of histopathological lesions such as hypertrophy, hyperplasia and fusion of gill lamellae. These observations are in good agreement with the results reported by Gupta and Dua (2002) in the air breathing freshwater fish *Channa punctata* treated with mercury; Thophan *et al*. (2003) and Rangsayatron *et al*. (2004) in *Lates calcarifer* and *Puntius gonionotus* treated with cadmium; Pane *et al*. (2004) in nickel administered *Oncorhynchus mykiss*. Further Al-Attar (2007) also observed such gill damages in nickel treated *Oreochromis niloticus*.

The present study showed that the gills of *Labeo rohita* exposed to cadmium for 30 days showed a higher occurrence of histopathological lesions such as hypertrophy, fusion of secondary lamellae, edema and mucus openings. These pathological changes may be a reaction to toxicants intake or an adaptive response to prevent the entry of the pollutants through the gill surface (Mohamed, 2009). The damages observed in the gills in terms of hypertrophy, fusion of secondary lamellae and necrosis could cause a decrease in free gas exchange, thus affecting the general health of fish (Skidmore and Tovell, 1972). Similar changes in gill epithelia were reported by Crespo (1982) in the dog fish, *Scyliorhinus canicula* subjected to zinc sulphate; Temmink *et al* (1983) in rainbow trout, *Salmo gairdneri* exposed to chromate; Gupta and Dua (2002), in the *Channa punctatus* intoxicated with mercury, Pane *et al*. (2004) in *Oncorhynchus mykiss* treated with nickel, and Acharya *et al*. (2005) in *Labeo rohita* treated with sublethal acidic (HCl) and alkaline (NaOH)

pH. Muthukumaravel *et al*. (2008) reported that copper exposure resulted in marked ultrastructural damage to the respiratory epithelium of gill in *Oreochromis mossambicus* including swelling and fusion of secondary lamellae. Palaniappan *et al*. (2008) observed hypertrophy, hyperplasia, alteration of lamellar surface and fused lamellae in Pb exposed *Catla catla*. The histopathological lesions of the gills of fish were reported in detail by Farzad Ghiasi *et al*. (2017). They observed histological changes like hypertrophy, fusion and telangiectasia in the secondary lamellae due to cadmium chloride toxicity. Aghamirkarimi *et al*. (2017) have also reported the histological alterations of gills due to copper nanoparticles toxicity in *Rutilus caspicus* and attributed them to the congestion of blood vessels in the primary gill filaments and detachment of secondary gill lamellae. Muthukumaravel *et al*. (2020) have also observed the histopathological changes in the gill of the fish *Mystus vittatus* due to toxicity of phenol. They observed fusion, malformation at the tips of secondary lamellae and lamellar disintegration.

Conclusion

In the present study, cadmium exposure during sub lethal treatment caused severe toxic effects on the respiratory organ of the freshwater fish *Labeo rohita*. The findings of the present study indicate that gill structural changes may serve as "biomarkers" for assessing heavy metal toxicity in aquatic environment.

References

Acharya S, Dutta T and Das MKR. (2005) Physiological and ultrastrcutural changes in *Labeo rohita* (Hamilton – Buchanan) fingerlings exposed to sublethal acidic and alkaline pH for long duration. Asian Fish Sci. 18: 295-305.

Aghamirkarimi S, Mashinchian M, Sharifpour JS and Ghavan MP. (2017) Sublethal effects of copper nanaoparticles on the histology of gill, liver and kidney of the Caspian roach, *Rutilus caspicus*. Global J Environ Sci Manag. 3(3): 323-332.

Al-Attar AM. (2007) The influences of nickel exposure on selected physiological parameters and gill structure in the teleost fish, *Oreochromis niloticus*. J Biol Sci. 7(1): 77-85.

Annon (1984) Water quality management in aquaculture (CMFRI special publication No. 22. CMFRI, Cochin).

APHA (American Public Health Association) (1998) Standard methods for the examination of water and wastewater, 20th edn., American Public Health Association, Washington, DC.

