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Article : Toxic Effects of Ammonia on Biochemical and Histological alterations in the Different organs of Freshwater mussel, Lamellidens marginalis

Author : Ramesh. V. [Annamalai University, Chidambaram]
B. Amanullah [Jamal Mohammad College, Trichirappalli]
A. Rameshkumar [Annamalai University, Chidambaram]

ABSTRACT

Aquatic organisms are susceptible to pollution by pesticide as well as by industrial effluents. Higher concentration of the pollutant cause histological changes and also collapses the biochemical composition. The present paper documents biochemical alterations with special reference to protein, carbohydrate and lipids and histological changes in gills, hepatopancreas and visceral mass of freshwater mussel, *L. marginalis* exposed to sub lethal concentrations of Ammonia.

Key words: *L. marginalis,* Ammonia, Histology, Gills, Hepatopancreas, Visceral mass.

INTRODUCTION

Fertilizers used in agricultural fields reach the aquatic body via surface run-off and affect aquatic organisms in several ways (Tilak,2006). Pesticide and insecticides used in Agriculture are further seriously aggravating the problem of pollution both from the point of view of public health as well as aquaculture. The knowledge of pertaining to the effect of the toxicants on aquatic organism would help us to predict the potential danger of various chemicals of the environment (Pickering and Thatcher, 1970). Khan and Sha (2000) reported that the effects of nitrogenous waste, pesticides, and fertilizer on aquatic organism under chronic condition reflecting adversely affecting behavior, survival, growth and reproduction, histopathology and biochemistry. When animals exposed to different concentrations of nitrogen compounds, accumulate nitrate in their blood. Aquatic

organisms are affected by pollutions both directly and indirectly in various ways (Amanulla, 1994).

Ammonia is a naturally occurring product of biological metabolism, but high concentrations are often associated with human sources such as sewage treatment plants, agricultural and feedlot runoff, coal coking and gasification plants, and fertilizer manufacturing plants (EIFAC, 1973). Ammonia and nitrate in ground water originates primarily from fertilizers, septic systems and manure storage or spreading operations. This run off of fertilizers makes the nitrogen unavailable for crops but also can elevate the concentration in water above the levels acceptable (Mclesland, 1996). The toxicity of ammonia is mainly due to the ability to oxidize haemoglobin to methemoglobin ("*Blue Baby*"). Methemoglobin reduces the capacity of blood to carry oxygen to all parts of the body.

Chemicals from environment cause damages to gill cells of aquatic organisms. In gills, the histological changes include disruption and loss of cilia, vacuolization, rupturing of capillaries, bulging the tip of gills and degeneration of gills, necrosis was already reported (Bigas and Durfort, 2001). Mussel gill cells were studied by Mendikute (2005) reported that some large cells with abundant cytoplasmic granules were presumably haemocytic granulocytes in the gills of *mytilus*. Based on a literature survey, this paper specifically discusses the biochemical and histological alterations in hepatopacreas, gill and visceral mass of *L. marginalis*due to toxic effect of Ammonia.

MATERIALS AND METHODS

The test organism freshwater bivalve molluses, *L. marginalis* were collected from river Cauvery near Tiruchirappalli and brought to the laboratory. They were acclimatized in the lab and culturing animals in the fibre glass culture tank. The series of sub-lethal concentration of ammonia were prepared. The Physico-chemical parameters such as water, pH, DO, TSS, TDS, TS, Calcium (Ca), Chloride (Cl), Phosphate, Ammonia in culture tank water were measured using standard methods described by Trivedy and Goel (1986). Estimation of Protein, Carbohydrate and lipid content was done by Lowry *et al.* (1951), Anthrone method and Sulfophosphovanillin method of Barnes and Blackstocle (1973) respectively. The histological analysis of gill, hepatopancreas and

visceral mass was carried out by standard microtome and slide preparation technique. Staining was done with hematoxylin and eosin.

RESULTS & DISCUSSION

The physicochemical parameters such as DO, TSS, TDS, PH, Calcium, Chloride, Nitrate, Ammonia, Silicate, and Phosphate, of tank water were determined. The dissolved oxygen of the tank water was 6.7 mg/l., the total suspended solids and total dissolved solids were 640mg/l. and 800mg/l. respectively. The pH of the water was 7.35 and turbidity was 20NTU. Calcium, chloride, ammonia, nitrate, silicate, urea, sulfate, magnesium, total hardness, phosphate were 6.0mg/l, 7.6mg/l, 10.14mg/l, 7.1mg/l, 4.8mg/l, 6.97mg/l, 0.91mg/l, 3.2mg/l, 13mg/l and 5.4mg/l. respectively. The fresh water mussel *L. marginalis* were exposed to series of different sub lethal concentration of ammonia ranging from 100ppb to 400ppb for a period of 24 hrs, 48 hrs, 72 hrs & 96 hrs. The lethal concentration of ammonia (LC50) was 176ppb.

