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Effect of bioaccumulation on fish histopathology in the *Mugil cephalus* and *Oreochromis mosambicus*, inhabiting lake Chakkamkandam, Kerala

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Abstract
Lakes are vital to the upkeep of the environment because they regulate river water flow and act as a source of water during dry seasons. Aquatic environments affected by sewage effluents usually show the release of a complex mixture of xenobiotics, which can impact important fish physiological aspects. Monitoring aquatic ecosystems, especially significant lakes, is crucial for ensuring the availability of aquatic resources. Kerala lakes are under anthropogenic stress, mostly as a result of the ongoing sewage influx and agricultural runoff that change the chemical composition of the water. To detect the presence of the pollutants, biomonitoring techniques employ a range of cellular, biochemical, and histopathological indicators. To ascertain the harmful consequences of contaminants, these biomarkers are included in monitoring programs. The current paper provides a summary of the evolution and use of fish histopathology as biomarkers. Because fish are primarily influenced by the water quality. The goal of the current study was to assess the variations in the histopathological alterations in the muscles and gills of fish in Kerala's Lake Chakkamkandam.Degeneration in muscle bundles with specific areas of necrosis, atrophy and edema of muscle bundles, were all observed histologically in the muscles of both fish. Proliferative, degenerative, and necrotic changes in the epithelium of gill filaments and edema in secondary lamellae, dilatation and congestion in gill filament and mucous cell proliferation were among the pathogenic abnormalities in the gills. The environmental contamination of Lake Chakkamkandam, it was determined, caused a number of histological changes in the tissues of Oreochromis mosambicus and Mugil cephalus. The results suggest that the selected biomarkers can be used to assess the consequences of pollution in naturally occurring aquatic ecosystems. The anthropogenic activities related to agriculture, human habitation, and tourism activities in the city have an impact on water quality. The morphological results of the current study emphasise the value of histopathology analysis in biomonitoring programmes for evaluating water quality and the environment.

Keywords: histopathological, biomarkers, chakkamkandam lake, Oreochromis mosambicus and mugil cephalus

Introduction

Wetlands are a unique type of ecosystem that has a high nutrient content, a large carrying capacity, and a lot of potential for production. They are valued as sources of food and fodder for humans and their associated allies. Wetlands offer a region a diversity of food chains and webs, which has significant ecological and economic importance. They also contribute to the hydrological cycle, groundwater recharge, energy storage, and plant and animal habitat. (Vincy *et al.*, 2012) [32]. Inland water bodies provide drinking water, irrigation for agriculture, hydropower generation, leisure, and the production of fish and other aquatic life that provide food security and protein. In some underdeveloped regions of the world, fishing in lakes, reservoirs, rivers, and other inland waters is the main source of food protein and revenue (Lynch *et al.*, 2016) [17]. They sustain fish ecosystems, which are important suppliers of essential protein. (Dirican, 2014) [5].

Because of the threats that pollutants provide to aquatic organisms as well as the effects that they have on human health, environmental contamination has become a subject of concern (Mustafa *et al.*, 2017) [21]. A lot of sediments, fertilizers, pesticides, heavy metals, and organic debris are concentrated in the drainage water that is released into the lake.

Due to the constant exposure to chemical and physical changes in water bodies, fish are particularly susceptible to them.The elements in their blood may exhibit these alterations (Sheikh and Ahmed, 2016; Witeska, 2022) [34]. Fish are widely used as biomonitoring species in ecotoxicological studies because they are sensitive to the potential risks of toxicants introduced to the aquatic environment (Srivastava and Reddy, 2020) [28].

To appropriately interpret the haematological findings in fish, it is crucial to take into account the physiological and biochemical effects of changes in hazardous environmental factors or pollutants. The physicochemical characteristics of water, and their changes also have an impact on fish health. (Sahiti *et al.*, 2018) [25].

