ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 08, Issue, 11, pp.6801-6806, November, 2017

RESEARCH ARTICLE

TOXICITY OF CADMIUM ON THE OXYGEN CONSUMPTION AND GILL HISTOLOGY ESTUARINE CLAM *MERETRIX CASTA*

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ARTICLE INFO

Article History:
Received 17th August, 2017
Received in revised form
26th September, 2017
Accepted 14th October, 2017
Published online 30th November, 2017

Key words:
Cadmium, *Meretrix casta*,
Oxygen consumption, Gill histology.

ABSTRACT

The toxic effect of the heavy metal cadmium on the oxygen consumption and histology of gill clam *Meretrix casta* was studied. The clam were exposed for 10, 20 and 30 days in 10% sub lethal concentration of 96 hr LC_{50} of cadmium (1.25 mg/l). Cadmium significantly decreased the oxygen consumption rate. The oxygen consumption rate decreases exponentially with increase in exposure periods. Appreciable changes were noticed in the gills of mussels within 7 days of 10% sub lethal exposure. Sloughing of basal epithelium, damaged epithelial cells and swelling of lamellae with haemocytes were observed. The damage was more severe and progressive after 14 days exposure. Vacuolization of epithelial cells and loss of inter lamellar junctions were prominent in the gills of mussels exposed to 21days.

INTRODUCTION

Heavy metals are dangerous because they tend to bio-accumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, estuaries, sea and groundwater. Cadmium derives its toxicological properties from its chemical similarity to zinc an essential micronutrient for plants, animals and humans. Cadmium is bio-persistent and once absorbed by an organism, remains resident for many years (over decades for humans) although it is eventually excreted. In humans, long-term exposure is associated with renal dysfunction. High exposure can lead to obstructive lung disease and has been linked to lung cancer, although data concerning the latter are difficult to interpret due to compounding factors. Cadmium may also produce bone defects (osteomalacia, osteoporosis) in humans and animals. In addition, the metal can be linked to increased blood pressure.

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Industrialization, intensification of agriculture and rapid growth of human population have led to the increased discharge of pollutants, which are pernicious to the biotopes. Radionuc1eotides, petroleum hydrocarbons, pesticides and heavy metals are the four principal categories of pollutants, which jeopardize the marine environmental resources. Among these, heavy metals are most dangerous because of their stability in the biological system. Metallic contamination in a stream may constitute a menace to public health if the water is to be used subsequently for drinking and other purposes. The effects of the different heavy metals on the aquatic ecosystems have been experienced in recent years. Rapid industrialization and urbanization have led to the utilization of heavy metals on a large scale. These metals ultimately enter in to the aquatic ecosystems directly as effluents or indirectly by precipitation, resulting in an alarming rate, there by causing harmful effects to aquatic life. In general trace metal due to their persistence, have a strong attraction to biological organisms and the slow acclimation of these chemical in the biological systems, have led to their accumulation in the body tissues, resulting in the stimulation, irritation and inhibition of a variety of body functions. The ecotoxicological studies on the effect of pollutants are very much essential to know the physiological consequences. Several workers studied the effect of heavy metals on biochemical composition (Sunda and Guillard 1976; Andrew *et al.,* 1977; Moore *et al.,* 1988; Sreekala Pillai and Menon, 1998) were studied. With respect to tissues of bivalves has been reported that heavy metals exert inhibitory effect on

physiological processes such as ciliary activity of the gills and oxygen consumption (Brown and Newell, 1972; Manely, 1983 and Calabrese *et al.,* 1984) studies on the histology and histopathology have proved to be a very useful tool in assessing the pollutant induced injury to whole animal. Pathological changes caused by various pollutants in the gills of some species of bivalves have been reported by Ansell, 1961; Barnes, 1980, Stainken, 1975 and Muthukumaravel *et al*., 2008. The bivalves feed and respire by means of water current drawn into its body under the influence of the ctenidial cilia. The filtration rate of bivalves i.e. their ability to clear the particles of a certain size in a unit of time, is known to be influenced by environmental factors such as salinity, temperature, DO and concentration of suspended matter (Cole and Hepper 1954; Badman, 1975; Foster and Smith, 1975 and Mane, 1975). Through much data are available on the acute toxicity of many species, information on sub lethal effects of metal on the rate of oxygen consumption and histopathology of gills are scanty. The present work has been carried out with a view to the effect of heavy metal cadmium on the rate of oxygen consumption and gill histopathology of the estuarine clam *Meretrix casta.*

MATERIALS AND METHODS

The acute bioassay was analysed following the method of Litchfield and Wilcoxen (1949). The 96 h LC_{50} value for cadmium in *Meretrix casta* was found to be 1.25 mg/l. The clams were maintained in four groups. Group1 served as controls, Groups 2, 3 and 4 were exposed to 10% sub lethal concentration of chromium for 7, 14 and 21 days respectively.

