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Histopathological changes in the gill of clam *Gafrarium divaricatum* exposed to sub-lethal concentrations of WSF of crude oil

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Abstract

The cytotoxic effect of the water-soluble fractions of crude oil in gills of *Gafrarium divaricatum* was studied histopathologically. The study, provides an insight into the extent of damage caused to the gill exposed for a period of 7, 14, 21 and 28 days to various dilution concentrations of 10% stock water-soluble fractions (WSFs) of Bombay High (BH) crude oil. The gill exposed to low sublethal concentrations of 5 and 10 percent did not show much morphological alteration during 7 day exposure. However, after 21 days, fusion of adjacent lamellae was prominent in same concentrations due to advanced stage of hyperplasia. In 25% dilution concentration gills showed widening of water tubes, vacuolisation, clumping and bending of lamellae, etc. after 7days of exposure. In higher concentrations of WSF (50%, 75% and undiluted WSF) sloughing of lateral cells from the chitinous rods of gill filaments, desquamation in lamellae, and haemocytic infiltration were observed within 7days. Various groups of ciliary arrangements in the gills were uprooted; only the chitinous supporting rods remained on 14th day in 75% WSF dilution concentration.

Keywords: Water-soluble fractions, crude oil, histopathological changes in gill, *Gafrarium divaricatum*

Introduction

The clam, *Gafrarium divaricatum* is widely found along the sea shores in Mumbai. They are exploited and consumed by the fishermen. Mumbai is a very busy port at the same time due to Bombay High pipeline the marine animals are chronically exposed to oil spill. Though attempts were made with individual hydrocarbon (Fondekar *et al.*, 1980; Deshmukh *et al.*, 1985; Gagne, 2001), studies related to oil pollution in relation to marine clams are scanty in India (Donde *et al.*, 2002). Marine animals vary greatly in their sensitivity to oil and to gain insight into this, the bivalves have been widely used as indicator species in pollution studies (Goldberg *et al.*, 1983).

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. Hence there is a need to study the histopathological changes in gills of bivalve in response to pollutants. Thus in the present study, observations were made on the cytological damage caused to gill of a clam *G. divaricatum,* following the exposure to sub lethal concentrations of WSFs of Bombay High (BH) crude oil.

Materials and methods

The thick-shelled clams *G. divaricatum* were collected from an unpolluted site along the intertidal zone of the North-eastern coastline of Mumbai. The animals were then acclimatized for about a week in laboratory. The average length and weight of the species with shell was 30 ± 2.5 mm and 16 ± 1.1 g respectively. Glass aquaria of size 60x30x30 cm each containing 2.0 l of seawater were used and 20 clams were introduced in each of the experimental containers. The water-soluble-fractions (WSFs) were prepared by mixing oil (Bombay High crude oil) and seawater (10%) for 24 h. The aqueous phase collected after settling down (12 h) was used as pure WSF stock (undiluted) and various dilution concentrations were prepared by adding seawater. The static bioassays for 28 days were carried out with 24 h test water replacement. Clams exposed to sub lethal concentrations of 5%, 10%, 25%, 50%, 75% WSF dilution concentrations and 100% (undiluted) WSF stock, were subjected to histological examinations at intervals of every 7 day (for 14 days in all sublethal concentrations and for 28 days in 5, 10, 25, and 50 percent dilution concentrations only, as clams of 75% and 100% showed high

percentage of mortality after 2nd week) to study the morphological alterations in the gill.

After each exposure period the tissue was fixed in Bouin's fixative and was processed as per the routine histological/microtechnique methods. The sections (8 μm thick) were stained using eosin and Ehrlich's hematoxylin stains. The tissue sections were observed under various magnifications. Criteria for cytotoxicity were evaluated by observing the structural integrity of gill and a comparison was done to discriminate between control batches and those treated by WSFs of BH crude oil.

Results

The normal gill filaments of *G. divaricatum* showed (Fig.1) ciliated epithelium surrounding water tube and contain blood sinuses. Gill epithelium is provided with several groups of cilia and chitinous rod. Each ctenidium is flattened and somewhat rectangular. Each lamina consists of two similar elongated flaps and the lamellae are held together by connective junction tissues. Lamellae are made up of ciliary filaments through which branchial blood vessels pass. Several partitions, the inter-lamellar junctions and a number of vertical compartments called water tubes divide the cavity between the two lamellae. All the water tubes of a lamina open into a common supra-branchial chamber, situated above the lamina. Each lamina is made up of several N-shaped gill filaments. The filaments are joined by inter-lamellar junctions. Wide interfilamental spaces and enlarged blood sinuses are prominent (Fig.2).

