

Histopathological Changes in the Gill and Intestine Tissues of Fish from Pichavaram Mangroves, South east Coast of India

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ABSTRACT

A study was conducted to analysis the Histopathological Changes in the Gill and Intestine Tissues of *Mystus gulio* and *Mugil cephalus* from Pichavaram Mangroves for different seasons. The maximum overall metal concentrations of Iron (Fe) recorded in the gills of *M. cephalus* was found to be maximum in S2 as 48.41 ± 0.72 mg/l, copper (Cu) is maximum at station 6 as 0.52 ± 0.02 mg/l, chromium (Cr) accumulation in the gills of *M. gulio* was highest in S5 as 5.23 ± 0.05 mg/l, the highest lead (Pb) accumulation in the gills of *M. gulio* was found to be maximum in S6 as 0.71 ± 0.03 mg/l. Likewise, the highest Iron (Fe) accumulation in the kidney of *M. gulio* was found to be maximum in S2 as 36.13 ± 0.41 mg/l followed by copper (Cu) in (S6) 0.41 ± 0.02 mg/l, Chromium (Cr) in (S6) records at 2.98 ± 0.06 mg/l and by lead (Pb) maximum in S5 0.64 ± 0.06 mg/l. The highest Iron (Fe) accumulation is found in the liver of *M. gulio* in S2 as 29.14 ± 0.34 mg/l, followed by copper (Cu) in (S3) 0.32 ± 0.01 mg/l, Chromium (Cr) in (S6 and S1) as 2.98 ± 0.06 & 2.98 ± 0.03 mg/l respectively and in lead (Pb) the highest concentration is recorded at S6 1.10 ± 0.06 mg/l. The highest Iron (Fe) accumulation is found in the intestine of *M. gulio* was found to be maximum in S6 as 29.11 ± 0.44 mg/l and by copper (Cu) in (S6) 1.61 ± 0.01 mg/l and chromium (Cr) in (S5) as 2.43 ± 0.03 mg/l and in lead (Pb) at S6 1.74 ± 0.03 mg/l. The highest Iron (Fe) accumulation is found in the muscle of *M. gulio* in S1 as 14.03 ± 0.37 mg/l, followed by copper (Cu) in (S2) 1.83 ± 0.06 mg/l, Chromium (Cr) in (S6) as 2.63 ± 0.03 mg/l and in lead (Pb) the maximum in S6 1.52 ± 0.03 mg/l.

Keywords: Pichavaram, *Mystus gulio*, *Mugil cephalus*, gills and heavy metals, Bio accumulation.

INTRODUCTION

Industrialization has led to an exponential increase in waste generation, often discharged or accumulated in water networks such as rivers and seas (Alesci et al., 2022). Water pollution represents an alteration of its original characteristics through the introduction of anthropogenic contaminants, various chemical and toxic pollutants, biocides, pesticides, and heavy metals, in such a way as to alter its use for human food and/or for the sustenance of biotic communities (Khan et al., 2020). The marine environment serves as a sink for contaminants including heavy metals (Agrawal et al., 2010 & Chouvelon et al., 2012) and organic contaminants (polychlorinated biphenyls (PCBs) and persistent pesticides) (Rajfur, 2013 & Mormede and Davies, 2003).

India's first and foremost occupation is agriculture and it's an agro-based country with more than 60–70% of its population totally reliant on agriculture. However, 30% of its agricultural produce is lost owing to pest infestation. In the absence of a better alternative, the consumption of pesticides becomes inevitable despite their known hazardous effects. The utilization of pesticides in India is about 3% of the total world consumption and is increasing at the rate of 2–5% per annum (Bhadbhade et al., 2002). Heavy metals are defined as any metal or metalloid having a relative atomic density greater than 4 g/cm³ or 5 g/cm³ that is dangerous even at very low concentrations (Nriagu, J.O.; Pacyna, 1988 & Lenntech, 2004). Heavy metals are ubiquitous in the environment; they are easily dissolved and carried by water, where they are quickly absorbed by aquatic biota. Due to their high toxicity, extended persistence, and non-biodegradable nature in the food chain, heavy metals are a core group of aquatic contaminants that cause cellular toxicity, mutagenicity, and carcinogenicity in animals; their presence in the aquatic environment can influence water quality parameters and all forms of aquatic life (Adeboyejo et al., 2018; Di Bella et al., 2015; Naccari et al., 2015 and Afonso et al., 2008).

Contamination of aquatic bodies with a vast array of pollutants has seriously increased worldwide attention. Anthropogenic activities resulting from modern agricultural practices, rapid urbanization and industrialization involve the increased release of various chemical pollutants and toxicants, such as industrial effluents, biocides, pesticides and heavy metals etc. which ultimately reach the aquatic environments and become responsible for their degradation (Wang, 2002; Dautremepuits et al., 2004). Among these pollutants, heavy metals have been recognized as strong biological poisons because of their persistent nature, toxicity, tendency to get accumulated in organisms and undergo biomagnification (Kamble & Muley, 2000; Dinodia et al., 2002). Heavy metals are considered the most hazardous of all environmental pollutants (Al-Attar, 2005) due to their bioaccumulation and toxicity tendency (El-Nagger et al., 2009). This is because heavy metals may precipitate and get absorbed on sediment particles, remain soluble or suspended in water and/or may be taken up by aquatic fauna upon their entry into water bodies (Mohamed, 2008). Metals are then absorbed through gills and skin and/or ingested through food to cause bio accumulative toxicity in fish where the intensity of the toxicity is influenced by the temperature, oxygen concentration, pH and hardness of the water (Forstner & Wittman, 1986).

Aquatic pollution is one of the most emerging issues occurring globally and now a day water resources have been widely exploited by the natural systems. 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 current scenario. Hence, the protection of the coastal and marine regions from continuing pollution becomes the most essential in coastal resources management (Lauriano et al., 2016). The evaluation of the health status of marine fish species is a crucial step in determining an environmental assessment (Feist et al., 2015; La Torre et al., 2020; Panebianco et al., 2021).

Fish are important organisms in the study of heavy metal pollution, because fish move freely and assimilate heavy metals in a myriad of ways, including ingestion of suspended particles in water, ion exchange of dissolved heavy metals through lipophilic membranes (gills), and surface adsorption tissues and membranes. The type of exposure (dietetic or aqueous) has an impact on the distribution of heavy metals in different fish tissues (Kaur et al., 2018). Histopathological alterations are used as biomarkers to assess the general health of fish exposed to pollutants (Adams, 2002). The liver, gills, and kidneys are all involved in the accumulation and biotransformation of xenobiotics, as well as excretion and respiration in fish (Gernhöfer et al., 2001). Because of its location, function, and blood supply, the liver is involved in detoxification and biotransformation. It is also one of the organs most vulnerable to damage caused by various toxic substances (Camargo & Martinez, 2007). The kidney is an important organ for maintaining water and salt balance, for excretion of metabolic waste from the blood, and for aspects of xenobiotic metabolism (Thophon et al., 2003 & Mabrouk, 2004). Gills are the initial target of waterborne contaminants and are extremely sensitive to heavy metal deposition due to constant contact with the external environment. The highly branching morphology of gill tissues, as well as the circulation of water through them, enable heavy metal accumulation (Shah et al., 2020). The histopathology provides a sensitive indicator of pollutant induced stress due to the central role that the organs play in the transformation of different active chemical compounds in the aquatic environment; particularly the gills, kidneys, and liver are considered key organs for toxicological studies (Reish et al., 1987).

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 (Austin, 1998). Austin (1998) reviewed the effects of marine pollution on fish health and Au (2004) reviewed the application of histocytopathological biomarkers in marine pollution monitoring. Additionally, histological biomarkers provide powerful tools to detect and characterize the biological endpoints of toxicant and carcinogen exposure (Omar Shaik, 2012) and oil exposure (Gusma et al., 2012).

The usage of pesticides is found to be increasing use in recent years since they are biodegradable and therefore persist in the environment only for a short time. Because of their low persistence, repeated applications of these pesticides are being practiced for the control of pests in agricultural fields and thereby large quantities find their way into water bodies (Sivaperumal, 2007). A large number of pesticides are commonly used to control various agricultural pests; however, their toxicological impact also extends to non-target species like fish (Vinodhini and Narayanan, 2008). Fish is good indicator of aquatic contamination because

its biochemical stress responses are quite similar to those found in mammals (Kalaiyarasi et al., 2017). The assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on data on the toxicity and effects of pesticide preparations to non-target organisms. Fishes are among the group of non-target aquatic organisms. Histological changes provide a rapid method to detect the effects of irritants, especially chronic ones, in various tissues and organs (Bernet et al., 1999). Very few histopathological studies have been reported on treatment with dichlorvos (Bhuyan et al., 2014).

