RESEARCH PAPER | Zoology | Volume : 5 | Issue : 3 | March 2015 | ISSN - 2249-555X

Histopathology of the gill and liver tissues of the fish Anabas testudineus exposed to heavy metals in tannery effluents

KEYWORDS: Anabas testudineus, gill, liver, heavy metals.

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ABSTRACT *The presence of heavy metals in our environment has been of great concern because of their toxicity when their concentration is more than the permissible level. These metals enter in the environment by different ways like industrial activities etc. In this study highlights the histopathological profile of gills seed and liver of Anabas testudineus exposed to tannery effluents. The fish samples were collected from the Bharath fish feed in Chennai. One hundred fingerlings of the fish were exposed to tannery effluent for four weeks. The liver and gill were removed every 7 days for histological examination. The results showed that the liver had the lowest metal concentrations compared with the gills.*

1. INTRODUCTION

Pollution has become a serious threat and has brought drastic changes and ill-effects to the growing population as well as the mother earth. Pollution of aquatic eco-systems is a major global problem since the past two decades. Rapid urbanization and industrialization has led to increased disposal of pollutants such as heavy metals, radio nuclides and various types of organics and inorganic into the aquatic environment. Extensive industrialization has measurably influences the quality of water of lakes, ponds and rivers all over the world (Anand chetna *et al* 2006).

Heavy metals constitute leather production is a major industry in India which makes a significant contribution to the country's foreign exchange earnings. During the process of leather making, several chemicals like $Cr (SO_4)_3$, NaCl, $Ca(OH)_2$, H_2SO_4, etc., are extensively used. Therefore the resultant effluent is enriched with Chromium (Cr) and salts (NaCl and SO_4). Chromium present in effluent is primarily in the less toxic trivalent form Cr (III).When this effluent is discharged into the soil, due to varying environmental condition, Cr (III) is oxidized to toxic hexavalent form which seldom remains as Cr (VI). Tannery industries produce great amounts of effluents of a different nature, most of them are highly pollutants. The toxic compounds discharged into air, water and soil get into food chain. When toxic substances accumulate in the environment and in food chains, they can greatly disrupt biological processes (Praveena and Jayantha Rao, 2013).

The organisms present in the aquatic environment may accumulate the toxic metals, which ultimately affect not only the productivity and reproductive capabilities of the organisms, but also the health of the protein. In aquatic animal species, fish are the inhabitants that cannot escape from the detrimental effects of the pollutants (Gbaruko and Friday 2007.). Studies carried out on various fish species have revealed that heavy metals may alter the biochemical parameters both in tissues and in the blood (Tilak *et al* 2001). Histology acts as an integrated parameter, providing a more complete evaluation of the organism's health, effectively mirroring the effects of exposure to environmental pollutants. Histopathological changes have also been widely used as biomarkers in the evaluation of the health of the fish exposed to contaminants both in laboratory and field studies (Das and Mukherjee, 2000). One of the advantages of the using histopathological bio markers in environmental monitoring is that this category of the biomarkers allows examining specific target organs including gills, liver, kidney, brain that are responsible for vital functions, such as respiration, detoxification, excretion and coordination.

Hence, the present study aims to investigate the histological alterations of gill and liver of fish *Anabas testudineus*, exposed to heavy metals in tannery effluents.

2. MATERIALS AND METHODS

2.1. Collection of sample

Healthy living specimens of *Anabas testudineus* weighing 0.6±0.5 gm were procured from Bharath Fish Seed India, Poondi, Thiruvallur District of Tamilnadu. They were transported to the laboratory in well-aerated containers, to avoid hyperactivity, physical injuries and stress to the fish. The tannery effluent for the present study was collected from a tannery at Ranipet, Vellore. Only the filtered effluent was used for the study.

2.2. Experimental Setup

The control and experimental fishes were maintained for 7, 14, 21 and 28 days to evaluate the long term effect of heavy metals in tannery effluent. The medium was changed once in two days and no mortality of fishes was recorded during the period of investigation.

