HISTOPATHOLOGICAL ALTERATIONS OF GILL, DIGESTIVE GLAND AND MANTLE OF *Telescopium telescopium*: AN INTERTIDAL GASTROPOD OF THE INDIAN SUNDARBAN ESTUARY UNDER THE EXPOSURE OF DIESEL

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Abstract: *Telescopium telescopium* is an intertidal gastropod which is abundantly distributed in the mudflats of Sundarban estuary of India. They play a vital role in the fragile ecosystem of Sundarban mangroves. These hard shelled molluscs are mud creepers and feed on the algal mats of the estuary. Motorized boats run by diesel are the chief mode of transport for the human population of this geographical region. A good number of such boats regularly ply along the creeks of Sundarban estuary for various purposes. Occasional spillage of diesel from these boats causes contamination of water and soil of this ecosystem. *Telescopium telescopium* along with other benthic organisms are chronically exposed to this vehicular diesel. Present study reports histopathological alterations of gills, digestive gland and mantle of *Telescopium telescopium* treated with environmentally realistic concentration (0.15%) of diesel for a period of 15 days. Histopathological damage of vital organs like gills, digestive gland and mantle render this species vulnerable to the risk of population decline in its natural habitat.

Keywords: *Telescopium telescopium*, diesel, histopathology, digestive gland, mantle, gills, Sundarbans

INTRODUCTION

Telescopium telescopium (Linnaeus, 1758), (Mollusca: Gastropoda: Potamididae) is an intertidal, benthic, detritivore, euryhaline mollusc evenly distributed along the mudflats of rivers and creeks of Sundarbans estuarine complex of the state of West Bengal, India. This species is a mud creeper and feeds on algal mat and organic detritus with its extendable proboscis during low tide. The natural habitat of *T. telescopium* is often contaminated by diesel (CAS number: 68476-30-2) which is used as fuel by the motorized boats and fishing trawlers.

Diesel or its components in different states may enter from the polluted surroundings into the body of *T. telescopium* as the emulsions and suspended forms with respiratory water through gills, from bottom sediments with food through alimentary canal, or, through mantle membrane, foot or other body parts exposed physically to oil films. Oil may pass through the intestinal barrier and is incorporated and stabilized in the lipid pool of the organisms (Blumer *et al.,* 1970). Histopathological studies of gill and digestive gland of *T. telescopium* have been reported to reflect the toxicological spectrum of heavy metal contamination and these vital organs are proposed as biomonitors of heavy metal contamination (Yap and Noorhaidah, 2011). According to Owen (1966), the digestive gland of gastropods is reported to perform the physiological functions like absorption of ingested food material, extracellular and intracellular digestion, secretion, excretion and osmoregulation. It also plays an important role in the internal defense mechanism (Bayne, 1974; Renwrantz *et al.*, 1981; Ottaviani, 1990), detoxification processes (Marigomez *et al.*, 1986), and in the storage of calcium (Sumner, 1965) and glycogen (Gabe, 1962; Kulkarni, 1981).

Gills are the principal organs for respiratory gaseous exchange in molluscs. The respiration in gastropods is generally achieved by gills located within the mantle cavity and regarded as a pallial organ. They are affected by different xenobiotics that flow through the mantle cavity (Oehlmann *et al.*, 2000). Besides being the principal respiratory organ, mucus-covered gills of bivalves and some snails are able to entrap, food particles and transport them to ciliated flaps, called palps, where particles are sorted, into the mouth; thus aiding in filter feeding.

The mantle or pallium is the dorsal body wall which covers the visceral mass and usually protrudes in the form of flaps well beyond the visceral mass itself. In many species of molluscs, the epidermis of the mantle secretes calcium carbonate and conchiolin and forms a shell. In this present investigation, detailed histopathological analyses of the digestive gland, gills and mantle of *T. telescopium* exposed to diesel have been carried out in order to ascertain organ toxicity due to diesel exposure.