Water quality is playing an important role and it's directly related to the safety of human health and the animals that inhabit these environments (Moiseenko et al., 2008). Among aquatic organisms, fishes are directly affected by an uncontrolled discharge of domestic and industrial sewage, agricultural chemicals, heavy metals, and other xenobiotics. So, the fishes are sentinel organisms that can be used as bioindicators of environmental stress from abiotic and biotic changes caused by pollutants. The first responses of these organisms to environmental stress are changes happened at the cellular and tissue levels (Svobodová, 1993). Because fish gills have a large surface area in contact with water, structural modifications in gills have been widely used as indicators of environmental contamination (Al-Ghanim et al., 2019).

Therefore, the present study is aimed to determine the histopathological effects on various tissues in catfish (*Mystus gulio*) and mullet (*Mugil cephalus*) by random fishing using cast nets in the Pichavaram mangrove forest.

MATERIALS AND METHODS

Study Area and Sampling Stations

This study was carried out in the Pichavaram mangrove forest (Lat. 11°20' N; Long. 79° 47' E) which is located between the Vellar and Coleroon estuaries, near Chidambaram, Tamilnadu, India. The mangrove covers an area of about 1300 ha, of which 50% is covered by mangrove forest, 40% by waterways and the remaining filled by sand flats and mud-flats (Krishnamurthy and Prince Jayaseelan, 1983). The Pichavaram mangrove is influenced by the mixing of three types of waters:

1. Neritic or costal water from the adjacent Bay of Bengal through a mouth called 'Chinnavaikkal',
2. Brackish water from the Vellar and Coleroon estuaries and,
3. Fresh water from an irrigation channel (Khan Sahib canal'), as well as from the main channel of the Coleroon river.



Fig.1. Map showing the Six fishes samples collection locations in Pichavaram Mangrove

Six sampling stations were fixed along the Pichavaram Mangrove Forest. The sampling locations were selected based on the properties of physico chemical parameters and the source of contaminations. The sampling locations were Backwater Zone – Mouth (N11°27'13.1", E079°47'40.1") followed by Shrimp Pond effluent site (N11°25'14.4", E079°45'56.9"), Degraded Mangrove site (N11°25'54.9", E079°47'18.3"), Natural site (N11°25'45.6", E079°47'43.2"), Freshwater Zone – Uppanar (N11°25'41.1", E079°46'15.7") and Freshwater Zone – Vellar (N11°25'04.2", E079°46'19.5"). The fishes were collected from six different locations of the Pichavaram Mangrove Forest during Post monsoon (February, 2018) and summer (May, 2018). The fish samples were collected by using cast nets and they are collected randomly at each site. The detail of the study area is mentioned in Figure 1. Totally, nineteen species were recorded in Pichavaram mangrove forest in that two fish species *Mystus gulio* (Fig. 1a) and *Mugil cephalus* (Fig. 1b) were selected based on the abundance and dominant fish catch in the four seasons.



Fig. 2a: *Mystus gulio*



Fig. 2b: *Mugil cephalus*

The fish samples were collected and various parts like gill, intestine, liver and muscle tissue samples were dissected from fishes immediately fixed in Bouin's fluid for 24 h. The fixed tissues were washed up in running tap water. Then, the tissues were dehydrated by a series of upgraded alcohol solutions (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 μ m sections) were taken from the processed tissues using a 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 a spirit lamp (Humason, 1979). Then the slides were stained with hematoxylin and eosin stain. Histological sections are observed under trinocular microscope. Further, the gills and intestines of the fish samples were analyzed for histological assessments by Bernet et al. (1999).

Heavy Metal Analysis in Fish Organs

Sixty-four samples of each tissue of each fish species were collected which can accumulate metals and yet tolerate heavy metal load. From each of the fish sample, about 15 g of gills, liver, kidney and muscles were collected and washed with distilled water, transferred into clearly marked polyethylene flexible bags, and stored at -20°C for further analysis. Before analysis, the tissue samples were thawed at room temperature. 1 g of sample was weighed carefully (gills, liver, kidney and muscle) and it was digested with 5 ml Perchloric acid and 15 ml HNO₃ on a hot plate until brown fumes ceased to evolve, then samples were cooled at room temperature, diluted with 50 ml distilled water by following (Saad et al. 2011).

Analysis of Fish Samples

Samples of gills, kidney, liver and muscle of the fishes were analyzed by following methods described in (Saad et al. 2011) for the detection of metals viz., Pb, Cu, Fe and Cr through an Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). The blanks and calibration standard solution were also analyzed in the same way as for the samples. The instrument calibration standards were prepared by diluting standard (1000 ppm) purchased from Merck, Germany. A known 1000 mg/l concentration of Pb, Cu, Fe and Cr standard solution was prepared from their salts.

Statistical Analysis

The mean values and standard error were calculated. The data were analyzed by using the statistical package Minitab 15. Level of significance was established at $P < 0.05$.

RESULTS

Various tests were conducted to detect the presence of heavy metals in the gills, kidney, liver and in their intestines of *Mystus gulio* and *M. cephalus* in Pichavaram Mangrove Forest, samples collected from six stations.

Levels of Heavy Metal Accumulation in *Mystus Gulio*

The maximum overall metal concentrations of Iron (Fe) recorded in the gills of *M. gulio* was found to be maximum in S2 as 48.41 ± 0.72 mg/l, followed by 26.28 ± 0.36 mg/l (S1), 24.07 ± 0.54 mg/l (S3), 23.34 ± 0.33 mg/l (S4), 22.11 ± 0.24 mg/l (S6) and minimum in 11.27 ± 0.13 mg/l (S5) mg/l. Likewise, the heavy metal concentration of copper (Cu) is maximum at station 6 as 0.52 ± 0.02 mg/l (fig.4) and minimum recorded in (S1) at 0.51 ± 0.01 mg/l. The highest chromium (Cr) accumulation in the gills of *M. gulio* was found to be maximum in S5 as 5.23 ± 0.05 mg/l, followed by 4.24 ± 0.06 mg/l (S6), 3.24 ± 0.32 mg/l (S4), 2.31 ± 0.08 mg/l (S3), 1.87 ± 0.04 mg/l (S2) and minimum in 1.24 ± 0.05 mg/l (S1) mg/l. The highest lead (Pb) accumulation in the gills of *M. gulio* was found to be maximum in S6 as 0.71 ± 0.03 mg/l, followed by 0.47 ± 0.04 mg/l (S2), 0.39 ± 0.06 mg/l (S3), 0.34 ± 0.02 mg/l (S4), 0.33 ± 0.09 mg/l (S5) and minimum in 0.20 ± 0.02 mg/l (S1) (Fig. 4).