2.3. Histopathological analysis

After exposure to the species *Anabas testudineus* fish were washed by tap water. The test fish were dissected out and different tissues like gill and liver tissues were fixed in Bouin's fixative for 24 hours. The tissues are cut into small pieces ($2mm^3$) and washed repeatedly in 70% alcohol and further dehydrated in graded alcohol in ascending order, cleared in xylene and finally embedded in paraffin then 4-6μ thick sections were cut. These sections were stained with haemotoxylin and eosin (Culling 1974). Light microscopy was performed and stained section was photographed.

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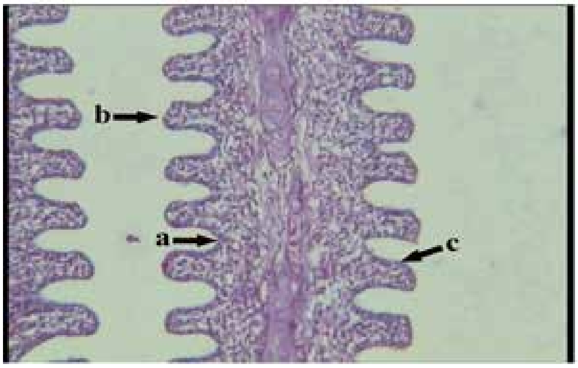
3. RESULTS

3.1. Gill histology

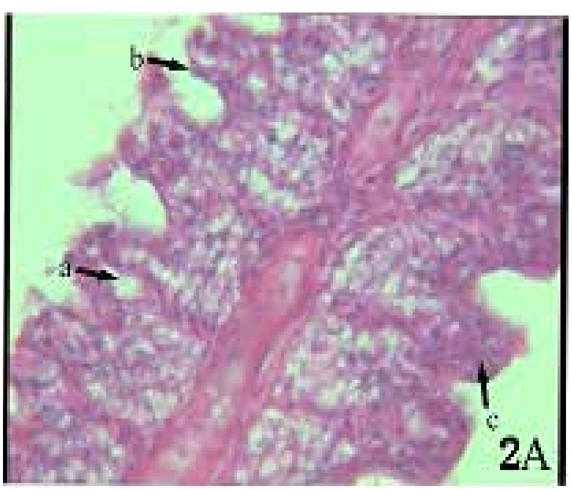
Control gills showed normal structure of lamella with wide interlamellar space [Figure 1]. Primary lamellae and secondary lamella were compact with very thin epithelium. Numerous semicircular secondary gill lamellae were lined along both sides of the gill filament. Hence, in the controlled set no histopathological changes were observed.

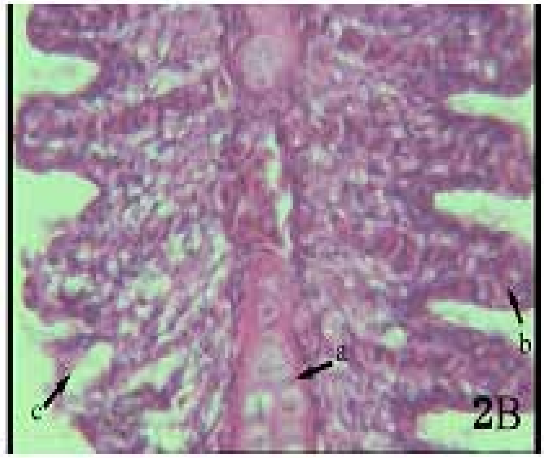
Figure 1: Microphotograph of normal structure of gill of control fish Anabas testudineus, showing inter lamellar cells (a), secondary lamellar (b), epithelial cells (c) [45X].



In tannery effluent treated gills [Figure 2] showed, the damages were much more pronounced after 28 days of exposuration. In 7 days of exposuration, the lifting of epithelium occurs. The epithelial necrosis and hyperplasia was also observed [Figure 2A]. In 14 days of exposuration, the epithelial lifting and hyperplasia occurs. Desquamation was also observed [Figure 2B]. In 21 days of exposuration, fusion of secondary lamellae and epithelial hyperplasia occurs. Shortening of secondary lamella was also observed [Figure 2C]. In 28 days very well distinct damages are occurred when compared to other days. The vaculation, hypertrophy of epithelial cells of secondary lamellae was also observed [Figure 2D].

