



Histological Alterations in Two Estuarine Fishes, (*Mugil cephalus* and *Sillago sihama*) from Ennore Creek, South East Coast, India

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ABSTRACT

Background: Two fish species namely the flathead grey mullet, *Mugil cephalus* and Silver sillago, *Sillago sihama* were selected for the histological studies to assess the impact of pollution on the vital organs of fishes.

Methods: The gill, liver and kidney tissues of *Mugil cephalus* and *Sillago sihama* of Ennore Creek and unpolluted reference site, Arambakkam were collected. Histological sections of tissues collected from the UPRS, Arambakkam showed normal histoarchitecture. However, there were several histological alterations in the above-mentioned organs of these two species collected from Ennore Creek due to high pollution in Ennore Creek.

Result: The present study showed that the fishes are exposed to pollution-induced stress at Ennore Creek, making the fishes anaemic, weak and vulnerable to diseases. The Ennore Creek is a coastal wetland affected by a major oil spill and other anthropogenic pollution sources. Histological analysis revealed the occurrence of prominent gill and liver lesions, especially in the tissues of *M. Cephalus*. This study provides scientific evidence for the biological impacts on the resident fishes in the Ennore creek and calls for further research on the impacts of coastal water pollution and potential management strategies.

Key words: Ennore, Gill, Histology, Kidney, Liver, Pollution.

INTRODUCTION

Fishes are considered as indicators of environmental and ecological changes within estuaries (Coulibaly *et al.*, 2012). Lagoons and estuaries are the most vulnerable ecosystems to oil contaminations due to oil spills in the open sea. The oil slicks can drift towards the shore and further into the estuaries and lagoons (Hinton and Lauren, 1990). Chemical monitoring of water helps to measure the degree of contamination. Fish is a suitable indicator for environmental pollution monitoring because they concentrate pollutants in their tissues directly from water and also through their diet, thus enabling the assessment of the transfer of pollutants through the trophic web (Fisk, 2001). The assessment and evaluation of pollution effects on various organisms, including fish can be studied using biomarker responses. Biomarkers are measures of sub-organismal responses in organisms or exposed biological systems which can demonstrate exposure to, or the effects of environmental contaminants (Peakall and Walker, 1994). A variety of molecular, biochemical, physiological, histo-cytopathological, organismal, population and community responses may be used to identify exposure to certain chemicals, provide information on spatial and temporal changes in the concentration of contaminants and indicate environmental quality or occurrence of adverse ecological consequences. Among the various biomarkers, histopathological changes in animal tissues are reliable and direct indicators to environment stressors. Histopathology has become a

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standard biomarker when defining toxicological effects, but it is invasive, time-consuming and expensive. Histopathological evaluation remains an important part of the assessment of the adverse effects of xenobiotics on the whole organism (Reddy and Rawat 2013). In addition, haematological and biochemical parameters are also used as indicators in the measurement of health conditions and toxicological symptoms of organisms. While providing information about the health status of organisms, these parameters may also indicate abnormal environmental conditions. Information about the existence, status and degree of possible sickness in organisms can be rapidly obtained by with use of haematological and biochemical parameters.

MATERIALS AND METHODS

Site description and fish collection

The Ennore Creek (13°13'25.483" N, 80°19'26.603" E) is located approximately 24 km in the northeastern part of Chennai City, Tamil Nadu, India at the coast of the Bay of Bengal. The creek or estuary is connected with Pulicat Lake in the north through Buckingham Canal and Kosasthalaiyar River in the northwest. The sampling site was chosen in Ennore Creek for the present research (Fig 1). A fishing village, Arambakkam (Fig 2), (13°33'16.9" N 80°04'40.3" E) is located along the coast of Pulicat Lake is a relatively Unpolluted Reference Site for the collection of test animals, water and sediment samples compared to Ennore Creek. There are no industries in the vicinity of this fishing village. Further, this location is a part of the Pulicat Lake Bird Sanctuary.

Collection of tissue samples

The tissue samples like gill, liver and kidney tissues were collected from *M. cephalus* and *S. sihama* from both the study site as well as from unpolluted reference site and the collected tissue samples were preserved in 10% buffered formalin.

