Histopathology of the Foot, Gill and Digestive Gland Tissues of Freshwater Mussel, *Lamellidens marginalis* Exposed to Oil Effluent

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**Abstract**

We investigated the histopathological alterations in the tissues of freshwater mussel, *Lamellidens marginalis* in response to oil effluent. Based on the previous acute toxicity, two sub lethal [1/4th (11.88ppt) and 1/10th (8.55ppt)] concentrations of oil effluent (hydrocarbon) were prepared and exposed to mussels. In a first series of experiment, animals were exposed/accumulated for 30 days [1st, 8th, 15th, 22nd and 30th days] by two sub lethal concentrations of oil. In a second series of experiment, oil exposed animals were thereafter transferred to clean water and kept in it up to 30 days [1st, 8th, 15th, 22nd and 30th days] to assess the recovery pattern (depuration). At seven-day intervals, histopathological alterations were analyzed in foot, gill and digestive gland tissues of mussel. First series of experimental observation showed remarkable damages in foot (disorganized outer epithelium, necrosis of the cell, the formation of luminal material) and also oil effluent inducement are confirmed detached glandular epithelium, vertical clefts, presence of leucocytes, dense edema formation, necrosis, dis-aggregated cilia) and in digestive gland (stoma, detached glandular epithelium, vertical clefts, presence of leucocytes, dense accumulation of luminal material) and also oil effluent inducement are confirmed with the aforementioned results. At second series of experiment, it was found that oil effluent tended to accumulate in tissues in a duration-dose-dependent manner. Tissue burden by oil effluent of mussels completely were restored at 30th day. The present experimental findings may be of early warning signals of oil effluent pollution. In conclusion oil effluent are highly toxic to the *Lamellidens marginalis*.

**Keywords:** Oil effluent; Accumulation and depuration; Histopathology of foot; Gill; Digestive gland; *Lamellidens marginalis*

**Introduction**

Researchers with different expertise have converged towards a common interest for understanding and solving the problems associated with the occurrence of toxic level of contaminants in the environment, giving raise to the spectacular development of research in the field of Environmental Contamination and Toxicology, which has emerged as a multidisciplinary science resulting from the integration of classical disciplines such as toxicology, cell and molecular biology, physiology, ecology, chemistry, etc [1]. Uptake and accumulation of xenobiotics in the tissues of aquatic organisms occur from the sediment, contaminated water column and food chain [2] that cause deleterious effects. Incorporation of even very low levels of toxics in the body of aquatic organisms causes various biochemical, physiological and hematological alterations in vital tissues [3]. Most common usage of the term biomarker has been for biochemical, physiological or histological indicators of their exposure to or the effects of xenobiotic chemicals at the sub-organismal or organismal level [4]. Most of the monitoring programmes are confined to the chemical analysis of accumulated substances, but sometimes include toxic responses, for instance histopathological effects [5,6] or physiological/biochemical responses [7]. When Polycyclic Aromatic Hydrocarbon (PAHs) exposed animals, several deleterious effects such as DNA damage [8]. As an indicator of exposure to contaminant, histology represents a useful tool to assess the degree of pollution, particularly for sub lethal and chronic effects [9]. Histological changes appear as a medium-term response to sub-lethal stressors, and histology provides a rapid method to detect effects tissues and organs [10]. Summary of some relevant earlier literature on marine as well as freshwater mussels histopathological observations with various toxicant exposure results are compiled (Table 1). In molluscs, especially in *Lamellidens marginalis* histological injuries, in response to the exposure to oil effluent, remains unexplored. Gills are the vital organs, which come into direct contact with water and are indicative of any environmental stress and also in fishes gills are the major vital respiratory organs [11]. The numerous lamellae along the double row of filament attached to the gill arch are affected by toxicants

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**Abbreviations**

EP: Epithelium; MU: Muscle Tissue; CI: Cilia; NE: Nucleus; BS: Blood Sinus; MU: Muscle Tissue; NE: Necrosis of Epithelial Tissue; GF-Gill Filaments: CR: Chitinus Rod; FL: Frontal Lateral Cilia; FC: Frontal Cilia; IS: Interlamellar Space; IJ: Interlamellar Junction; WC: Water Chamber; MU: Muscle Tissue; SC: Supra Brachial Chamber; GF: Gill Filaments; FL: Frontal Lateral Cilia; DD: Digestive Diverticula; LU: Lumen; ST: Stomach