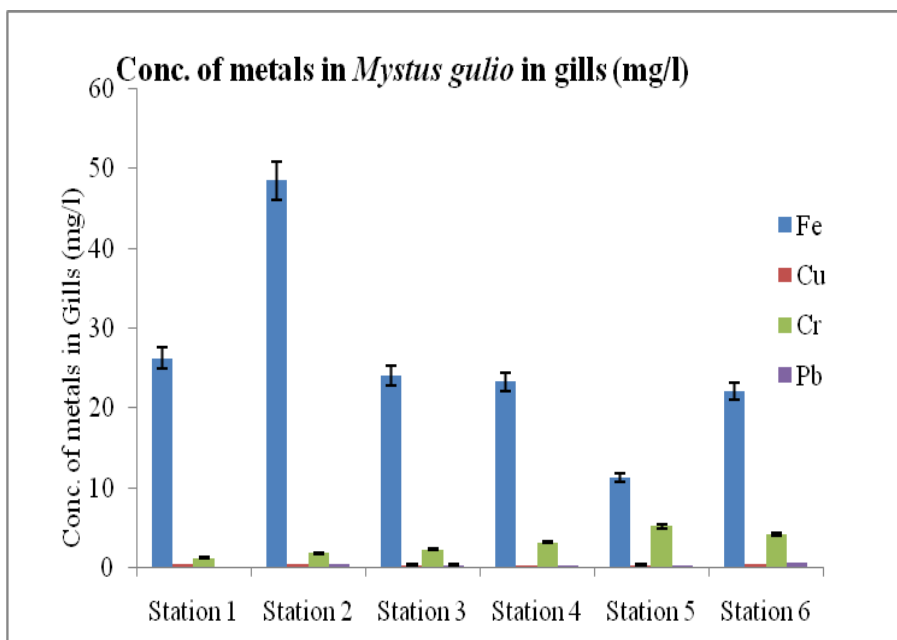


Fig. 4. Concentration of heavy metal accumulation in gills of *Mystus gulio*

The highest Iron (Fe) accumulation in the kidney of *M. gulio* was found to be maximum in S2 as 36.13 ± 0.41 mg/l and minimum in S5 as 9.78 ± 0.11 mg/l, followed by copper (Cu) maximum in (S6) 0.41 ± 0.02 mg/l and minimum in 0.23 ± 0.04 mg/l (S3). In the case of chromium (Cr), highest in (S6) 2.98 ± 0.06 mg/l and lowest in S1 as 1.15 ± 0.05 mg/l and followed by lead (Pb) maximum in S5 0.64 ± 0.06 mg/l and minimum in 0.46 ± 0.04 mg/l in Station 1 (Fig. 5).

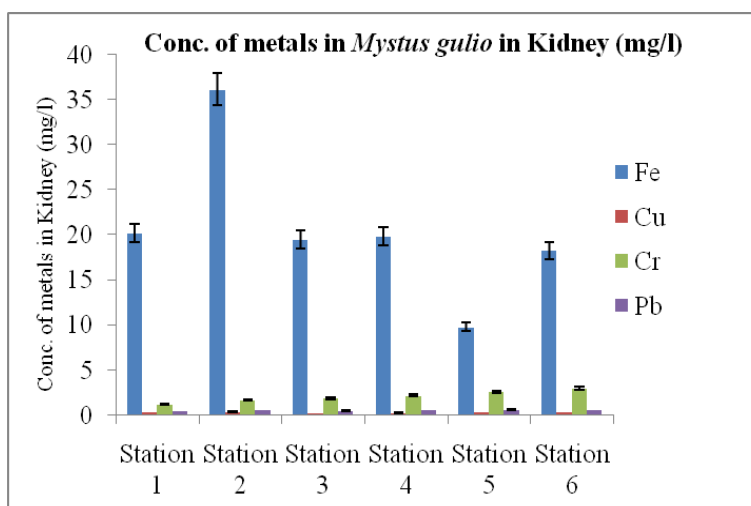


Fig. 5. Concentration of heavy metal accumulation of kidney in *Mystus gulio*

The highest Iron (Fe) accumulation is found in the liver of *M. gulio* was found to be maximum in S2 as 29.14 ± 0.34 mg/l and minimum in S5 as 10.38 ± 0.11 mg/l, followed by copper (Cu) maximum in (S3) 0.32 ± 0.01 mg/l and minimum in 0.03 ± 0.01 mg/l (S5). In the case of chromium (Cr), highest recorded in (S6 and S1) as 2.98 ± 0.06 & 2.98 ± 0.03 mg/l respectively and minimum

in S4 as 2.72 ± 0.06 mg/l and in lead (Pb) the maximum in S6 1.10 ± 0.06 mg/l and minimum in 0.45 ± 0.03 mg/l in Station 1 (Fig. 6).

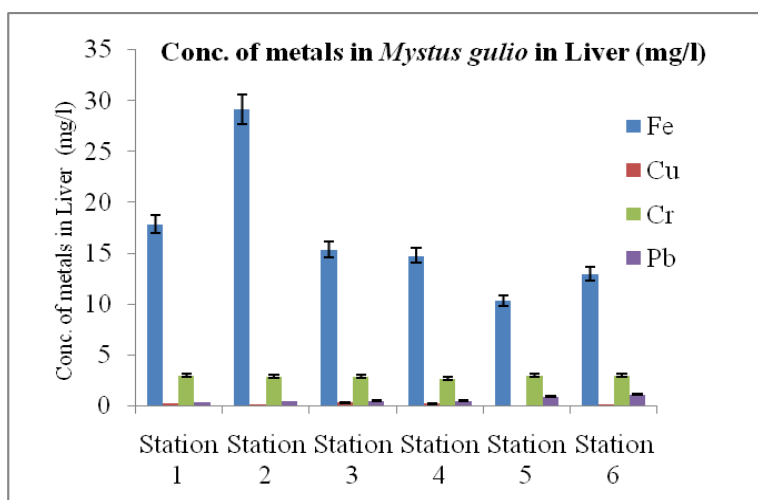


Fig. 6. Concentration of heavy metal accumulation of liver cells in *Mystus gulio*

The highest Iron (Fe) accumulation is found in the intestine of *M. gulio* was found to be maximum in S6 as 29.11 ± 0.44 mg/l and minimum in 9.21 ± 0.11 mg/l (S5), followed by copper (Cu) maximum in (S6) 1.61 ± 0.01 mg/l and minimum in 1.14 ± 0.06 mg/l (S1) shown in fig.7. In the case of chromium (Cr), highest recorded in (S5) as 2.43 ± 0.03 mg/l and minimum in S1 as 1.08 ± 0.06 mg/l and in lead (Pb) the maximum in S6 1.74 ± 0.03 mg/l and minimum values were recorded in 0.41 ± 0.02 mg/l in Station 1&2 respectively (Fig. 7).

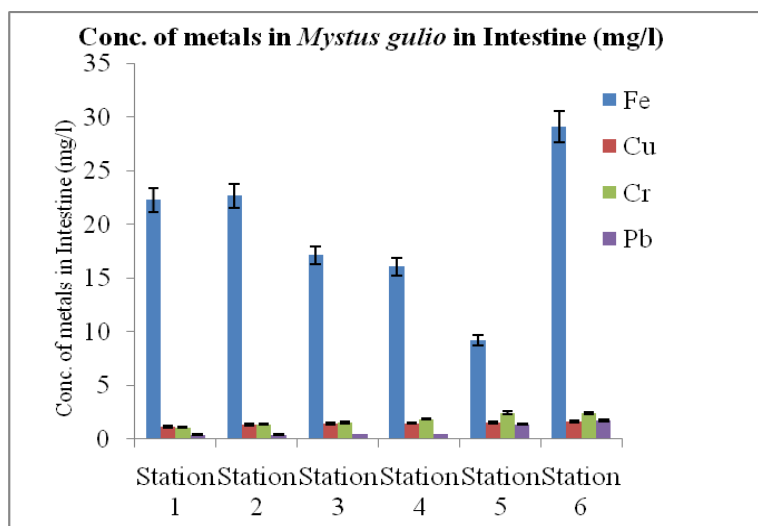


Fig. 7. Concentration of heavy metal accumulation in *Mystus gulio* intestines

The highest Iron (Fe) accumulation is found in the muscle of *M. gulio* was found to be maximum in S1 as 14.03 ± 0.37 mg/l and minimum in 12.00 ± 0.24 mg/l (S4), followed by copper (Cu) maximum in (S2) 1.83 ± 0.06 mg/l and minimum in 1.32 ± 0.03 mg/l (S4). In the case of chromium (Cr), highest recorded in (S6) as 2.63 ± 0.03 mg/l and minimum in S1 as 1.21 ± 0.06 mg/l and in

lead (Pb) the maximum in S6 1.52 ± 0.03 mg/l and minimum values were recorded in 0.38 ± 0.03 mg/l in Station 3 (Fig. 8).