Figure 2: Microphotograph of 7 days (2A), 14 days (2B), 21 days (2C) and 28 days (2D) exposed gill of fish Anabas testudineus, showing (2A) a-Epithelial lifting b- Epithelial necrosis c- Hyperplasis (2B) a-Hyperplasia of primary epithelial cells b- Hyperplasis c- Epithelial lifting (2C) a- Fusion of secondary lamella b- Shortening of secondary lamella c- Hyperpalsis (2D) a- Vaculation b- Shortening of secondary lamella c- Hyper trpopy[45X].





3.2 Hepatic histology

The liver of control fish showed large polyhedral cells within a network of minute canaliculi between the liver cells [Figure 3].The nuclei were vesicular with large nucleolus. Irregular distribution of bile duct, blood capillaries and sinusoids filled with erythrocytes were seen. Hepatocytes surrounding the central vessels were clearly observed. So in the control set no histopathological changes were observed.

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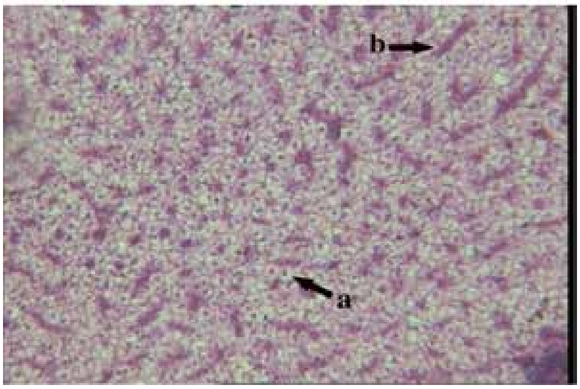
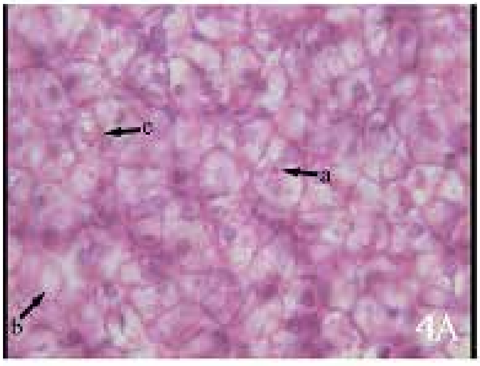
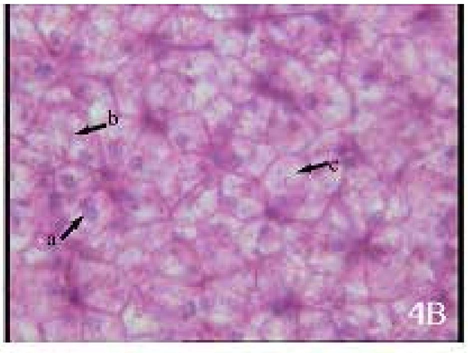
Figure 3: Microphotograph of normal structure of liver of control fish Anabas testudineus, showing hepatocyte (a), sinusoid (b) [45X].