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### Our Results

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**Table 1: Summary of Relevant Earlier Literatures on Freshwater and Marine Mussels Histological Changes with Various Xenobiotic Exposures and Comparison with Our Results**

| Marine Mussels | Pollutants | Tissues | Responses/Effect | Year | Reference |
|----------------|------------|---------|------------------|------|-----------|
| *Mytilus galloprovincialis* | Metals | Gill, digestive gland | brown cells, metal burden in tissues | 1997 | [42] |
| *Perna indica* | Heavy metals | Gill, digestive gland | Clumping of cilia, damage of gill filaments, dislodged epithelial cells, disintegration of digestive tubules | 2005 | [46] |
| *Crenomytilus grayanus* | Heavy metals and pesticides | Digestive gland | Heavy vacuolization of digestive cells, desquamation of digestive cells of tubules, necrosis, Edematous lysis of vesicular cells and of muscle filaments | 2006 | [17] |
| *Perna viridis* | Heavy metals | Gill | Loss of cilia, epithelial cell damage, swollen lumen, elongation of gill filaments. | 2008 | [40] |
| *Mytilus edulis* | Heavy metal | Gill, Digestive gland and adductor muscle | Inflammation and necrosis. | 2010 | [44] |
| *Gaffarium divaricatum* (clams) | xylene, benzene and gear oil-WFS | Hepatopancreas | Cell debris, fusion of nuclei, interruption of lumen line, disintegration of epithelial cells, necrosis, iated epithelial layer, detachment of epithelial cells. | 2011 | [53] |
| *Perna viridis* | Heavy metal | Gill, Digestive gland and adductor muscle | digestive epithelium, hemocytic infiltration in the gills and myodegeneration in the muscle tissue, necrosis and digestive tubule thickness | 2012 | [45] |
| *Mytilus galloprovincialis* | PAH | Digestive gland | Altered diverticula, damages in digestive tubule | 2013 | [47] |
| *Ruditapes decussatus* | Anthropogenic activities | Gill, Digestive gland | intestinal tissue necrosis, lesions such as digestive tubule (diverticula) | 2013 | [52] |
| *Mytilus galloprovincialis* | Industry Effluent (Iron, paper Harbour, cement etc...) | Gill, Hepatopancreas | degeneration, cell loss and necrose, lumen enlargement, Cilia loss and fusion, haemocytic infiltration, vacuolar degeneration | 2016 | [48] |
| *Mytilus galloprovincialis* | Cadmium | Digestive gland | lumen of digestive tubules, increase of the atrophic tubule | 2016 | [51] |
| *Mytilus spp.* | Mixture of Microplastics | Gill, Digestive gland | Necrosis, atrophies, lumen enlargement | 2019 | [39] |
| *Mytilus galloprovincialis* | Insecticide | Gill, Digestive gland | Vaccumulation, epithelial alterations, lipofuscin aggregates, presence of brown cells, digestive tubule alterations, hypertrophy, hyperplasia | 2020 | [36] |