Histology procedure

Gill and liver tissues of the fishes were preserved in neutral buffered formalin and were processed using standard histological methods (Hinton and Lauren 1990). The processed samples were embedded in paraffin wax and sections of 0.5 µm thickness were cut and stained with hematoxyline and eosin. The stained sections were observed under the bright field microscope for the examination of the histological structure of the gills and liver of fish in comparison to the normal histological

appearance in the fish tissues and severity of the histological alterations was also recorded categorically.

The fixed tissues were washed up in tap water. Then, the tissues were dehydrated with a series of upgraded alcohol solution (30% alcohol for 20 min; 50% for 20 min; 70% for 30 min; 90% for 30min; 95 % for 30 min; Absolute alcohol I for 45 min and Absolute alcohol II for 45 min) and the alcohol was cleared by a series of alcohol and xylene mixture [Alcohol (2) : Xylene (1) for 45 min; Alcohol (1) : Xylene (2) for 45 min; Xylene I for 1hr and Xylene II for 1 hr] with using an Automatic Tissue Processor and a Cold Plate. Thin sections (5 µm sections) were taken from the processed tissues using microtome. The sections were floated in the tissue flotation bath maintained at 60°C and collected on clean slides applied with Mayer's albumin. The sections were fixed on the slides at 60°C using spirit lamp.

RESULTS AND DISCUSSION

Histopathological changes in *M. cephalus* and *S. sihama* of the study site Ennore and relatively unpolluted reference site were observed under the microscope were given in (Fig 3-6). The histology of various organs of two species collected from selected site, Ennore and Arambakkam. The results were compared with respective organs of the same species collected from two different sites, Ennore and Arambakkam and the histological changes were marked.

Gills, are considered as a primary target of the contaminants as they participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment and particularly sensitive to changes in the quality of the water (Poleksic and Mitrovic, 1994; Fernandes



Fig 1: Sampling station.

and Mazon, 2003). The fish gill is a multi-function organ that is directly exposed to its environment and is an excellent model for the study of fish diseases and toxicology, which focus on gill alterations and responses (Samanta *et al.*, 2018). Histology of control gill showed normal filaments and well-shaped lamella separated by large interlamellar space with well-differentiated primary lamella and secondary lamella, normal pillar cells (Badroo *et al.*, 2020). Marked variations like primary lamellae, secondary lamellae, primary lamellar epithelium, blood vessel, interlamellar region, cartilaginous core were noticed in gill tissues of *M. cephalus* and *S. sihama* collected from the polluted site Ennore.

The histological alterations were found in *M. cephalus* and *S. sihama* collected from the polluted site (Fig 3 and 5) exhibited abnormalities like erosion of secondary gill lamellae, shortening of secondary gill lamellae, lifting up of epithelium, blood congestion in the secondary lamellae, curling of secondary lamellae, hyperplasia, excessive mucus secretion, hemorrhage at secondary lamellae and cartilage tissue hypertrophy. Similar histopathological observations were made in *Terapon jarbua* and *Arius maculatus* inhabited in a sewage polluted site along

Thoothukudi Coast of Gulf of Mannar, South India (Kalaifarasi, *et al.*, 2017). The present study agreed with findings of Rajeshkumar *et al.* (2015) the histopathological damage in *Mugil cephalus* collected from heavy metal polluted Berbice-Corentyne coast of Guyana. Through the gills, as the main site of xenobiotic transfer, the toxins are distributed through their bodies accumulating in tissues and organs and may have deleterious effects (Vasanthi *et al.*, 2013). According to Karan *et al.* (1998), gill histopathology changes are the fastest and easiest adaptations to low water quality and have the purpose of decreasing the respiratory surface and increasing diffusion and result from exposure to a number of inorganic and organic pollutants (Viana *et al.* 2013).

In their study, *Anabas testudineus* groups exposed to paraquat herbicide exhibited lamellar epithelial lifting, hyperplasia and fusion in their gills. The lamellar epithelial lifting is the separation between epithelial cells and lacuna in the secondary lamella. This separation increases oxygen diffusion distances between the epithelial cell and lacuna (Maharajan *et al.*, 2016). Mallatt (1985) reviewed that most common gill lesions found under several stressful conditions and reported the common changes like