Arellano JM, Blasco J, Ortiz JB, Capeta-Da SD, Navarro A, Sanchez-Del PMJ and Sarasquete C. (2000) Accumulation and histological effects of copper in gills and liver of *Seneqales sole, Solea seneqalensis* and Toad fish, *Halobatrachus didactylus*. Ecotoxicol Environ Res. 1: 22-28.

Athikesavan S, Vincent S, Ambrose T and Velmurugan B. (2006) Nickel induced histopathological changes in the different tissues of freshwater fish, *Hypophthalmichthys molitrix* (Valenciennes). J Environ Biol. 27(2): 391-395.

Baker JTP. 91969) Histological and electron microscopical observations on copper poisoning in the winter flounder (*Pseudopleuronctes americanus*). J Fish Res Bd Can. 26: 2785-2793.

Bansal SK, Verma SR, Gupta AK and Dalela RC. (1979) Physiological disfunction of hemopoietic system in a freshwater telecost *Labeo rohita* following chronic chlordane exposure I: Alterations in certain haematological parameters. Bull Environ Contam Toxicol. 22: 666-673.

Baskaran P, Palanichamy S and Balasubramanian MP. (1989) Effect of pesticides on protein metabolism in *Mystus vittatus.* J Ecobiol. 1(2): 90-97.

Bengeri KV, Shivaraj KM and Patil HS. (1984) Toxicity of dimethyl parathion to freshwater fish, *Labeo rohita* and oxygen uptake rate of exposed fish. Env Ecol. 2: 1-4.

Campagna AF, Fracacio R, Rodrigues BK, Eler MN, Fenerich-Verani N and Espindola ELG. (2008) Effect of the copper in the survival, growth and gill morphology of *Danio rerio* (Cypriniformes, Cyprinidae). Acta Limnol Bras. 20(3): 253-259.

Crespo S. (1982) Surface morphology of dog fish (*Scyliorhinus canicula*) gill epithelium and surface morphological changes following treatment with zinc sulphate: A scanning electron microscope study. Mar Biol. 67: 159-166.

Das VL, Jeewaprada PN and Veeraiah K. (1999) Toxicity and effect of cypermethrin on biochemical constituents of freshwater teleost, *Channa punctatus*. J Ecotoxicol Environ Monit. 9(3/4): 197-203.

Devos E, Devos P and Cornet M. (1998) Effect of cadmium on the cytoskeleton and morphology of gill chloride cells in parr and smolt Atlantic salmon (*Salmo salar*). Fish Physiol Biochem. 18: 15-27.

Dutta HM, Munshi JSD, Roy PK, Singh NK, Adhikari S and Killus J. (1996) Ultrastructural changes in the respiratory lamellae of the catfish, *Heteropneustes fossilis* after sublethal exposure to malation. Environ Poll. 92(3): 329-341.

Dutta HM, Munshi JSD, Roy PK, Singh NK, Mortz L and Adhikari S. (1997) Effects of diazinon on blue gill sunfish, *Lepomis macrochirus* gills: Scanning electron microscope observations Exp Biol. 2(17): 1-11.

Espina S, Salibian A and Diaz F. (2000) Influence of cadmium on the respiratory function of the grass carp *Ctenopharyngodon idella*. Water Air Soil Pollut. 119: 1-4.

Farzad G, Seyed SM and Javad A. (2017) Histological effects of cadmium on hepatopancreas and gill in *Cyprinus carpio*. Iranian J Toxicol. 11(5): 1-4.

Ferguson DE, Ludke JL and Muphy GG. (1966) Dynamics of endrin uptake and release by resistant and susceptible strains of mosquito fish. Trans Amer Fish Soc. 95: 335.

Finney DJ. (1978) Statistical method in biological assay. 3rd edn., Cambridge University Press, London, pp. 508.

Forstner U and Prosi F. (1979) Heavy metal pollution in freshwater ecosystem. In: Biological aspects of freshwater pollution, (ed.) Ravera O., Pergamon Press, Oxford, pp. 129-161.

Gardner GR and Yevich PP. (1970) Histological and haematological responses of an estuarine teleost to cadmium. J Fish Res Bd Can. 27: 2185-2196.