This accumulation of fish is influenced by the species and chemical concentrations, the level of environmental pollution, the length of exposure, the channel of metal uptake, the environment, the age and food intake by the fish, and the processing of metabolite organs (Elwasify *et al.*, 2021) [7].

The alteration in the target organs' histological properties may be brought on by contaminants' direct negative effects on edible organs. (Ibemenuga *et al.*, 2019; Mohamed, 2019 and Hadi, Al-hamadawi, 2021) [18, 12]. Therefore, studies assess the level of contamination by histological changes and serve as useful tool to mark the period of impacts of pollution in water bodies (Pirbeigi *et al*. 2016; Hussain *et al*., 2021) [22, 14].

In biomonitoring investigations, morphological findings have been significantly considered as early responses or detectable biological events caused by exposure to contaminants. (Liebel *et al*., 2013) [16].

Histopathological events were previously researched by several researchers. They are considered to be quick and effective tool for identifying impacts on fish and may also reveal the health of the organism exposed to contammination. Because they are constantly in contact with water, gills are a significant target organ for contaminants dissolved in water. Gas exchanges and osmotic control depend on this organ (Ayas *et al*., 2007) [2]. Gill morphology can serve as an indicator of adaptation strategies for the maintenance of various physiological processes or as a resistance of exposure to changes found in water and sediment over different periods (Tkatcheva, *et al*., 2004) [31]. Therefore, this research was to examine the impact of various pollution levels on histological characteristics in certain organs of Oreochromis mosambicus and Mugil cephalus.

Materials and methods

Study area

Chakkamkandam is a hamlet area close to Chavakkad and Guruvayur in the Thrissur district of Kerala, on the southwest coast of India. The area was renowned for its beauty, aquaculture, and Pokkali system, which provided locals with a means of subsistence. The lake is no longer a resource that could bring in money; it has been transformed into a waste dump. Lake Chakkamkandam's water is mixed with the sewage from Guruvayoor Town, a well-known pilgrimage site. The Lakshadweep Sea is reached once it continues forward to lake Chettuva. Current aquaculture restrictions have been placed on a section of the lake.

The geographical position of the lake is 10º 31′ 4″ N latitude and 76º 2′ 28″ E longitude.