MATERIALS AND METHODS

Collection, transport, laboratory acclimation and maintenance of *T. telescopium*

Adult healthy *T. telescopium* with similar range of shell length (7-10 cm) were manually collected during low tide, from selected oil-free intertidal mudflats of Sundarbans estuary of West Bengal, India. Collected specimens were transferred to rectangular plastic containers (30× 20 ×15 cm) with adequate volume of freshly collected estuarine water procured from their habitat and were transported to the laboratory for acclimation and experimentation. All the containers used for transportation bore air passages on the top lead to facilitate aeration during transportation.

At laboratory, the shells of snails were washed thoroughly with estuarine water and placed on mud beds of specially designed tide simulating aquaria for proper acclimation. Temperature of water was maintained at a range of 29 ± 3º C and the specimens received a uniform exposure of 12 hours of dark-light cycle. In the laboratory, the specimens were fed with leaf detritus of selected mangrove plants like *Heritiera fomes*, *Nypa fruticans* and *Ceriops decandra*. The water of the glass aquaria was routinely replenished in every 12 hours to avoid residual toxicity due to generation of excretory products and metabolites. Dissolved oxygen, hardness, salinity, acidity and alkalinity were estimated at every 24 hours. Specimens exhibiting the symptoms of disease and morbidity were not considered for experimentation. Temperature was monitored routinely by a thermometer and dissolved oxygen, hardness and salinity were estimated after APHA *et al.* (1998).

Histopathological analyses of selected tissues like- gill, digestive gland and mantle of diesel exposed (0.15% of diesel for 15 days) *T. telescopium* were carried out along with control.

Procedure

T. telescopium were exposed to 0.15% of diesel for 7 days of duration in tidal simulating aquaria. Gill, digestive gland and mantle of control and diesel treated specimens were undergone autopsy and the dissected tissues were placed in Bouin's fixative at 4°C *in situ* and fixed for 12 hours. The fixed tissues were passed through the ascending grades of ethanol for dehydration and transferred to absolute ethanol with dual changes. The dehydrated tissues were transferred to a mixture of alcohol-acetone solution at a ratio of 1:1 (v/v) for 30 minutes followed by transfer in acetone and kept for 45 to 60 minutes. Then the tissues were placed into a mixture of acetone- xylene (1:1) and kept for 30 minutes. Finally, the tissues were transferred to xylene and kept in it for 60 minutes. Tissues were then immersed into a mixture of equal volume of xylene and tissue grade paraffin and left in a paraffin bath in an oven for 30 minutes at 56ºC. Paraffin infiltration was allowed for 45 minutes at 58-62ºC with two changes of molten paraffin followed by embedding in paraffin at 58-60ºC. After 2 hours of infiltration, paraffin blocks with tissues were prepared in a dice and trimmed tissue blocks were sectioned at a thickness of 5 - 7 µm in a ribbon using a microtome (Leica Reichert-Jung – 2030). The resulting paraffin ribbons were thermally stretched and affixed to the glass slides using Mayer's albumen. Sectioned tissues were stained with hematoxylin and eosin following standard protocol and mounted in DPX. Histopathological observation and analyses were carried out under bright field microscope (Olympus BX51, Germany) and the images were documented digitally by a camera (ProgRes C5) fitted with the microscope.

RESULTS

Gill

Gill acts as a major organ functionally involved in respiration and filter feeding in aquatic gastropod. It is also reported as an organ of immunosurvillence of molluscs under toxic challenge. Gill filaments are

plate like vascularised structures, arranged antero-posteriorly on either side of the visceral mass. Gill filaments bear cilia which generate currents that transport food particles and water. In transverse section, the gill exhibits elongated lamellae with well connected lamellary processes (Figure 1: A,C,E). Upon treatment with 0.15 % of diesel for 7 days, gill exhibited water channel with enhanced tissue swelling and infiltration of hyperchromatic anaplastic cells. A state of tissue rupture and clogging of water channel was recorded along with formation of dense fibrosis and cellular disintegration (Figure 1: B,D,F).