Biochemical analysis such as protein carbohydrate and Lipid were estimated in gill, visceral mass, and hepatopancreas of control and experiment bivalve mollusc in (20ppb) sub lethal concentration of ammonia. The estimation of protein content revealed that decreased in gill, visceral mass and hepatopancreas at 96 hrs treated ammonia when compared to that of control. Out of three organs, gill contains more protein (6.0 mg/g) then visceral mass (2.0 mg/g). The protein content of organs decreased twice (2mg/g) when the animal exposed at the higher concentrating of ammonia 20ppb (Fig.1) when compared to that of control (6.0mg/g). Decreased trend of protein at the higher concentration of ammonia confirmed the results obtained by Arunachalam *et al*., 1999. Results of carbohydrate analysis revealed that the decreased in gill, visceral mass and hepatopancreas of *L. marginalis* in 96 hrs exposed in the 20ppb sub-lethal concentrations when compared to that of control. Among the three organs, the carbohydrate content was higher in the visceral mass than hepatopancreas and gills. The carbohydrate content of hepatopancreas decreased (36 mg/g) more than control (80 mg/g) when the animal exposed 20ppb concentrations (Fig.1). The present findings were in conformity to those of Murthy and Priyadevi (1982) reported that the tissue glycogen may be converted into glucose to meet the energy required for the active movement of animals exposed to pesticides. The reduction in carbohydrate in the present study might be due to its utilization at the time of high energy demand warranted by the altered metabolism because of the toxic stress.

Estimation of lipid content shows that decreased in visceral and hepatopancreas of *L. marginalis* in 96 hrs exposed in 20ppb sub-lethal concentrations when compared to that of control. The lipid content among the organ showed that decreased trend (Fig.1). The lipid content of hepatopancreas decreased (28.8 mg/g) at 20ppb than the control (107.2 mg/g). The decrease in tissue lipid and protein might be partly due to their utilization in cell repair and tissue organization with the formation of lipoprotein, which is important cellular constituents of the cell membrane and cell organelles present in cytoplasm (Harper, 1983).