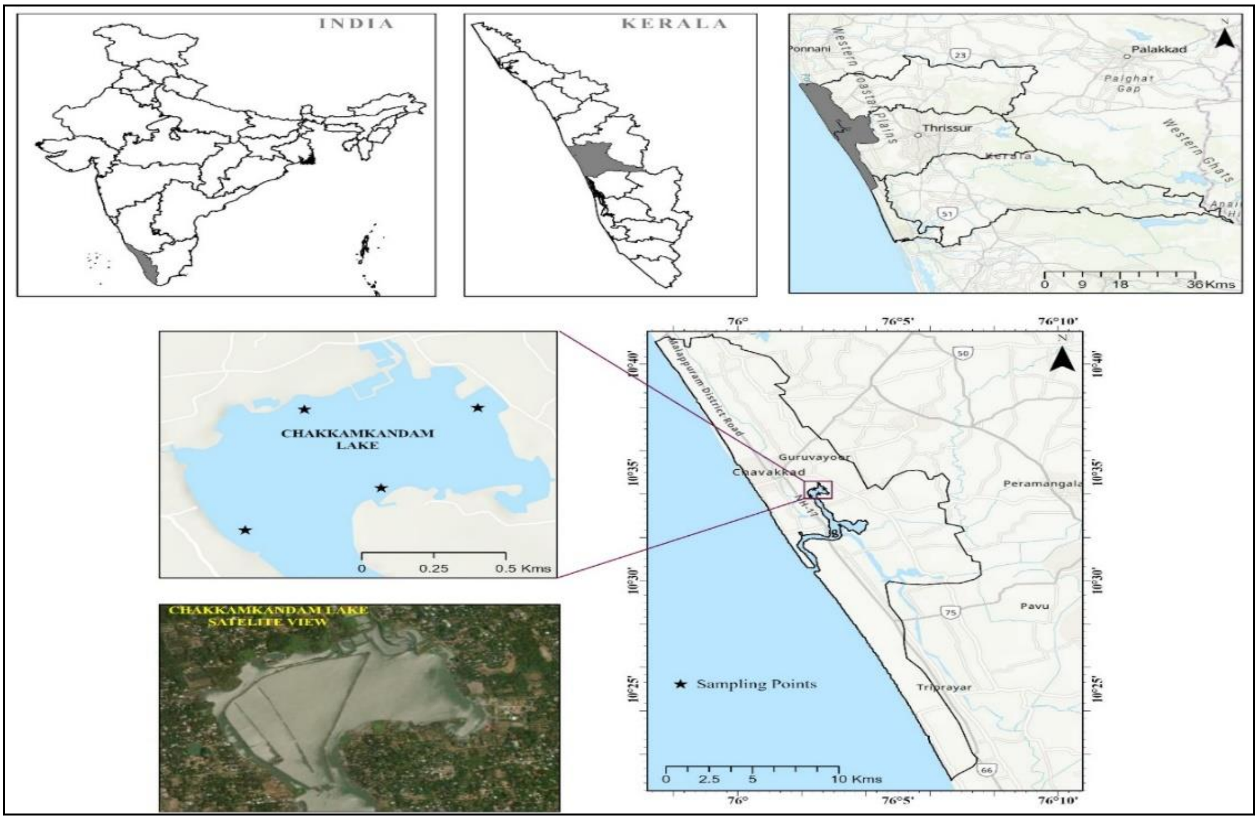
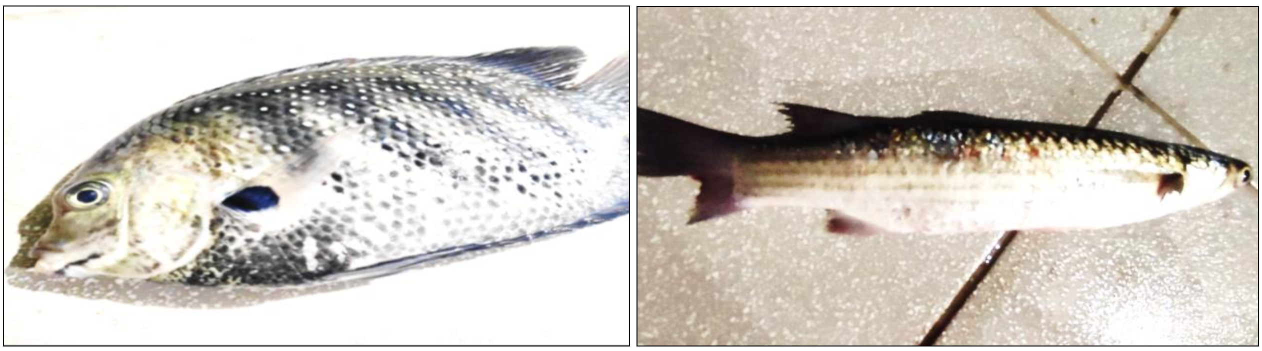


Fig 1: Study area

The geographical location and sampling areas of the Chakkamkandam lake are presented in Figure 1.

Fish

Live specimens of both sexes of fish *Mugil cephalus* (n = 10; 8.2 ± 0.7 cm; 7.1 ± 0.41 g) and Oreochromis mosambicus (n = 10; 9.3 ± 0.5cm; 8.1 ± 0.31 g) were collected from a fish farm and polluted lake (polluted site) of the Chakkamkandam lake during pre-monsoon season in 2021 with the help of local fisherman. The fish were immediately transported to the laboratory.



