Fishery Technology 51 (2014) : 162 - 166

Effect of Urea, an Agrochemical on the Histology of Black Clam, *Villorita cyprinoides*

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Abstract

The indiscriminate use of agrochemicals results in run off contamination of water bodies and it adversely affects the aquatic flora and fauna in many ways. Urea is a nitrogenous fertilizer, widely used in agriculture. Toxicological studies were conducted to assess the extent of urea stress on the black clam, *Villorita cyprinoides.* Bivalves are filter feeders and can accumulate toxins in their soft tissues which form the edible portion of the animal. These pollutants cause cellular and tissue alterations, irregular reproductive patterns and also produce variety of lesions. In the present study, clams were collected from Vembanad Lake, Kerala, India and exposed to different concentrations of urea. Histology of *Villorita cyprinoides* was assessed after exposing the clams to sub-lethal concentration of urea, which is 0.7 g l^{-1}, for about 14 days along with a control. For histopathological studies, different tissues of clam *viz.,* foot, mantle and gill were excised, processed and stained in haematoxylin and eosin stain. In the experiment conducted, an acute histological response of the tissues to urea was observed in the exposed organisms. The results of the study show that the clams were affected by urea exposure and that it had caused severe damage to various organs.

Keywords: Histopathological effects, urea, fertilizer, black clam, *Villorita cyprinoides*

Received 20 July 2013; Revised 19 November 2013; Accepted 19 November 2013

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Introduction

Aquatic ecosystem is under pressure of anthropogenic pollutants originating from various point and non point sources. Discharge of partially treated or untreated wastes from factories, sewage and chemicals from agricultural operations are the main factors contributing to water pollution in the aquatic ecosystem. Animals inhabiting water bodies are unable to cope up with the changes the pollutants cause in the biological systems. The signs of stress become evident at different functional levels. Information on the responses of organisms with respect to pollutant stress is a necessary prerequisite for proper management of aquaculture. Bivalves have been extensively used as biomonitors as well as bio-indicators (Auffret, 1988; Bhamere, 1993; Pillai, 1993; Ittop et al., 2006; Dove & Sammut, 2007; Andhale et al., 2011) because of their sedentary nature and abundance. Besides, bivalves are very easy to sample, resistant to handling stress and tolerant to environmental variations to some extent.

Chemical fertilizers are extensively used in agriculture and fish culture practices. The indiscriminate use of these chemicals has got the potentialities of changing the aquatic media affecting the tolerance limit of aquatic flora as well as fauna, thereby posing danger to the ecosystem (Palanichamy et al., 1985). But studies on the toxicity of fertilizers on aquatic fauna are limited.

The nitrogenous fertilizer, urea is highly soluble in water and capable of easy penetration into the tissues of organisms (Srivastava & Sriwastawa, 1984). Overuse of urea can cause pollution. Srivastava & Srivastava (1981) studied urea-induced histopathology in kidney of the freshwater teleost *Channa punctatus*. Srinivasan et al. (1991) analysed the effects of urea on acid and alkaline phosphatase activities in a freshwater crab O*ziotelphusa senex senex*. But not many studies are available on urea toxicity in clam. The present study is aimed at investigating the histopathological manifestations of urea, on the black clam *Villorita cyprinoides.* Assessment of these alterations provides an insight into the degree of stress, susceptibility and adaptive capability of the stressed organisms.

Materials and Methods

Black clam, *V. cyprinoides* were collected from the Vembanad Lake, Kerala, India and transported to the laboratory. They were washed to remove debris and allowed to acclimatize for one week. Animals of almost same size (3.5 cm) were used for toxicity studies. Feeding was stopped 24 h prior to commencement of the experiment.

Toxicity bioassay experiments were conducted by exposing the clams to wide range of concentrations *viz.*, 6, 6.5, 7, 7.5, 8, 8.5 and 9 g l^{-1} of urea and later in narrow range concentrations *viz.*, 6.2, 6.4, 6.6, 6.8, 7 and 7.2 g l^{-1} of urea for 96 h. Mortalities were recorded every 24, 48, 72 and 96 h and dead clams were instantly removed. Probit analysis was run by using the SPSS version 16.0 statistical system (Finney, 1971). The 96 h LC_{50} was found to be 6.98 g l^{-1}.

Sublethal toxicity was conducted by exposing the clams to sublethal concentration, 0.7 g l^{-1} of urea for 14 days. A control was also included in the experiment. After the period of exposure, the clam shell was prised apart and the tissues *viz.*, foot, mantle and gill were excised for histopathological study. Tissues were immediately fixed in Bouins fixative and dehydrated in alcohol series. Tissue blocks were prepared, cut and stained in haematoxylin and eosin as per Weesner (1960).