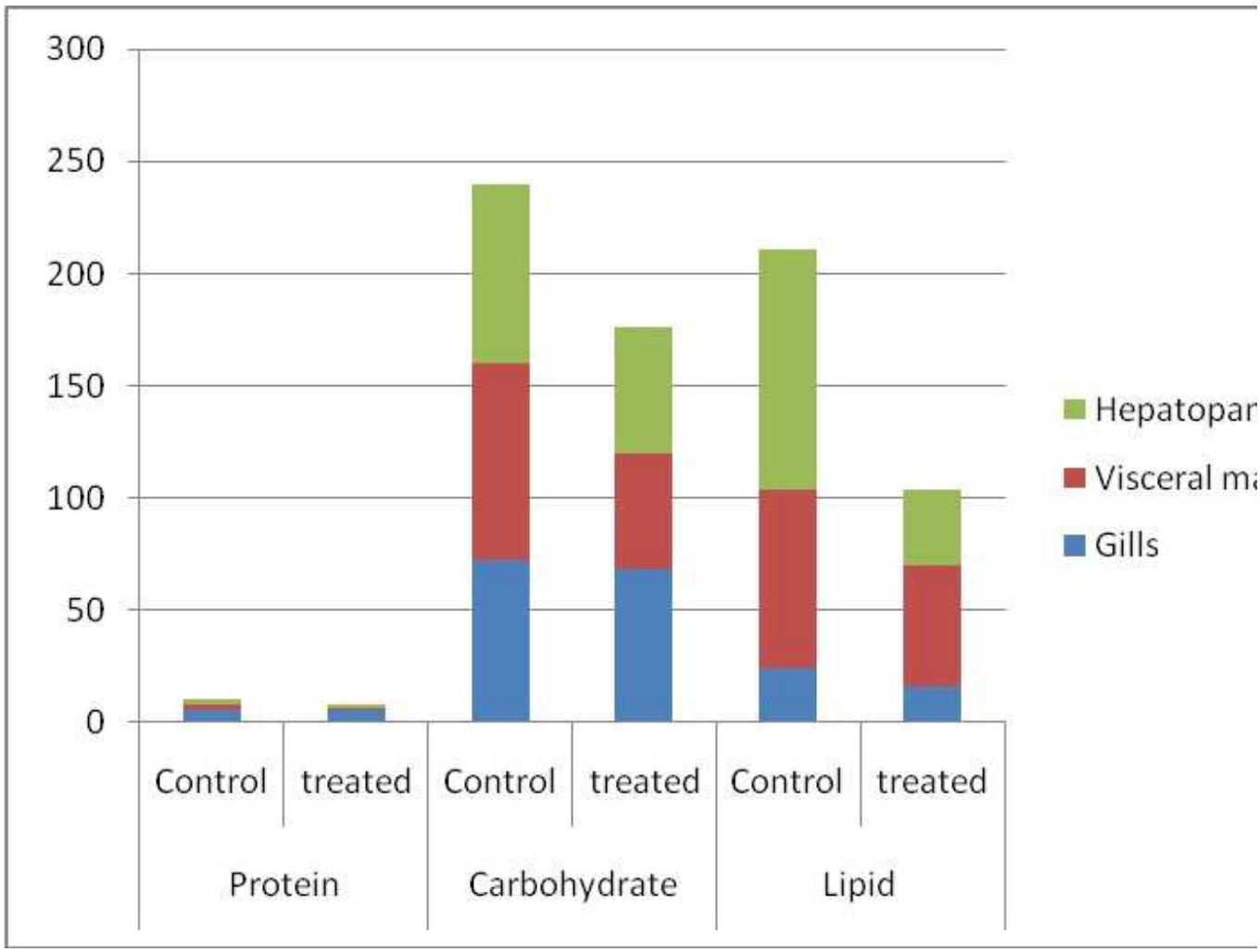
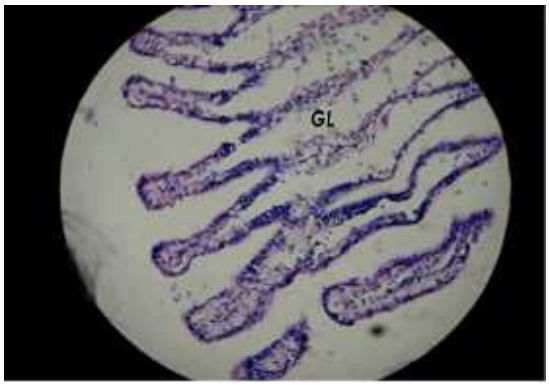


Fig. 1 Comparisons of protein, carbohydrate and lipid content in gills, visceral mass and hepatopancreas by the effect of ammonia.

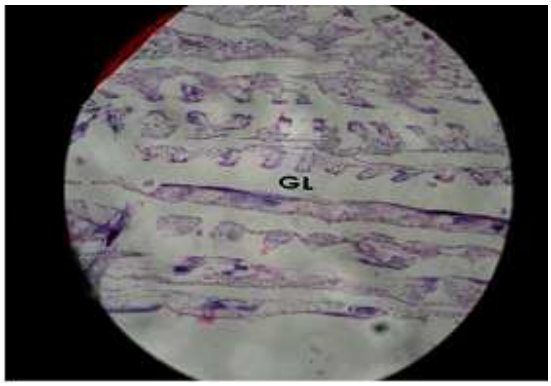
The histological studies revealed that changes occurred in different organs of *L. marginalis* when treated with (20PPB) sub lethal concentration of ammonia. Gills of *L. marginalis* treated with sub lethal concentration of ammonia were revealed that the changes of disruption and loss o cilia, vacuolization with dense fluid under the layer, mucous coating on the surface of gill lamellae (Bigas and Durfort 2001). The toxic nature of ammonia also affects the gill lamellae by bulging the tip of gill, degeneration of gill filaments, and necrosis of gill epithelium cells (Plate I a & b). The similar trend was obtained by Migliort (1990) who reported that the gill nuclei of mytilus affected by polluted marine water. In case of hepatopancreas, showed changes in the glandular cells and they are secretary in nature, swelling of hepatocytes, dilation and congestion of sinusoids, cytoplastic degeneration, nuclear disarray and excretion of nuclei in hepatocytes. Beyond that disruption of reticular pattern of hepatopancreas cells were observed (Plate I e & f). Visceral mass of treated animal showed that the apical region of epithelium of gut walls showed necrosis and vacuolization, the gut epithelium were fused and created spaces among the cell. The separation of epithelium from mussel strand was also observed. The degeneration of peritrophic membrane and muscular layer, losses of absorptive cells were observed in this analysis (Plate c & d).