Digestive gland

Structurally, the digestive gland is a spirally coiled compact mass which is dark grey in colour. A single digestive duct conveys the secretory materials of this digestive gland to the alimentary canal. The digestive gland is composed of numerous fairly large lobules with considerable interlobular spaces. Each lobule contains four types of cells i.e. large, wedge shaped calcic cells; excretory cells with excretory spherules; large, elongated secretory cells and large, ovoid vesicular cells (Das and Manna, 1993). During feeding by filtration, the organism gets into contact with water with suspended food matter or dead or decaying organic matter. Digestive tubules of untreated *T. telescopium* were devoid of any histopathological signs (Figure 2: A,C,E). Upon exposure of 0.15 % diesel for 7days, the digestive gland of *T. telescopium* exhibited cytoplasmic vacuolation associated with damage of the digestive cells. The digestive cells appeared to be disrupted and ruptured at multiple anatomical points (Figure 2: B,D,F).

Mantle

Mantle of mollusc provides a protective cover of the visceral organs. It shields the internal organs from mechanical, chemical and biological stressors. Histopathological examination revealed the existence of cuboidal epithelial cells and muscular fibers in untreated *T. telescopium* (Figure 3: A,C,E). Treatment with 0.15 % diesel for 7 days, resulted in rupture of cuboidal epithelial cells and subsequent damage of the muscular fibers. Cellular disruption and disintegration of mantle appeared to be prominent in *T. telescopium* exposed to diesel (Figure 3: B,D,F).

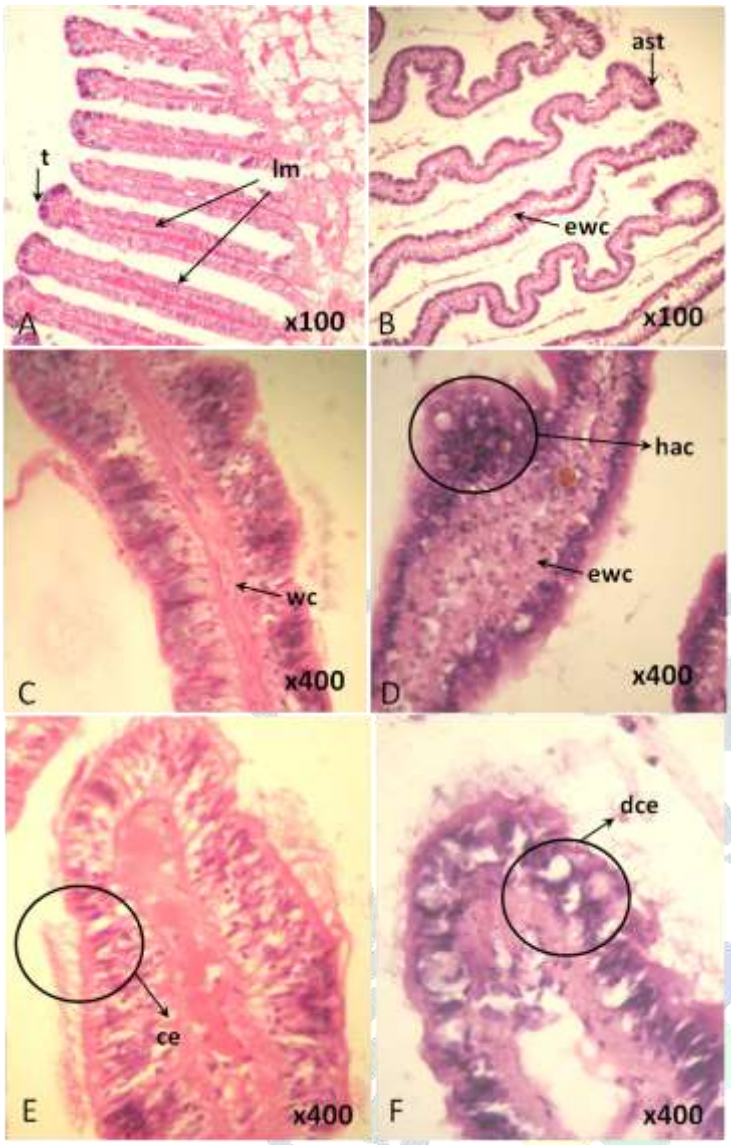

Figure 1: Histopathology of gill of *T. telescopium*. Transverse sections of gill filaments of control *T. telescopium* exhibiting elongated lamellae (lm) with regular tips (t) arranged equidistantly exhibiting ciliary epithelium (ce) and water channel (wc) (A, C, E). treatment of diesel (0.15% / 7 days) resulted in altered shape of the tips of gill lamellae (ast), enlarged water channel (ewc), infiltration of hyperchromatic anaplastic cells (hac) and damaged ciliary epithelia (dce).

