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Histopathology of copper toxicity in the Indian edible oyster, *Crassostrea madrasensis* (Preston)

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Abstract

Indian edible oysters (*Crassostrea madrasensis*) of average size 6.4 ± 1.2 cm x 4.3 ± 0.8 cm were exposed to two sublethal concentrations of copper viz. 0.5 ppm and 1 ppm at a salinity of 12 ppt for a period of four weeks. Adductor muscle, mantle and gill tissues were dissected out and subjected to histological examination. The results revealed extensive damages to the adductor muscle, mantle and gills of the copper treated oysters. The maximum damage occurred in the epithelial cells of all the tissues studied in the oysters exposed to copper. It was also observed that the physiological responses such as opening and closure of the shell valves and feed filtration were impaired in the animals exposed to copper.

Keywords: Edible oyster *Crassostrea madrasensis*, histopathology, copper toxicity

Introduction

Copper is an ingredient in a number of fungicides that are used in agriculture. These fungicides reach the water bodies through run off water. Copper contamination of aquatic environment has been a subject of concern as early as 1970's (Helz *et al.*, 1975). Although this metal is an essential trace element (Adelstein and Vallee, 1962), it has been shown to be lethal for many species even at very low concentrations (Roesijadi, 1980). Histopathological changes due to copper pollution have been reported in different species of molluscs. The present study was intended to reveal the histopathological changes caused by copper in important tissues of the Indian edible oyster, *Crassostrea madrasensis*.

Materials and methods

Maintenance of edible oysters: *C. madrasensis* (mean size 6.4 ± 1.2 cm x 4.3 ± 0.8 cm) collected from the backwaters of Kochi around Vypeen Island were stocked in 50 liter fiberglass tanks holding 30 liters of filtered and aerated seawater at a salinity of 12 ppt. The experimental tanks were cleaned after siphoning out dirt and faecal matter and 50% of water was exchanged daily. Water quality parameters were maintained at optimum range throughout the course of the experiment.

Experimental design: The lethal concentration of copper (LC_{50}) has been determined to be 5 ppm at a salinity of 12 ppt for *C. madrasensis* (Gijo, 2004). Animals were exposed to two sub-lethal doses, viz., 0.5 ppm and 1.0 ppm of copper for a period of four weeks along with a control. Appropriate amount of $CuSO_4$ $5H_2O$ was dissolved in water of 12ppt salinity to get the respective concentrations of copper ion. Each treatment had three replicates of 15 animals each. Throughout the course of the study the animals were fed algae (*Chaetoceros* sp.) *ad libitum*.

On termination of the experiment, the adductor muscle, gills and mantle tissues of animals were carefully dissected out and fixed in Bouin's fixative for 24 hours. The tissues were processed, embedded in paraffin wax and 5 μm sections were cut and stained by hematoxylin and eosin (Sanders, 1974). The sections were observed under a light microscope.

Results

The adductor muscle fibres of the control animals were enclosed by fascial sheaths of fibrous tissue, the epimysium (Fig. 1). The epimysium was covered with a layer of ciliated pseudo-stratified epithelial cells and below this there was a layer of glandular cells. In the animals exposed to 0.5 ppm copper, the epimysium was thickened and wavy, with a lot of fibrous tissue growth in the sub-epithelial layer (Fig. 2). In the perimysial area, tissue growth was seen. The muscle fibres appeared fragmented and hyalinized (Fig. 3). There were areas of muscle fibre necrosis with fibrous tissue growth (Fig. 4). In 1 ppm copper exposed animals, the surface epithelial cells of epimysium became shrunken and cilia were lost (Fig. 5). There was extensive necrosis and loss of muscle

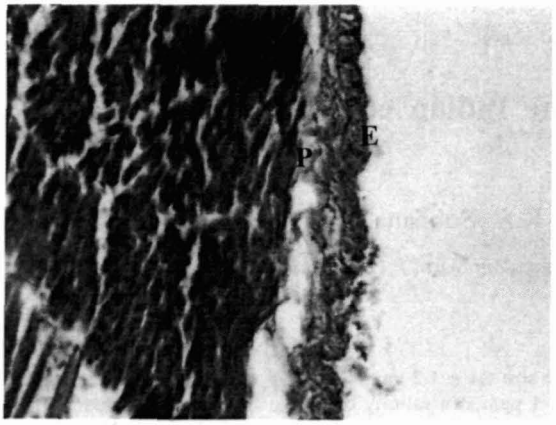

Fig. 1. Section of adductor muscle of normal, healthy C. madrasensis *showing epimysium (E) and perimysium (P) (X400; H & E)*