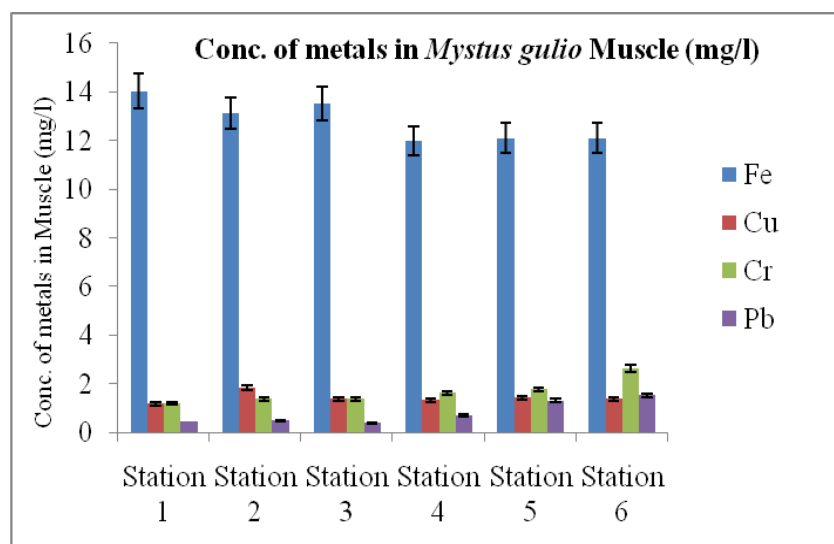


Fig. 8. Concentration of heavy metal accumulation in the muscles of *Mystus gulio*

Levels of Heavy Metal Accumulation in *Mugil Cephalus*

The maximum overall metal concentrations of Iron (Fe) recorded in the gills of *M. cephalus* was found to be maximum in S3 as 15.36 ± 0.10 mg/l, followed by 14.48 ± 0.09 mg/l (S1), 14.01 ± 0.14 mg/l (S2), 11.47 ± 0.24 mg/l (S6), 10.24 ± 0.24 mg/l (S4) and minimum in 8.34 ± 0.08 mg/l (S5) mg/l. Likewise, the heavy metal concentration of copper (Cu) is maximum in station 1 as 0.84 ± 0.03 mg/l and minimum recorded in (S4) 0.21 ± 0.01 mg/l. The highest chromium (Cr) accumulation in the gills of *M. cephalus* was found to be maximum in S4 as 1.04 ± 0.05 mg/l and minimum in 0.53 ± 0.05 mg/l (S6). The highest lead (Pb) accumulation in the gills of *M. cephalus* was found to be maximum in S2 & S4 as 0.38 ± 0.04 mg/l, and minimum in 0.18 ± 0.02 mg/l (S1) (Fig. 9).

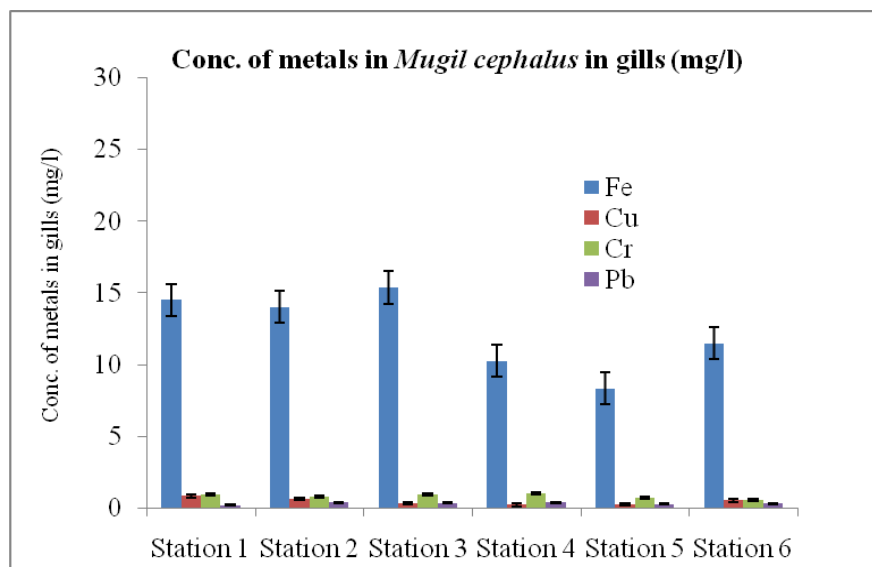


Fig. 9. Concentration of heavy metal accumulation in the gills of *Mugil cephalus*

The highest Iron (Fe) accumulation in the kidney of *M. cephalus* was found to be maximum in S2 as 22.19 ± 0.34 mg/l and minimum in 7.63 ± 0.14 mg/l (S5), followed by copper (Cu) maximum in (S6) 0.32 ± 0.02 mg/l and minimum in 0.12 ± 0.04 mg/l (S1). In the case of chromium (Cr), highest in (S5) 0.64 ± 0.05 mg/l and lowest in S3 as 0.24 ± 0.03 mg/l and followed by lead (Pb) maximum in S2 0.56 ± 0.05 mg/l and minimum in 0.26 ± 0.03 mg/l in Station 5 (Fig.10).

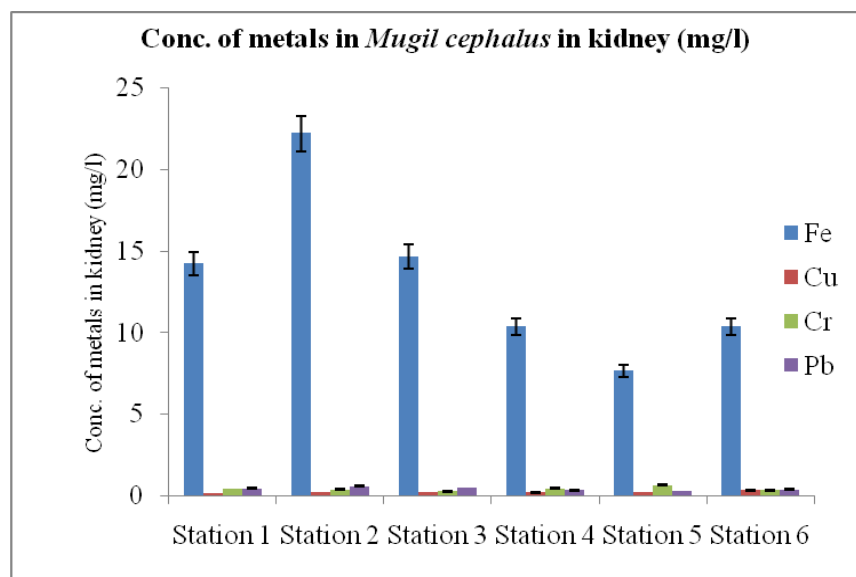


Fig. 10. Concentration of heavy metal accumulation in *Mugil cephalus* in kidney

The highest Iron (Fe) accumulation is found in the liver of *M. cephalus* was found to be maximum in S2 as 14.36 ± 0.41 mg/l and minimum in 7.89 ± 0.05 mg/l (S5), followed by copper (Cu) maximum in (S3) 0.32 ± 0.01 mg/l and minimum in 0.09 ± 0.01 mg/l (S5 & S6). The levels of chromium (Cr) is highly recorded in (S3) as 0.96 ± 0.05 mg/l and minimum in S5 as 0.35 ± 0.03

mg/l and in lead (Pb) the maximum in Station 60.46 ± 0.05 mg/l and minimum in 0.15 ± 0.03 mg/l in Station 4 (Fig. 11).

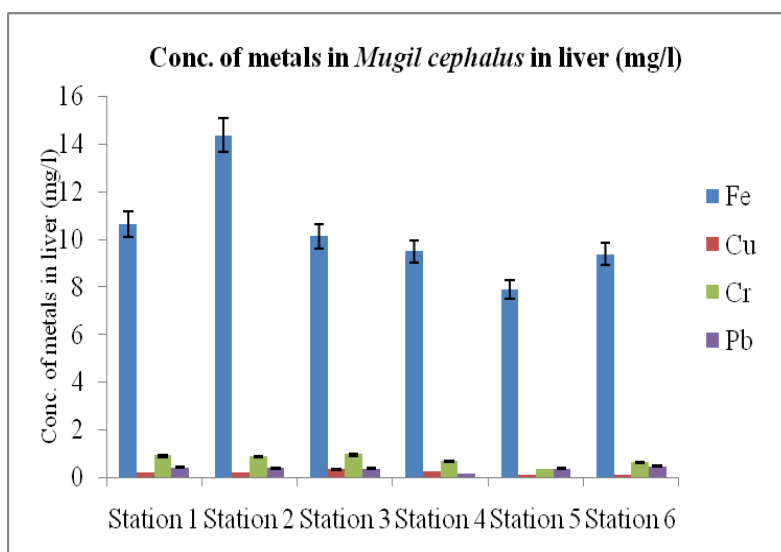


Fig. 11. Concentration of heavy metal accumulation in liver of *Mugil cephalus*

The highest Iron (Fe) accumulation is found in the intestine of *M. cephalus* was found to be maximum in S6 as 14.67 ± 0.34 mg/l and minimum in 04.11 ± 0.06 mg/l (S5), followed by copper (Cu) maximum in (S4 & S5) 0.94 ± 0.04 mg/l and minimum in 0.67 ± 0.08 mg/l (S1). In the case of chromium (Cr), highest recorded in (S5) as 0.99 ± 0.05 mg/l and minimum in S6 as 0.37 ± 0.02 mg/l and in lead (Pb) the maximum in S6 0.78 ± 0.05 mg/l and minimum values were recorded in 0.37 ± 0.02 mg/l in Station 4 (Fig.12).