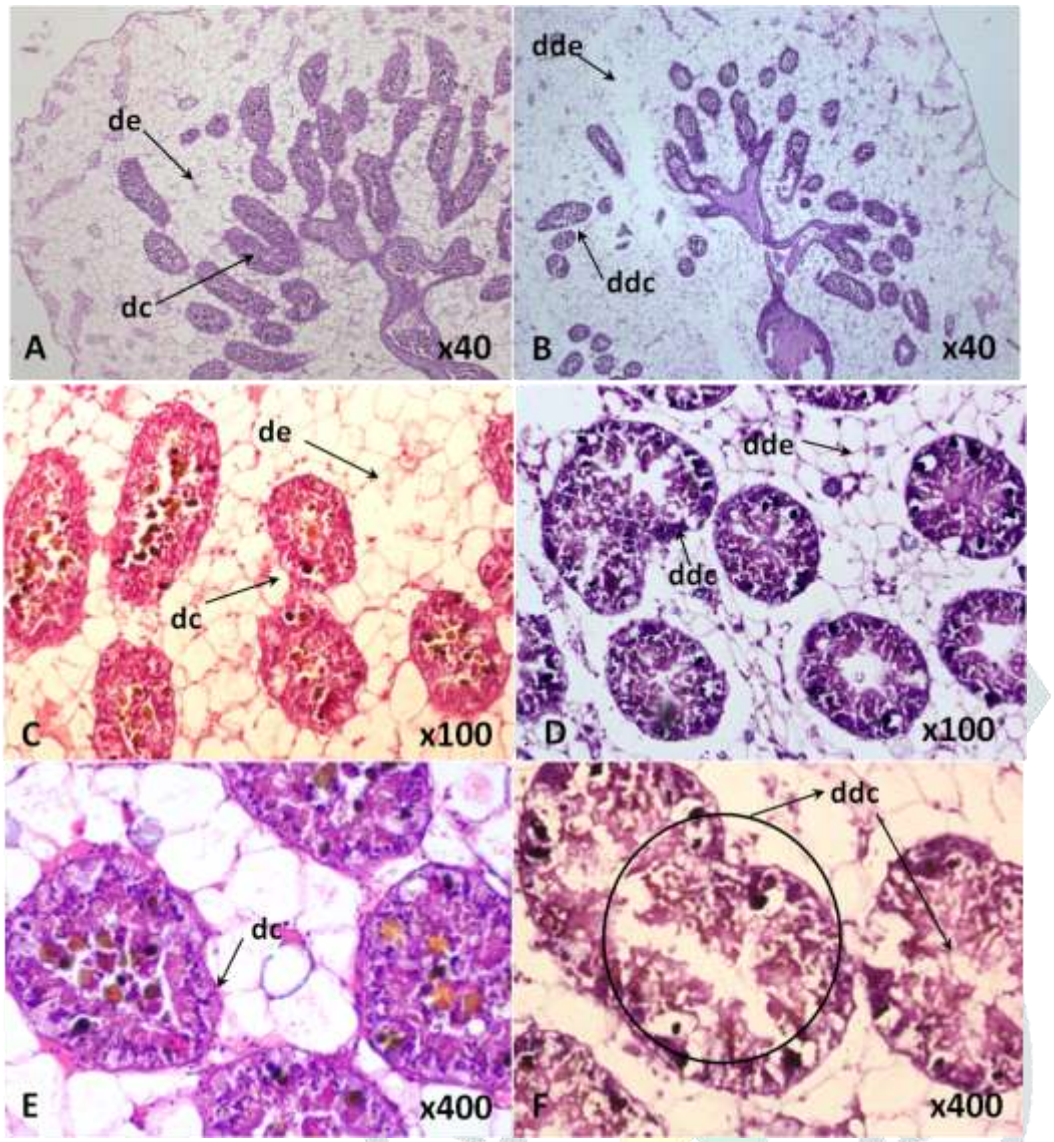

Figure 2. Histopathology of digestive gland of *T. telescopium*. Transverse sections of control (A, C, E) specimens exhibiting digestive cells (dc) and digestive epithelium (de) and that of diesel treated (0.15% / 7 days) specimens (B, D, F) exhibiting defective digestive cells (ddc) and damaged digestive epithelium (dde).