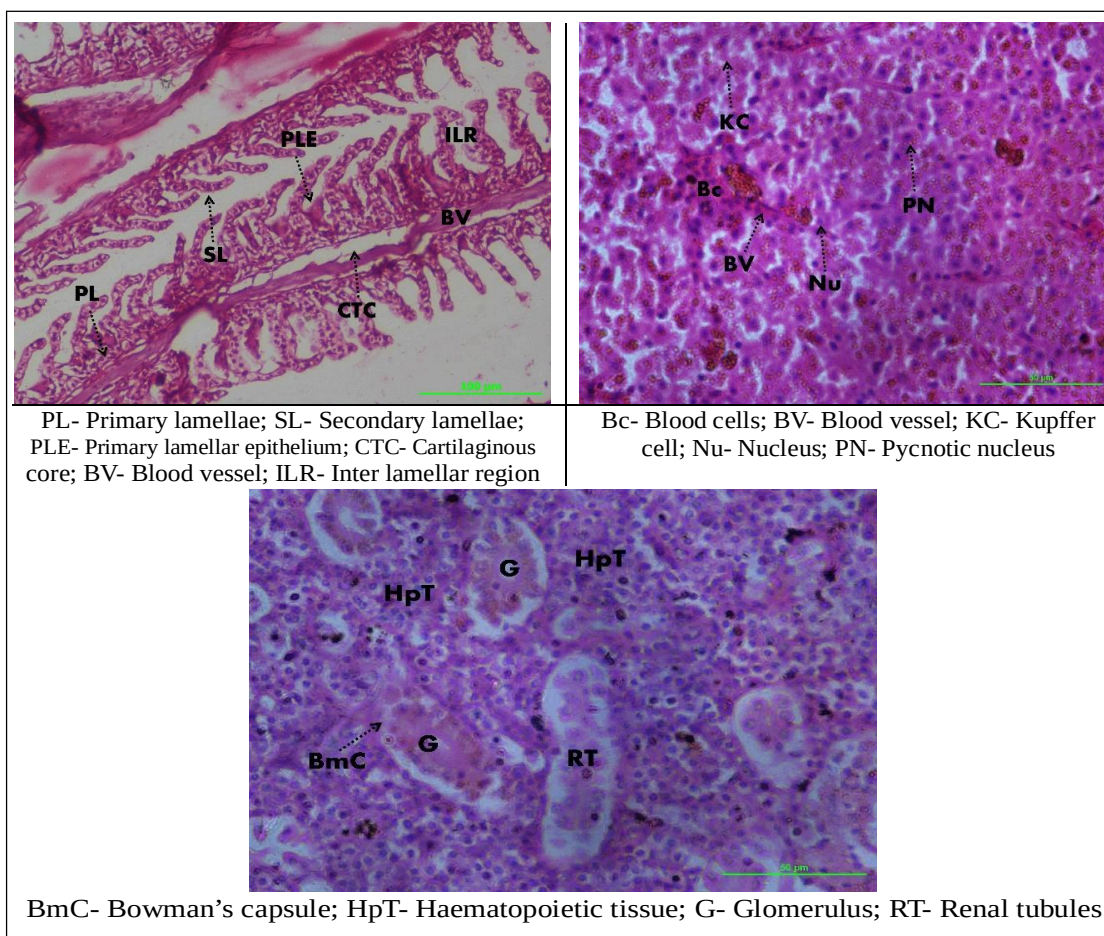


Fig 2: Photomicrograph of histological section of gill, liver and kidney of Mullet collected from reference site (Arambakkam) [section – 5 µm thickness; H and E staining: Gill (200 X); Liver and Kidney (400 X)].

epithelial lifting, necrosis, hyperplasia, hypertrophy and hemorrhage as a of lethal conditions. A typical chronology of damage from acute exposure to the test chemical is first a lifting of outer layer of lamellar epithelium usually starting in the area of chloride cells. Edematous spaces are formed between the layers of epithelium and these may become infiltrated with leukocytes. Eventually, the whole epithelium sloughs off and the lamellar loses rigidity on the blood side of the lamellar, the central space collapse, but the marginal channel often remains normal until the rest of the lamella is essentially destroyed (Heath, 1987). The proliferation of chloride cells are thought to be a compensatory response to iron loss. And therefore, chloride cells hyperplasia may therefore be a good biomarker of adaptation. Hyperplasia of undifferentiated epithelial cells, which results in clubbing and lamellar fusion is a much less specific lesion associated with a wide variety of unrelated insults (Hinton and Lauren, 1990).

