



Histopathological Alterations and Bioaccumulation in Mussel *Perna viridis* Exposed to Sub-lethal Concentrations of Cadmium

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Abstract

Bioaccumulation of heavy metal, cadmium and its histopathological effects on gills and hepatopancreas in the Indian green mussel *Perna viridis* were assessed on the basis of 96 h LC₅₀. The mussels were exposed to sublethal concentrations of cadmium (0.01, 0.025, 0.05, 0.1, 0.2 and 0.4 ppm) for 21 days. The whole tissue of mussels was dried at 65°C for 72 h and was subjected to acid digestion and analysed for cadmium content using AAS. A dose and time dependent increase in accumulation of cadmium was observed for 15 and 21 days of exposure. Higher concentration (0.4 ppm) showed an abrupt increase in cadmium content compared to other lower concentrations. Histopathological analysis showed significant alterations in the structure of gills and hepatopancreas on cadmium exposure for 21 days. Loss of cilia, disruption of epithelial cells and hyperplasia were observed. Hepatopancreas showed bulging and enlargement of digestive tubule and disruption of collagenous layer.

Keywords: *Perna viridis*, cadmium, bioaccumulation, histopathology, hepatopancreas, gills

Introduction

Marine invertebrates accumulate trace metals to varying degrees and their body concentrations may reach high levels (Bryan, 1979). Molluscs have been used widely for monitoring trace metal pollution (Phillips, 1977; 1980; Bryan et al., 1980) and concentrations of metals in whole or parts of these

organisms and taken as a measure of ambient concentration. Moreover, the studies reveal that a simple linear relation exists between metal concentration in water and in marine organisms.

Mussels of the genus *Perna* have tremendous potential to accumulate potentially toxic trace metals in their tissues far in excess of the environmental levels particularly in warm waters (Rainbow, 1995). The green mussel, *P. viridis* has a wide distribution along the west coast of India and this species has been proposed as sentinel organism for marine pollution monitoring (Krishnakumar & Pillai 1990; Krishnakumar et al., 1998). Based on the International Mussel Watch approach developed a few decades ago, many studies proposed the use of *P. viridis* as a potential biomonitoring agent for heavy metals in the aquatic environment (Ismail et al., 2000; de Astudillo et al., 2005; Yap et al., 2007; Jalius et al., 2008; Soumady & Asokan, 2010). It fulfills the important criteria to be a good biomonitor because of its wide geographical distribution, sedentary lifestyle, reasonable abundance, availability throughout the year, easy identification and sampling and importance both ecologically and economically. Knowledge of accumulation and distribution of metals in the soft tissues may help us to understand the process involved in the uptake and excretion of metals by mussels.

Cadmium is widely distributed in earth's crust and it is principally used as stabilizer and pigment in plastics and in electroplating. It is being used routinely in different industrial processes and its potential hazard to life forms is documented. Molluscs can accumulate large concentration of cadmium ranging from 1900-2000 ppm dry weight (Clark, 1992). It was also reported that cadmium concentration is almost ten times higher in shell fishes than in fin fishes (Ololade et al., 2008).

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Therefore the present investigation was aimed at monitoring bioaccumulation of heavy metal cadmium by *P. viridis* and histopathological examination of three important tissues namely, gills, hepatopancreas and digestive gland.

Materials and Methods

Green mussel, *P. viridis* (length 3 to 4 cm) were collected from rocky shores of Chellanam, Ernakulam district. Samples were transported in plastic containers with sea water of ambient salinity. On reaching the laboratory, the samples were cleaned to remove algae, mud or other fowlers and then washed in a jet of water and acclimatized in the laboratory conditions for 48 h in filtered sea water. Salinity of sea water ranged between 30-35 ppt and pH between 8.15 and 8.30.

For LC₅₀ studies, ten mussels were randomly selected and exposed to cadmium chloride (CdCl₂) at a dose range of 0.05, 0.1, 0.5, 1 and 4 ppm for a period of 96 h. Sea water (2L/mussel) was changed every day and algal suspension as feed was given throughout the experiment. The number of mussels dead and alive was recorded on each day for each concentration. The experiment was done in triplicate and the average mortality for each concentration was taken and the LC₅₀ value was estimated by Finney's Probit Analysis (Finney, 1971).