Mugil cephalus Oreochromis mosambicus

Fig 2

Histopathological study

Samples of the gills and muscles were taken after the fish had been dissected, and they were preserved in alcoholic Bouin's solution for at least 24 hours. In order to identify diseased tissue alterations using hematoxylin and eosin and a light microscope, the tissue was subjected to this procedure. (Pearse, 1968). Gills and muscles were taken from the exposed fish and the control fish, respectively, and fixed in a 10 percent formaldehyde solution for 48 hours. The method developed by Bernet *et al*. (1999) [3] was used to prepare tissues for histopathological research. Tissues were dehydrated using various ethanol. Embedding in paraffin wax is done after clearing using xylene. Then, using a rotary microtome, the sections (6 m thickness) were cut and mounted on glass slides. For general histological analyses, sections were deparaffinized in xylene, hydrated in alcohol, and stained with haematoxylin and eosin (HE). Under a light microscope, photomicrographs of stained section were taken.

Result and discussion

Muscle

Muscle samples from Oreochromis mosambicus and Mugil cephalus that were taken from Chakkamkandam Lake and examined histopathologically revealed edoema, atrophy, and necrosis (Plate – A and C). The fish specimens displayed intramyofibril space. Muscle fibre degeneration with nuclear loss is visible in sections examined from the obtained (Plate A and C).

“The majority of the fish's body is made up of muscle tissue (Yacoub *et al*., 2021) [35] Its general activities include movement, blood pumping, coordinated skeletal movement, peristaltic constriction of visceral organs and associated tissues, and locomotion. (Soliman *et al*., 2022) [27]. The muscle tissue from the control locations' histological analysis revealed a typical histological structure for muscle and evenly spread muscle bundles, indicating the fish was not under any stress. It denoted muscle fibres that are sheathed in a thin layer of areolar endomysial connective tissue (Plate 1 B and D).

While the fish were taken from contaminated areas displayed balloon necrosis (Bn) and vacuolar degeneration in muscle bundles (Plate 1 A and C). Additionally, melano-macrophage centres and muscle fibre splitting. Additionally, muscular bundle atrophy was noticed. These fibres were discovered to have several nuclei and be formed like lengthy spindles. One micrometer-diameter myofibrils were discovered to be composed of several alternately thick and thin myofilaments (plate – B and D).

“The histological changes in the muscles of the Siganus rivulatus are consistent with those seen by other researchers who have examined how various contaminants affect fish muscles.” (Shahid *et al*., 2022).