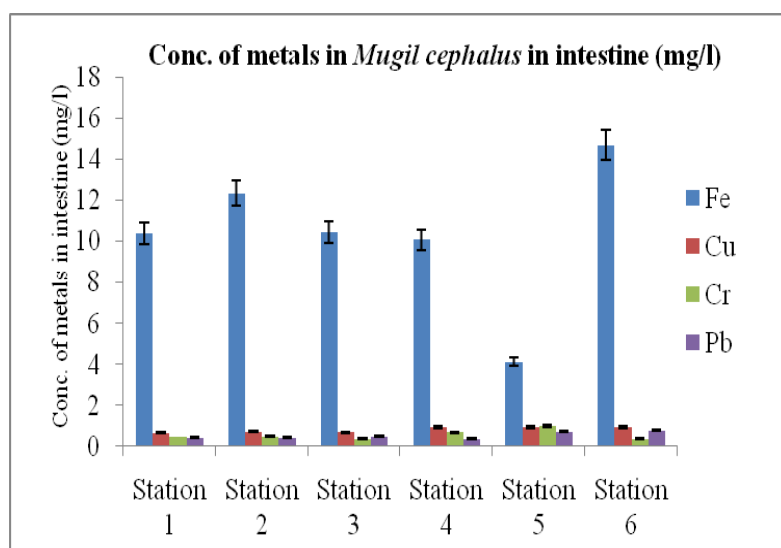


Fig. 12. Concentration of heavy metal accumulation in the intestine of *Mugil cephalus*

The highest Iron (Fe) accumulation is found in the muscle of *M. cephalus* was found to be maximum in S1 as 14.03 ± 0.14 mg/l and minimum in 12.01 ± 0.05 mg/l (S6), followed by copper

(Cu) maximum in (S6) 0.98 ± 0.05 mg/l and minimum in 0.06 ± 0.03 mg/l (S2). In the case of chromium (Cr), maximum recorded in (S5) as 0.87 ± 0.05 mg/l and minimum in S1 as 0.06 ± 0.01 mg/l and in lead (Pb) the maximum in S5 0.91 ± 0.04 mg/l and minimum values were recorded in 0.31 ± 0.05 mg/l in Station 3 (Fig. 13).

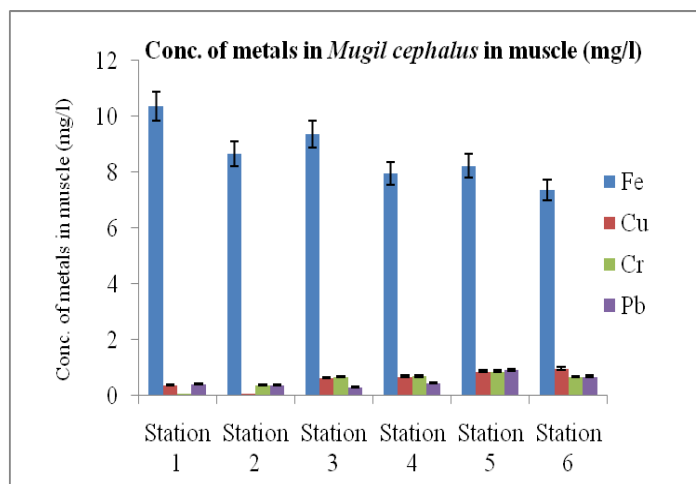


Fig. 13. Concentration of heavy metal accumulation in the muscle of *Mugil cephalus*

Histological Analysis of *Mystus gulio* and *Mugil cephalus*

The normal fish gill of *Mystus gulio* was shown in fig. 14 viewed under microscope of 10x and 40x. Histology of *Mystus gulio* gill tissue was collected from the different locations in Pichavaram mangrove site i.e., shrimp pond, fresh water flow areas, degrading areas of mangroves were selected for the study showed normal histoarchitecture (Fig. 14). The gills and intestine (Fig. 15) were consisting of Inter lamellar cell and they are composed of undifferentiated mass of cells, justifying their high proliferative rate and supporting the epithelial regeneration and proliferation of new mitochondria rich cells.

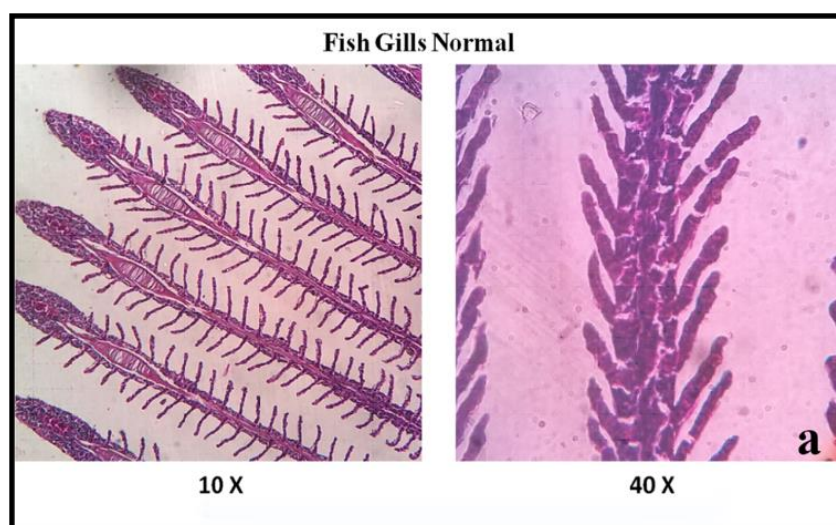


Fig. 14: Normal fish gill of *Mystus gulio*

Summer and winter seasons showed several histological alterations like infected gills of *Mystus gulio* (Fig. 15) and *Mugil cephalus* (Fig. 16) Infected fish intestine in *Mystus gulio* (Fig 18ab). The result from the present study clearly indicates that fish gills, intestines were infected and noticeable morphological changes were observed in their internal organs and fishes caught nearby shrimp pond in Pichavaram Mangrove Forest are found to be slightly accumulation of the heavy metals (18 a&b &19 a&b)

