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# Heavy Metal Pollution Induced Histopathological Changes in *Anabas testudineus* collected from Periyar River at Ernakulam district and the Recovery Responses in Pollution free water.

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**Abstract:** Heavy metal (lead, nickel, zinc, arsenic and cadmium) pollution status in water, sediment and the corresponding bioaccumulation in biomass of Anabas testudineus from two different stations of Periyarriver at Ernakulam district (Station I- Eloor Industrial area, Station II- Irumbanam, Ernakulam) of two year period analysis showed that the bioaccumulation rate depends on pollution status in the environment. The histopathological changes in liver, gills and muscle varied based on the pollution load. The recovery responses were studied in fishes kept in aquaria maintained at controlled laboratory conditions for 30 days and from the results it was apparent that the pollution induces stress in fishes that has been reflected in the histological characteristics of vital organs. However by keeping them in pollution free waters positively influenced towards recovery from stress.

**Key Words**: Periyarriver, Ernakulam, *Anabas testudineus*.

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## I. INTRODUCTION

Heavy metals undergo metabolic activation that provokes a cellular change in the affected fish. The tissue lesions and apoptosis arise from bioaccumulation stimulate necrotic alterations in the fish with an inflammatory defensive reaction [1]. Disturbance of living processes at the molecular and subcellular levels of biological organization by xenobiotics can lead to cell injury, resulting in degenerative and neoplastic diseases in target organs [2]. Several studies have also reported on the response of fish to sediments contaminated by pesticides, heavy metals, and persistent organic pollutants [3, 4, 5, 6].

## II. MATERIALS AND METHODS

The methodology adopted to attain the objectives like seasonal variation of heavy metal pollution in water, sediment and biomass from Periyar, pollution induced changes in histology of vital organs in *Anabas testudineus* collected from the same site and the rate of recovery in bioaccumulation and histopathology is as follows.

2.1 **Study Area**:A stagnant water body located in an unpolluted area of Cherthala, Alappuzha district was Selected as a reference site (Control station). Based on specific geographical features, water flow regimes and anthropogenic activities, two sampling locations (Station I and Station II) were selected in Periyarriver. One of the sampling areas flowing through the Eloor industrial Estate (Station I). The other one, Station II is located at Irumbanam. Three separate samples were collected from all the stations.

**2.1.1 StationI** – ELOOR: Station one is a portion of Periyar river passing through the Eloor-Kalamassery, the study area, is 1.5 Km upstream to Eloor ferry is between 10°08'54.46"N latitudes and 76°28'51.66"E east longitudes. The study area is located downstream of industrial belt well known for large- and small- scale industries. The major industries include FACT, TCC, IRE, BZL, etc and are on the banks of the Periyarriver[7].

**2.1.2 Station II- Irumbanam**:Station two is located at Irumbanam, Trippunitura. It is considered as a site locatedat Chithrapuzha, a tributary of Periyar river. A station in between 9°59'00.1"N latitude and 76°20'44.6"E longitude was selected for the material collection. Factory out lets of BPCL, FACT, IOCL etc were near to this site.

2.2 **Study period**:The study period extended for two years from 2012 to 2014.

2.3 **Heavy metals analyzed for the study**: The heavy metals like Lead (Pb), Nickel (Ni), Zinc (Zn), Arsenic (As) and Cadmium (Cd) were selected to study the seasonal variation of heavy metal pollution in Periyar.

2.4 **Experimental materials**: Water, sediment and *Anabas testudineus*were selected as the experimental materials.

Heavy metal analysis of the water and sediments from the study area during the study period will give the idea about the seasonal fluctuation of heavy metal pollution load in the river and that in the selected organs viz., liver, gill and muscle of the fish will give the information about its bioaccumulation rate. The concentration of antioxidant enzyme activity in liver, gills and muscle will be considered as an indication of stressful conditions.

