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Kalaiyarasi T
Department of Fisheries Biology and Resource Management Fisheries College and Research Institute, Tamil Nadu Fisheries University Thoothukudi, Tamil Nadu, India

Jayakumar N
Department of Fisheries Biology and Resource Management Fisheries College and Research Institute, Tamil Nadu Fisheries University Thoothukudi, Tamil Nadu, India

Jawahar P
Department of Fisheries Biology and Resource Management Fisheries College and Research Institute, Tamil Nadu Fisheries University Thoothukudi, Tamil Nadu, India

Ahilan B
Department of Fisheries Biology and Resource Management Fisheries College and Research Institute, Tamil Nadu Fisheries University Thoothukudi, Tamil Nadu, India

Subburaj A
Department of Fisheries Biology and Resource Management Fisheries College and Research Institute, Tamil Nadu Fisheries University Thoothukudi, Tamil Nadu, India

Correspondence
Jayakumar N
Department of Fisheries Biology and Resource Management Fisheries College and Research Institute, Tamil Nadu Fisheries University Thoothukudi, Tamil Nadu, India

Histological changes in the gill and liver of marine spotted catfish, *Arius maculatus* from sewage disposal site, Therespuram off Thoothukudi, Southeast coast of India

Kalaiyarasi T, Jayakumar N, Jawahar P, Ahilan B and Subburaj A

Abstract
The study evaluated the histological changes in gill and liver of marine spotted catfish, *Arius maculatus* collected from the sewage disposal site at Therespuram off Thoothukudi, Southeast coast of India. Specimens of *Arius maculatus* were collected from the study site bimonthly from Sep’ 2016 to June’ 2017 with the help of local fishermen by gillnetting. Similarly, specimens of the same species were collected from the relatively unpolluted site (Vembar, Southeast coast of India). Then, the histological sections of the gill and liver were prepared. Observation of the histological sections revealed several abnormal changes in the gill and liver of the fishes collected from the study site whereas the same was normal in the case of fishes collected from the reference site. Histopathological picture of the organs can corroborate with the biochemical changes accounting for the functional disruptions in the activity of the organs due to cellular damage. Hence, it becomes necessary to take appropriate measures to prevent the entry of raw sewage in to the sea.

Keywords: Sewage, coastal pollution, catfish, histology, gill and liver

Introduction
Coastal and marine ecosystems are one of the most productive ecosystems in the world, provide many services to human society and are of great economic value (UNEP, 2006). 75% of commercially harvested fish and shellfish depends on estuary and nearby coastal water for some part of their life cycle. The water quality of the coastal areas, estuaries and major river around the world is getting rapidly degraded due to massive discharge of industrial water of diverse origin, domestic sewage, mine drainage, oil spills, and extensive use of agrochemicals [35].
Aquatic pollution is one of the current global environmental issues. Water resources have been the most exploited natural systems since man strode the earth. With the rapid development of industrialization, unplanned urbanization and an increase in human population, the pollution of water bodies has become a universal phenomenon in the present day world. The discharge of industrial, agricultural and domestic waste water into the environment results in the pollution of these aquatic systems. The coastal pollution has seriously affected the exploitable living resources, recreational and commercial uses of coastal areas and the overall integrity of the marine and coastal ecosystems. Hence, the protection of the coastal and marine regions from continuing pollution becomes the most essential in coastal resources management [10].
Histopathological characteristics of specific organs express condition and represent a time-integrated endogenous and exogenous impact on the organism stemming from alterations at lower levels of biological organization [6, 33]. Histological changes take place in advance to reproductive changes. Further, these changes are more sensitive than growth and reproductive factors. In addition, as histology is an integrative parameter, it provides a better assessment of the health of the organism than a single biochemical parameter [31]. In this regard, Austin [6] reviewed the effects of marine pollution on fish health and Au [5] reviewed the application of histo-cytopathological biomarker in marine pollution monitoring. Additionally, histological biomarkers provide powerful tools to detect and characterise the biological end points of toxicant and carcinogen exposure [13, 14, 23, 24] and oil exposure [3, 9, 24].
Fishes are considered as early warning for the degradation of environmental quality but also specific measures of the existence of toxic carcinogenic and mutagenic compounds in the biological materials. Liver and gills are the main organs for metabolism and respiration and

these organs are also target organs for contaminant accumulation resulting in structural damage to organs and tissues. Untreated sewage effluents are reported to increase significantly the impact on aquatic ecosystems, decreasing the species diversity in different regions of the world [30]. The tolerance of wild organisms to toxicants in domestic effluents may vary among species and their integrative effects may lead to reproductive failure or reduction of the fish species number. Many researchers have reported the degenerative changes in selected tissues of the fishes in response to different kinds of toxicants [1, 18, 23, 28].