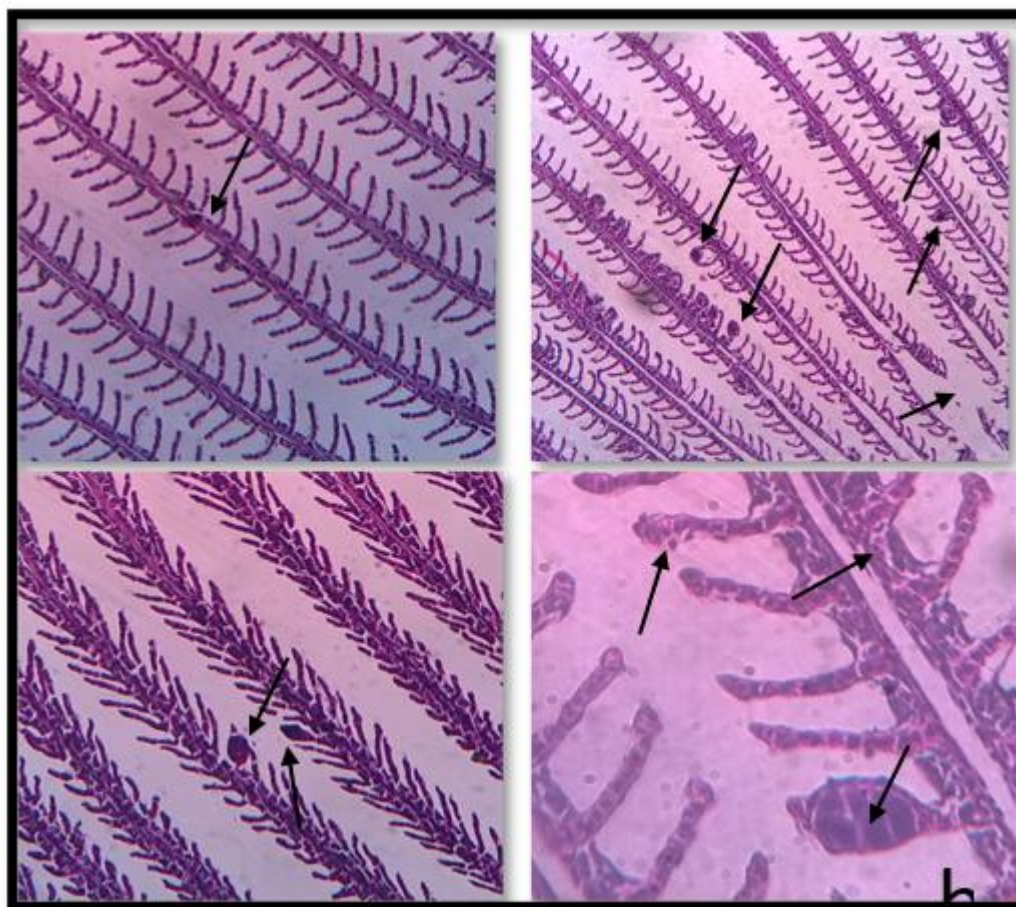


Fig. 15: Infected fish gills of *Mystus gulio* observed in 10 X and 40 X

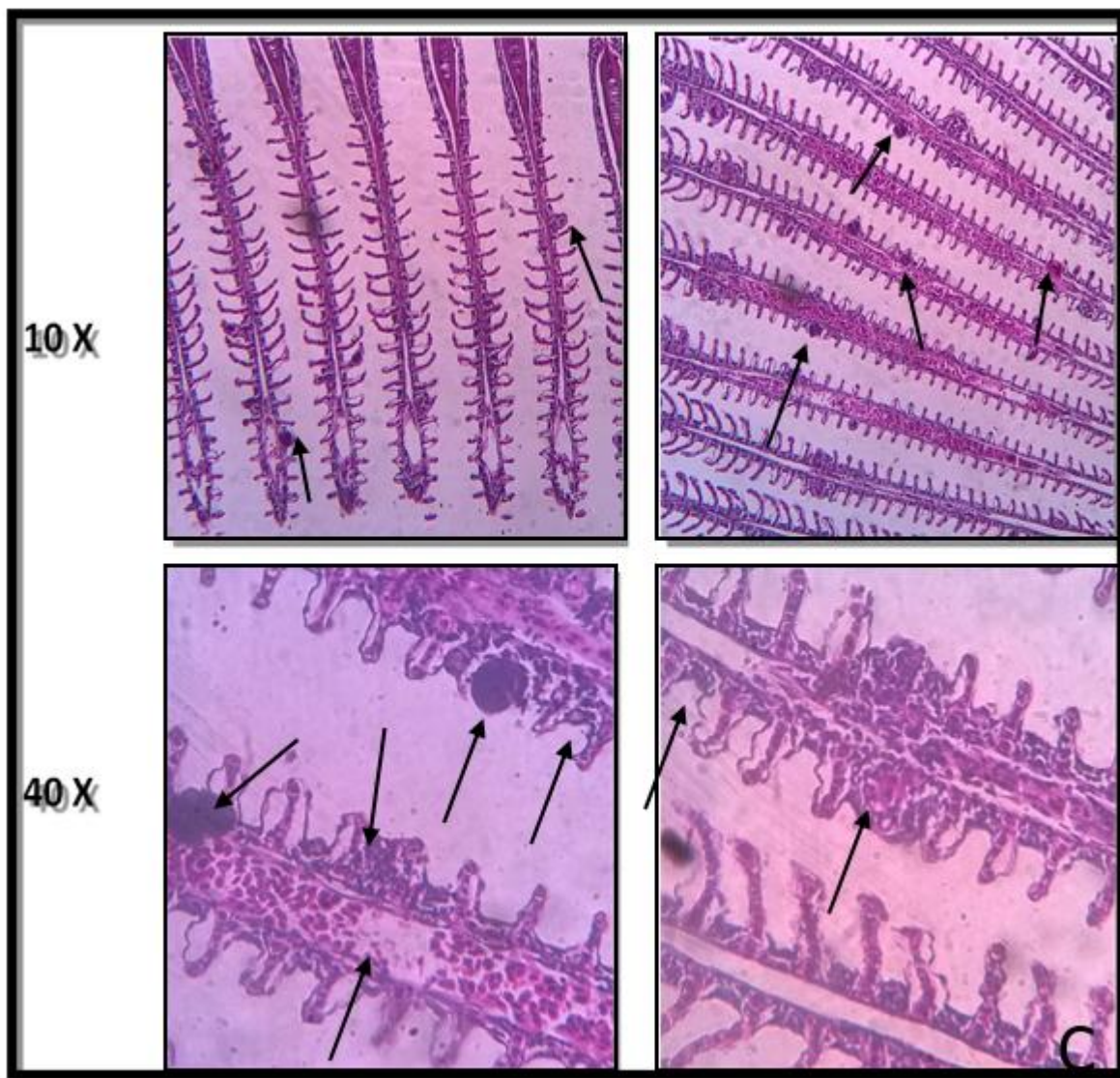


Fig. 16: Infected fish gills of *Mugil cephalus*

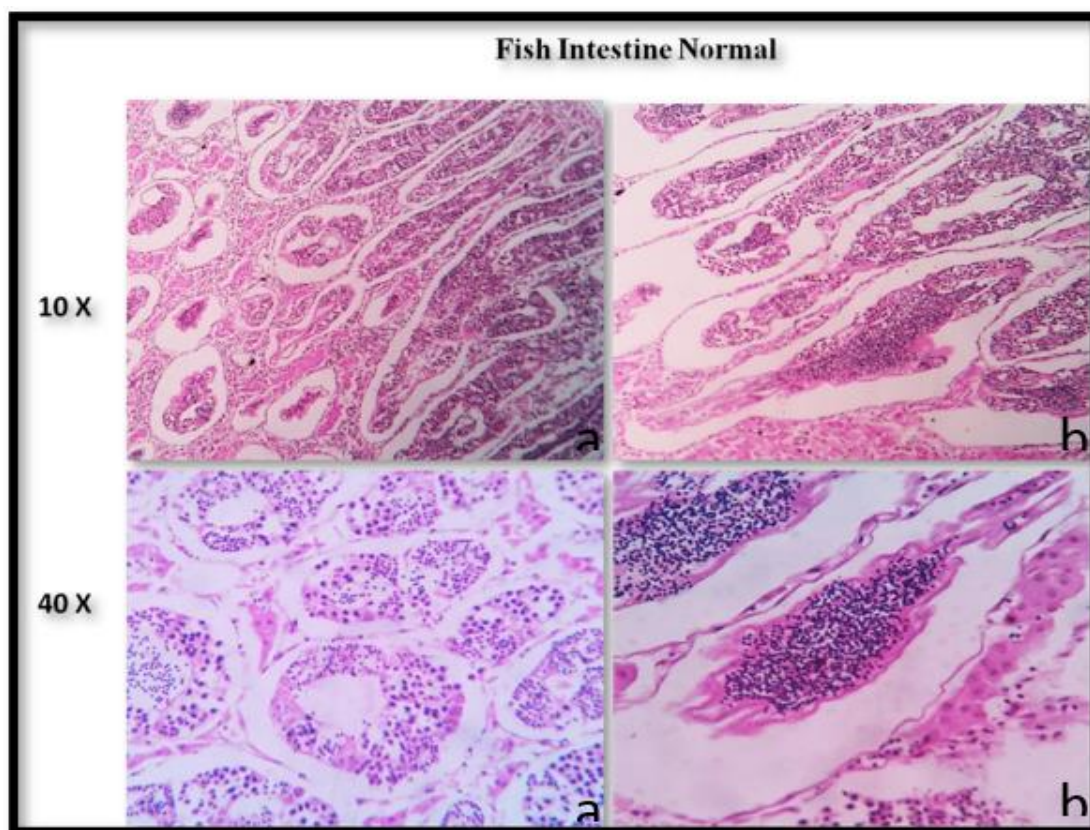


Fig. 17. Normal fish intestine observed at 10X and 40X in a. *Mystus gulio* & b. *Mugil cephalus*