**Experimental design:**Three samples of water and sediments and fish coming under similar size rangewere collected from each station (Control, Station I and Station II) during the study period. The collected materials were prepared variously.

**2.4.1 Sampling and storage**:Surface water samples were collected in a 2-litre conventional polyethylene container. Three samples were collected from the same location. A stainless steel plastic-lined Van Veen grab was used to collect sediment samples. The top 5 cm layer was carefully skimmed from the grabs using a polyethylene scoop, homogenized and stored in polyethylene containers. Fish sample (*Anabas testudineus*) were collected using Cast net with the help of local fishermen. All the sample materials were taken to the laboratory without delay.

On reaching the laboratory the fish were categorized in two groups; one group was introduced into aquarium that has been set in laboratory conditions to carry out the recovery studies. The second group of fish were anesthetized and dissected to collect the organs (liver, gills and muscle). Then water, sediment and the organ samples were processed variously for heavy metal analysis and antioxidant enzyme assay.

**2.4.2 Sample processing for heavy metal analysis:**All glassware and plastic ware used in the experiments werepreviously washed, soaked in dilute nitric acid and then rinsed with double distilled water. Reagents and standard solutions were prepared with double distilled water. The preparation of various samples for heavy metal analysis was done following the method suggested by [8].

**2.4.2.1 Preparation of water sample for heavy metal analysis**:Five $cm^3$ of concentrated hydrochloric acid were added to 250 $cm^3$ of water sample and evaporated to 25 $cm^3$. The concentrate was then diluted to 50 $cm^3$ using de-ionized water. The processed water sample were then filtered through the Whatmann No.1 filter paper then labeled and stored for analysis.

**2.4.2.2 Preparation of sediment sample for heavy metal analysis:**One gram dried, sieved sediment samples were placed in a clean 20 ml glass vial and one ml de-ionized water, two ml 70 % $HNO_3$ and one ml 65% $HClO_4$ were added. The open vials were placed in $80^0$C temperature for 12 hours. The digested samples were diluted to 50 ml using de-ionized water. The processed sediment sample were then filtered through the Whatmann No.1 filter paper then labeled and stored for heavy metal analysis.

**2.4.2.3 Preparation of biomass sample for heavy metal analysis**:One gram tissue sample (wet mass) was weighed and placed into a clean glass vial and one ml de-ionized water, two ml 70 % $HNO_3$ and one ml 65% $HClO_4$ were added. The open vials were placed in $80^0$C temperature for 12 hours. The digested samples were diluted to 50 ml using de-ionized water. The processed biomass sample were then filtered through the Whatmann No.1 filter paper then labeled and stored for heavy metal analysis.

2.5 **Heavy metal analysis**: The processed water, sediment and biomass sample were sent to STIC (Sophisticated Test and Instrumentation Centre, Kochi, Ernakulam) for the analysis of selected heavy metal (Pb, Ni, Zn, As, Cd) concentration in it using ICP-AES system.

2.7**Preparation of tissue sample**: The *A. testudineus*collected from the field were immediately transferred to the lab and anesthetized by giving a deep blow to the head region and were dissected immediately. The organs (liver, gills and muscle) were carefully dissected out from fish and the intact organs were wiped thoroughly with blotting paper to remove the blood and body fluid and then fixed in 10% neutral buffered formaldehyde for 24 hours.

2.8**Steps involved in histological procedure**: The major steps involved in histopathological analysis are fixation, tissue processing, decalcification, section cutting and staining [9].

## III. RESULTS

The alterations in the histology of different organs of fish from polluted waters were compared with that of fish from less polluted reference station. Some changes were observed and are described under the following heads.