Sub-lethal concentrations were selected on the basis of lethal toxicity studies of CdCl₂ on mussels. Ten mussels of shell length 3 to 4 cm were used for each sub lethal concentration in the range of 0.01, 0.025, 0.05, 0.1, 0.2 and 0.4 ppm and were inspected every 12 h for 21 days. During the course of experiment, sea water was changed daily and the animals were given algal diet.

The level of cadmium in the whole tissue of mussels was determined using the method of AOAC (2002). Whole tissue (edible portion) from the shells of mussels was excised out using a stainless steel scalpel. Tissues were dried in the oven at 65°C for 72 h and finely ground using a mortar and pestle and a portion (1 g) of it was subjected to acid digestion. The digested samples were filtered and made up to appropriate dilutions. The solutions obtained was filtered and analyzed using Air/Acetylene Flame Atomic Absorption Spectrophotometer (UNICAM 696 AA Spectrometer).

Gills and hepatopancreas from mussels exposed to different concentrations of CdCl₂ were dissected out and immediately fixed in 10% formalin. Tissues were treated with progressive series of absolute alcohol and then stained with haematoxylin-eosin and photographed using light microscope.

LC₅₀ value for CdCl₂ toxicity in mussels was calculated by Probit analysis. One-way ANOVA was conducted to find significant differences in cadmium accumulation between 15 and 21 days experiment using SPSS version 14.

Results and Discussion

The LC₅₀ value for cadmium in mussel was found to be 0.5 ppm. Cadmium concentration ranged from 0.077 to 1.466 µg g⁻¹ dry weight (Fig. 1) after 15 days of exposure. Post-treatment analysis of mussels exposed to sub-lethal concentrations of cadmium, showed an overall gain of 1.46 µg g⁻¹ and 1.981 µg g⁻¹ for higher concentrations, than the level detected in control animals over the 15 and 21 days period of observation respectively. Mussels exposed to sub lethal concentrations of cadmium accumulated 19 and 14.2 times higher level than which was detected in the lower concentration over the 15 and 21 days period respectively. It was also found that there was an abrupt increase in accumulation of cadmium from 0.2 to 0.4 ppm on 15 and 21 days of exposure. Significant (p<0.001) difference in cadmium accumulation was found by 15 and 21 days of exposure.

Many authors have reported that cadmium accumulates in the soft tissues of *P. viridis* (Yap et al., 2003; Soumady & Asokan, 2010; Kamaruzzaman et al., 2011). In the present study, the rate of accumulation

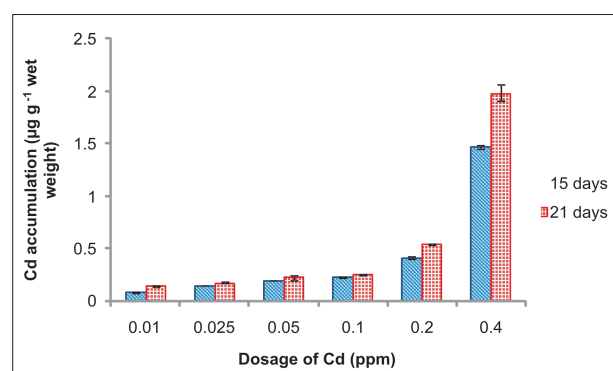


Fig. 1. Cadmium concentration in *P. viridis*.

of cadmium showed an increasing trend from 15th to 21st day of exposure. Similar observations were also made by Chan (1988) where a corresponding increase in the accumulation of cadmium was noticed with that present in seawater. According to Li et al. (2006), bivalves do not regulate cadmium and usually accumulate this element.