Georgieva E, Arnandou A and Velcheva I. (2010) Clinical, haematological and morphological studies on ex-situ induced copper intoxication in crucian carp (*Carassius qibelio*) J Central European Aqri. 11(2): 165-172.

Gopalakishna Reddy T and Gomathy S. (1977) Toxicity and respiratory effects of pesticide thiodon on catfish, *Mystus vittatus*. Indian J Environ Hlth. 19: 360-363.

Gupta AK and Kumar A. (2006) Histopathological lesions in the selected tissues of *Cirrhinus mrigale* (Ham.) fingerlings exposed to a sublethal concentration of mercury. J Environ Biol. 27(2): 235-239.

Gupta AK and Rajbanshi VK. (1995) Mercury poisoning: Architectural changes in the gills of *Rasbora daniconius* (Ham.) J Environ Biol. 16(1): 33-36.

Gupta N and Dua A. (2002) Mercury induced architectural alterations in the gill surface of a fresh water fish, *Channa punctatus*. J Environ Biol. 23: 383-386.

Gurr E. (1959) Methods of analytical histology and histochemistry. Leonard Hill Ltd., London, pp. 45-49.

Hameed SVSA, Kumarasamy P, Amsath A and Muthukumaravel K. (2005) Effect of cadmium on oxygen consumption and histopathological changes in the gill of *Oreochromis mossambicus.* J Exp Zool. 8(2): 405-410.

Heath AC. (1995) Water pollution and fish physiology, 2nd edn., Lewis, Boca Raton, FL.

Hingorani HG, Diwan AD and Naidu CS. (1979) Oxygen consumption in fish *Labeo rohita* under exposure to different concentrations of industrial effluents. Comp Physiol Ecol. 4(1): 272-276.

Holt SJ. (1967) The contribution of freshwater fish production to human nutrition and well being. In: The biological basis of freshwater fish production, (ed.) Gerking S.D., Blackwell Scientific Publication, Oxford, pp. 455-457.

Huner JV, Bowdon BF and Bennett HJ. (1967) The effects of endrin on the oxygen consumption of the blue gills, *Lepomis machrochirus*. Proc Nat Acad Sci. 30: 80-86.

Koundinya PR and Ramamurthi R. (1980) Toxicity of sumithion and sevin to the freshwater fish, *Sarotherodon mossambicus* (Peters). Curr Sci. 49(22): 875-876.

Kumar A, ManiRam P, Srivastava K, Tripathi S and Srivastav Ajai K. (2010) Branchial histopathological study of catfish *Heteropneustes fossilis* following exposure to purified neem extract, azadirachtin. World J Zool. 5: 239-243.

Lee EL. (1969) Measurement of pesticide toxicity by fish respiration rate. D.Sc. thesis, Washington University.

Maina JN. (1998) The gas exchangers, structure, function and evolution of the respiratory processes. Springer, New York, pp. 498.

Mathivanan R. (2004). Effects of sublethal concentration of quinophos on selected respiratory and biochemical parameters in the fresh water fish *Oreochromis mossambicus.* J Ecotoxicol Environ Monit. 14(1): 57-64.

Matthiessen P and Brafield AE. (1973) The effects of dissolved zinc on the gills of the strickle back *Gasterosteus aculeatus* L. J Fish Biol. 5: 607-613.

Mazon AF, Cerqueira CCC and Fernandes MN. (2002) Gill cellular changes induced by copper exposure in the south American tropical fish *Prochilodus scrofa*. Environ Res. 88(1): 52-63.

McGlashan DJ and Hughies JM. (2001) Genetic evidence for historical continuity between populations of the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae) east and west of the Great Range. J Fish Biol. 59: 55-67.

Mohamed FAS. (2009) Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from lake Qarun, Egypt. World J Fish Mar Sci. 1(1): 29-39.

Muthukumaravel K, Murthy A, Kumarawamy P and Amsath A. (2008) Light and scanning electron microscopic evaluation of effects of copper sulphate on the gill architecture of freshwater fish *Oreochromis mossambicus*. Poll Res. 27(4): 715-719.