**Freshwater Mussel**

| Lamellidens marginalis | Oil effluent (hydrocarbon) | Foot, Gill, Digestive gland | Foot-disorganization of outer epithelium, necrosis of the cell, formation of lumina, disorganisation of tissue bundle, Gill-disruption of the epithelium, oedema formation and necrosis, cilia appeared disaggregated | 2020 | *Our Results* |
| Lamellidens marginalis | Heavy metals | Foot, Hepatopancreas | splitting of muscle bundles, loss of connective tissue, oedema, atrophy of muscle bundles, Cell necrosis, damage to the intestinal connective tissue | 2008 | [26] |
| Lamellidens marginalis | Insecticide | Gill | The bulging of primary filament gill tips, curling of secondary filament, fusion of gill lamellae, hyperplasia, necrotic and clavate glabate lamellae of the gills | 2011 | [33] |
| Lamellidens marginalis | Dimethoate | Hepatopancreas | Disruption in digestive tubules, disrupted epithelial lining and necrotic tissue in the lumen, Hypertrophic nucleus, Necrotic tissue | 2011 | [54] |
| Lamellidens marginalis | Dimethoate | Gill | Disruption in epithelium, damage in epithelial lining, nuclear hypertrophy etc. | 2012 | [34] |
| Lamellidens marginalis | Pesticide | Gill, Digestive gland | gill exhibiting reduced space between water channel and interlamellar junction, intense infiltration of hyperchromic anaplastic cells , tissue swelling, irregular shaped branchial filaments, digestive gland exhibiting hepatic tubules with disintegrated epithelial cells and infiltrated basophilic cells | 2012 | [35] |
| Dreissena polymorpha | Fluoride | Gill, Digestive gland | scattered pyknotic nuclei, condensed nuclei, altered morphology of the cells | 2012 | [50] |
| Lamellidens marginalis | Monocrotophos | Foot | Hyperplasia of marginal pedal glands, disruption of nuclei of the epithelial glands | 2015 | [27] |
| Lamellidens marginalis | Mercury chloride | Gill | Hypoplasia of epithelial cells, gill filaments altered, oedematous, necrotic and vacuolated epithelium | 2016 | [41] |
| Anodonta cygnea | Heavy metals | Gill, digestive gland and gonads | Gills-lamellar fusion, dilated hemolymphatic sinus, clumping, and generation of cilia and hemocytic infiltration digestive gland-inflammation, hydropic vacuolation, and lipofuscin pigments, and gonad- atresia, necrosis, granulocytoma, hemocytic infiltration | 2018 | [49] |
| Unio Pictorum | Pesticide | Gill | damaged gill, epithelium rupture, damaged epithelium | 2019 | [37] |
| Lamellidens marginalis | Pesticide | Gill | Damaged ciliated epithelium, Elongated gill filament, Delaminated ciliated epithelium, gill epithelium ruptured with damaged ciliary lining | 2019 | [38] |
Molluscs are widely used in different biomonitoring projects and their histopathological analysis provides information about the general health of the animals and contaminant-specific changes in the tissues. Although laboratory as well as field studies suggest that pollutants cause toxic effects to molluscs, the histopathological effects of chemical contaminants have not generally been measured [13]. Blue mussels can retain on their gills, including oil particles have observed [14]. Like numerous bivalves, they concentrate many xenobiotics in their tissues and have been used extensively for biomonitoring of pollutants [15] but there is inadequate contribution on freshwater mussels. Gills [16] are suitable organs for histological examination in order to determine the effect of pollution. Histological changes occurs in the bivalves especially in the hepatopancreas (digestive gland) as they are the metabolically active sites and are responsible for food collection, absorption, digestion, enzymatic activity as well as accumulation and biotransformation (detoxification) of various organic and inorganic toxic substances upon exposure to the organic and inorganic contaminants in the water. Pathological changes in the vital tissues of bivalves have been reported after pollutant exposure [17,6]. Owing to their poor existence and meagre information about organic and inorganic pollutants [15] but there is inadequate contribution on freshwater mussels, their tissues and have been used extensively for biomonitoring of pollutants [15] but there is inadequate contribution on freshwater mussels. Gills [16] are suitable organs for histological examination in order to determine the effect of pollution. Histological changes occurs in the bivalves especially in the hepatopancreas (digestive gland) as they are the metabolically active sites and are responsible for food collection, absorption, digestion, enzymatic activity as well as accumulation and biotransformation (detoxification) of various organic and inorganic toxic substances upon exposure to the organic and inorganic contaminants in the water. Pathological changes in the vital tissues of bivalves have been reported after pollutant exposure [17,6]. Owing to their poor existence and meagre information about the histopathology in invertebrates remarkably in freshwater mussels.

This present study attempts to understand the pathological injuries in mussels. Therefore, present investigation were examined during This present study attempts to understand the pathological injuries in mussels. Therefore, present investigation were examined during exposure experiment to detect the accumulated oil effluent (hydrocarbon) in this species.

Materials and Methods

Animals
Almost uniform size fresh water mussel Lamellidens marginalis (total length 6-7 cm and weight 25-27 g) were collected from the River Cauvery (Tiruchirappalli, India) and maintained in the laboratory.

Acute toxicity experiment
The aqueous oil effluent originated from the coal conversion plant, turbine section of boiler units in the Boiler plants of Bharat Heavy Electricals Limited (BHEL) situated 14 km away from Tiruchirappalli, are collectively released into a drainage canal. It consisted mainly of hydrocarbons. Initial experiments were conducted to assess the minimum concentration of oil effluent to obtain maximum mortality, for freshwater mussel, Lamellidens marginalis, over a 96-hr exposure. After confirming the minimum concentration, 10 animals in 5L of tubs (each) and exposed to various concentrations of oil effluent, ranging from 4ppt to 16ppt for a period of 96-hr to ascertain LC50 concentration. In addition, a control was also maintained. The 96-hr LC50 values with 95% confidence limits were calculated using National Crop Production Centre Technical Bulletin [18].