Estimation of oxygen consumption

A series of rectangular glass jars, each with one L capacity were used as aquarium. They were filled with water. Care was taken to avoid trapping of air bubbles. Only one mussel was introduced into each aquarium and a thick layer of coconut oil was spread on the surface of the medium to prevent the contact of the medium to the atmosphere and to prevent the mussel from reaching the atmospheric air. Before starting the experiment, the initial oxygen content of water used for the preparation of animal chambers was estimated by collecting a sample into a narrow mouth, glass stoppered sample bottle of known volume following the Winkler's method (Annon, 1984). A healthy mussel was allowed to respire for one hour in animal chambers. After one hour, samples from respiratory chamber were taken into the sample bottle of known volume through siphon system and the dissolved oxygen was estimated.

Determination of oxygen content of the sample

The initial oxygen content of water was determined by collecting the sample in a narrow mouthed glass stoppered sample bottle of known volume. To this 1 ml of manganous sulphate solution was added followed by addition of 1 ml of alkaline iodide solution. The bottle was stoppered and shaken vigorously and kept in a dark place to prevent any photochemical reaction for about 15 minutes. A few drops of conc. sulphuric acid were added into the sample bottle in order to dissolve the precipitate. The precipitate was completely dissolved by shaking vigorously.

Twenty ml of the sample was taken in a clean conical flask and the liberated iodine was titrated against sodium thiosulphate using four to five drops of starch as indicator. The disappearance of blue colour was taken as end point. The burette values were tabulated. The final oxygen content of the respiratory chamber was also determined in the same manner. Oxygen consumed by the fish was calculated by finding out the difference between the initial and final oxygen content in the animal chambers. Also the rate of oxygen consumption per gram weight of the mussel per hour was calculated and the values were expressed as ml O_2/gm/hour.

The dissolved oxygen content in the water was calculated using the following formula:

$$O_2 \text{ content ml/litre} = \frac{K \times 200 \times \text{volume of } Na_2S_2O_3 \text{ consumed} \times 0.698}{\text{Volume of the sample titrated}}$$

$$\text{Where } K = \frac{\text{Volume of sample bottle}}{\text{Vol. of sample bottle} - \text{Vol. of reagents added}}$$

(0.698 is the conversion factor to convert parts per million to ml/litre)

(200 is the constant which is obtained by multiplying the equivalent weight of oxygen and normality of $Na_2S_2O_3$ and 100 ml).

Histology

Meretrix casta were exposed to 10% sub lethal concentration of Cadmium for 21 days. On 7,14 and 21 days, clam were taken out sacrificed and the tissues of gills were excised out. The tissues were fixed in Bouin's fluid and then they were processed adopting the usual procedure (Gurr, 1959) and embedded in paraffin wax (58 – 60ºC). Serial sections of 8 µm thickness were cut and deparafinised sections were stained in haematoxylin and counter stained with aqueous Eosin for microscopic observation.

RESULTS

Rate of oxygen consumption

The rate of oxygen consumption of *M. casta* after chronic exposure to cadmium showed significant alterations (Table 1& Fig.1). In the present study an acceleration in respiratory rate was observed in bivalves exposed to sublethal concentration of 10% on 7 days of exposure. However a decline in the rate of respiration was recorded when they were exposed for a prolonged period of 14 and 21 days. Maximum decline (-70.8%) in the rate of O_2 consumption was recorded on 21 days of exposure.

Table 1. Rate of oxygen consumption of *M. casta* after chronic exposure to cadmium (ml O_2 consumed / g / hr)

S.No.	Medium	Period of exposure (days)		
		7	14	21
1.	Control	0.367 ± 0.027	0.361 ± 0.042	0.367 ± 0.032
2.	10% SLC	0.499 ± 0.034	0.202 ± 0.061	0.107 ± 0.068
3.	Variation (%)	+35.96	-44.5	-70.8

Each value is the mean ± SD of five individual observations.
+ or - denotes % decrease over control

