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International Journal of Current Research
Vol. 6, Issue, 01, pp.4739-4742, January, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

IMPACT OF FERTILIZER AMMONIUM SULPHATE ON THE HISTOLOGY OF GILL AND LIVER OF FRESHWATER FISH *OREOCHROMIS MOSSAMBICUS*

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ARTICLE INFO

Article History:
Received 25th October, 2013
Received in revised form
16th November, 2013
Accepted 10th December, 2013
Published online 31st January, 2014

Key words:

Fertilizer, Ammonium sulphate,
Fish, Histology, Gills and Liver.

ABSTRACT

The toxic effect of ammonium sulphate on the histology of gill and liver of *Oreochromis mossambicus* was studied. The fish were exposed for 10, 20 and 30 days in 10% sublethal concentration of 96 h LC_{50} of ammonium sulphate (148mg/l). The gills exposed to sublethal concentration of ammonium phosphate showed mild histological alterations during 10 days of exposure. However after 30 days, fusion of gill lamellae, hypertrophy and degeneration of epithelium were prominent. Liver lesions consisted of vacuolation, degeneration of hepatocytes and disintegration of cell boundaries of hepatocytes. These changes occurred predominantly in the 30 days exposure.

INTRODUCTION

Farmers use various types of fertilizers to the crops for its growth. No doubt the crops may yield high quantity of grains, but on the other hand it causes environmental contamination like presence of toxic chemical residues in soil and water. Rainfall washes away fertilizers and other agricultural chemicals from widespread area. Natural waters are the ultimate recipients of fertilizer residues used for agricultural purposes which are transferred from land to water. Nitrogen pollution from agricultural sources is now considered to be a major problem in many regions of the world (Vidal *et al*., 2000) The aquatic organisms are sensitive to environmental changes. Sub-lethal concentrations of fertilizers may cause ecological imbalance of these organisms after sufficiently long time of exposure probably as a result of cumulative impact of impaired metabolic functions (Cheng and Chen, 2002). Fish 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. 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. That's why it must be included in human diet at least 1.3 kg per week (FAO, 1989). However, the fish habitats are being contaminated alarmingly through a number of aquatic pollutants (Rajathy, 1991). Among these pollutants fertilizers are most injurious to fish. These pollutants have not only depleted the fish stock but also have threatened the human health by incorporating into food chain (Thurston and Russo, 1983). In the present work, an attempt was made to evaluate the long term exposure effect of ammonium sulphate on the histology of gill and liver of the freshwater fish *Oreochromis mossambicus*.

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MATERIALS AND METHODS

Animal maintenance

The fish, *Oreochromis mossambicus* (Weight :30g ; Length 8 cm) were collected from the Udhayamarthandapuram Lake (N10° 26' 49.4" - E 79° 33' 12.8") is a bird sanctuary and it is located in Tiruthuraipoondi Taluk in Tiruvarur district, Tamil Nadu. They were acclimatized for 15 days in large cement tanks (Temperature – 28 ± 2°C**;** total hardness – 518 ± 23 mg/l; DO - 5.6 ± 0.2 mg/l; salinity - 1.2 ± 0.13 ppt and pH - 7.8 ± 0.04) previously washed with 1% potassium permanganate. The water as renewed every 24 h. The LC_{50} of urea for 96h was found out by using Probit method (Finney, 1971).

Preparation of stock solution and determination of 96 h LC_{50} value of Ammonium sulphate

Stock solution of ammonium sulphate was prepared by dissolving 1 g of ammonium sulphate in an appropriate amount of water (1ppt). For the determination of median tolerance limits or LC_{50} different concentrations of ammonium sulphate

were prepared from the stock solution. Three replicates were maintained for each concentration and 10 fishes of equal size and weight were introduced. The test water was renewed at the end 24 h and freshly prepared ammonium sulphate was added to maintain the concentration of ammonium sulphate at a constant level. The mortality was recorded after 24, 48, 72 and 96 h, and median lethal concentration (LC_{50}) values were calculated by the Finney method (1978). $1/10^{th}$ value of the LC_{50} value for 96 h was taken as the sublethal concentration (Sprague, 1971).

Sublethal studies

For sublethal toxicity tests 80 fishes were selected and divided into four groups (one control and three experimental) with 20 fish in each aquarium filled with water. The desired concentration (1/10 of 96h LC_{50} – 148 mg/l) 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.