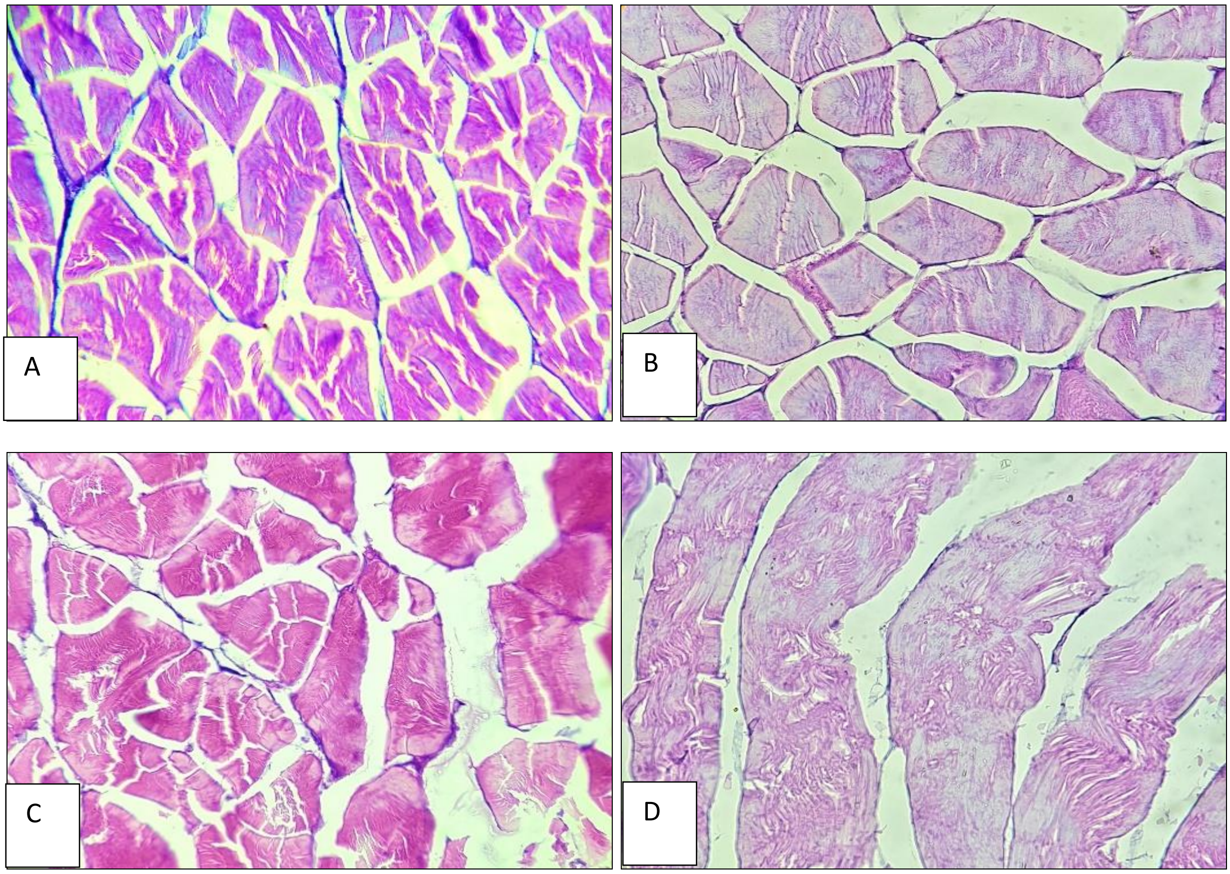


Plate 1: Pathological alteration in the muscle of Oreochromis mosambicus and Mugil cephalus collected from Chakkamkandam lake compared with control fish. A- Mugil cephalus (lake water), B- Mugil cephalus (control), C-Oreochromis mosambicus (lakewater) and D-Oreochromis mosambicus (contro)

Gills

The gills of Oreochromis mosambicus and Mugil cephalus specimens taken from Chakkamkandam Lake showed signs of vacuolization, hypertrophy, and fusion of secondary lamellae. Fish taken from a lake showed inflammatory cell infiltration and hypertrophy while elevating primary epithelial and fused gill lamellae (plate 2-A and C). Sections studied from the received biopsy show degeneration of gills with the feature of congestion (plate 2 A and C).

A control specimen of Oreochromis mosambicus and Mugil cephalus was observed with normal histology of gills indicating primary lamellae of gills consisting of mucous, epithelium and gill arch and epithelium consisting of cells (Plate 2 – B and D). The secondary lamellae indicated the presence of blood capillaries and pillar cells. The current study showed that the greater pollution concentration in the gills may be related to that organ's proximity to the environment, while the other organs such as kidney and liver showed that the rising levels may be related to those organs' essential detoxifying and excretion functions. The current findings are consistent with observations made by Ghanem (2006), Asante *et al*. (2014), Hadi and Al-hamadawi (2020), and others noted that the gills are the first location of water interaction and the focus of accumulation.