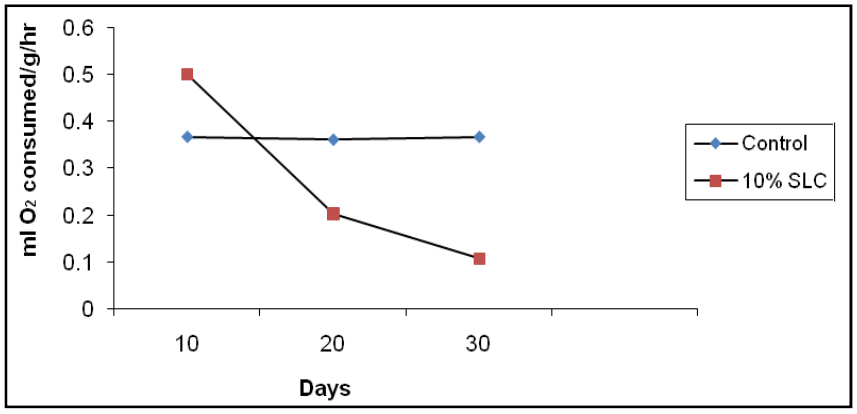
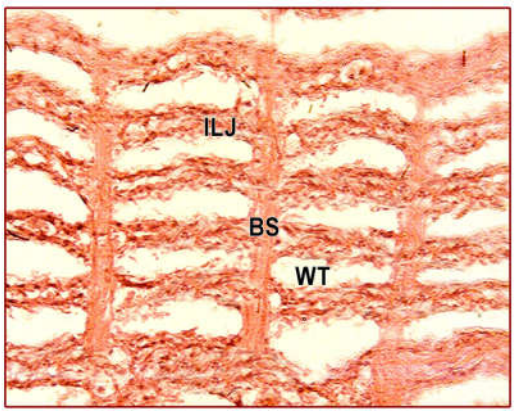


Fig. 1. Rate of Oxygen consumption of *M. casta* after chronic exposure to Cadmium

Plate I.

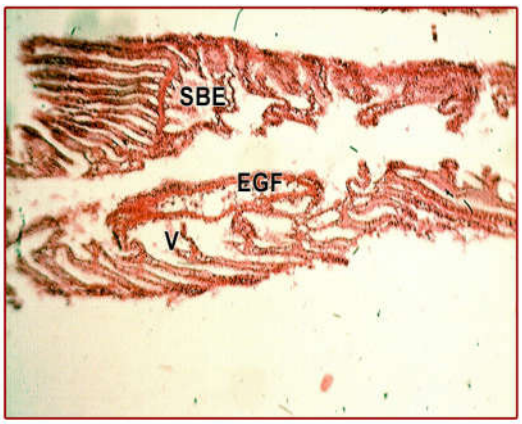


ILJ - Inter Lamellar Junction

WT - Water Tubes

BS - Blood Sinus

Fig. a. Control clam gill



SBE - Sloughing of Blood Epithelium

V - Vacuolization

EGF - Enlargement Gill Filament

Fig. b. 10% SLC of Cadmium treaded clam gill for 7 days

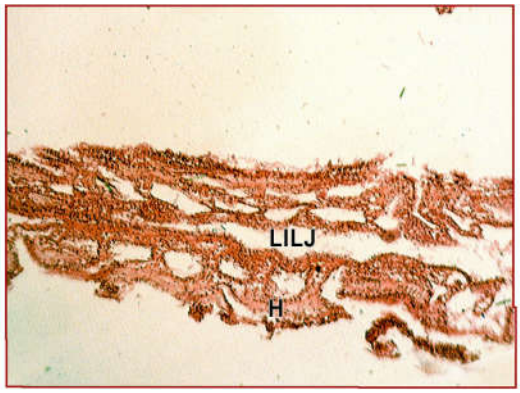
Histology of control gill

In estuarine clam, gill consists of plate like structure called laminae or gill plate. Each lamina is formed of two vertical plates called lamellae. The lamellae are made of a number of vertical filaments called gill filaments (Plate I & Fig.a). Normal gill filaments showed ciliated epithelium and are provided with several groups of cilia and chitinous rod. Between the two lamellae of a gill plate a space is divided by vertical bars of vascular tissue forming inter lamellar junctions. The interlamellar junctions between two lamellae divide the space into distinct compartments called water tubes.

Histology of gill of *M. casta* exposed to 10% sub lethal concentration of cadmium

Appreciable changes were noticed in the gills of mussels within 7 days of 10% sub lethal exposure. Sloughing of basal epithelium, damaged epithelial cells and swelling of lamellae with haemocytes were observed (Plate 1 & Fig.b). The damage was more severe and progressive after 14 days exposure. Clumping and enlargement of filament, sloughing of gill filament and vacuolization of basal epithelium were observed (Plate II & Fig.c). Higher degree of hypertrophy in the gill lamellae, Vacuolization of epithelial cells and loss of inter lamellar junctions were prominent in the gills of clams exposed to 21days (Plate II & Fig.d).

Plate II.