Histology of liver tissue of both fish species collected from a reference site (Arambakkam) (Fig 3 and 5) showed normal histoarchitecture. The liver tissue of fish species collected from Ennore site showed several abnormal

changes in the organs due to pollution. The liver is a detoxification organ that serves as a biomarker for environmental quality (Gartner *et al.*, 2014). Moreover, liver plays a major role in complex enzymatic process that are responsible for vital functions, such as accumulation and biotransformation of xenobiotics in the fish. Some distinct changes like rupture of hepatocytes, melanomacrophages, cytoplasmic vacuolization, blood congestion, degeneration of hepatocytes, cellular necrosis, increased pycnotic nucleus, nuclear degeneration were observed in the liver of fish of polluted Ennore creek (Fig 5). Since liver is involved in detoxification of pollutants (Afifi, *et al.*, 2014), it is susceptible to a greater degree of disruption in its structural organization due to toxic stress. Macrophage aggregates have been suggested as potentially sensitive histological biomarkers and or immunological biomarker of contaminant exposure.

Histopathological alterations in the form of irregular shape, vacuolation, pycnotic nuclei and focal necrosis of hepatocytes, ruptured sinusoids with hemorrhages, perivascular fibrosis of liver cells, disposition of yellow-brown grains on hepatic tissues were found in

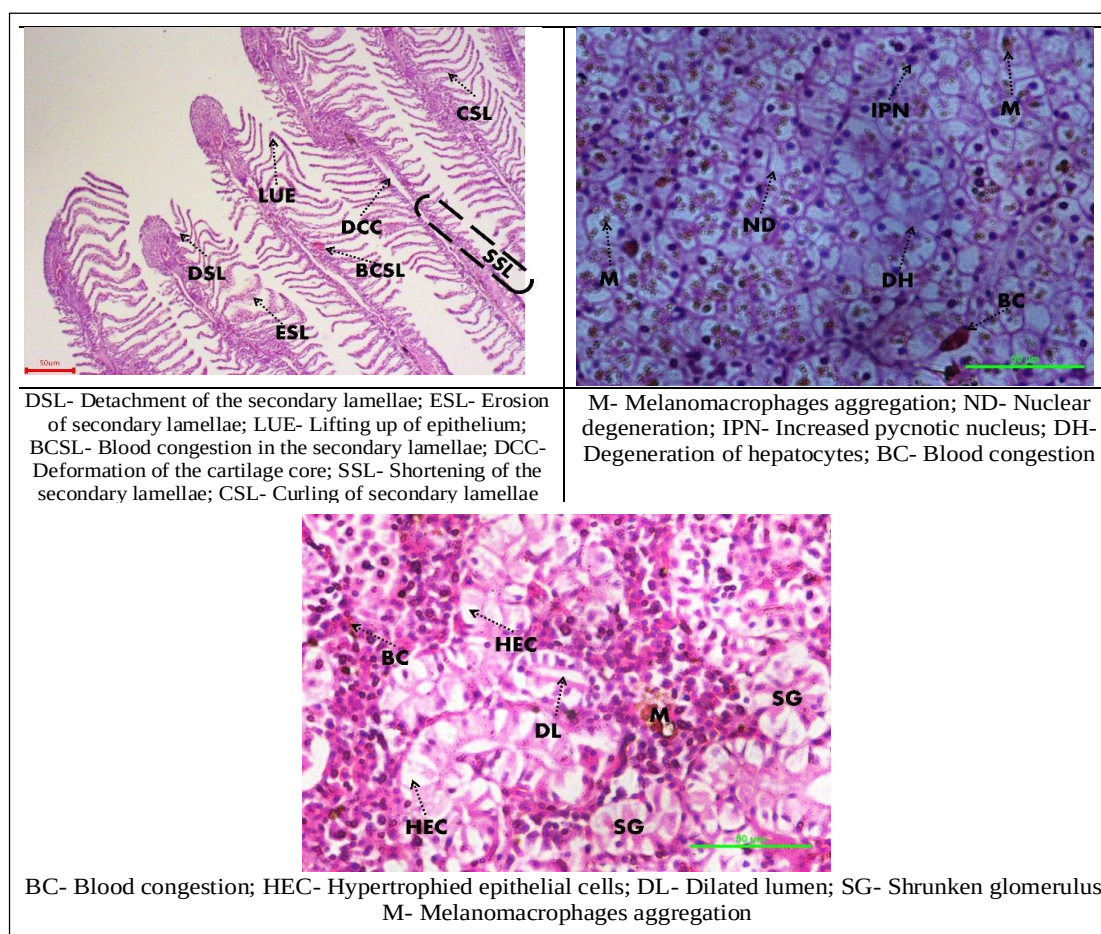


Fig 3: Photomicrograph of histological section of gill, liver and kidney of Mullet collected from Polluted site (Ennore Port) [section – 5 µm thickness; H and E staining: Gill and Liver (200 X); Kidney (400