In case of visceral mass, changes in vacuolization, fusion of gut epithelium, separation of gut epithelium from muscles and degeneration of peritrophic membrane are reported due to the ammonia. Similarly changes have been identified in hepatopancreas by swelling of hepatocyte, dilation and congestion of sinusoids and cytoplasmic degeneration (Mendikute, 2005) and they were all proved that lot of histological changes occurs in animals when they were exposed to stress conditions.

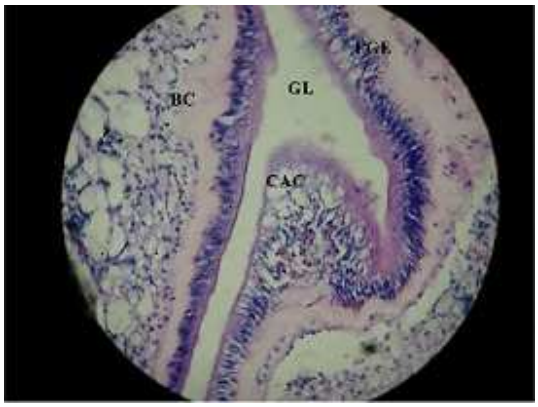
Fig. 2 Pictures showing T.S of Gill, Visceral mass and Hepatopancreas of Freshwater mussel, *Lamellidens marginalis*



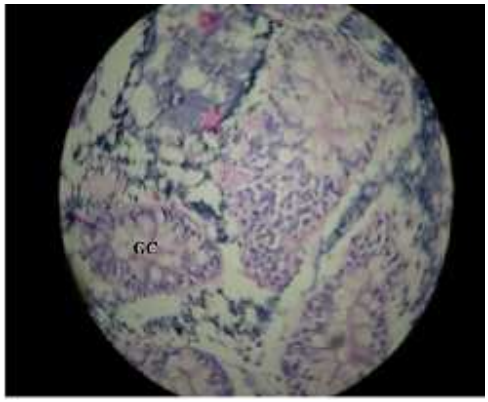
a) T.S OF GILL SHOWING NORMAL LAMELLAE (STANDARD)



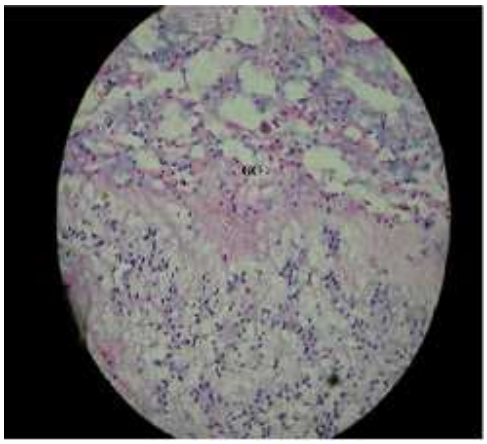
b) T.S OF GILL SHOWING LAMELLAE WITH ABNORMAL CONDITION (TREATED)



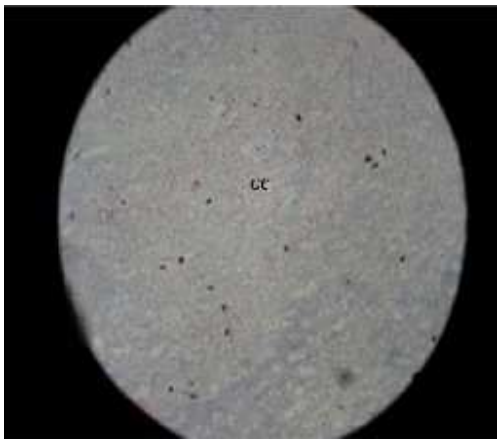
c) T.S OF PORTION OF VISCERAL MASS SHOWING THE GUT LUMEN IN NORMAL CONDITION (STANDARD)



d) T.S OF PORTION OF VISCERAL MASS WITH BLOOD CELL (TREATED)



e) T.S OF HEPATOPANCREAS SHOWING GLANDULAR CELLS WITH RICH SECRETION (STANDARD)



f) T.S OF HEPATOPANCREAS SHOWING GLANDULAR CELLS WITHOUT ANY VACUOLES (TREATED)

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