**3.1 Liver**

In the present study the liver of *A. testudineus*from control station, comparatively less polluted waters show the normal architecture of liver with large polyhedral cells within the network of minute canaliculi, irregularly distributed bile duct, blood capillaries and sinusoids that are filled with erythrocytes. Hepatocytes surrounding the central vessels appeared to be lightly arranged in a rosette pattern with 10 to 12 cells in each group. Hepatic cells are roundish polygonal, containing clear spherical nucleus. They are located among sinusoids forming cord like structures, known as hepatic cell cords. In fish, these structures are generally obscure [10] (Plate 2). However the liver of fish from Station I showed some pathological changes like Clear Cell Foci (CCF) and Necrosis (NC) (Plate 3) and the fish from station II showed distortion of hepatic cells (DH) and Necrosis (NC) (Plate 4). The signs of the recovery responses were noticed during recovery studies and the Necrosis (NC) persist (Plate 5 and Plate 6)

3.2 GILLS

Light microscope examination of the photomicrograph of the vertical section of the gills showed the arrangement of primary and secondary lamellar processes of which the former was thicker than the latter. The primary gill lamellae are flat leaf like structures with a central rod like supporting axis and a row of secondary gill lamellae on each side of it. The secondary lamellae were equally spaced along the columnar structures with intact cellular layer attached at their bases with the primary lamellae and free at their distal ends. The normal secondary lamellar epithelium was simple, consisting of a thin single or double sheet of epithelial cells, blood vessels and a row of pilaster cells. The region between the two adjacent secondary gill lamellae is known as interlamellar region [10] (plate7). In the gills of fish from Station I and II, severe changes were noticed unlike the case of liver. The damages include architectural loss, lamellar shortening to complete loss of secondary lamella, epithelial desquamation and lamellar clubbing, etc (Plate 8, 9, 10, 11). The symptoms get restricted to architectural loss after recovery (Plate 12, 13).

3.6 MUSCLE

The photomicrograph of the muscle depicted the presence of normal myotomes with equally spaced muscle bundles (Plate 14). The present study results did not come across with any histopathological changes (Plate 15, 16). After the recovery period the muscle appeared similar to the control (Plate 17, 18).

## IV. DISCUSSION

In the present study the liver of *A. testudineus*from Station I showed some pathological changes like Clear Cell Foci (CCF) and Necrosis (NC). The Clear Cell Foci are the indications of altered staining pattern and are considered as precursors to hepatocellular neoplasm. Necrosis is the symptom of cell death. The homoeostatic capacity may be altered. The leakage of lysosomal marker enzymes like Acid Phosphatase and increased Alanine aminotransferase in liver observed in the study can be connected with it. The changes were not severe, may be due to the nektonic habit of fish they try to avoid the uncomfortable immediate surroundings and occupy at a less polluted area. The liver of fish from Station II also registered some histopathological signs in the form of distortion of hepatic cells, necrosis, etc. Pollution induced alterations of enzyme activity and the related cell damage may be the reason behind. Lead was found to inhibit the impulse conductivity by inhibiting the activities of monoamine oxidase and acetylcholine esterase to cause pathological changes in tissue and organs [11]. In the work of [12] observed striking histological changes in the liver tissues exposed to single and combined doses of $CdSO_4$ and $PbNO_3$ when compared to the control fishes. These included distortion of hepatic cells as indicated by the presence of spaces, aggregation of blood cells among others. Some investigators like [13] carried out histological examination of the liver sample of the fish collected from heavy metal polluted river and showed fatty degeneration, degeneration and necrosis in hepatic cells, congestion, haemolyses, parasitic forms, oedema, hemosiderin, haemorrhage and branching in blood vessels.