Injury of gill epithelium is common in response to extreme level of heavy metal exposure. Gill tissue of mussel exposed to sub lethal concentrations of cadmium showed major alterations like loss of cilia, hyperplasia, enlarged lumen and infiltration of haemocytes (Fig. 2A - 1F). Cells with a vacuolated appearance was observed which might be due to desquamation of epithelial cells, (Fig. 2D & E). Gill filament was highly elongated with a swollen lumen. Complete desquamation of epithelial cells with loss of cilia and clubbing of cells were the other striking alterations brought about by cadmium exposure (Fig. 2G). Severe loss of epithelium along with loss of structure was noticed in gills of animals exposed to 0.4 ppm cadmium (Fig. 2H). Sloughing of gill epithelium, swollen lamellae with haemocytes, loss of inter lamellar junction and hypertrophy were observed in mussels exposed to sub lethal concentrations of cadmium. Similar observations were also reported by Sunila (1988).

Gills are shown to be most susceptible to histological alterations resulting from toxic effects of metals (Moore 1985). Toxicants come in contact with gills first and animal responds by secreting excess quantities of mucus. Increased mucous secretion and its deposition on gills are known to be the effect of heavy metal toxicity (Cusimano et al., 1986). This may serve as a protective barrier to pollutants, especially heavy metals as a binding site to capture them before they can cause damage to the tissues, or as a means of expelling pollutants absorbed by secretory and other cells (Jose et al., 2005). Loss of cilia was a general observation. Denudation of cilia cause impairments in the functions such as intake of water, collection of food material and its transportation and can adversely affect the growth of mussel.

Presence of large number of haemocytes in the gill indicates internal haemorrhage. Choi et al, (2003) suggested that enlargement of gill cells is often accompanied by the presence of haemocytes, indicating tissue damage as well, mainly in the form of inflammation. Detachment and desquamation of

epithelial cells were common in all the samples. Some earlier studies have reported that epithelial tissues are the major sites for metal accumulation in molluscus (George et al., 1978; Marigomez et al.1990; Nigro et al. 1992; Abd Allah & Moustafa, 2002).

Histopathological examination of the hepatopancreas of cadmium-exposed mussels showed loss of digestive tubules, and became bulged and enlarged (Fig. 3 A & B). The flattened epithelial layer and presence of wandering haemocytes in the lumen of diverticula were significant changes observed in mussels exposed to lower concentrations of cadmium (Fig. 3 B). A sporadic sloughing off of the digestive tubule and extensive disruption of collagenous layer was noticed in exposure to higher concentration of cadmium (Fig. 3 C-E). Prolific vacuolisation and obliteration of lumen and wandering haemocytes were observed in mussels exposed to higher sub lethal concentrations (Fig. 3 F).

In gastropods and bivalves, digestive gland is the major site of heavy metal storage (Simkiss & Mason, 1983). Molluscs possess an efficient detoxification mechanism chelating the heavy metals especially cadmium with specific proteins called metallothioneins. Lipid rich digestive gland plays a central role in the accumulation and detoxification of various substances and considered to be a target organ in environmental pollution assessment (Klobucar et al. 1997). The present study noticed pathological changes like disruption of integrity of digestive tubules, separation of digestive cells from the basement membrane, reduced epithelium height, and tubular atrophy. Similar observations were reported by Salinas, (2012). Moreover, Sarasquete et al. (1992) reported degenerative processes in the digestive gland ranging from inflammatory responses to extreme vacuolation, particularly in cadmium exposed *Mytilus galloprovincialis* and clams, *Ruditapes philippinarum*.

From the study it can be concluded that the exposure of mussels to sub lethal concentrations of cadmium can severely affect the normal morphology and structure of vital organs like gills and hepatopancreas. The study also proved that the accumulation of cadmium is time and concentration dependent. Further studies on molecular biomarkers are needed to establish the effect of cadmium on the biological processes of mussel.