Exposure experiment
Based on the 96-hr LC50 value of oil effluent, sublethal concentrations of 1/4th (11.88ppt) and 1/10th (8.55ppt) of LC50 were prepared and used for histopathology. In this study, two sets of 10-L plastic tubs were used. In each tub, mussels were exposed to 11.88ppt or 8.55ppt of oil effluent. A control was also run simultaneously without the addition of oil effluent. At seven days interval, mussels were sacrificed for histological analysis. After 30 days, the treated mussels were released into freshwater 30-day depuration (recovery) study was conducted. Four mussels were randomly chosen and removed from each of the two tubs (n=8) for dissection.

Preparations of tissue samples
Control set of animals tissues were sacrificed for 1st, 8th, 15th, 22nd and 30th days of foot, gill and digestive gland tissues of mussels. At every seven days intervals, accumulation and depuration (recovery) period of 1st, 8th, 15th, 22nd and 30th days of foot, gill and digestive gland tissues of mussels from both exposures were sacrificed for the evaluation of histological analysis.

Paraffin method: For the paraffin method, the above specified tissues were fixed in Bouin’s fluid, and embedded in paraffin. Serial sections were obtained at 3-5 μm thickness using a Leica (Germany) microtome with provision of disposable blade. Serial sections stained in haematoxylin and eosin [19] and mounted in DPX mountant for microscopic observations.

Microscopic analysis: For light microscopic observation Carl-Zeiss (Germany) Axioskop 2-research microscope was used, and the images were captured in a computer using Carl-Zeiss (Germany) Axiovision Software and the images processed using the same software. The histopathological changes in the tissues of experimental as well as control mussels were recorded and compared.

Results

Histology of foot
Foot is the locomotory organ chiefly employed for burrowing and is formed of an outer dense epithelium, which is grown into tall folds (villous); the epithelium lies top of the cells the musculature, variety of protractor, retractor muscles. Blood sinuses are found between the fine muscles. The muscular wall of the foot surrounds the coelomic phase which itself is lined by coelomic epithelium. In the outer epithelium, the cells differ in height and length of the nucleus. In some of the cells the nucleus is extremely elongated. The outer borders of the epithelium have dense cilia (Figures 1-4).

Histopathology of foot
During the exposure of both sublethal concentrations (1/4th and 1/10th) of oil effluent little changes were observed in the foot tissues of mussel at 1st day. However, from the 8th day onwards the outer borders of the epithelium have dense cilia (Figures 1-4).

Figure 1: Haematoxylin and eosin stained paraffin sections of control mussel Lamellidens marginalis foot tissue. (X100, 400).
epithelium was thoroughly disorganized resulted in necrosis of the cell and formation of lumina both on top of the musculature, with exposure for longer durations the muscle bundle themselves were disorganized in both sublethal concentration of oil effluent exposure (Figures 5-11). During the depuration (recovery) process, the fresh water mussel brought about almost complete restorations of the histo-architecture of the foot tissues (Figures 12-21).

**Histology of gill**

The gill of the mussel is formed of an outer and inner lamellae called ctenidium, each folded to form the outer and inner lamellae. The lamellae are connected by inter-lamellar junctions, which contain blood vessels. The gill filament constitutes a longitudinal array and

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**Figure 2:** Haematoxylin and eosin stained paraffin sections of control mussel *Lamellidens marginalis* foot tissue. (X100, 400).

**Figure 3:** Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-8th, 8th and 15th days) foot tissue. (X400).

**Figure 4:** Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-8th, 8th and 15th days) foot tissue. (X400).

**Figure 5:** Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-8th, 8th and 15th days) foot tissue. (X400).

**Figure 6:** Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-8th, 8th and 15th days) foot tissue. (X400).

**Figure 7:** Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-22nd and 30th days) foot tissue. (X400).

**Figure 8:** Haematoxylin and eosin stained paraffin sections of treated mussel (1/4th accumulation-22nd and 30th days) foot tissue. (X400).

**Figure 9:** Haematoxylin and eosin stained paraffin sections of treated mussel (1/10th accumulation-8th, 15th and 22nd days) foot tissue. (X400, X400, X100).