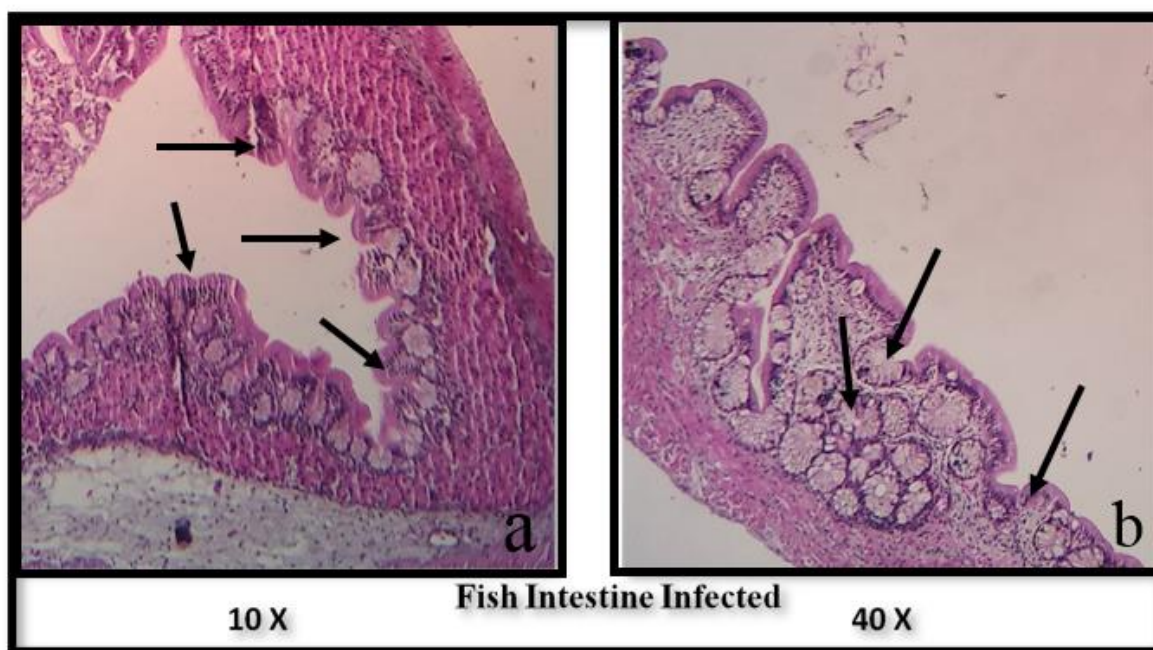


Fig. 18 a&b: Infected fish intestine observed at 10X and 40X in *Mystus gulio*

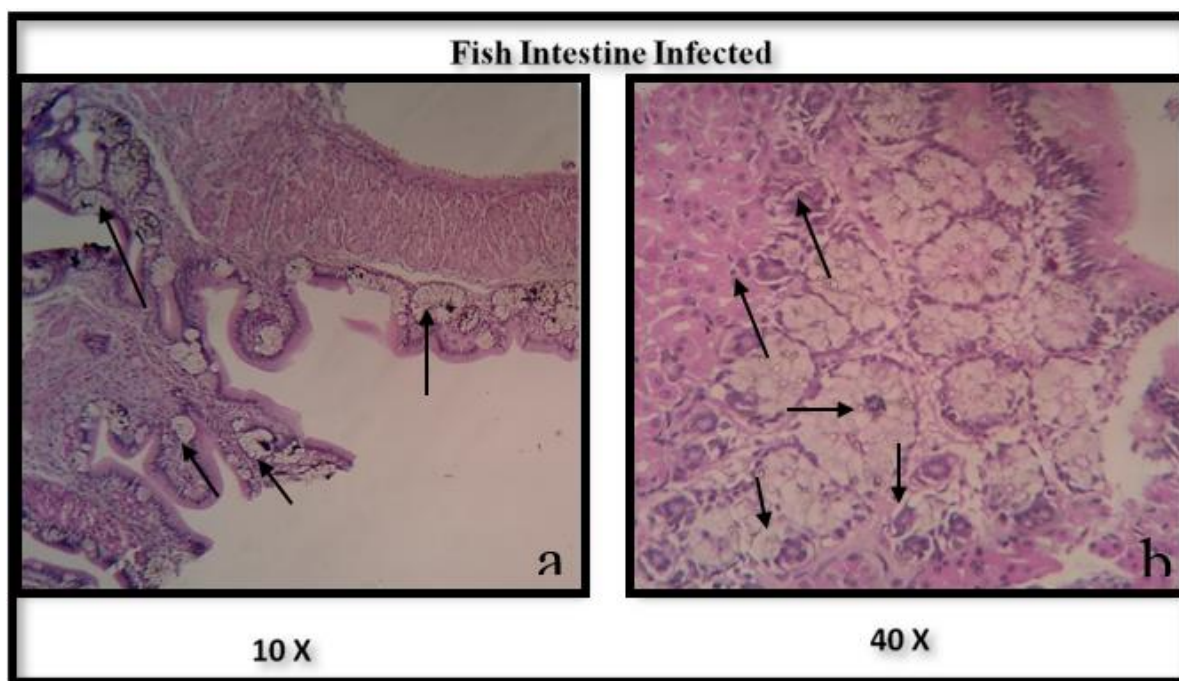


Fig. 19 a&b: Infected fish intestine observed at 10X and 40X in *Mugil cephalus*

DISCUSSION

Fish are considered one of the most important biomonitors in the aquatic ecosystem for assessing heavy metal pollution (Abou El-Gheit et al. 2012). Furthermore, fish are at the top of the food chain and can accumulate metals that are transferred to humans, aquatic mammals and birds through consumption of fish, causing acute or chronic diseases (Al-Yousuf et al. 2000).

Accumulation of pollutants disrupts the physiology of fish tissues. The endpoint in assessing the risk of pollutants in the environment is the microscopic examination of target tissues through histopathological parameters (Fatima *et al.* 2015). Histopathological changes can be used as indicators of the impact of various anthropogenic pollutants on organisms and as a measure of the overall health of the entire aquatic ecosystem (Saad et al. 2011). Harmful effects of pollutants can be manifested in fish tissues before consequential changes in the external appearance and behavior of fish (Mahboob et al. 2020).

In addition to different species, differences in metal concentrations depend on the types of tissues analyzed (Abarshi et al. 2017), with gills containing a higher level of the studied metals compared to the muscles in all tilapia species. The tissue of gills in all fish has the ability to accumulate significant levels of metals compared to other tissues and their surface has a negative charge and therefore provides a possible site for positively charged elements (Shovon et al. 2017). Iron is the most important metal for biological life. It plays a greater biological role than any other heavy metal. Its toxicity causes diarrhea, hemorrhagic gastroenteritis, liver necrosis and leads to death by hepatic coma (Clarke et al. 1981).

The concentration of metals, especially zinc was more elevated in the gills than in the muscles because the gills are the main entrance for metals into the fish. They are taken up by fish directly from water, especially through mucus and gills (Skidmore, 1964). The high concentration of zinc may be due to domestic, sewage and agricultural wastes discharged into the lake through different drains and affecting the entire area of the lake (Abdel-Satar 2008; Abdel-Satar & Geneid 2009; El-Naggar et al. 2016; Elmorsi et al. 2019).

Mn deficiency causes reproductive and skeletal abnormalities. Daily intake of small amounts of Mn is recommended for growth and good health of children. However, excess consumption of Mn can lead to neurologic and psychological disorders (Ahmed et al. 2016). Pb and Cd play no role in biological processes of living organisms and are highly toxic non-essential elements even at low concentrations (Dimari et al. 2008). They are also potent mutagenic and carcinogenic agents (Markman & Horsfall 2016). Pb inhibits impulse conductivity by inhibiting the activity of acetylcholine esterase and monoamine oxidase, leading to pathological changes in organs and tissues (Rubio et al. 1991). It also impairs the larval and embryonic growth of fish species (Dave & Xiu, 1991).

These changes in the muscles may be attributed to the accumulation of heavy metals and/or inorganic fertilizers that are discharged from different drains into the lake with a large amount of wastes (Mahmoud & El-Naggar 2007; Tayel et al. 2018) and to parasitic infections (Saad et al. 2011; Abou El-Gheit et al. 2012). The gills are the most delicate structure of the teleost body, having an external location.