Materials and Methods

Thoothukudi which is present in Gulf of Mannar has a greatest interest due to an industrial belt having many major industries, involved in the production of chemicals, petrochemicals and plastics. The human activities around Thoothukudi have altered the coastal ecosystem greatly. The Thoothukudi municipality generates sewage of 14 MLD capacity. This sewage is discharged into the sea without any treatment (Tamil Nadu Fisheries Statistics, 2004). Untreated sewage is discharged to the sea at Therespuram, off Thoothukudi, southeast coast of India. Sampling of *Arius maculatus* was conducted at sewage disposal site, Therespuram, off Thoothukudi during Sep' 2016 – June' 2017 bimonthly with the help of local fishermen using gill net. Similarly, fish sampling was conducted at Vembar (V) a relatively unpolluted reference site. The fishes were collected and brought to the laboratory. Parts of gill and liver tissue samples were dissected out from fishes collected from study site and reference site and fixed in Bouin's fluid for 24 h. The fixed tissues were washed up in tap water. Then, the tissues were dehydrated by a series of upgraded alcohol solution (30% alcohol for 20 min; 50% for 20 min; 70% for 30 min; 90% for 30 min; 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 1 h and Xylene II for 1 h] with using an Automatic Tissue Processor and a Cold Plate (Thermo Scientific). 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 (Humason, 1972). Then the slides were stained with heamotoxylin and eosin stain. Histological sections are observed under trinocular microscope.

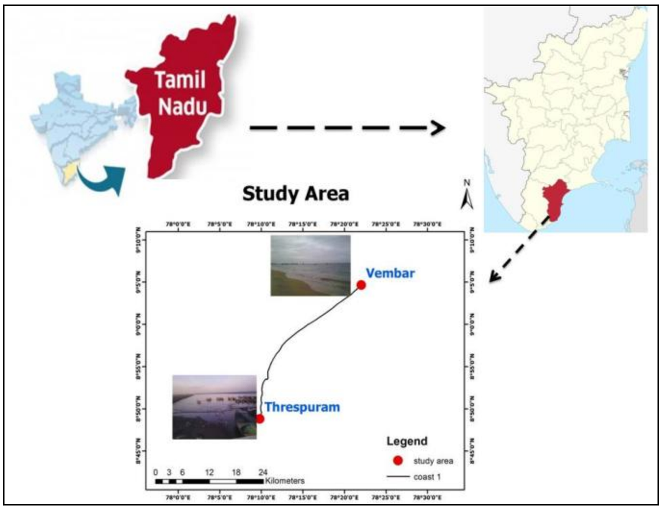


Fig 1: Map showing the study site and reference site

Result and Discussion

Histopathology of fish gill

Histology of gill tissue of the fish specimens collected from the reference site (Vembar) showed normal histoarchitecture (Plate 1.a). The gill tissue of fish specimens collected from polluted site during the summer season and winter season showed several histological alterations due to sewage. However, the intensity of the alterations was observed to be the highest in the specimens collected during the summer season and the lowest in fish specimen during winter season.

The gills, are considered the 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 [8, 26]. Marked variations like hyperplasia, vacuolation, deformation of cartilage core, bubbling of gill filament, epithelial lifting, lamellar fusion; secondary lamellar damage, shorter secondary lamellae and erosion of secondary lamellae were noticed in the gill tissues of *A. maculatus* collected from the polluted sites (Plate 1.b i, ii). Similar results were obtained by several workers. The notable ones are discussed here. Histological changes in gill like epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, partial fusion of some secondary lamellae are examples of defense mechanisms as these changes result in increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants [8, 14, 19, 32].

Rajeshkumar *et al*. [27] assessed the heavy metal pollution in east Barbic-Correntyne, Guyana by using histology of *Mugil cephalus* gill tissue and the study revealed alterations like aneurysm, mucous deposition, hypertrophy, fusion of secondary lamellae, ruptured epithelial layer, lifting of primary lamellae, lamellar swelling and necrosis. 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 [34].

Mallatt [19] reviewed that most common gill lesions found under several stressful conditions and reported the common changes like epithelial lifting, necrosis, hyperplasia, hypertrophy and hemorrhage as a result of lethal conditions.

A typical chronology of damage from acute exposure to the test chemical is first a lifting of the 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 [11]. The proliferation of chloride cells is 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 [12].