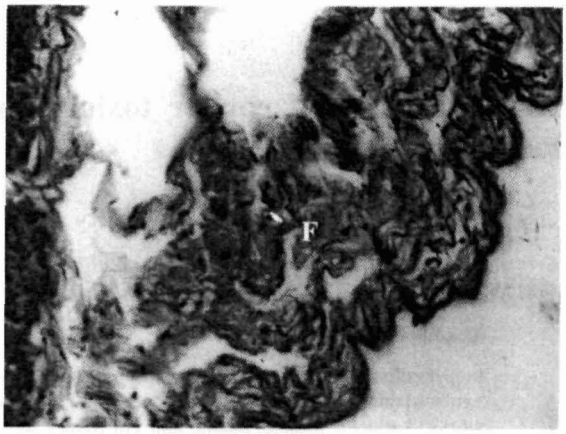

Fig. 2. Section of the epimysium showing thickening and wavy appearance and sub-epithelial fibrous tissue growth (F) in the animals exposed to copper (X400; H & E)

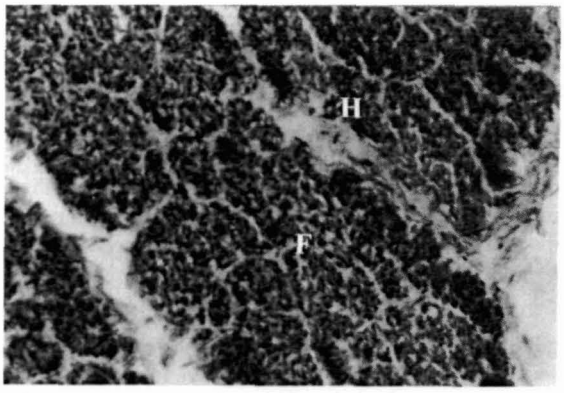

Fig. 3. Section showing muscle fiber fragmentation (F) and hyalinization (H) in the animals exposed to copper (X400; H & E)

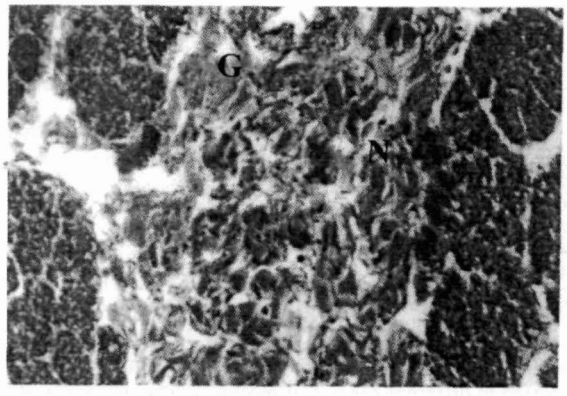

Fig. 4. Section showing muscle fiber necrosis (N) and fibrous tissue growth (G) in the animals exposed to copper (X400; H & E)

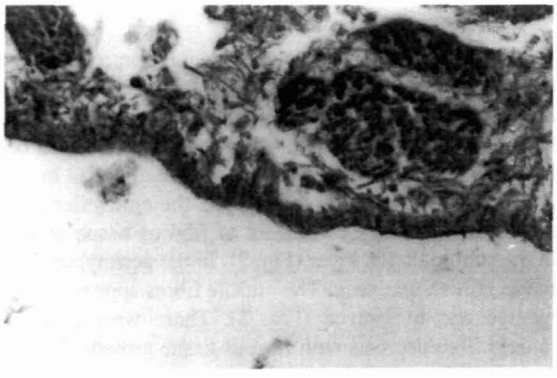

Fig. 5. Section showing loss of cilia in the epithelial cells of epimysium in the animals exposed to copper (X400; H & E)