**Figure 10:** Haematoxylin and eosin stained paraffin sections of treated mussel (1/10th accumulation-8th, 15th and 22nd days) foot tissue. (X400, X400, X100).

**Figure 11:** Haematoxylin and eosin stained paraffin sections of treated mussel (1/10th accumulation-8th, 15 and 22nd days) foot tissue. (X400, X400, X100).
The gill filament is lined by an epithelium formed of a single row cells, short cuboidal adiphase and tall columnar towards the tip. Three types of cilia are associated with gill filament, they are, tall lateral-cilia, tall latero-frontal cilia and short frontal -cilia at the tip. The stroma underlying the epithelium bridges of connective tissue containing adjacent filaments are connected by inter-filamentor junctions.
Histopathology of gill

When exposed to both sublethal concentrations of oil effluent caused disruption of the epithelium of the gill filaments and all the versions of the cilia. The epithelium indicated severe pathological changes of the oedema formation and necrosis. Disaggregated cilia were appeared (Figures 24-32). During the recovery period, brought about partial to almost complete restoration of the histo-architecture of the gill filaments. The cilia appeared normal. The epithelium shows almost free from oedema formation and necrosis (Figures 33-42).

connecting rods for support and also blood vessels (Figures 22,23).

Figure 22: Haematoxylin and eosin stained paraffin sections of control mussel gill tissues (X400, X1000).

Figure 23: Haematoxylin and eosin stained paraffin sections of control mussel gill tissues (X400, X1000).

Figure 24: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-1st day) (X400).

Figure 25: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

Figure 26: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

Figure 27: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

Figure 28: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

Figure 29: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/4th accumulation-8th, 15th, 22nd and 30th days and 1/10th accumulation-1st day and 8th day). (X400).

Figure 30: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/10th accumulation-22nd and 30th days). (X400).

Figure 31: Haematoxylin and eosin stained paraffin sections of treated mussel gill tissues (1/10th accumulation-22nd and 30th days). (X400).
Histology of digestive gland

The digestive gland consists of diverticula and their ducts, which connected to be, and inter diverticula tissue (Figure 43). The glandular epithelium is formed of different types, most of which are tall columnar. The different heights of the cell, render the epithelium appears live villous folds, lamellae propitious underlies the epithelium. The lumen of diverticulate contains a few materials, which are likely the ingested food (Figure 44). The nucleus of epithelium is elongated,
spindle shaped and darkly staining, it is located at different heights of the cells. The folds of epithelium produce pockets in the profile of the lumen (Figure 45).

**Histopathology of digestive gland**

When exposed to both sublethal concentrations of oil effluent, produced gross changes in the epithelium of the glands as well as the stroma. The glandular epithelium was invariably detached from the stroma (Figure 46-65). This stroma adds dense accumulation leucocytes from 1st day of both sublethal concentrations. The integrity of the epithelium was thoroughly disrupted and a major change was consisted of vertical clefts. Another important feature was noticed towards the dense accumulation of the luminal material, which also
contain leucocytes, which were otherwise confined to stroma. During the recovery (depuration) period, the fresh water mussel brought about a gradual restoration of the organization of the digestive gland and nature of the epithelium. The day 15th onwards the glandular

Figure 52: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-Ist, 8th, 15th and 22nd days) (X400).

Figure 53: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-Ist, 8th, 15th and 22nd days) (X400).

Figure 54: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-Ist, 8th, 15th and 22nd days) (X400).

Figure 55: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissues (1/10th accumulation-30th days). (X400).

Figure 56: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-Ist, 8th, 15th, 22nd and 30th days) (X1000).

Figure 57: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-Ist, 8th, 15th, 22nd and 30th days) (X1000).

Figure 58: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-Ist, 8th, 15th, 22nd and 30th days) (X1000).

Figure 59: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-Ist, 8th, 15th, 22nd and 30th days) (X1000).

Figure 60: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/4th accumulation-Ist, 8th, 15th, 22nd and 30th days) (X1000).