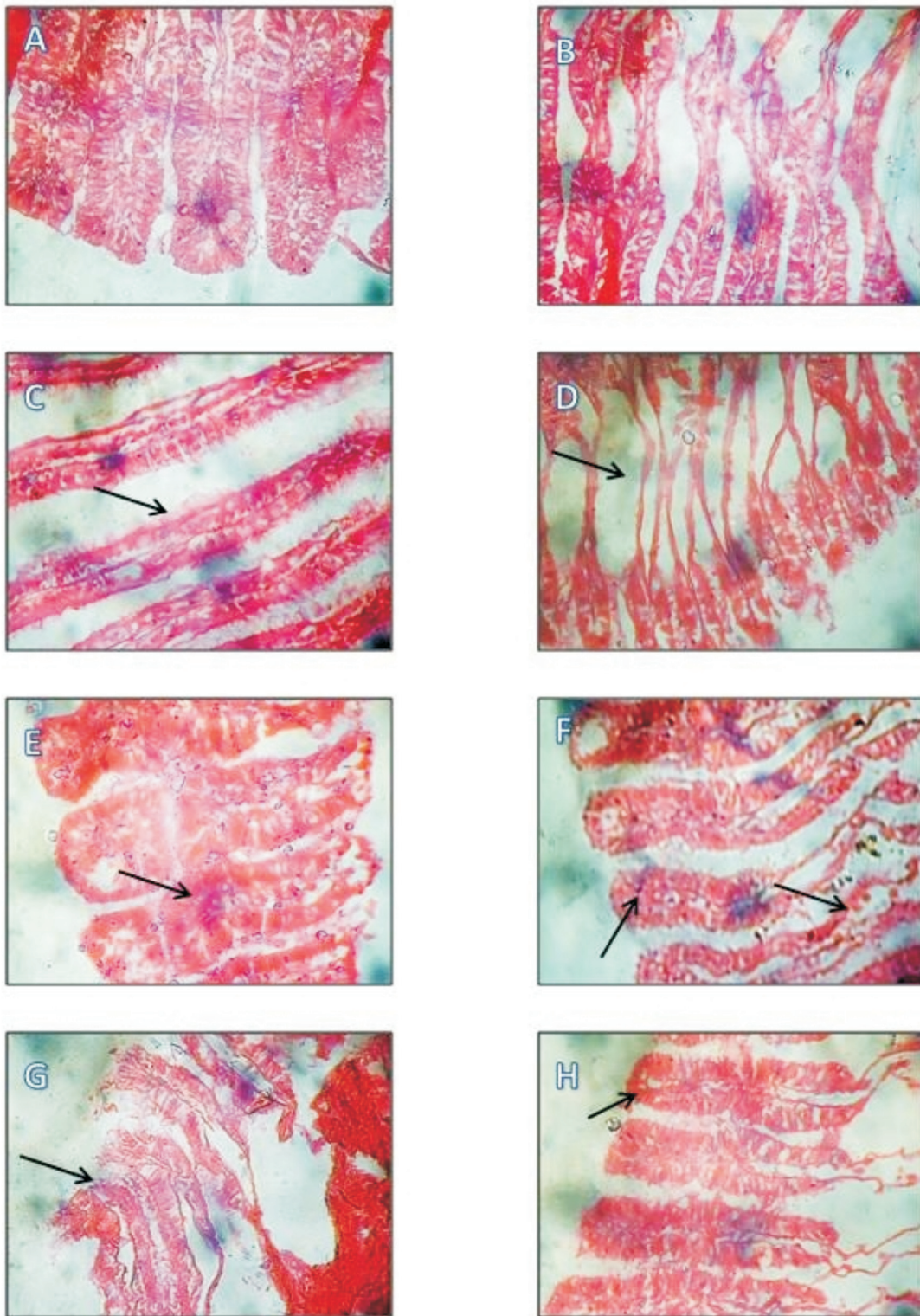


Fig. 2. Effect of sub-lethal concentrations of Cd on the gills of mussel *P. viridis* (400 X). A & B) Gills of control mussels (not exposed to Cd) showing intact gill lamellae. C) Gills of mussels exposed to Cd showing loss of cilia. D) Enlarged lumen. E) Gills showing hyperplasia and hypertrophy of cells. F) Haemocyte infiltration. G) Desquamation of epithelial cells. H) Massive necrosis of gill tissue.