Histology

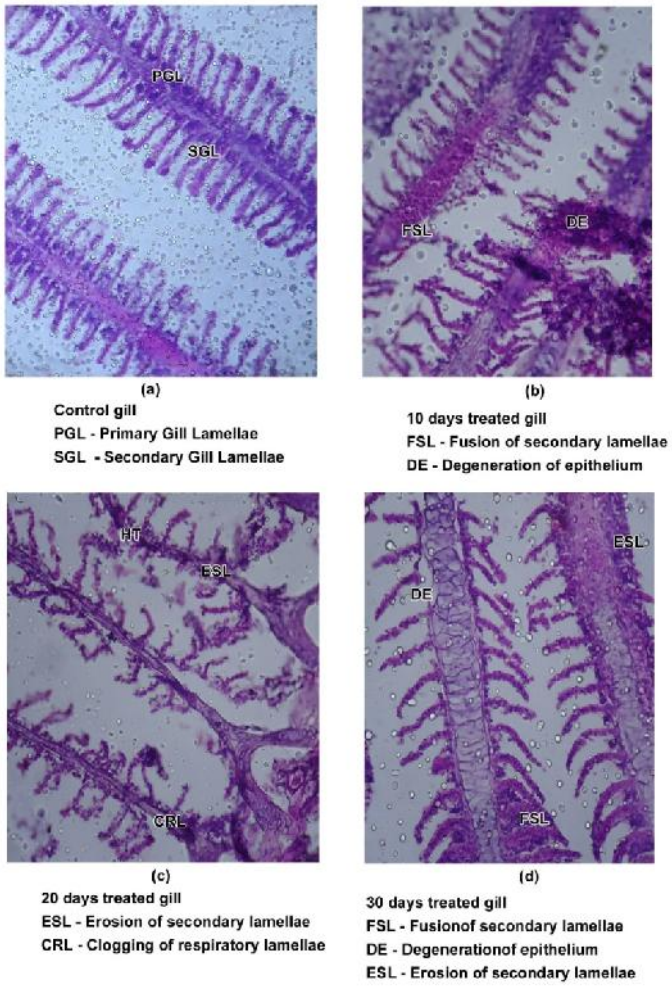
Light Microscopic Studies

On 10,20 and 30^{th} day fish were taken out, sacrificed and the gill and liver was excised out. The tissues was fixed in Bouin's fluid and then they were processed (Gurr, 1959) and embedded in paraffin wax (58 – 60^0C). Serial sections of 8 µm thickness were cut and deparafinshed sections were stained in haematoxylin and counterstained with aqueous eosin. The stained slides were examined for histopathological changes and were photomicrographed.

RESULTS AND DISCUSSION

In control fish, the secondary gill lamellae (SGL) appeared as finger –like structures. The SGL were thin, slender and attached on either side of the primary gill lamellae (PGL). The secondary gill lamellae are highly vascularised and surrounded by a thin layer of epithelial cells (Plate 1; Fig.a). The overall observed results in the present investigation indicates that marked histopathological changes have been found in the gill of fish *O. mossambicus* under sublethal concentrations of ammonium sulphate in chronic exposure. Fusion and shortening lamellae, hypertrophy, degeneration of epithelium and necrosis were found in the gills of ammonium sulphate treated *O. mossambicus* (Plate 1; Fig.b&c). Higher degree of hypertrophy and fusion of gill lamellae were prominent in the gills of fish exposed to 30 days (Plate 1; Fig.d). Hemalatha and Banerjee (1977a) and Ashok Kumar Gupta and Ashani Kumar (2006) noted similar types of gill lesions in zinc treated *Heteropneustes saccobranchus fossilis* and mercury treated *Cirrhinus mrigala* respectively. Kaud *et al.* (2010) observed severe hyperplasia in secondary gill lamellae which lead to complete embedding in adjacent lamellae in copper, cadmium, lead and mercury treated *Oreochromisniloticus*. In the present study, hypertropy and degeneration of secondary lamellae were apparent in *O.mossambicus* exposed to ammonium sulphate (Plate 1; Fig.a). These observations are quite comparable to pathological lesions induced in gills by mercuric chloride in *Acipenser persicus* fry (Zahara Khoshnood *et al*., 2011), by lead and cadmium treatment in Cyprinuscarpio(Bharat BhusanPatnaik *et al*., 2011), *Lates calcarifer* (Thophon *et al*., 2003), *Brachydanio rerio* and *Salmo gairdneri* (Karlson-Norgren *et al*., 1985). Patel and Bahadur (2010) also noted severe gill lesions in copper treated *Catla catla*. In the present investigation the gill epithelium of ammonium sulphate treated fish was completely desquamated, fuion and shapeless secondary lamellae and were broken at several places ((Plate 1; Fig.c,d). Daoust *et al*. (1984) also observed similar pathological lesions in the gill of copper treated rainbow trout, *Cyprinus carpio* respectively. Further, Hemalatha and Banerjee (1997a) and Al-Attar (2007) also observed such gill damages in zinc chloride and nickel treated *Heteropneustes fossilis* and *Oreochromis niloticus*.