The gills exposed to sub lethal concentrations exhibited sloughing of basal epithelium, damaged epithelial cells with distinct chitinous rods and swollen lamellae

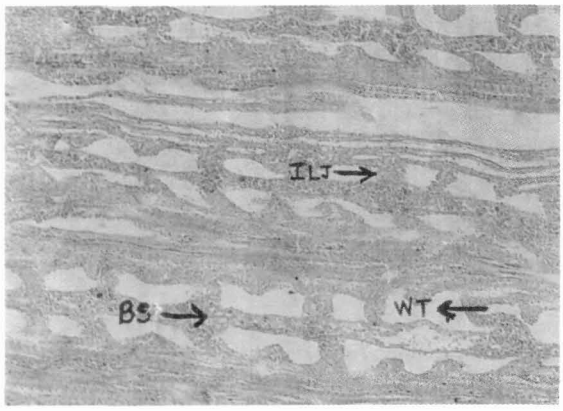

Fig. 1. (100 x) : Normal gill of control *G. divaricatum* (7th day) : (ILJ) interlamellar junctions. (WT) water tubes. (BS) blood sinuses

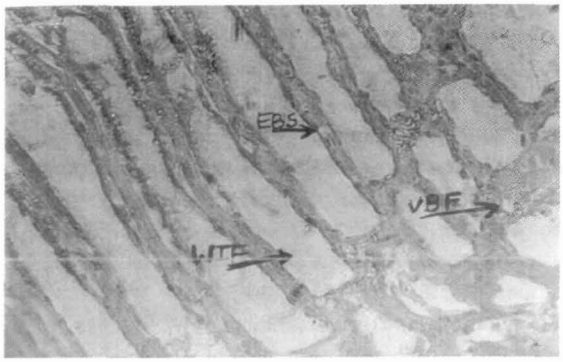

Fig. 2. Normal gill (28th day): (WIF) wide interfilamentous spaces. (EBS) enlarged blood sinuses.

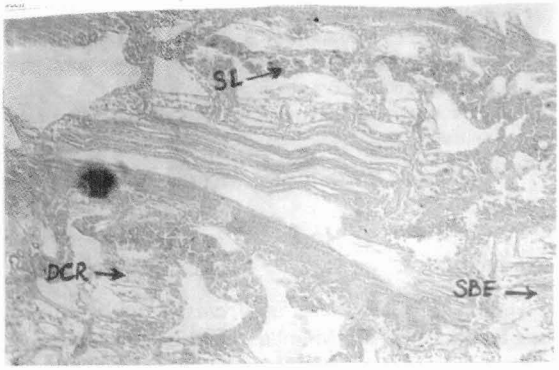

Fig. 3. (100 X): Exposed gill of *G. divaricatum* in 75% WSF dilution (7th day): (SBE) sloughing of basal epithelium, (DCR) damaged epithelial cells with distinct chitinous rods. (SL) Swollen lamellae with haemocytes and initation of desquamation of hyperplastic epithelium.

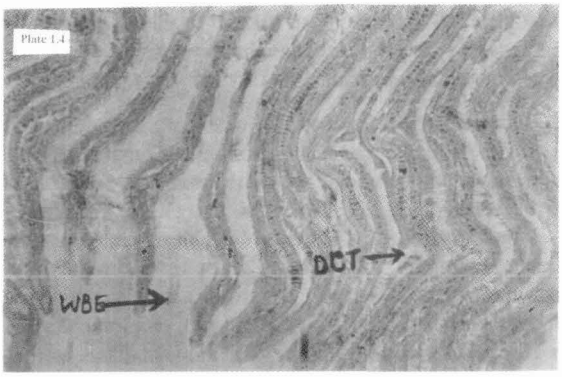

Fig. 4. Exposed gill in 10% WSF dilution (7th day): (WBE) widening of basal epithelium, (DCT) discontinuous inter-lamellar junctions. Clumping and bending of lamellae seen

with haemocytes in clams exposed to 75% WSF dilution for 7 days (Fig.3). In gills exposed to higher concentrations (100% undiluted WSF) a general disorganization is seen and some of the lamellae are detached from the chitinous rod (Fig.5). The eulaterofrontal cirri, terminal cilia and the frontal cilia are observed to be severely damaged in higher concentrations (75% and 100% dilution concentrations) in a week period (Fig.8). Marked effect on the sensory cilia is noticed indicating that the oil exposure exerts a different effect on the activities of lateral cilia depending on the concentrations and exposure period. Terminal cilia perhaps become more active especially at higher concentrations and get affected faster. Widening of interfilamental space is prominent. The infiltration of haemocytes in the gill lamellae is also observed.