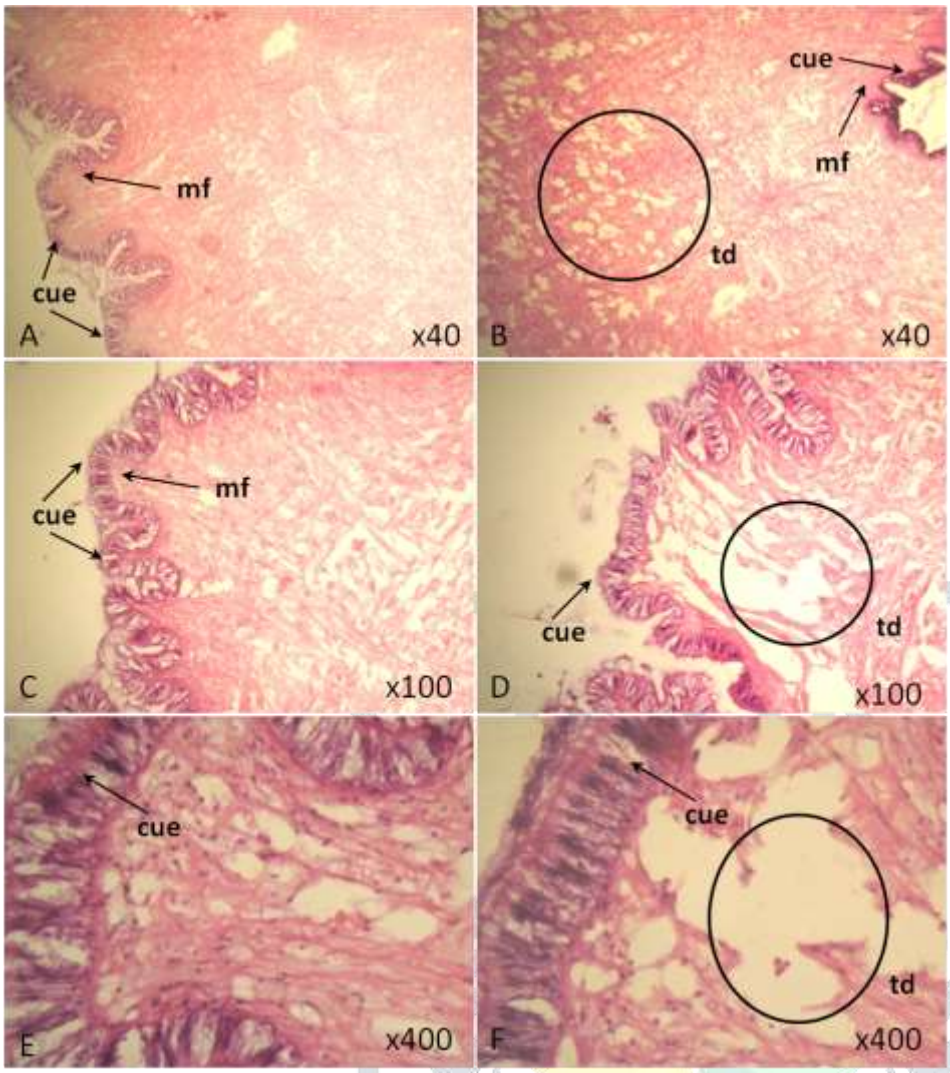

Figure 3. Histopathology of mantle of *T. telescopium*. Transverse sections of the mantle (A, C, E) of control exhibiting cuboidal epithelium cells (cue) and muscle fibres (mf) and mantle of diesel treated (0.15% / 7 days) specimens (B, D, F) exhibiting histological signs of tissue damage (td).