Results and Discussion

Cumulative percentage mortality of *V. cyprinoides* observed in the experiment showed 6.98 g l^{-1} of urea as 96 h LC_{50} value (Fig. 1). The three tissues selected for the study exhibited alterations.

Foot, the highly muscular lower part of the body, is composed of outer multi-folded epithelial layer, thin connective tissue layer and inner muscular layer. The well developed muscular layer is composed mainly of collagen fibres and smooth muscle fibres (Fig. 2). After exposure of clam to urea, alterations like desquamation of epithelial cells, disruption of columnar muscle fibre and changes in the number of mucous cells were observed (Fig. 3). Muscle fibre degeneration and increased gap between muscle fibres in foot in Cd-treated blood clam, *Tegillarca granosa* has been reported earlier (Liu et al., 2012)

Mantle, a thick cellular wall inner to the shell, is structurally homogeneous. The mantle proper, formed of scattered skeletal muscle fibres, lies between a columnar epithelium layer on the side towards the shell and simple cuboidal epithelial layer lining the mantle cavity (Fig. 4). Internal and external lining of the mantle contains epithelial cells capable of secreting mucus. In bivalves, mantle plays a role in the bioaccumulation of organic and inorganic contaminants, although gills, kidney and digestive glands are considered as more important sites. Detachment of the external lining of the mantle, appearance of intramantle spaces, marginal disintegration of the external lining and presence of highly vacuolated cells were the conspicuous histopathological variations noticed during the study (Fig. 5). Disruption of columnar muscle fibres and alterations of epithelial layers were detected in

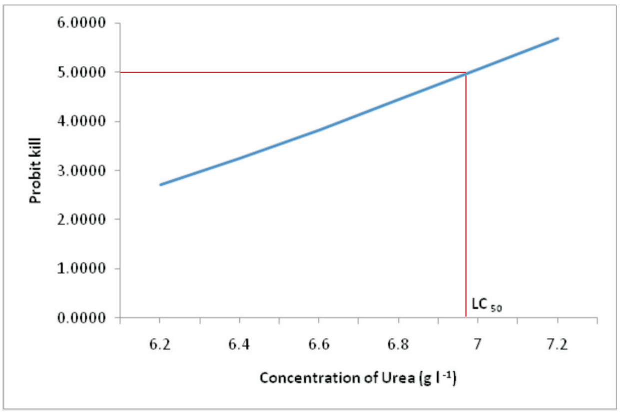

Fig. 1. Median lethal concentration of *Villorita cyprinoides* exposed to urea

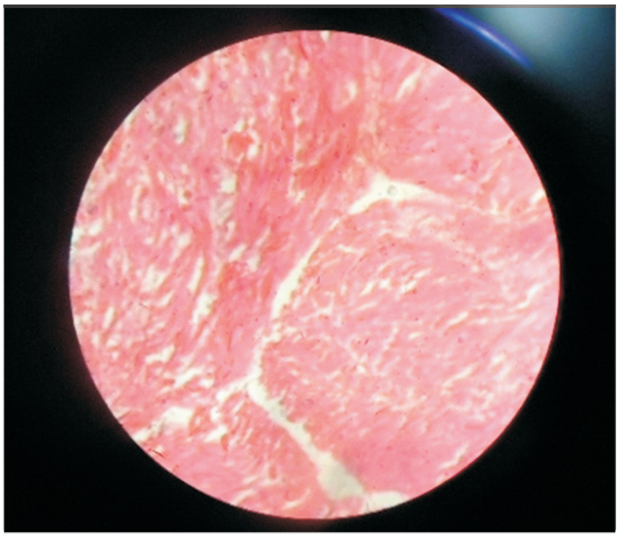

Fig. 2. Foot of *Villorita cyprinoides* (control) showing thickly packed muscle fibres arranged in bundles