Muthukumaravel K, Vasanthi, N, Arumugam S, Lubna A, Bharathi S and Mohamed SM. (2020) Sublethal effects of phenol on histology of selected organs of freshwater fish *Mystus vittatus.* Environ Sci Pollut Res. 28(1): 1-9.

Nagarathinama R and Ramamurthi R. (1982) Metabolic depression in the fresh water teleost *Cyprinus carpio*, exposed to an organophosphate pesticide. Curr Sci. 51(13): 660-669.

Natarajan GM. (1984) Effects of sublethal concentration of metasystox on selected oxidative enzymes, tissue respiration and haematology of the fresh water air breathing fish *Channa striatus*. Pesticide Biochem Physiol. 21(2): 194-198.

Nath K and Kumar N. (1989) Nickel induced histopathological alterations in the gill architecture of a tropical freshwater perch, *Colisa fasciatus* (Bloch & Schn.). Sci Total Environ. 15L 293-295.

Nazeemual Khane KMA, James R and Manjula M. (1992) Sublethal effect of deltamethrin on the rate of metabolism in *Oreochromis mossambicus*. J Ecobiol. 4(2): 81-85.

Oliveira Ribeiro CA, Belger L, Pelletier E and Rouleau C. (2002) Histopathological evidence of inorganic mercury and methyl mercury toxicity in the arctic charr (*Salvelinus alpinus*). Environ Res. 90(3): 217-225.

Palaniappan PLRM, Sabhanayakam S, Krishnakumar N and Vadivelu M. (2008) Morphological changes due to lead exposure and influence of DMSA on the gill tissues of freshwater fish *Catla catla*. Food Chem Toxicol. 46: 2440-2444.

Pandey BN, Chanchal AK and Singh MP. (1976) Effects of Malathion on oxygen consumption and blood parameters of *Channa punctuates.* J Zool. 27: 95-100.

Pane EF, Haque A and Wood CM. (2004) Merchanistic analysis of acute, Ni induced respiratory toxicity in the rainbow trout (*Oncorhynchus mykiss*): An exclusively branchial phenomenon. Aquat Toxicol. 69: 11-24.

Patel JM and Bahadu A. (2010) Histopathological alterations in *Catla catla* induced by chronic exposure of copper ions. J Cell Tissue Res. 10(3): 2365-2370.

Pruski AM and Dixon DR. (2002) Effects of cadmium on nuclear integrity and DNA repair efficiency in the gill cells of *Mytilus edulis*. Aquat Toxiol. 57(3): 127-137.

Radhika R and Krishnamoorthy R. (2010) Effect of copper sulphate on histological changes in the fresh water fish *Oreochromis mossambicus*. J Ecotoxicol Environ Monit. 20(5): 431-435.

Ramakrishnan R and Sivakumar AA. (1993) Effect of quinolphos on the fish *Oreochromis mossambicus*. J Ecobiol. 5(1): 45-50.

Ranganatha Koundinya P and Ramamurthi S. (1979) Haematological studies in *Sarotherodon mossambicus* exposed to Atrazine. Poll Res. 13(4): 375-380.

Rangsayatron N, Pokethitiyook P, Upatham ES, Lanza GR and Singhakaew S. (2004) Ultrastructural changes in various organs of the fish *Puntius gonionotus* fed cadmium enriched cyanobacteria. Environ Toxicol. 19L 585-593.

Rao DMR, Devi AP and Murthy AS. (1981) Toxicity and metabolism of exdosulphan and its effect on oxygen consumption and total nitrogen excretion of the fish, *Macrognathus aculeatum*. Pesticide Biochem Physiol. 15(3): 282-287.

Ravindran K and Swami KS. (1987) Effect of DDT on some physical parameters of body and on tissue cations of the fish *Tilapia mossambica*. J Environ Biol. 5(4): 651-654.

Roy PK and Munshi JSD. (1991) Malathion induced structural and morphometric changes of gills of a freshwater major carp *Cirrhinus mrigala* (Ham.). J Environ Biol. 12(1): 79-87.