Santos *et al*. [29] reported that the histology of fishes like *Centropomus undecimalis* and *Sardinella* sp collected from Jansen lagoon that receive domestic sewage water, the gill tissue of the above mentioned specimen showed similar histological alterations. Afifi *et al*. [2] studied the histological alterations in gill tissue of *Siganus canaliculatus* and *Epinephalus morio* caught from oil polluted Jeddah coast and observed congestion of blood vessel, focal hemorrhage, edema, epithelial swelling, and fusion of secondary lamellae, necrosis and aggregation of mucous cells.

Histopathology of fish liver

Histology of liver tissue of the fish species collected from the reference site (Vembar) displayed normal histoarchitecture (Plate 2.a). The liver tissue of fish specimens collected from polluted site during the summer season and winter season showed several histological alterations due to sewage pollution (Plate 2.b. i and 2.b. ii). Like gill tissue alterations, the intensity of the alterations was observed to be the highest in the liver tissues of fish specimens collected during the summer season and the lowest during winter season.

Fish liver is a very interesting model for the study of interactions between environmental factors and hepatic structures and functions. Liver of fish can be considered as a target organ to pollutants, microorganisms *etc*, and alterations in its structure can be significant in the evaluation of fish health [21]. Moreover, the liver plays a major role in complex enzymatic processes that are responsible for vital functions, such as accumulation and biotransformation of xenobiotics in the fish (Plate 2.b. i and 2.b. ii).

Fish liver histopathology is an indicator of chemical toxicity and it is a useful way to study the effects of exposure of aquatic animals to toxins present in the aquatic environment [8]. The concentration of pollutants is more important in bringing the histological changes in the liver of fish; hence these changes could be used as a tool for assessing the toxic effects of the pollutants in the aquatic environment [16]. The extent of liver damage observed in the present investigation indicates that chronic exposure always causes impairment to the architecture of the tissue. Since liver is involved in detoxification of pollutants [18], it is susceptible to a greater degree of disruption in its structural organization due to toxic stress.

Some distinct changes like rupture of hepatocytes, melanomacrophages, increased Kupffer cell, increased pycnotic nucleus, vacuolation, ruptured nucleus, Blood congestion, cytoplasmatic vacuolation and nucleus disorganization were observed in the liver of fish (Plate 2.b. i and 2.b. ii).

Macrophage aggregates have been suggested as potentially sensitive histological biomarkers and or immunological biomarker of contaminant exposure [30]. Histological changes observed in various studies in liver taken from the fishes exposed to pollutants include increased vacuoles in the cytoplasm, changes in nuclear shapes, focal area of necrosis (death of cells in a localized area), ischemia (blockage of capillary circulation), hepatocellular shrinkage, and regression of hepatocytic microvilli at the bile canaliculi, fatty degeneration and loss of glycogen.

Liver of fish 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, or in other organs [14]. 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. The liver tissues of *Mugill cephalus* collected from polluted Ennore estuary liver tissues showed histological alterations like vacuolization in the hepatocytes, fibroblast proliferation vacuole formation granular degeneration, and necrosis [34].

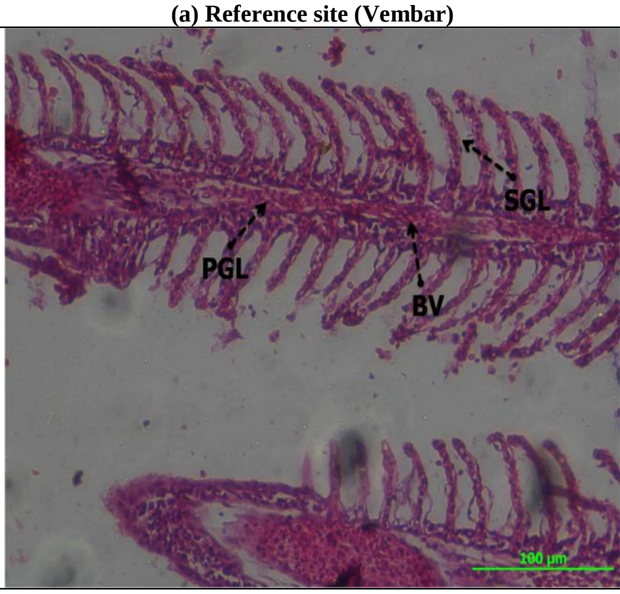
Marchand [20] reported that histopathological changes of fish liver from polluted freshwater system shows structural alterations in hepatic plates or cords, multiple focal areas of cellular alterations leading to a loss of uniform hepatocyte structure, steatosis, cytoplasmic and nuclear alterations (hypertrophic and pyknotic nuclei) of hepatocyte, increase in the size of melanomacrophage centers (MMCs), and focal areas of necrosis.