These changes may be due to fertilizers, salts and sewage discharged into Lake Al-Manzalah. Tayel et al. (2018) and Mahmoud & Abd El Rahman (2017) found similar histopathological changes in the liver of *Mugil* species and *Clarias gariepinus* caught in the same lake. Degeneration and necrosis of hepatocytes may be due to the accumulation of heavy metals.

The gills and intestine of the two fish species (*Mystus gulio* and *Mugil cephalus*) collected from mangrove areas during winter, summer, Monsoon and Post monsoon seasons the results are exactly coincides to previous reports of Kalaiyarasi et al. (2017). Gills are the first line of defense against waterborne toxins, and they are particularly vulnerable to heavy metal deposition due to their constant contact with the outside world. They are also the primary site for heavy metal uptake (Shah et al, 2020; Hermenean et al., 2017). Heavy metal intake in the gills damages the lamella, which is involved in the ion exchange mechanism during osmoregulation (Raju, 2013). According to Fonseca *et al.* (2017), metals have been linked to filament epithelium growth, lamellar fusion, and epithelial necrosis, and their effects can be significant (Mladin et al., 2021). Hyperplasia, lamellar fusion, epithelial necrosis, and edema have all been detected in gills and ascribed to heavy metal toxicity (Ayoola and Alajabo, 2012).

Summer and winter seasons showed several histological alterations like infected fish gills of *Mystus gulio* and infected fish gills of *Mugil cephalus* due to the discharge of sewage. However, the intensity of the alterations was observed to be the highest in the specimens collected during the summer season and the lowest during the winter season. The gills are considered to be primary target of the contaminants as they are involved in many important functions 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 *M. gulio* and *M. cephalus* collected from the polluted sites.

The gills and intestines of *M. gulio* and *M. cephalus* are drastically affected and it is clearly noticed in histopathological studies that were observed at 10 & 40 X respectively. The gill is playing a vital role in remodeling and it is directly related to oxygen demand and it can be considered as a defense response that leads to a decrease in the entry of toxic compounds through the gills (Mendes Almeida et al., 2014). Thus, it is possible that the histological changes observed in gills follow a common induction pattern that is independent of the type of fish and stressor agent. The increased thickness of gill filaments can act as a barrier to xenobiotics present in water since this process could increase the distance between the capillary and the lamellar surface, reducing the absorption of pollutants (Fig. 3b&c) (Camargo and Martinez, 2007). But, the reduced thickness of gill filaments may lead to accumulate the pollutants in lamellar surface thereby increasing the distance between the capillary and lamellar surface.

Numerous authors have reported that increase in the numbers of these cells it may be due to toxins exposure (Kaur et al., 2016); however, whether the origin of these histological findings is cell division or differentiation remains unclear. The predominant pathological response of a common fresh water fish called yellow perch (*Perca flavescens*) where the gills were involved in proliferation of their basal epithelial cell and extensive proliferation in mucous cells following changes were occurred due to increase in their salinity. This is consistent with observations in the proliferation of gill mucous cells and basal epithelial cells following exposure to organic contaminants (Kaur et al., 2018). Mucous cell proliferation of yellow perch from oil sands reclaimed environments following longer residency periods has also been observed (Nero et al., 2006), suggesting that this type of response may be a long-term adaptation. Mucous cells contain mucins, polyanions composed of glycoproteins that can be effective in trapping toxicants and aid in the prevention of toxicant entry into the gill epithelium (Kaur et al., 2018). Although mucous cell proliferation may be beneficial in reducing toxicant entry, the consequence is an increase in the distance for gas exchange along the secondary lamellae, potentially reducing the efficiency of gas exchange and causing hypoxic conditions (Raju et al., 2013).

When aquatic animals are exposed to toxic concentrations of heavy metals, their internal organs may accumulate the element (Kumari et al., 2012), which may lead to biochemical and morphological changes, particularly in the liver, intestine, gills and kidney (Abdel-Satar et al., 2008). The present study also proves that the intestines of both the fishes were found to be affected due to one of the reasons mentioned above. Likewise, Dar et al., 2011 reported that catfish *Clarias batrachus* are exposed to 4×10^{-6} and 8×10^{-6} CdCl₂ for 30 and 60 days, it includes in the alterations in hepatocytes, eccentric positions of nuclei, enucleation, development of cytoplasmic vacuoles, and necrosis of hepatic tissue. The uptake of metals occurs mainly through the gills but it may also occur by means of intestinal epithelium (Mohamed, 2008).

Toxic pollutants enter the digestive tract of fish via the food and water they consume, causing structural and functional deterioration of the intestine (Kole et al., 2001). The present study showed that significant damage to the intestines of both the fishes was found and shown in Fig. 4a&b. Similar findings were reported by Kaoud et al. (2011) who reported pathological changes in the intestine of *O. niloticus* that were exposed cadmium, including: atrophy in the muscularis mucosa; degenerative and necrotic changes in the mucosa and submucosa with necrotized cells aggregated in the intestinal lumen; and edema and atrophy in the submucosa (Younis et al., 2014). Previously by Hanna et al., 2005 reported that the changes observed in the intestine of *O. niloticus* were severe degenerative and necrotic changes are noticed in intestinal mucosa. Likewise, the present study results of the two common fish intestines (i.e., Cat fish & Mullet) were found and the edema observed between the submucosa and mucosa may also due to the absorption of toxic heavy metals (Hanna et al., 2005). The order of bioaccumulation of metals in both the fishes were found to be gills was $Fe > Cr > Pb > Cu$; kidney and muscles were; $Fe > Cr > Cu > Pb$; liver as $Fe > Cu > Cr > Pb$ (Table 3). An increasing trend of concentration of iron, copper, chromium and lead was observed in all the tissues. The increase of iron accumulation is very higher in all the seasons it's due to the discharge of wastes from the shrimp ponds and other external factors. The level of lead, chromium and copper are in the permissible limit as suggested by FAO (2001) are found in the tissues of fishes.

The cat fish *Mystus gulio* and common mullet *Mugil cephalus* can be suggested as a biological model for investigating possible adaptations to pollutants. This study has confirmed the toxic effect and the pollutants released in waters are may be due to usage of chemicals or pesticides, etc., which are released from shrimp pond effluent sites or it may be used for agricultural practises. Further research is in progress to check the heavy metal accumulation in the aquatic organisms.

This is consistent with the findings of Authman & Abbas (2007) who stated that the liver is involved in a detoxification of toxins such as heavy metals. Accumulation of hemosiderin in liver cells may contribute to the rapid and continuous destruction of red blood cells (Hashem et al. 2020; Tayel et al. 2018; Ibrahim & Mahmoud 2005). Degeneration of hepatocytes can be caused by oxygen deficiency due to intravascular hemolysis and vascular dilation (Gaber & Gaber 2006). Toxins secreted by microorganisms in sewage water may cause necrosis and hemorrhage (Saad et al. 2011). Fatty degeneration can be caused by an increased rate of utilization of energy reserves or an induced imbalance between fat utilization and production (El-Naggar et al. 2009). The liver is an organ that excretes and binds proteins such as metallothionein. Metal-binding proteins, which are present in the nuclei of hepatocytes, increase the cell damage (Mela et al. 2007). The present study evidence that the sample collected from station 6 (nearby aquaculture pond) shown high amount of heavy metal accumulation in the internal organs of the two fish species from Pichavaram waters. These fishes are abundantly available in all the seasons and are edible, consumed by various living organisms including human beings and having a greater chance of accumulation of heavy metal transfer from one trophic level to another trophic level; shortly called as Biological Magnification. Further studies have been planned to study the toxicity of these heavy metals in human beings residing in and around the Pichavaram.

Acknowledgements

We are very thankful to DST-SERB for the financial support (No. SB/EMEQ-060/2013; dt. 12.07.2013), Principal Chief Conservator of Forest & District Forest Officer Cuddalore Division, Tamil Nadu Forest Department for the permission to collect the samples and Mr. R. Narendran & Mr. P. Dinesh, Ph. D Research Scholar, Centre for Advance Studies in Marine Biology, Annamalai University for assisting in sample collection, and for the authorities of Bharathiar University for providing necessary laboratory facilities.

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