cypermethrin treated *O. niloticus* (Majumder and Kaviraj, 2022). The liver tissues of *Mugil cephalus* collected from polluted Ennore creek showed histological alterations like vacuolization in the hepatocytes, fibroblast proliferation, vacuole formation, granular degeneration and necrosis (Vasanthi *et al.*, 2013). Afifi *et al.* (2014) studied the histological alterations in liver tissue of *Siganus canaliculatus* and *Epinephalus morio* caught from oil polluted Jeddah coast and reported that lipid aggregation, hydrophic degeneration, necrosis, pyknosis and karyorrhexis with cholangitis of proliferated bile duct epithelium, spongiosis hepatis, Hepatocyte with enlarged nuclei with prominent nucleoli (megalocytes). Congestion of hepatoportal blood vessels, intravascular hemolysis, hemorrhage and intravascular vacuolated leukocytes are seen in the liver tissue histology. A study conducted by (Javid and Usmani 2013), in *Mastacembelus armatus* for the assessment of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution in effluent dominated water. The result showed severe lamellar fusion, hyperplasia, hypertrophy and epithelial lifting, swelling and deformed lamella, in some parts sloughing off and curving of lamellae. Rajesh kumar *et al* (2015) assessed the heavy metal pollution in east

Barbic-Correntyne, Guyana by using histology of *Mugil cephalus* liver tissue. The result showed the alterations like vacuolization in the hepatocytes, fibroblast proliferation, vacuole formation, granular degeneration and necrosis. Bakhiet (2015) reported that fishes collected from sewage contaminated Nile river stretch showed histological changes in liver tissues due to bioaccumulation of heavy metal. The result revealed degeneration of the hepatocytes, congestion of central vein and nuclear pyknosis in the majority of hepatic cells. Similar results were observed by Van Dyk (2003). The fish's liver is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment, other organs (Heath, 1987). These findings were apparent as the liver considered the organ of detoxification, excretion and binding proteins such as metallothionein. The metal-binding proteins present in the nuclei of hepatocytes suggested that the increase in the cell damages.

In the present study, kidney of both the fish species suffered damages due to the exposure to the pollutants. Some distinct changes like hypertrophied epithelial cells, melanomacrophages aggregation, narrowing lumen,