Agamy [3] investigated the histological alterations in liver tissue of *Siganus canaliculatus* following the exposure to crude oil. The alterations included microvesicular lipid vacuolation, lipid accumulation, blood vessel congestion, nuclear and cellular degeneration, megalocytosis, karyomegally, bile stagnation, cholangitis, bile duct proliferation, lymphocytic infiltration, and spongiosis hepatis were noted. Afifi *et al*. [2] 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 on the impacts of heavy metal (Cu, Ni, Fe, Co, Mn, Cr, Zn) pollution on the gill tissues of *Mastacembelus armatus* inhabiting an effluent dominated water body showed severe lamellar fusion, hyperplasia, hypertrophy and epithelial lifting, swelling and deformed lamella, in some parts sloughing off and curving of lamellae was also observed [15].

Feist [7] investigated the histology of *Hoplostethus atlanticus* and *Coryphaenoides rupestris* collected from continental slope of northwest Atlantic Ocean and the study revealed histological alterations of liver tissues like hepatocellular and nuclear pleomorphism and enlarged hepatocyte with loose granular cytoplasm, hypertrophied nuclei with coarse chromatin and granuloma. Rajeshkumar *et al*. [27] 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.

1. Photomicrograph of histological sections of gill of *Arius maculatus* collected from polluted site (Therespuram) and reference site (Vembar) during monsoon and summer season [Section - 5 µm thickness; H & E staining [gill (200 X)]

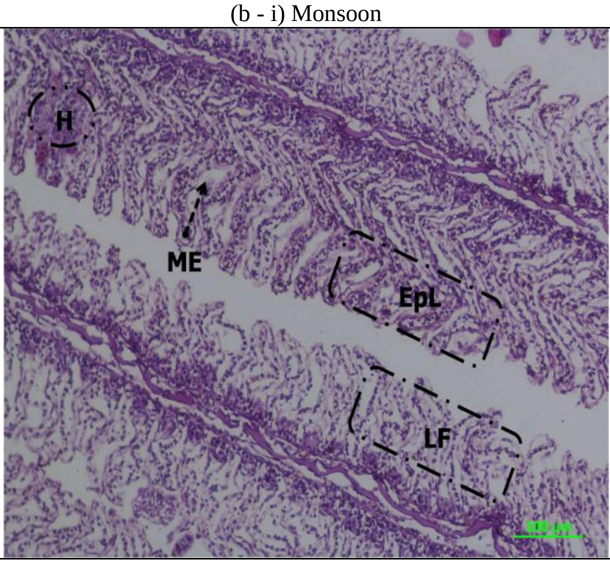
(a) Reference site (Vembar)



PGL - Primary Gill Lamella, SGL - Secondary Gill Lamella, BV - Blood Vessels

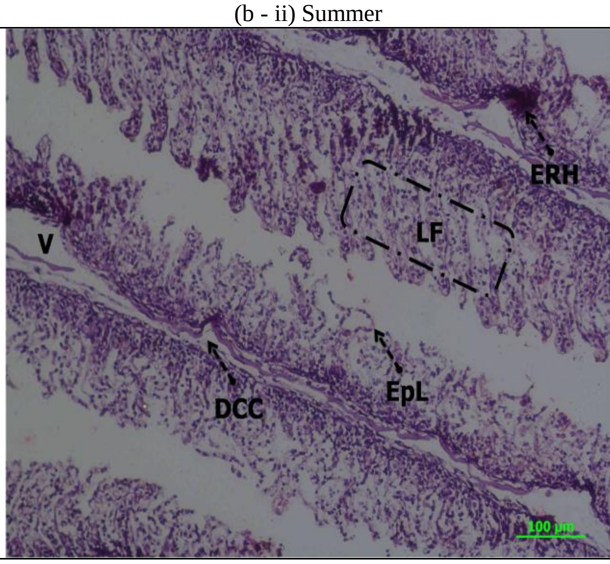
(b) Polluted study site (Therespuram)

(b - i) Monsoon



H - Hyperplasia, ME - Mucous Exudate, EpL - Epithelial Lifting, LF - Lamellar Fusion