In the gills of fish from Station I and II, severe changes were noticed unlike the case of liver. The damages include architectural loss, lamellar shortening to complete loss of secondary lamella, epithelial desquamation and lamellar clubbing, etc. The gill lesions may appear either due to the direct effect of pollutants or the defence mechanisms from the fish. The observed lamellar damages and desquamation may be the result of direct effect of the pollutants. Lamellar shortening, loss of secondary lamellae directly affect the oxygen exchange efficiency of gills. Fish exposed to copper shows several histological alterations, namely lamellar epithelium lifting, epithelium proliferation, lamellar axis vasodilation, oedema in the filament, fusion of lamellae and lamellar aneurisms (Figureueiredo-Fernandes et al. 2007). Some studies revealed that interstitial oedema is one of the more frequent lesions observed in gill epithelium of fish exposed to heavy metals [14]. The lifting of lamellar epithelium is the histological change observed, probably induced by the incidence of severe oedema[15, 16, 17].Oedema with lifting of lamellar epithelium could be serve as a mechanism of defence,

because separation of epithelia of the lamellae increases the distance across which waterborne pollutants must diffuse to reach the bloodstream [15]. These changes also can be due to the exposition to different kinds of pollutants, such as endosulfan[18], arsenic [19], drugs [17] and other heavy metals, such as aluminium[20], cadmium [21], and nickel [16]. Thus, this signifies that these alterations are not specifically induced by copper or other heavy metals. In fish collected from polluted waters [10] have found that the gill showed cytoarchitectural distortion of the lamella with overlapping of the primary and secondary lamella. Considerable mucous and granulated eosinophilic cells were witnessed in their cytoplasm. Extensive vacuolization were observed with prominent disruption of epithelium. Completely disrupted primary gill structure, marked hyperplasia of the branchial arch, pilaster cell vacuolization and congestion of blood vessels were well marked. The main response of gill epithelium was reduction in permeability.

The photomicrograph of the muscle of fish from control and polluted stations of Periyar depicted the presence of normal myotomes with equally spaced muscle bundles. The dilution of pollutant on reaching the muscle and the comparatively large area of muscle than liver and gills may be the reason behind the absence of any noticeable damages in the muscle of fish from polluted waters. But [10] reported that in the muscle of fish from polluted waters marked thickening and separation of muscle bundles, haemolysis, necrosis, lesions with reduced compactness was observed. Sub lethal concentration of cadmium led to pronounced intramuscular oedema with minor dystrophic changes was also noticed. However the present study results did not come across with any such observations as mentioned earlier.

Recovery study revealed that the damages occurred on the liver and gills get reduced or showed the signs of recovery but not completely after the treatment in clean waters. In the case of liver the symptoms of clear cell foci haven't noticed in the liver of recovered fish but the symptoms of necrosis persist but the frequency get reduced significantly. Even though the histopathological changes in the gills were very prominent, the recovery treatment considerably reduced the histopathological changes except architectural loss. Since the pollution did not induced any prominent damage in the case of muscle of fish from polluted waters, there were no significant difference between the histology of the muscle of fish from polluted waters and their corresponding recovery. Some investigators [22] studied the effects of cadmium on the gills of the African freshwater cichlid *Oreochromismossambicus* in water with normal and relatively high calcium concentrations for periods up to 35 days. Changes in the ultrastructure of the gill epithelium upon exposure to cadmium in the ambient water indicated degeneration of pavement cells and chloride cells, and acceleration in the turnover of the chloride cells. Macrophages, lymphocytes, rodlet cells and neutrophilic granulocytes infiltrated the filament epithelium. These authors observed recovery of the gills was observed after 35 days of treatment in high water calcium concentration.

The sub-lethal effects of copper on the humoral, enzymological and histopathological parameters in the teleost fish, *Anabas testudineus*and the curative capacity of vitamin C were investigated by [23]. The liver of exposed fish showed drastic architectural disruption such as hepatocyte degeneration, cell necrosis, inflammation with sinusoid dilation and thrombus formation. Their investigation clearly reveals toxicity of copper even at sub-lethal concentrations on the physiology and histology of the fish. The betterment of histological aspects in vitamin treated fish illustrates the curative and prophylactic role of the vitamin against copper intoxication.