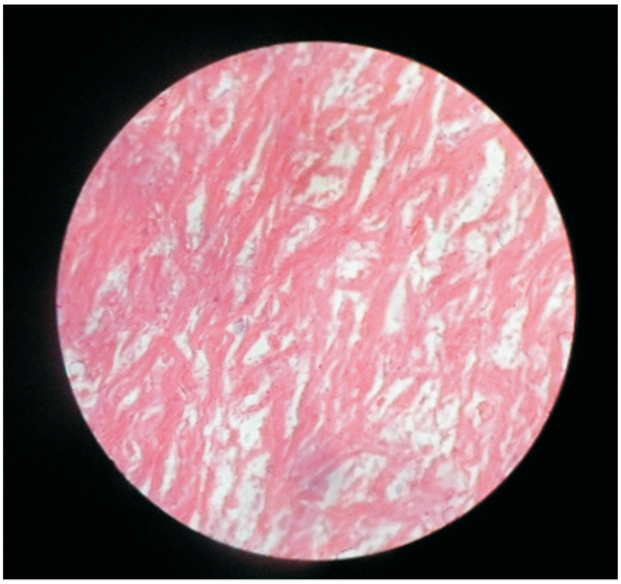

Fig. 3. Foot of *Villorita cyprinoides* exposed to sublethal concentration of urea.

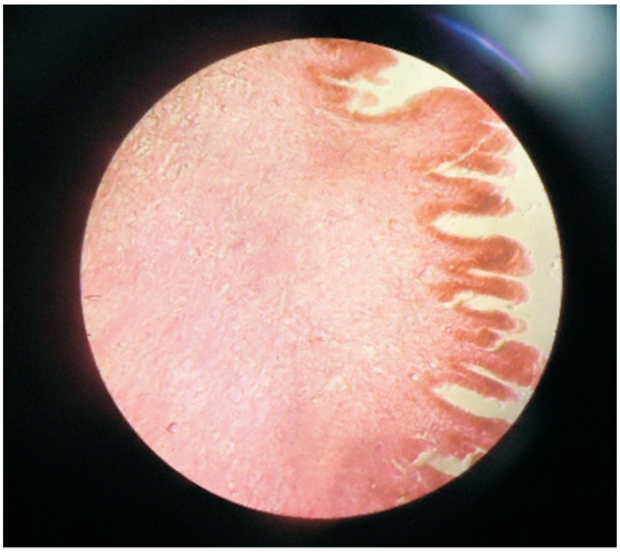

Fig. 4. Mantle of *Villorita cyprinoides* (control) showing the normal arrangement of muscle fibres and mantle edge

the foot and mantle of the urea-treated specimens. Alterations in tissues such as changes in the number and size of the mucous cells have been reported in foot and mantle of freshwater snail, *Galba truncatula* exposed to thiodan (Cengiz et al., 2005). Similarly, El-Shenawy et al. (2009) observed necrosis in the glandular cells of the mantle in clam, *Ruditapes decussatus* exposed to organophosphorous pesticides.

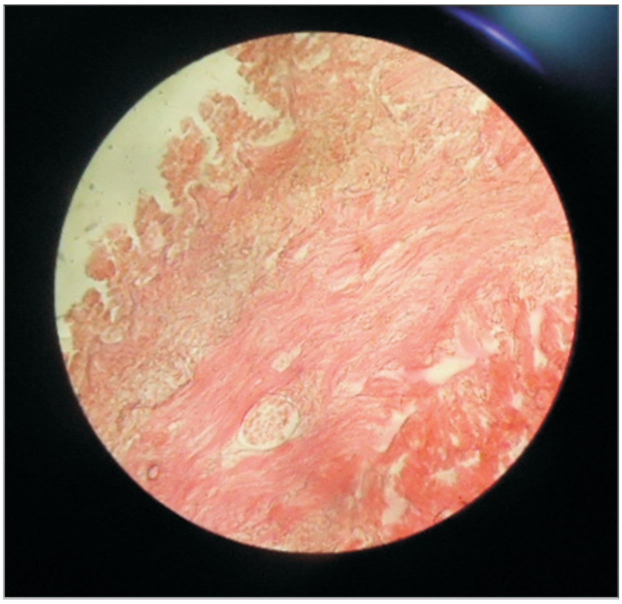

Fig. 5. Mantle of *Villorita cyprinoides* exposed to sublethal concentration of urea.

Gill filaments of the gill plates on either side of the body have the lining of ciliated columnar epithelial cells and a core of loose connective tissue with interlamellar tissue. The gill filaments are covered by different kinds of cilia and are supported by two chitinous rods (Fig. 6). Gills are the most suitable structure to study the acute histological response of the tissues due to its direct contact to pollutants. It is described as the target organ of accumulation of pollutants (Eknath, 1978). Since gills come in direct contact with urea in water, different levels of damage have been observed in the present investigation. Gills showed shrunken and flattened gill filament with damaged frontal cells devoid of frontal cilia, partial destruction of the interfilamentar junction, partial disappearance of the inner parts of the filaments, broken chitinous rod and detached lamellae at some places, damaged abfrontal muscles and broken epithelium (Fig. 7). Similar results were observed in the clam, *Ruditapes decussatus* exposed to organophosphorous pesticides (El-Shenawy et al., 2009). Gills of *Anadara antiquata* exposed to acute level of ammonium sulphate exhibited swollen lamellae, shrunken water tubes, loose inter-lamellar junction, unlimited mucous secretion and decrease in cilial length (Matojo & Pratap, 2009). Fusion of secondary lamellae, decrease in space between water tubes and interlamellar junction due to hypertrophy, severe damage to cilia, chitinous rods