Figure 61: Haematoxylin and eosin stained paraffin sections of treated mussel digestive gland tissue. (1/10th accumulation-Ist, 8th, 15th, 22nd and 30th days). (X1000).
architecture was comparable to that of the control mussels, though the dense accumulation of leucocytes between the stroma and epithelium continued till the 30th day, but the stroma was absent i.e. completely restored (Figure 66-75).
Discussion

Biological, physiology and morphological structure of molluscan systems were described [20-22]. The normal structure of mussels gill, foot, digestive gland has been well-described [23,24]. In some publications, lesions have only been described morphologically [25]. Summary of some relevant earlier literature on marine as well as freshwater mussels histological observations with various toxicants and comparison with our present results are compiled (Table 1). In the present study, when exposed to both sublethal concentrations of oil effluent little changes were observed in mussels foot tissue on 1st day. From 8th day onwards, outer epithelium of the mussels’ foot tissue were disorganised and resulted in necrosis of the cell. When exposed to the sublethal concentrations of oil effluent, for longer duration’s muscle bundle themselves were also disorganised in mussel foot tissue. This could lead to the failure of a number of biochemical activities as well as osmo and ionic-regulatory functions of the foot. Present histopathological findings may explain a defensive reaction from the mussels under investigation as similar results were observed by molluscan researchers. Thus, the present results are in agreement with [26] who exposed to heavy metals splitting of muscle bundles in foot tissue were observed in the fresh water mussel, Lamellidens marginali. Similar findings [27] were observed disruption of nuclei of the epithelial glands in foot tissue when exposed to pesticide. The histopathology of foot indicated that concentration and duration of exposure period resulted in massive destruction in normal architecture of foot tissue of mussel. Similarly, the disruption of the epithelium of the gill filaments and all the versions of the cilia were observed when exposed of both sublethal concentrations of oil effluent. The epithelium indicated severe pathological changes of the oedema formation and necrosis. The cilia appeared disaggregated during accumulation of both sublethal concentrations of oil effluent. The gills (cetenidia) of lamelibranch bivalves play a dominant role in controlling the interaction between the individual and its environment. A great deal of literature is available on the mechanisms of food particle retention, as well as on the nature and activities of the ciliary systems of such organs [28]. Histological alterations and biochemical changes of gill tissues produced by the chemical stress causes disturbed metabolism, enzyme inhibition, retardation of growth, fecundity reduction and longevity of the organism, which will affect balance in the ecosystem [29]. An exhaustive review of toxicant/ irritant induced changes in the gill, stated that the inflammatory changes tend to be largely non-specific, and seen to reflect physiological adaptation to stresses were made [16]. Tributyltin (TBT) treated mussel Mytilus galloprovincialis the structure of gill was destroyed, interfilament junctions and cilia disappeared and lateral and endothelial cells were changed [30]. A wide variety of histopathological and physiological responses to naphthalene exposure were described in the mummichog Fundulus heteroclitus [31]. Structural changes and proliferate lesions of gills in Salmo trutta, Oncorhynchus mykiss, was observed [32] when exposed to sewage plant effluents. When exposed to pesticides, fusion of gill lamellae in Lamellidens marginalis [33], gill epithelium lining and disruption in Lamellidens marginalis [34], gill exhibits reducing space between channels, tissue swelling in Lamellidens marginalis [35], vacuolation, epithelial alterations in Mytilus galloprovincialis [36], damaged epithelium, cilia, epithelium ruptured in gill of Unio pictorum [37], elongated gill filament, gill epithelium ruptured with damaged ciliary lining in Lamellidens marginalis [38] were observed and when exposed to microplastics, epithelial alterations, necrosis were observed in Mytilus spp. [39]. When exposed to heavy metals loss of cilia, epithelial damage, swollen lumen, elongation of gill filaments in Perna viridis [40]; gill filaments altered, oedematic, necrotic and vacuolated epithelium in Lamellidens marginalis [41] were observed. Hence, the changes in the histopathological structure of the gill can be use as biomarkers of exposure in the aquatic environment and the freshwater bivalve Lamellidens marginalis can be considered as a bioindicator organism to assess the water quality. Similarly, the digestive gland produced gross changes in the epithelium of the glands as well as the stroma. The glandular epithelium invariably
tube thickness, lumen enlargement, necrotic tissues by heavy metals and our present study are in agreement with the following findings of gland of mussels to various pollutants exposure of previous results accumulation of the luminal material, which also contain leukocytes, another important feature were observed towards the dense architecture of the gill and digestive gland tissues due to oil effluent and definitely disturbs its normal functions and storage of nutrient materials. Particularly, the digestive gland is also helpful for metabolism of xenobiotics in freshwater mussel *Lamellidens marginalis*. These histopathology might be due to the possible utilization for metabolic purpose and may be of early warning signals of environmental pollution.

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