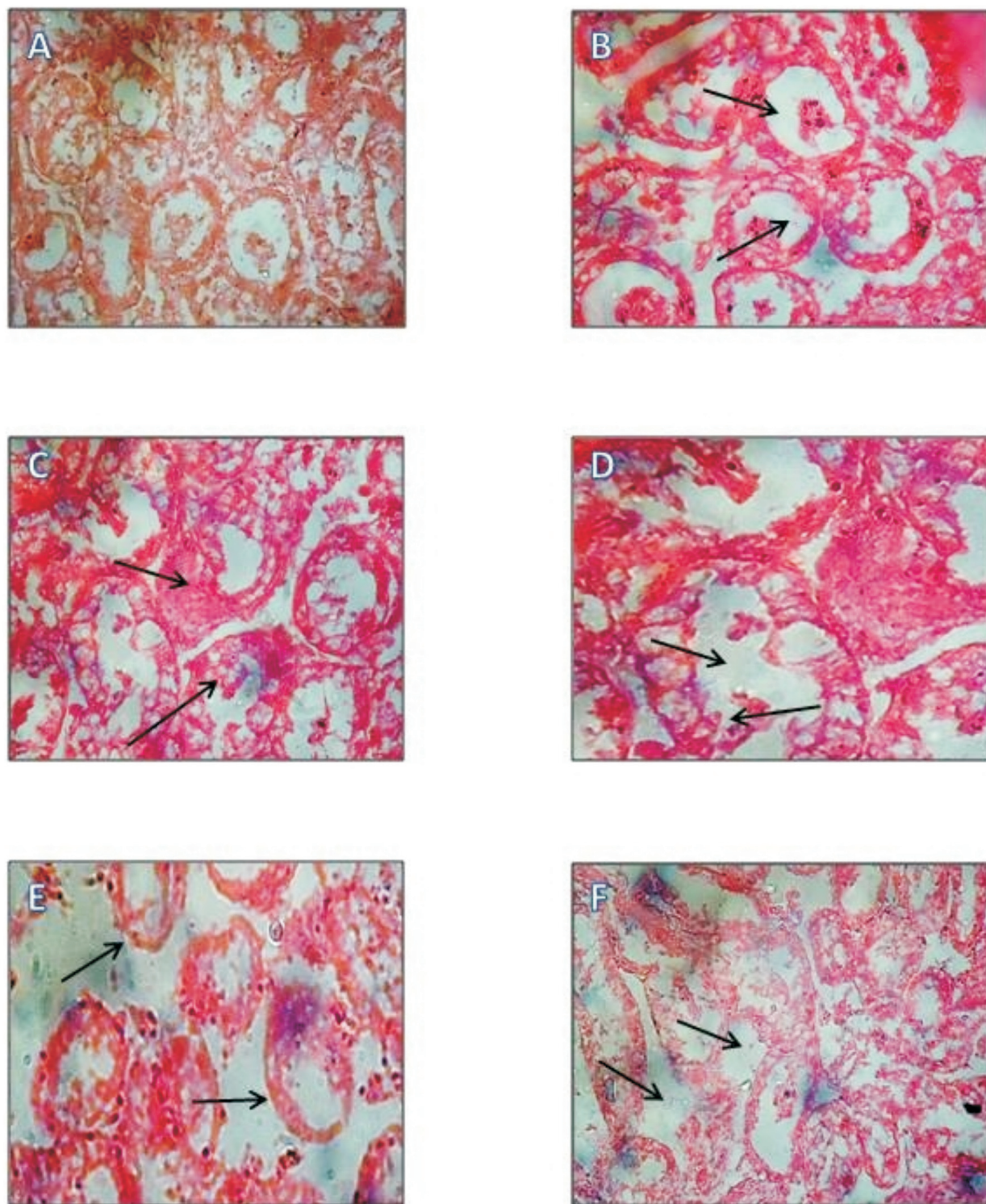


Fig. 3. Effect of sub-lethal concentrations of Cd on the hepatopancreas of mussel *P. viridis* (400 X). A) Hepatopancreas of normal control mussels (not exposed to Cd) with intact digestive tubules. B) Enlarged lumen, wandering haemocytes and flattened epithelial cells. C) Presence of sloughed off cells. D) Obliterated lumen and epithelial cells. E) Dislodged cells and disintegrated collagenous layer. F) Heavily vacuolated cells and wandering haemocytes.

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