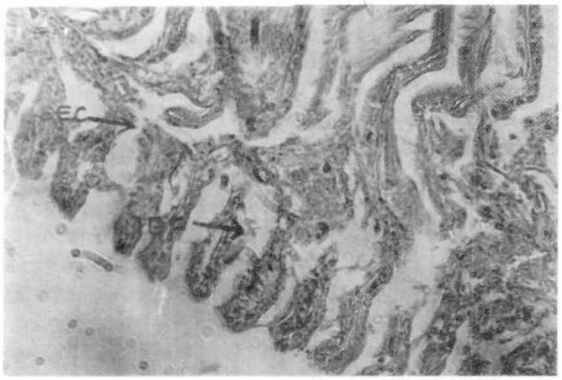

Fig. 5. (400 X) : Exposed gill in 100% WSF dilution (7th day): (NGF) necrotic gill filaments, (SEC) sloughing of epithelial cells. Hypertrophy of secondary lamellae is seen. Inter-lamellar spaces filled with hyperplastic epithelium and lamellar structure is lost

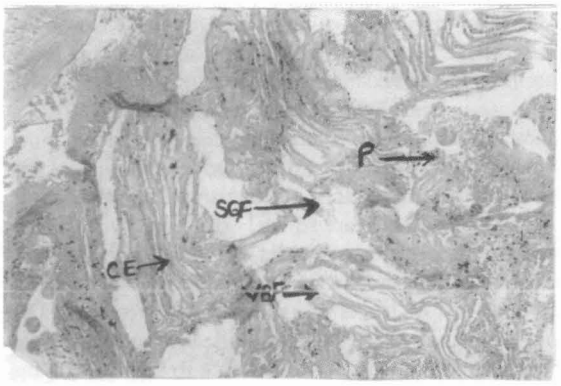

Fig. 6. (100X) : Exposed gill of *G. divaricatum* in 25% WSF dilution (7th day): (P) phagocyte, (CE) clumping and enlargement of filament. (SGF) sloughing of gill filament and (VBF) vacuolization of basal epithelium

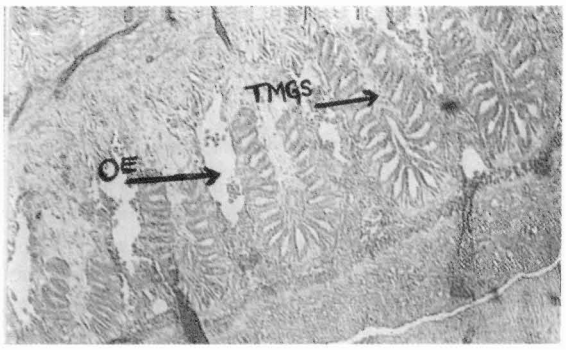

Fig. 7. (400 X): Exposed gill of *G. divaricatum* in 10% WSF dilution (14th day): (OE) ostium expanded. (TMGS) tightly meshed gill structure to prevent finest sediment from entering the suprabrachial cavity can be seen.

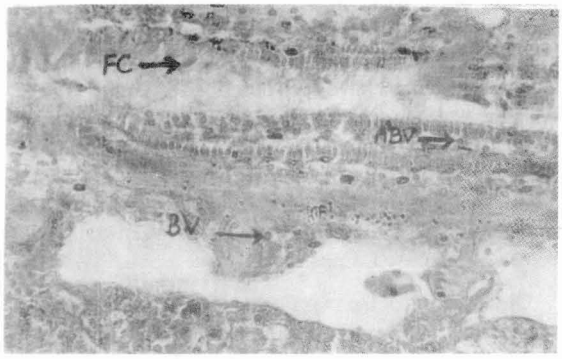

Fig. 8. (400 X): Exposed gill of *G. divaricatum* in 50% WSF dilution (14th day): (FC) loss of frontal cilia. (ABV) secondary lamellae transformed to globular structure. (BV) necrotic blood cells