## V.FIGURES AND PLATES

Systematic position of experimental animal, *A. testudineus*selected for the study is as follows (Plate 1)

| | | |
|---|---|---|
| Kingdom | : | Animalia |
| Phylum | : | Chordata |
| Class | : | Actinopterygii |
| Order | : | Perciformes |
| Family | : | Anabantidae |
| Genus | : | *Anabas* |
| Species | : | *A. testudineus* |

**3.1**The pollution induced variation in liver histology of *Anabas testudineus*from Station I and Station II and the respective recovery**.**

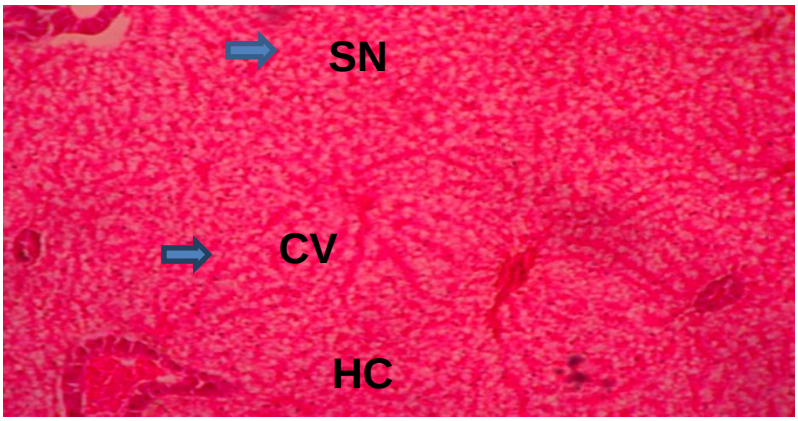


**Plate 2**: Photomicrograph of control liver of *A. testudineus*showing normal architecture with Hepatocytes (HC), sinusoids (SN), central vein (CV).

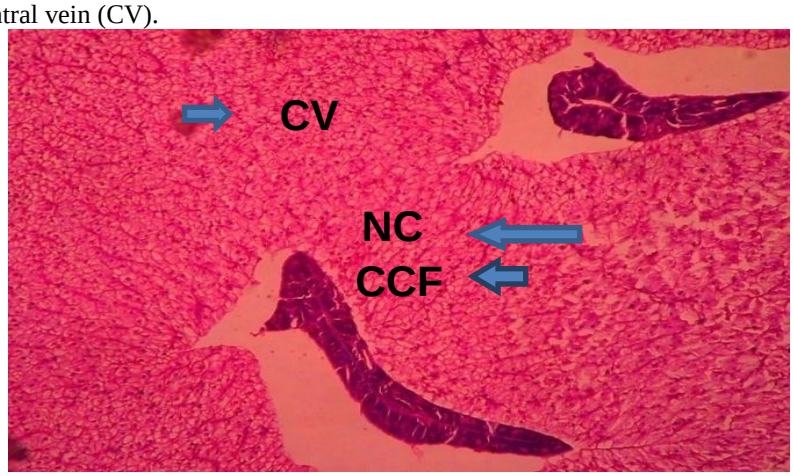


**Plate 3:** Photomicrograph of liver of *A. testudineus*from Station I showing Clear Cell Foci (CCF) and Necrosis (NC).

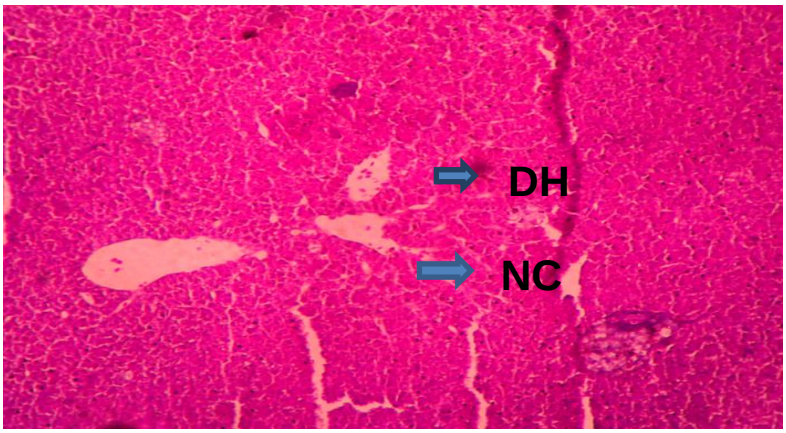

Plate 4: Photomicrograph of liver of *A. testudineus*from Station II showing distortion of hepatic cells (DH) and Necrosis (NC).