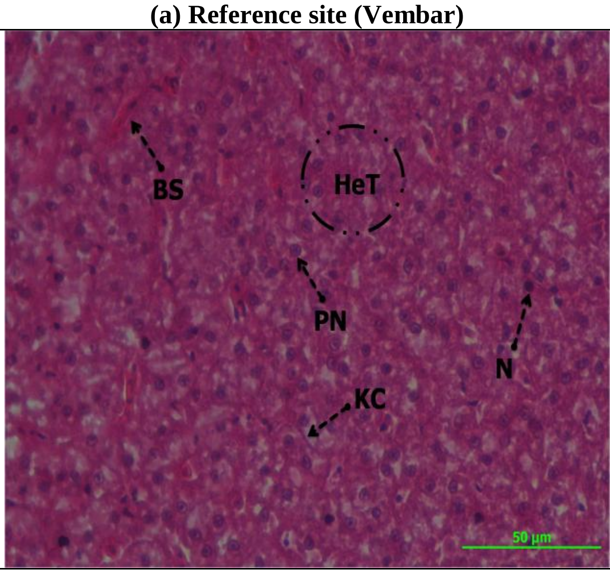
(b - ii) Summer



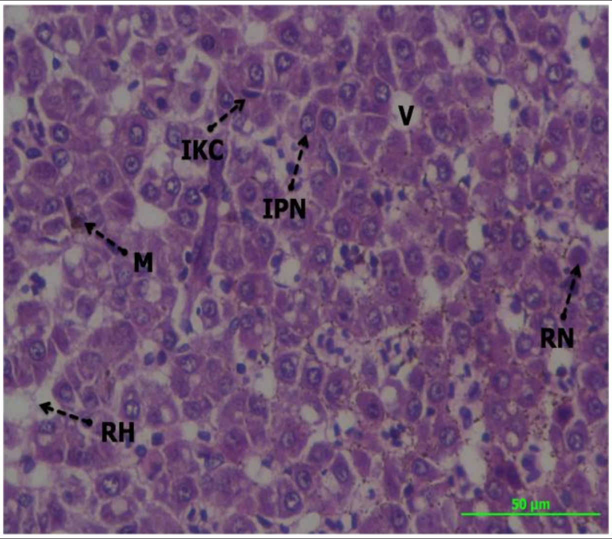
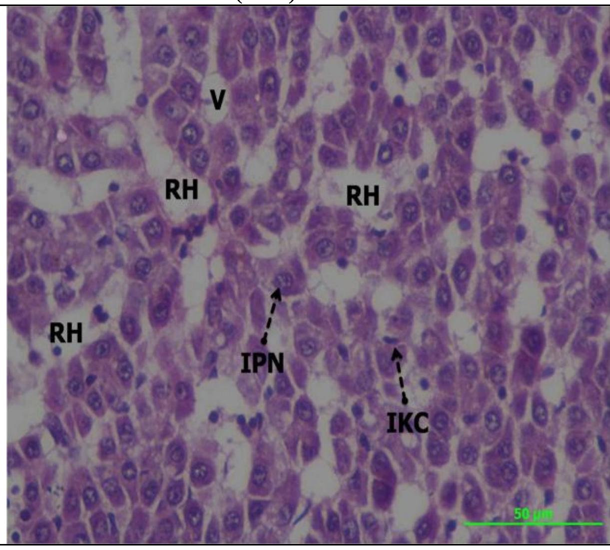
V - Vacuolation, DCC - Deformation of Cartilage Core, EpL - Epithelial Lifting, LF - Lamellar Fusion, ERH - Epithelium Rupture and Hemorrhage

2. Photomicrograph of histological sections of liver of *Arius maculatus* collected from polluted site (Therespuram) and reference site (Vembar) during monsoon and summer season [Section - 5 µm thickness; H & E staining [liver(400 X)]

(a) Reference site (Vembar)



BS - Blood Sinusoid, KC - Kupffer Cell, PN - Pycnotic Nucleus, HeT - Hepatic Tissue, Nu - Nucleus

(b) Polluted study site (Therespuram)	
(b - i) Monsoon	(b - ii) Summer
	
RH - Rupture of Hepatocytes, M - Melanomacrophages, IKC - Increased Kupffer Cell, IPN - Increased Pycnotic Nucleus, V - Vacuolation, RN - Ruptured Nucleus	RH - Rupture of Hepatocytes, V - Vacuolation, IPN - Increased Pycnotic Nucleus, IKC - Increased Kupffer Cell

Conclusion
The severity of histological changes in any particular organism is directly proportional to the concentration of a pollutant in the medium. Moreover, the histopathological picture of the organs can corroborate with the biochemical changes accounting for the functional disruptions in the activity of the organs due to cellular damage. Hence, appropriate measures need to be taken to treat the sewage before discharging into the sea.

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