DISCUSSION

Gill or ctenidium of aquatic molluscs are involved in physiological process of respiration, filter feeding and immunosurveillance (Chakraborty *et al*., 2010) and acts as a target organ of various pollutants (Ogundiran *et al*., 2009). The gastropod gill is monopectinate in organization, bearing parallelly arranged gill lamellae (Aksit and Mutaf, 2007). Aquatic and amphibious molluscs are capable of sieveing particulate food matters of water column by the gill lamellae and discriminate food from other substances. Vasanthi *et al*. (2012) reported that the histology gill serves as an important pathological marker of heavy metal toxicity. The histomorphology of gill is an established indicator of water quality parameters and the overall health status of test organism (Peters *et al*., 1984). Multiple xenobiotics have been reported to alter the structural integrity of gills of the organisms inhabiting polluted environments. Gill hemorrhage and respiratory disorder were recorded in fish *Clarias gariepinus* against alkylbenzene sulphonate exposure and is indicative to physiological threat on aquatic life (Ogundele *et al*., 2004).

Gills of untreated *T. telescopium* presented distinct water channels with integrated gill lamellae signifying the normal functional status of the tissue. Exposure of 0.15% of diesel for 7 days resulted in morphological alterations of gill lamellae associated with cellular disintegration (Figure 1). This is indicative of a state of toxicity affecting the efficacy of gaseous exchange, decrease in filtration ability and other functions under the exposure of diesel. Such a situation may lead to decreased ecological fitness and population size of the *T. telescopium* distributed in the diesel contaminated habitat.

The morphology of digestive gland was reported to be altered in the members of multiple invertebrate Phyla due to the exposure of several environmental stressors (Lomte*et al*., 1989; Sontakke *et al*., 1992). Das and Manna (1991) reported that the digestive gland of *T. telescopium* consisted of multiple lobules, each with four types of cells, i.e. calcic, excretory, secretory and vescicular types. Histological studies on the gastropod digestive gland indicated that the structure and functional status of its cellular components may vary even within the members of the same group (Kress *et al*. 1994). Diesel induced histological damage of the digestive gland of *T. telescopium* (Figure 2) is suggestive of a state of degeneration and necrosis of the digestive epithelium leading to the possible impairment in the digestive physiology in the same species.

Mantle of molluscs is characterised by flaps of muscular tissue that covers the visceral mass externally. The perimeter of the body cavity is formed by the mantle membrane and it is functionally associated with the process of shell synthesis (Ojima, 1952; Nakahara and Bevelander, 1971; Dix, 1973), secretion of periostracum (Verheken, 1989) and thus providing nonspecific protection. Treatment with 0.15% of diesel for 7 days resulted in rupture of the cuboidal epithelial cells and muscular fibers of mantle. Cellular disruption and disintegration of mantle appeared to be prominent in *T. telescopium* (Figure 3) under diesel treatment. Heavy metals have been reported to disrupt the histological architecture of mantle of mussel *Anodonta cygnea* (Moëzzi *et al*., 2013). Present data were indicative to a loss of cellular integrity in the tissue leading to a possible interference in shell formation process and protective function of the organ against mechanical and other stress. Additionally, the mantle is assumed to act as a physiological barrier that restricts the entry of pathogen and toxin and constitutes the first line of physiological defense of molluscs. Thus, diesel induced histological alterations of mantle was assumed to affect adversely the immunological efficiency of *T. telescopium* to limit the toxicity of environmental contaminants and pathogen invasion.

Unrestricted diesel pollution is thus apprehended as a prominent ecotoxicological threat for *T. telescopium* and other inhabitants of Sundarbans biosphere reserve of India. Adequate environmental awareness and appropriate impact assessment are thus recommended to protect this invaluable bioresource in its natural habitat. Restriction should be imposed on the number of the diesel run motorised boats and fishing trawlers in the Sundarbans mangroves by implementing proper legislature and surveillance.

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