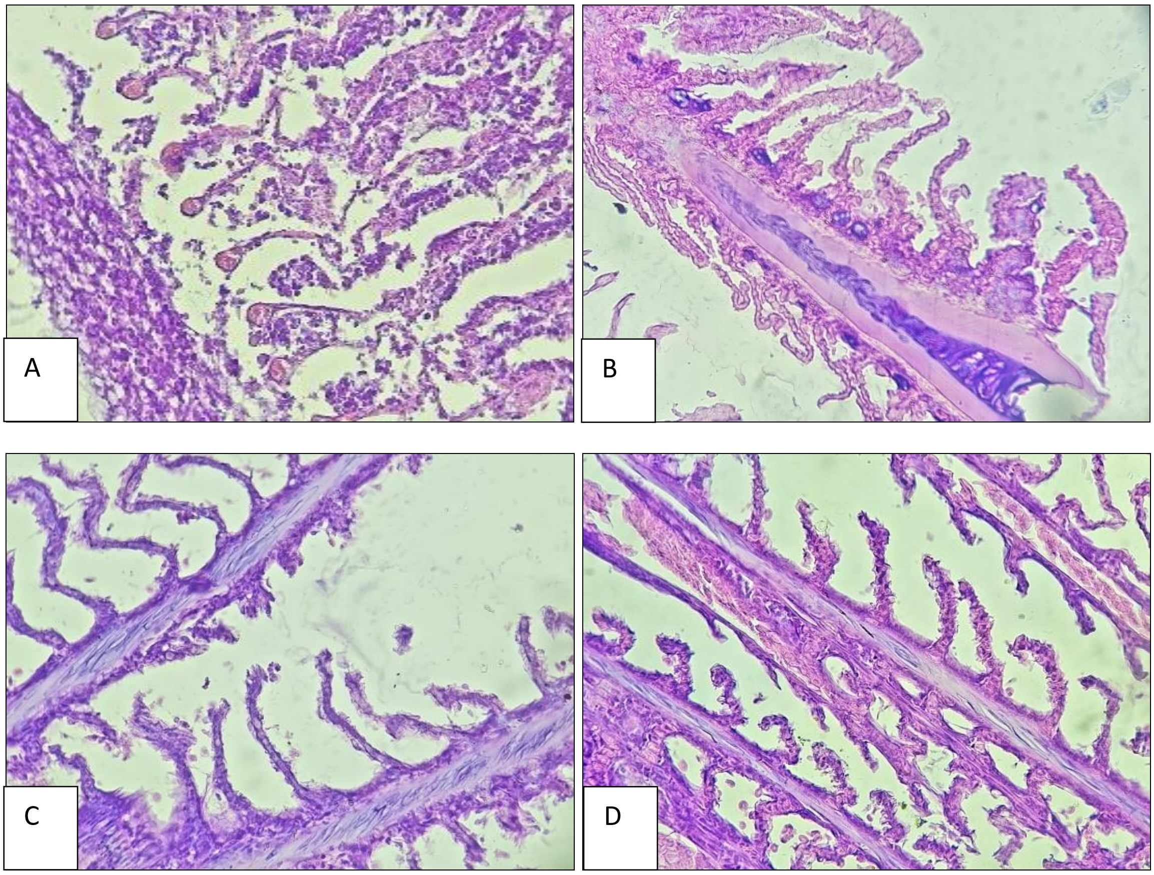


Plate 2: Pathological changes in the gills of Oreochromis mosambicus and Mugil cephalus collected from Chakkamkandam lake compared with control fish. A- Oreochromis mosambicus (lake water), B- Oreochromis mosambicus (control), C- Mugil cephalus (lake water) and D- Mugil cephalus (control)

Histopathological alterations of muscle

Body motion is specific to muscle tissue. It is made up of individual muscle cells that are stretched out into long fibres and held together by connective tissues. (Ghanem *et al*., 2015) [11]. In fish, the body musculature is rather simple. In muscles, fibres are arranged into bundles and separated by connective tissue fragments. The nerves enter a muscle from the side, penetrate the connective tissue, and then branch out. (Ghanem, 2019 and Elwasify *et al*., 2021) [10, 7]. The lateral muscles of fishes vividly display the segmentation or metamerism of vertebrate musculature. Myotomes, or muscle segments, are used to classify them. A groove that runs along the side of the fish separates each myotome into an upper (epaxial) and a lower (hypaxial) portion. Obliquely oriented connective tissue partitions divide successive myotomes (myosepta). an antero-posterior, fibrous septum divides the epaxial component of the myotome from the hypaxial myotome. Every fibre is protected by a skinny sarcolemma (specialized cell membrane). Five longitudinal myofibrils are present in the fiber's sarcoplasm, or protoplasm.