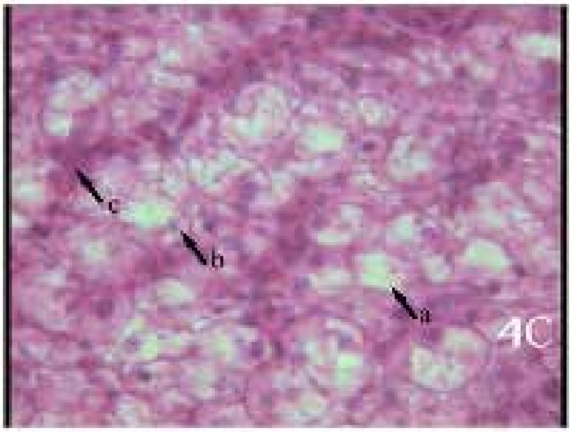


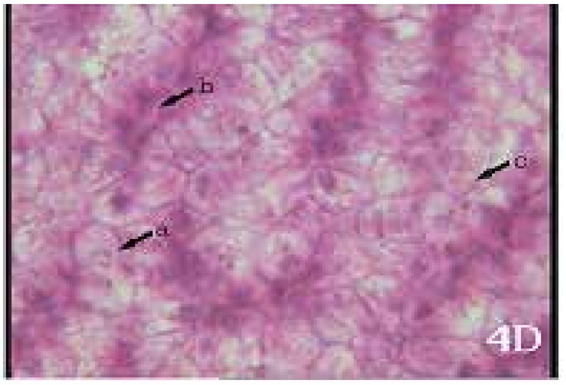
In tannery effluent treated liver [Figure 4] showed, to be compact and tightly arranged with no necrotic region. In 7 days treated section the cloudy, swelling of hepatocytes were seen [Figure 4A]. In 14 days treated section binucleated hepatocyte and cloudy degeneration of hepatocytes were evident [Figure 4B]. In 21 days treated section lipid vacuolation, karyolysis and dilation of sinusoids were visible [Figure 4C]. In 28 days treated section pycnotic nuclei, congestion, focal necrosis, cloudy, swelling of hepatocytes were observed [Figure 4D]. The more impact is seen in long day's exposuration.

Figure 4: Microphotograph of 7 days (4A), 14 days (4B), 21 days (4C) and 28 days (4D) exposed liver of fish Anabas testudineus, showing (4A) a- Cloudy of hepatocytes b-Vaculation c- Pycnotic nuclei (4B) a- Binucleated hepatocyte b- Cloudy degeneration of hepatocyte c- Lipoid vacuole (4C) a- Vaculation b- Karyolysis c- Dilation of sinusoids (4D) a- Pycnotic nuclei b- Dilation of sinusoids c- Congestion [45X].









4. DISCUSSION

Pollutant from tannery effluent causes deleterious effects on non-target organisms resulting imbalance of an ecosystem. Heavy metals caused the serious impairments in the metabolic, physiological and structural system which is present in high concentration. The severity of damage depends on the toxic potentiality of a particular compound or toxicant accumulated in the tissue and therefore, exposure to this heavy metal may adversely affect various organs/system in fish which ultimately could lead to overall toxic impact on organs like gill, kidney and liver may seriously affect the metabolic as well as physiological activities and could impair the growth and behavior of fish (Mazon *et al* 2002).

Histological studies helps to understand the pathological conditions of the fish. The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water, are considered the primary target of the contaminants. The present finding are in agreement with (Velmurugan *et al* 2007) who also observed the same histopathological changes in the gills of various fishes on exposure to different toxins resulting in necrosis, vacuolar degeneration, dystrophy, desquamation, epithelial lifting, shorting of secondary lamellae.

In the present study, the lesions were comparatively most severe in liver. The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position and blood supply (Vander oost *et al* 2003) it is also one of the organs most affected by contaminants in the water. Several studies have reported that chronic accumulation of some heavy metals in fish livers causes hepatocyte lysis, cirrhosis and eventually death (Varanka *et al* 2001). Some heavy metals accumulate in the food chain, causing long term health effects to aquatic life and eventually to humans (David 2002).

The present study showed that, heavy metals in tannery effluents altered the changes in the structures of the tissues like gill and liver of the fish *Anabas testudineus*. The toxicity of the tannery effluent was higher due to concentration of the chemical constituents which were more in tannery effluent. Hence, the environmental awareness becomes more necessary, since fish forms delicious component of human food and further it is poor man's dish, it may be concluded that the effluents from tannery factories presently evaluated cause lethal effects found in the surrounding area whether terrestrial or aquatic. So proper treatment of effluents is a necessary prerequisite system or environment.

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