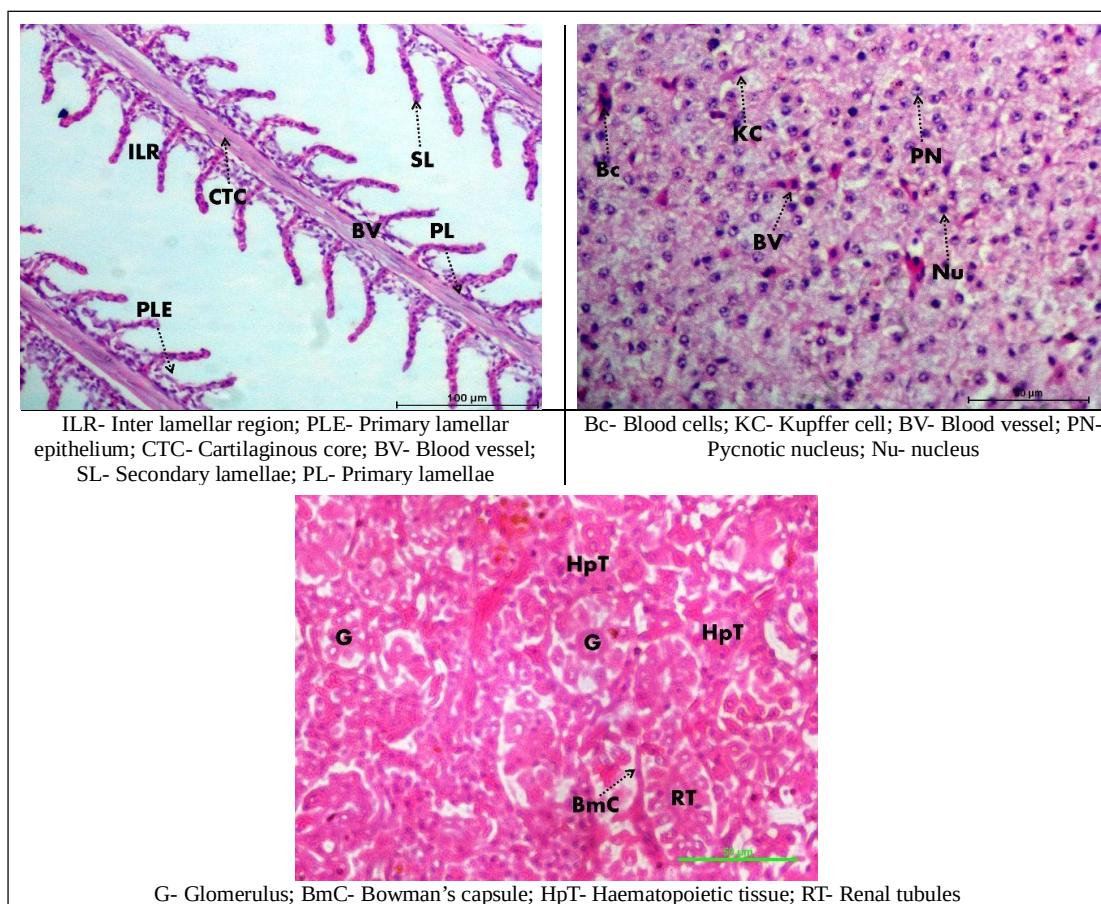


Fig 4: Photomicrograph of histological section of gill, liver and kidney of *Sillago* collected from reference site (Arambakkam) [section – 5 µm thickness; H and E staining: Gill (200 X); Liver and Kidney (400 X)].

dilated lumen, shrunken glomerulus, degenerated tubule, degenerated glomerulus, blood congestion were observed from both the fish species from the study area. Histology of kidney tissue of both the fish species from a reference site (Arambakkam) showed normal histoarchitecture (Fig 3 and 5). The kidney tissue of fish species collected from the polluted site Ennore showed several abnormal changes due to pollution. The kidney of fish receive the vast majority of post branchial blood and because of that, renal lesions can be expected in the fish when toxicant agents exist in the environment. Majumder and Kaviraj (2022) reported that the cypermethrin treated *O. niloticus* also exhibited glomerular shrinkage and expansion of the space between glomerulus and Bowman's capsule. Moreover, intracytoplasmic vacuolation in the renal tubular epithelium, hyaline degeneration of tubular epithelium and dilation of tubular lumen were also noted due to cypermethrin exposure.

Coulibaly (2012), noted the histological changes in kidney of black finned tilapia from heavy metals contaminated Bay Bietri and reported vacuolization, dilation

in renal cells, severe dilation with renal cells degeneration, hemorrhage, parasitic cyst, vacuolar degeneration in the epithelium of renal tubules, parasites, dilation in renal blood vessels and cyst were observed. Gusma *et al.* (2012) reported that the larvae of Marine Pejerrey *Odontesthes argentinensis* exposed to water-soluble fraction of petroleum resulted in histological alterations, like enlargement of glomerulus, intercellular spaces and abnormal nucleus of epithelial cells and hypertrophy were noticed. Non-specific kidney histopathological lesions e.g. degenerative changes in tubular epithelium, dilation of tubular lumina, proteinaceous or cellular casts within tubular lumina, tubular necrosis and/or epithelial desquamation and necrosis of interstitial hematopoietic tissues were observed following exposure of fish to organochlorines, petroleum compounds, organo phosphate, herbicides and heavy metals (Gartner and Hiatt 2014). Kidney histopathology in some feral fish shows promise as an indicator of organic contaminant exposure (Myers *et al.*, 1992).