Changes observed in the intermediate sub-lethal concentrations (25% and 50% dilution concentrations) do not differ conspicuously with the gills of low-level concentrations (5 and 10 percent dilution concentrations) up to 7 days. At some places detachment, clumping and bending of lamellae are observed in gills exposed to 10% WSF dilution concentration (Fig. 4). This is probably due to turbidity. At 25% sublethal concentration, the gill shows sloughing of lateral cells from the chitinous rods of gill filaments and the ciliary filaments of the lamellae show clumping tendency, slight enlargement and change in regular shape (Fig. 6). Other changes like widening of ostial spaces (Fig.7), lysis and vacuolization of epithelial cells and loss of interfilamental junction and swelling and degeneration of mucous secretory cells also occur along with progressive enlargement of gill lamellae in lower

concentrations after 2 weeks of exposure (Fig.8). Higher degree of hyperplasia (Fig.3) and hypertrophy (Fig.5) are very prominent in the gills of clams exposed to higher concentrations of WSF.

Discussion

Bivalves in their natural environment are frequently exposed to single spill or chronic discharges of petroleum oil. 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 WSF concentrations of BH crude oil. 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 clam 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).

Observations of the marginal groove in exposed clams 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). Observations in the gill exposed to WSF dilutions show a conspicuous increase in mucus production with increasing concentration of WSF. It indicated that the ciliary system of gills might have acted upon the oil droplets in the same manner as on food particles. Stainken (1975) in his studies on *Mya arenaria* has shown that oil droplets are carried by gill ciliary system from frontal surface of the gills to the mouth where they are then ingested. In the present study after 21 days, the gill of the WSF dilution exposed clams reveal colour change in the increasing order of the concentrations. After 28 days of exposure, blackening of the gills is observed. Damage to tissues of gill of *G. divaricatum* results in probable reduction in clearance rate, as there may be sluggish activities with less rapid effective strokes of various cirri after exposure to WSF dilutions. Such a sluggishness and immobility in sensory cilia have been observed by Axiak and George (1987) after exposure of mussels to WAF of Kuwait crude oil. Similar is the case of the present test clam also, even though the cilia are found to be affected, the rate of accumulation of PAH reduced but continuous accumulation of the toxicant was recorded (Donde *et al.*, 2002). This may be due to the action of frontal cilia. However, in lower concentration group accumulated hydrocarbons are found to drop after a period of 21 days along with conspicuous increase in mucus production. The damaged eulaterofrontal cirri observed in the higher concentrations exposure group indicates decreased filtration rate. A study by Jorgensen (1975) with *Mytilus edulis* suggested that the particle retention is not only determined by the action of eulaterofrontal cirri but also by the degree of coordination of their activities with frontal and lateral cilia. Moreover, the present study indicates that long-term exposure to concentrations of WSF of oil may lead to much significant reductions in retention efficiencies, as evidenced by drop in accumulation of hydrocarbon noted in the final week of exposure in clams of lower concentrations (Donde *et al.*, 2001). This probably indicates a resultant decrease in pumping rates in exposed clams. The infiltration of haemocytes in the gill lamellae is also observed. All together high activity of ciliary system may lead to a reduced or negative energy balance in the clam. The reduced filtration rates in *G. divaricatum* exposed to WSF dilutions confirm these observations (Donde, 2002). Gills are the respiratory and osmoregulatory organs in shellfish and any damage occurring in this tissue may impair respiration. The decline in polyaromatic hydrocarbon (PAH) level recorded can be attributed to its reduced uptake due to gill damage coupled with its continued elimination from the gill.

The observations of the damaged eulaterofrontal cirri exposed to WSF concentrations 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. The end result is that the clams tissues are severely damaged by hydrocarbon present in exposure media. However, the clams are observed to live more than five weeks in WSF with no mortality at low levels of exposure. This result may be considered to be of ecological significance since it implies that exposed individuals will be able to feed less on the normally abundant, naturally occurring food particles due to the resultant loss in the coordination of ciliary activities (Mortan, 1983; Axiak and George, 1987). It can be concluded that while crude oil may not be actually toxic

to intertidal hard clam *G. divaricatum,* exposure to high sublethal WSF concentrations may result in long term deleterious effects.

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