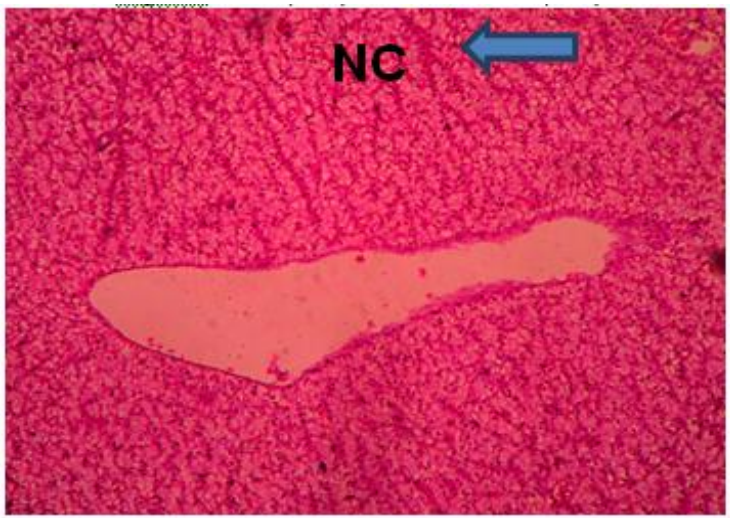


Plate 5: Photomicrograph of liver of *A. testudineus*after recovery from Station I showing Necrosis (NC).

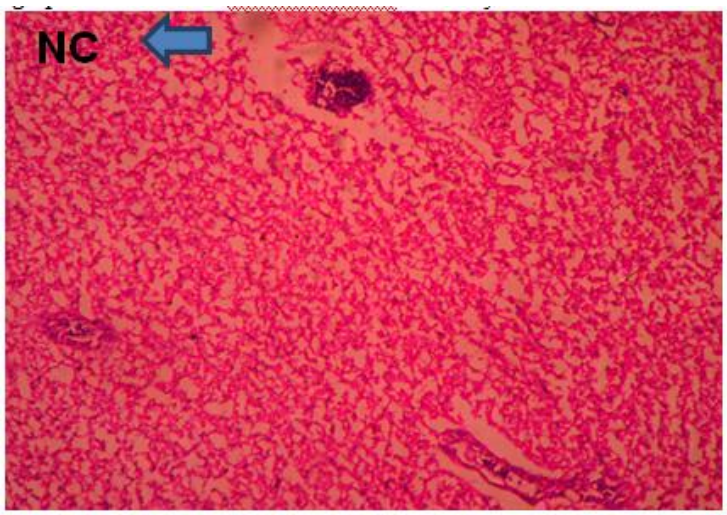


Plate 6: Photomicrograph of liver of *A. testudineus*after recovery from Station II showing Necrosis (NC).

**3.2** The pollution induced variation in gill histology of *Anabas testudineus*from Station I and Station II and the respective recovery