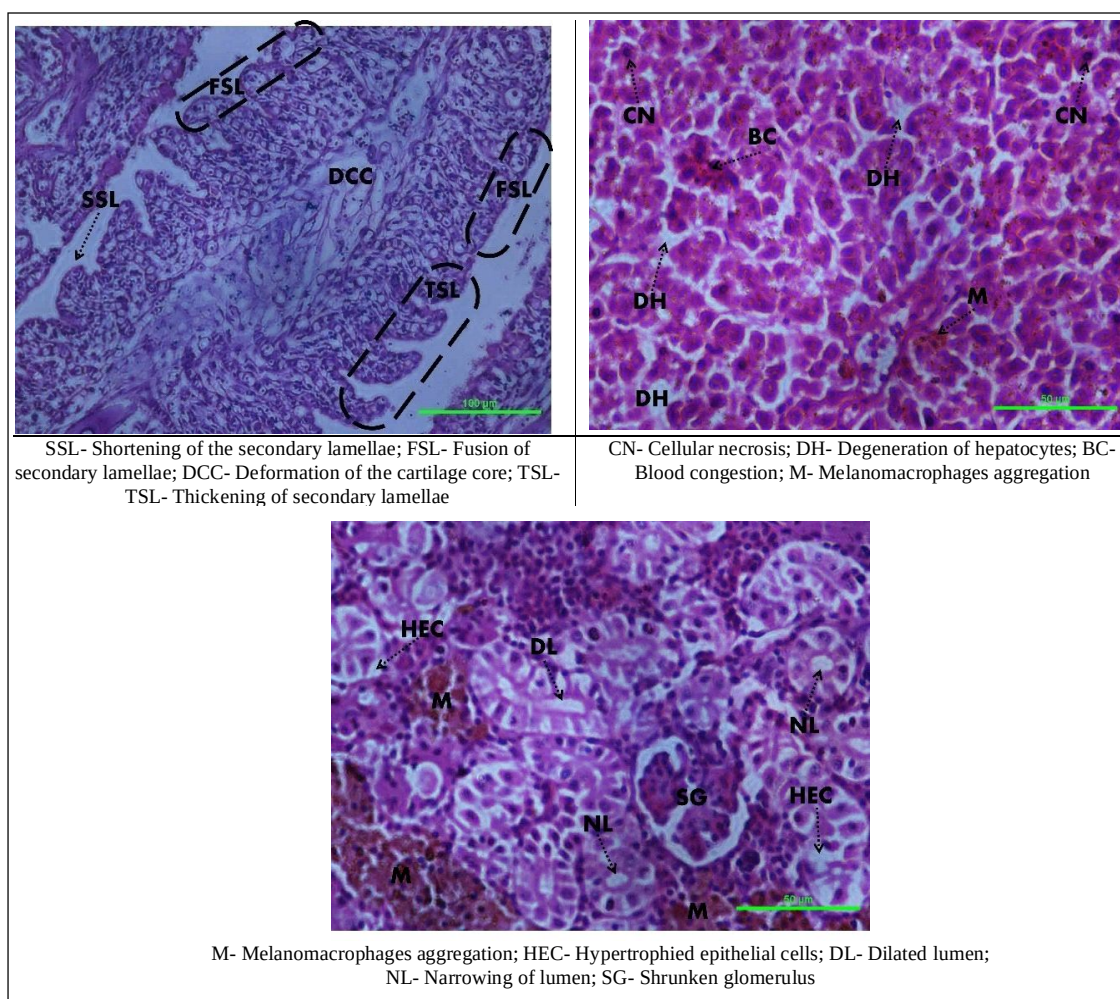


Fig 5: Photomicrograph of histological section of gill, liver and kidney of *Sillago* collected from Polluted site (Ennore Port) [section – 5 μ m thickness; H and E staining: Gill and Liver (200 X); Kidney (400 X)].

In more severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibiya, 1995). The presence of tubule degeneration, coupled with the absence of necrosis in the kidney in the present study indicates that the kidney suffered damage after exposure to pollutants. Some distinct changes like blood congestion, increased periglomerular space, increased peritubular space, glomerulus, degenerated tubules, shrunken glomerulus, necrosis, vacuolation, melanomacrophage, degenerated glomerulus, loss of cytoplasm were observed in the kidney of fish.

CONCLUSION

In the present study concluded that, the results of histopathological changes in gill, liver and kidney tissues of *M. cephalus* and *S. sihama* from the study site Ennore creek were showed several alterations in the structure which might be due to major oil spill and other anthropogenic pollution sources in the study area and relatively unpolluted reference site Arambakkam were showed normal histoarchitecture of *M. cephalus* and *S. sihama*. This study provides scientific evidence for the biological impacts on the resident fishes in Ennore creek on the impacts of coastal water pollution and potential management.

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Conflict of interest

All procedures followed were in accordance with the ethical standards of the responsible, the authors declare that they have no conflict of interest.

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