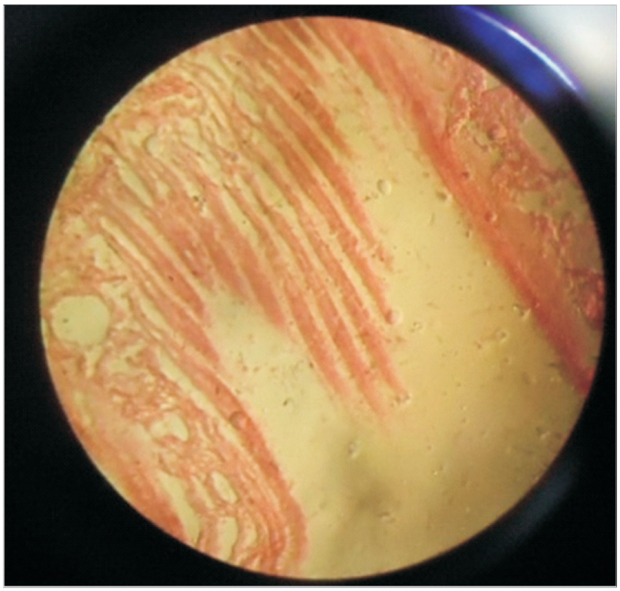

Fig. 6. Gill of *Villorita cyprinoides* (control) showing gill filaments.

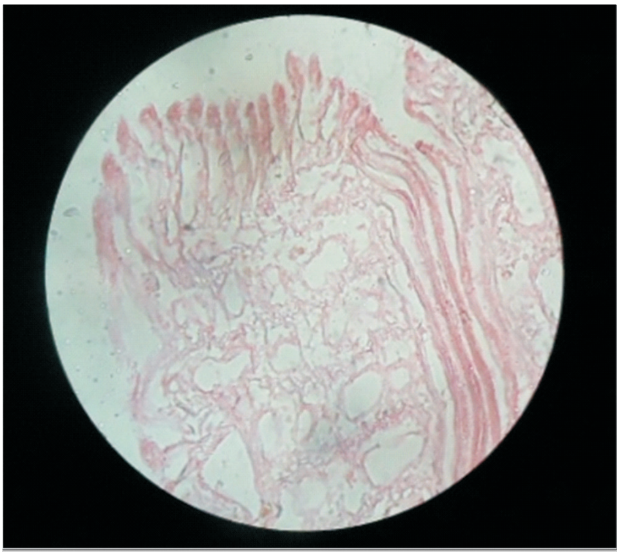

Fig. 7. Gill of *Villorita cyprinoides* exposed to sub-lethal concentration of urea.

and epithelia were reported in *Lamellidens marginalis* exposed to nickel (Andhale, et al., 2011)

Histopathological alterations in animal tissues are manifested as meaningful indications of cellular responses to pollutant induced stress. It is known that there will be structural and functional alterations in individual cell types or group of cells at an early stage of response before alteration in cellular structure could manifest at organismic level (Moore, 1980). Normally injured cells undergo structural alterations which are reversible or irreversible (Auffret, 1988). The ability of bivalve molluscs to concentrate pollutants in different tissues and apparently to survive and reproduce indicates that they have evolved control or tolerance mechanisms at the cellular level and organismic level (El-Shenawy et al., 2001). The extent of severity of tissue damage is a consequence of the concentration of toxicant and is time dependent. Also, the severity of damage depends on the toxic potentiality of particular compounds accumulated in the tissues (Rao, 1984).

From the study it is understood that urea, though not as toxic as heavy metals and pesticides, has deleterious effects on the histology of the clam *V. cyprinoides*. Thus urea pollution affects adversely the molluscan fishery both by contaminating the flesh, there by making it inedible to human beings and by reducing the population density of the mollusc. Quantification of toxicity of urea would help to adopt procedures for proper handling and use of this agrochemical.

Acknowledgement

Authors express sincere gratitude to the Principal, Sree Narayana College, Cherthala for providing facilities and the University Grants Commission for the financial assistance.

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