PLATE - 1



(a) Control gill
PGL - Primary Gill Lamellae
SGL - Secondary Gill Lamellae

(b) 10 days treated gill
FSL - Fusion of secondary lamellae
DE - Degeneration of epithelium

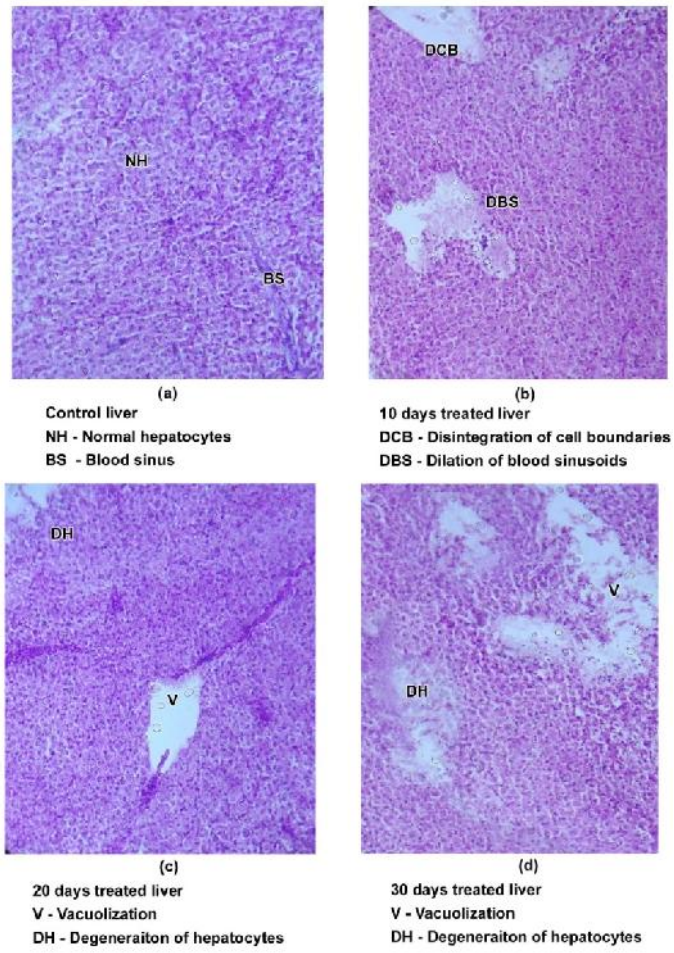
(c) 20 days treated gill
ESL - Erosion of secondary lamellae
CRL - Clogging of respiratory lamellae

(d) 30 days treated gill
FSL - Fusionof secondary lamellae
DE - Degenerationof epithelium
ESL - Erosion of secondary lamellae

Liver of fish is responsible for digestion, filtration and storage of glycogen (Atif M El-Naggar, 2009). The liver also produces many enzymes that stored in the gall bladder. The liver functions to store food energy (Tayel *et al*., 2008).The normal liver is made up of continuous mass of hepatocytes arranged in irregular cords. The hepatic cells are polygonal in shape with distinct nuclei. Large number of blood sinusoids were also seen around the hepatocytes (Plate 2; Fig. a). The fish exposed to the sublethal concentrations of ammonium sulphate showed the

vacuolation, loose arrangement of hepatic cells, histolysis and disintegration of cell boundries (Plate 2; Fig. b-d).

PLATE - 2



(a) Control liver
NH - Normal hepatocytes
BS - Blood sinus

(b) 10 days treated liver
DCB - Disintegration of cell boundaries
DBS - Dilation of blood sinusoids

(c) 20 days treated liver
V - Vacuolization
DH - Degeneraiton of hepatocytes

(d) 30 days treated liver
V - Vacuolization
DH - Degeneraiton of hepatocytes

The damage as more severe and progressive after 30 days exposure. Histological changes in the liver of fishes have been extensively reported. Athikesavan *et al*. (2006) reported the histological lesions in the liver of *Hypophthalamichthys molitrix* exposed to nickel. They observed marked changes like degeneration of blood vessels hypertrophy, vacuolisation necrosis and pyknotic nuclei of the exposed fish. The histological lesions due to cadmium and zinc poisoning were reported by Van Dyk *et al*. (2007) in *Oreochromis mossambicus*. Loganathan *et al*. (2006) and Radhakrishnan and Hemalatha (2010) have also observed the histological changes in the liver of zinc treated *Labeo rohita* and cadmium chloride treated *Channa straitus*. They observed that the cytoplasmic vacuolization of hepatocytes, congestion of blood vessel, leucocytic infiltration and necrosis. The results of the present observations in *O.mossambicus* exposed to ammonium sulphate were in agreement with those of the earlier workers especially in the vacuolization and necrosis in hepatic tissue. Intracellular vacuolation, necrosis and shrinkage of nuclei were also apparent in the present study in ammonium sulphate treated *O. mossambicus* (Plate 2; Fig.d). Similar changes are observed by Loganathan *et al*. (2006) in zinc treated *Labeo rohita* and by Karuppasamy (2000) in phenyl mercuric acetate treated *Channa punctatus*. The development of necrosis, congestion of hepatic blood vessels and vacuolization in ammonium sulphate treated *O.mossambicus* were mainly due to large scale accumulation of these metals in liver. Liver is the vital organ for detoxificationof unwanted and toxic substances (Soufy *et al*., 2007). Accumulation and elimination processes of metals ions in the liver may lead to hepatic lesions (Pragatheeswaran, 1987).

Conclusion

The results in the present study showed that the fertilizer ammonium sulphate has been proved to be harmful to fishes. Therefore, the information obtained may be useful for management and monitoring of fertilizer contamination in the aquatic environment.

Acknowledgements

The authors wish to acknowledge the Principal, Khadir Mohideen College, Adirampattinam for making available all the necessary facilities.

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