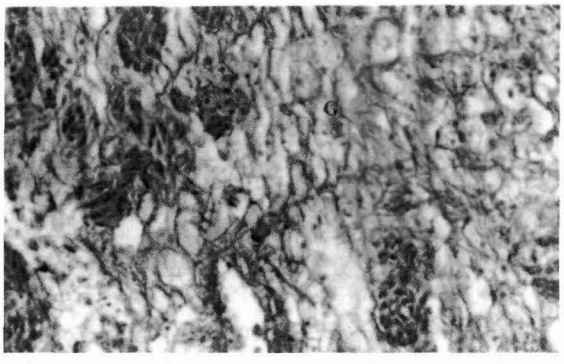

Fig. 6. Section showing complete necrosis of muscle fibers and growth of fibrous tissue (G) in the animals exposed to copper. A few islands of degenerating muscle tissue can also be seen in the section (X400; H & E)

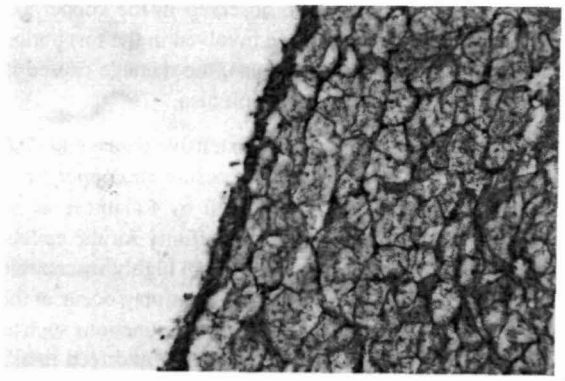

Fig. 7. Section of the mantle tissue of a normal animal showing columnar epithelium and sub epithelial connective tissue (X400; H & E)

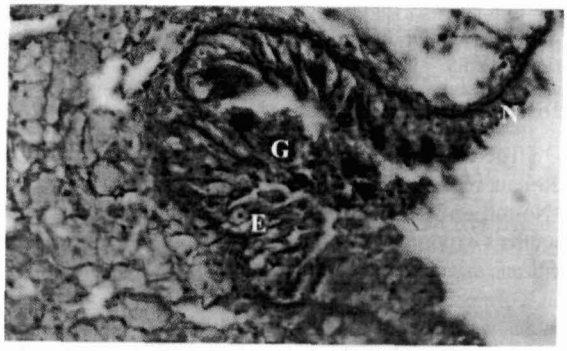

Fig. 8. Mantle section showing areas of necrosis (N), increased number of goblet cells (G) and area of epithelial proliferation (E), in the copper exposed animals (X400; H & E)

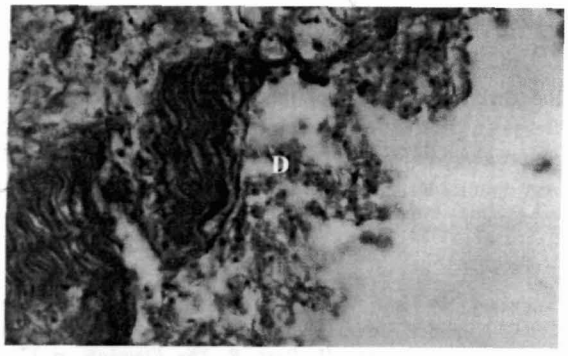

Fig. 9. Mantle epithelium showing extensive desquamation (D) in copper treated animals (X400; H & E)

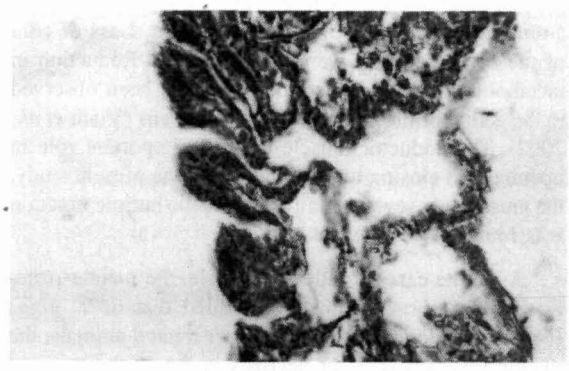

Fig. 10. Section of normal gill of C. madrasensis *(X400; H & E)*

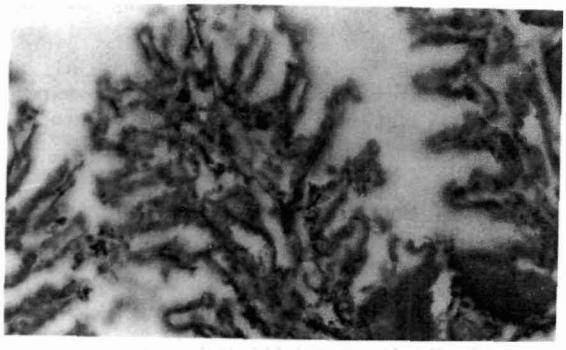

Fig. 11. Gills exposed to copper showing detachment of frontal, post lateral and abfrontal cells (X400; H & E)

fibres with growth of fibrous tissue (Fig. 6). The reflex response of the animals to physical disturbance by closing the shell valves was found to be impaired in the treated groups.