The results coincide with early researchers by Ahmed (2007) [1], Yacoub *et al*. (2008) [36], and Saad *et al*. (2012) [23] who observed hemorrhage, and hemosiderin. As well as, fatty degeneration and necrosis in the connective tissue of hypodermal layer and also degeneration, necrosis, and edema in the muscle fiber were recorded. In Tilapia zillii and Solea vulgaris obtained from Lake Qarun, Mohamed (2009) [20] examined the histological alteration was shown that muscular bundles had atrophy and vacuolar degeneration. Muscular fibre breaking and edoema within the muscle bundles were visible. Tayel *et al*., 2013 [29] observed Hemorrahge, hemosidrin, fatty degeneration and necrosis in the connective tissue of hypodermal layer and degeneration in the muscles of T. zillii.The current findings are consistent with those of Mohamed (2019) [18], who found alterations in the muscles of Tilapia sp., in Lake Qarun, including marked muscle bundle degeneration and atrophy and splitting of the muscle fibers. Muscles, are less exposed and are not directly exposed to contaminants, according to Shahid *et al*., (2021).

Histopathological alterations of gills

A typical structural structure of the lamellae was seen in the control group without any pathological abnormalities, according to a histopathological investigation of the gills. Hyperplasia of the epithelium and increased lifting of the lamellar epithelium were the predominant abnormalities seen in polluted lake areas.

Histopathological research has been a crucial tool in environmental monitoring since it enables the examination of certain target organs. The present study's histological findings in every tissue of Oreochromis mosambicus and Mugil cephalus show that levels of anthropogenic pollution in the lake led to moderate to severe changes in gill structure and muscle fibers. The gills are a crucial organ that performs essential functions like respiration, detoxification, osmoregulation, etc.

This investigation revealed varying degrees of histological alterations in the gills, including necrosis, edoema, telangiectasis, the fusion of secondary lamellae, epithelial hyperplasia, and epithelial lifting. Epithelial lifting and hyperplasia reactions could cause the fish's gills to stop working, which would then cause the fish to suffocate. These pathological alterations are likely brought on by increased capillary permeability, but they could also be explained as a general defence mechanism to stop contaminants from entering via the surface of the gills.

These changes in the gill epithelia of Lates calcarifer were noticed after exposure to cadmium (Thophon *et al*., 2003) [30]. These findings concur with those of Hadi and Alwan (2012) [13], who described aluminum-exposed tilapia zillii. Figueiredo-Fernandes *et al*. (2007) [8] found comparable outcomes in Nile tilapia, Oreochromis niloticus, exposed to waterborne copper. These results were also observed in freshwater fish (Puntius gonionotus, Oreochromis niloticus), which had been treated to the insecticides paraquat and dimethoate, respectively (Cengiz and Unlu, 2006; Elezaby *et al*., 2001) [4, 6].

The histopathology of different organs may therefore be a highly sensitive and accurate tool to assess the effects of xenobiotic compounds in the field and in laboratory research, which represent the health of the overall aquatic environment (Thophon *et al*., 2003) [30]. This study was carried out to learn more about the effects of pollution on Chakkamkandam Lake and the histological characteristics of the organs (the gills and the muscle) of Oreochromis mosambicus and Mugil cephalus.

Conclusion

A crucial indicator of fish's poor health status may be the growing bioaccumulation and histological alterations in edible organs. Additionally, it is vital to exercise greater caution while using aquaculture's water resources in order to decrease or prevent future pollution while ensuring good fish production and preventing any harm that could endanger the health of the end consumers.

The findings of this study demonstrated the value of histopathology as a biomarker for evaluating environmental quality and identifying pollutants that may degrade the quality of the aquatic environment.

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