H - Hypertrophy

LILJ - Loss of inter Lamellar Junction

Fig. c. 10% SLC of cadmium treaded clam gill for 14days

V - Vacuolization

LILJ - Loss of Inter lamellar Junction

WOS - Widening of Ostial Spaces

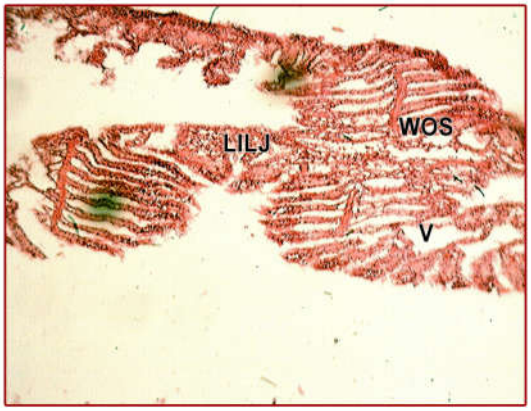


Fig. d. 10% SLC of cadmium treaded clam gill for 21 days

DISCUSSION

Oxygen consumption is a very sensitive physiological process in aquatic animals and therefore, alteration in the respiratory activity is considered as an indicator of stress of animals exposed to heavy metals (Sarkar, 1999). Billinski and Jones (1973) have suggested that decrease in oxygen consumption is brought about by severing of links between oxidative and phosphorylative processes. Lomte and Jadhav (1982) have reported that copper sulphate and mercuric chloride decrease the oxygen consumption in *L. corrianus*. Akberali and Earnshaw (1982) reported that copper, lead and cadmium at high concentration inhibited respiration at the cellular level. Brown and Newell (1972) reported that long term exposure to copper salts resulted in the histopathological damage to the gill epithelium. The various report showed that the gill accumulated higher metal content (Pringle *et al.,* 1968; Rajendran *et al* 1988 and Chidambaram, 1992).Therefore this may be the probable reason for the reduced oxygen consumption of bivalves of the present study. The present investigation is in confirmation with the trend observed in earlier investigations of significant drop in the rate of oxygen consumption in the fresh water bivalve, *Corbicula striatella* exposed both copper and mercury salts (Mahajan and Zambare, 2001). In clams, the gills have been identified as excellent biological indicators of the effects of toxic materials in the ambient environment. Several changes in the gills have been noted at the cellular level after exposure to sub lethal concentration of Cadmium. Physiological functions are largely dependent on structure and morphological changes.

In filter feeding bivalves, the gill is involved in food sorting and respiration (Galtsoff, 1964). In a normal mussel during feeding, the lateral cilia beat inwards in the beginning and draw water into the inhalent siphon and this water enters the vertical water tubes through the ostia. The structure and ciliary activities of gills, of the family veneridae in general have been described by Ansell (1961). When the water is relatively clean, the gills are expanded and the upward-moving tracts are largely in operation. When there is lot of turbidity or suspended particles in the water, the gills are stimulated to contract, placing the principal filament (filament that lies between the folds) deep within folds (Barnes, 1980). Histopathological studies help in understanding the stress caused to the animal exposed to pollution. This is a universal method for the assessment of the impact of xenobiotics on the tissues of animal. The ability of any tissue to regulate its normal physiological function is extensively related to its structural integrity. Any damage to the tissues usually results in altered and frequently abnormal metabolic activities. Observations of the marginal groove in exposed mussels indicate that it always remained open and there is a conspicuous increase in the mucus production. Bivalves rapidly accumulate organic compounds such as petroleum hydrocarbons from their environment and the dynamics of uptake and depuration have been studied (Donde *et al.,* 2001). Damage to tissues of gill of *L. marginalis* results in probable reduction in clearance rate, as there may be sluggish activities with less rapid effective strokes of various cirri after exposure to heavy metal dilutions. The reduced filtration rates in *L. marginalis* exposed to Zn confirm these observations (Donde,

2002). The observations of the damaged eulaterofrontal cirri exposed to Cadmium concentration reveal that the angle of beat of such cirri might have reduced, apparently causing loss of their normal co-ordination rhythm. Similar observations have been reported by Axiak and George (1987) on gill functions and ciliary activities of a marine bivalve *Venus verucossa*. Loss of cilia and epithelial cells from filaments may reduce filtering efficiency. The structural damage in gills thus leads to reduced respiration rate and general loss of regulatory mechanism. In general, the heavy metal selected were found to cause damage to the tissues of vital organs namely gill. In the gills, disintegration of inter-filamenter junctions, dilation and shrinkage of branchial vein were observed. Similar branchial vein damage and breakdown of interfilamenter junctions were observed by Donde (2006) in *G. divericatum* exposed to WSF of crude oil. The gills are the vital organs for respiration and feeding and are exposed directly to the surrounding test water. The loss of lateral cilia would affect the circulation of water through the gill filaments and the transport of food particles to the mouth. Present study shows that histopathological changes in the respiratory epithelium of gill would ultimately affect the rate of oxygen uptake. Thus, direct contact of the respiratory surface area with polluted water may lead to its alteration as well as its diffusing capacity leading to the death of the clam in due course.

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