Saradhamani N, Shanthi K, Manimegalai M and Binu Kumari S. (2009) Efficacy of herbicide glyphosate on oxygen consumption of a freshwater fish, *Catla catla*. Indian J Environ Ecoplan. 16(1): 239-243.

Sarkar SK. (1999) Effects of two heavy metals (copper sulphate, cadmium sulphate) on the oxygen consumption of the fish *Cyprinus carpio* (Linn.). Uttar Pradesh J Zool. 19(1): 13-16.

Sax NI. (1974) Industrial pollution. Van Nostrand Reinhold Company, New York.

Schwaiger JK, Fent H, Stecher H, Ferling H and Negele RD. (1996) Effects of sublethal concentration of triphenyltinaceate on rainbow trout (*Oncorhynchus mykiss*). Arch Environ Contam Toxicol. 30: 327-334.

Shahul Hameed P and Vadamalai P. (1986) Effect of sublethal concentration of Dimethoate EC30 on feeding, growth, oxygen consumption and activity in

Macrones keletius (Dumeril). J Environ Biol. 7(4): 277-284.

Skidmore JF and Tovell PWA. (1972) Toxic effects of zinc sulphate on the gills of rainbow trout. Water Res. 6: 217-228.

Sprague JB. (1971) Measurement of pollution toxicity to fish.III. Sub – lethal effects and 'safe' concentration, Water Res. 5: 245-266.

Srivastava SK, Tiwari PR and Srivastav Ajai K. (1989) Chlorpyrifos induced histological change in the gill of freshwater catfish, *Heteropneustes fossilis*. Bolm. Fisiologia 13: 23-28.

Sriwastwa VMS and Maurya RS. (1991) Effect of cadmium stress on gill and intestine of *Mystus vittatus* (Bloch): Scanning electron microscopic study. J Ecobiol. 3(1): 69 -71.

Sunderman FW, Plowman MC and Hopfer SM. (1991) Embryotoxicity and teratogenicity of cadmium chloride in *Xenopus laevis*, assayed by the FETAX procedure. Ann Clin Lab Sci. 21(6): 381-391.

Teh SJ, Adams SM and Hinton DE. (1997) Histopatologic biomarkers in feral freshwater fish populations exposed to types of contaminant stress. Aquat Toxicol. 37: 51-70.

Temmink JHM, Bouwmeister PJ, De Jing P and Vandenberg JHJ. (1983) An ultrastructural study of chromate induced hyperplasia in the gill of rainbow trout (*Salmo gairdneri*). AquatToxicol. 4: 165-179.

Thophon S, Kruatrachue M, Upatham ES, Pokethiriyook P, Sahaphong S and Jaritkhuan S. (2003) Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environ Pollut. 121: 307-320.

Tont S. (1977) Food sources of the oceans: An outline status of potential. Environ Conserv. 4: 243.

Toor HS and Kamal Deep K. (1974) Toxicity of pesticides to the fish *Cyprinus carpio communis*. Indian J Exp Biol. 12(4): 334-336.

Vasanthi M and Ramasamy M. (1987) A shift in metabolic pathway of *Sarotherodon mossambica* (Peters) exposed to thiodan (Endosulfan). Proc Indian Acad Sci (Animal Sci.) 96(1): 56-61.

Venkatesan R and Subramanian N. (2007) Effect of copper sulphate on histopathological changes in the freshwater fish *Oreochromis mossambicus*. J Ecotoxicol Environ Monit. 17(4): 353-361.

Wendelaar Bonga SE and Lock RAC. (2008) The osmoregulatory system. In: The toxicology of fishes, (eds.) Di Giulio R.T. and Hinton D.E., CRC Press, Taylor & Francis Group, Boca Raton, F.L., pp. 401-415.

Wobeser G. (1975) Acute toxicity of methyl mercury chloride and mercuric chloride for rainbow trout *Salmo gairdneri* fry and fingerlings. J Fish Res Bd Can. 32(1): 2005-2013.

Yigit S and Altindag A. (2006) Concentration of heavy metals in the food web of lake Eqirdir, Turkey. J Environ Biol. 27(3): 475-478.