In control oysters, the mantle tissue comprised muscular and connective tissue network. The columnar epithelium covered the mantle (Fig. 7). The mantle surface epithelium in the 0.5 ppm treated animals revealed focal areas of necrosis and appearance of increased number of mucous secreting cells. In some areas, focal proliferation of the epithelial cells was also noticed (Fig. 8). In the animals maintained at 1 ppm copper, extensive desquamation of epithelial cells and in some areas signs of tissue regeneration were also seen (Fig. 9).

Gills of *C. madrasensis* consisted of filaments that were joined to each other by ciliary interfilamentar junctions. A branchial vein ran through each filament. The epithelium was composed of ciliated and unciliated cell types. The frontal cells and lateral cells were ciliated. The abfrontal cells were not ciliated. There were numerous glands in the abfrontal area (Fig. 10). The gills exposed to 0.5 ppm as well as 1 ppm copper showed detachment of frontal, post lateral and abfrontal cells (Fig. 11). The

feed intake was found to be markedly less in the treated groups which indicated impaired filtration of algal particles by the gills.

Discussion

The adductor muscle of normal animal revealed a structure similar to that described for *Tridacna gigas* (Norton and Jones, 1992). In the adductor muscle of animals exposed to 0.5 ppm of copper, thickening due to fibrous tissue growth was observed in epimysium as well as perimysium. Fragmentation and necrosis of muscle fibres were also noticed. In animals exposed to the highest dose of copper, the epimysium was shrunken, cilia were lost and further necrosis of the muscle fibres was also noticed. The changes were very prominent. A similar change in the muscles of the digestive diverticulae of *Mytilus edulis* was observed by Calabrese *et al.* (1984). In their study, varying degrees of myodegeneration and atrophy of muscle bundles were recorded. Loss of cilia of the epithelial cells were also observed. Reduction in metabolic rate in response to copper has been observed in the adductor muscle of *Haliotis rufescens* (Viant *et al.*, 2002). The adductor muscle plays an important role in opening and closing of shell valves. In the present study, the muscle was severely damaged and the muscle function was found to be affected.

As in the case of adductor muscle, the mantle structure of the normal animal resembled that of *T. gigas* (Norton and Jones, 1992). In copper treated animals, the mantle revealed areas of necrosis in the epithelium and increase in the number of goblet cells. Focal proliferation of the epithelial cells was also noted. In the higher dose (1 ppm), extensive necrosis was observed and signs of regeneration were also noticed. These changes indicate that copper produces mild to moderate irritation, which causes proliferative and secretory responses. The increased response of mucous secreting cells is reported in response to copper pollution (Viant *et al.*, 2002).

The structure of gills observed in *C. madrasensis* is very similar to that of *M. edulis* (Sunila, 1988). The gills of the copper exposed animals revealed detachment of frontal, post lateral and abfrontal cells and there was vacoulation of the post lateral cells in the present study. Necrosis and sloughing of the gill epithelium were noted in the whelk *Busycon canaliculatum* (Betzer and Yevich, 1975). Sunila (1986, 1988) has also reported the above changes in *M. edulis*. However, in *M. edulis* there was hemocytic infiltration in the gills of the animals exposed to copper. This was not observed in our study. Impaired feed filtration by the gills can be suggested as a reason for the reduced feed intake observed in the copper exposed animals. Since gills are involved in the respiration and gas exchange of the animals, the damage caused is likely to influence the respiration also.

The present study revealed extensive damage to vital tissues of *C. madrasensis* on exposure to copper for a period of four weeks. As reported by Calabrese *et al.* (1984) copper seemed to have an affinity for the epithelium of various organs rendering them highly susceptible to various opportunistic pathogens that may occur in the coastal environment. The physiological functions such as opening and closure of the shell valves and feed intake were found to be affected in copper exposed animals. The damages to the vital tissues will definitely have serious repercussions in other physiological functions also, thus lowering their general health and defense capacity.

Acknowledgements

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