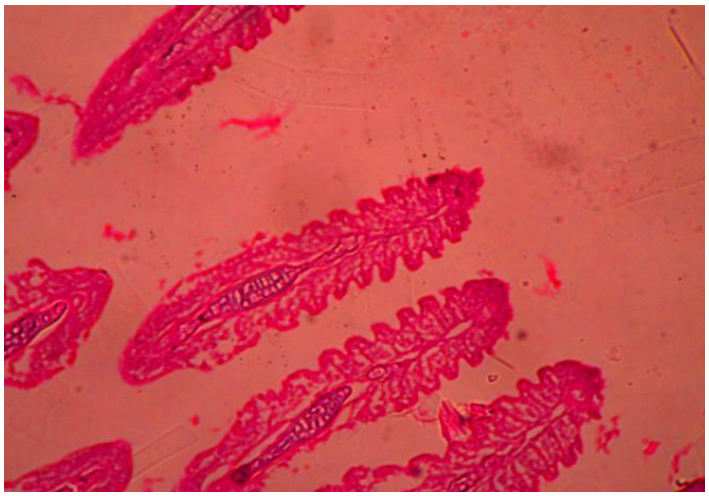

Plate 7: Photomicrograph of control gills of *A. testudineus*showing normal architecture with primary lamellae and secondary lamellae (10X).

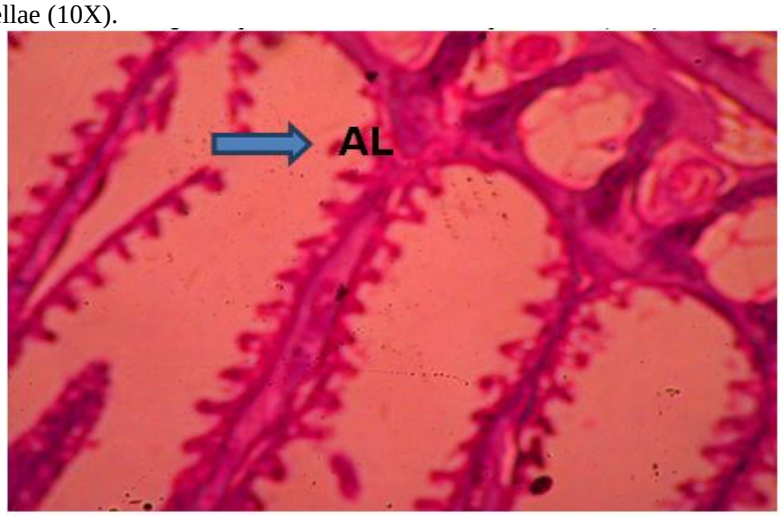


Plate 8: Photomicrograph of gills of *A. testudineus*from station I showing architectural loss (AL) (10X).

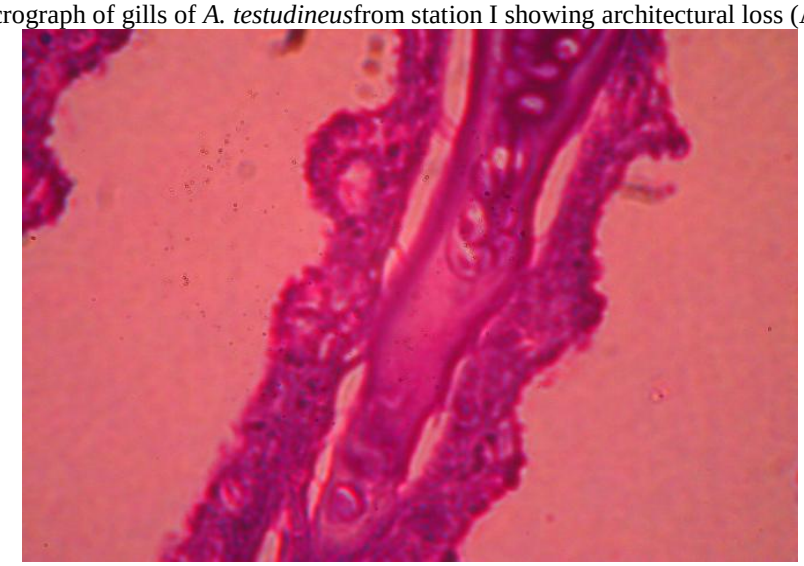

Plate 9: Photomicrograph of gills of *A. testudineus*from Station I showing complete loss of secondary lamella (40X).

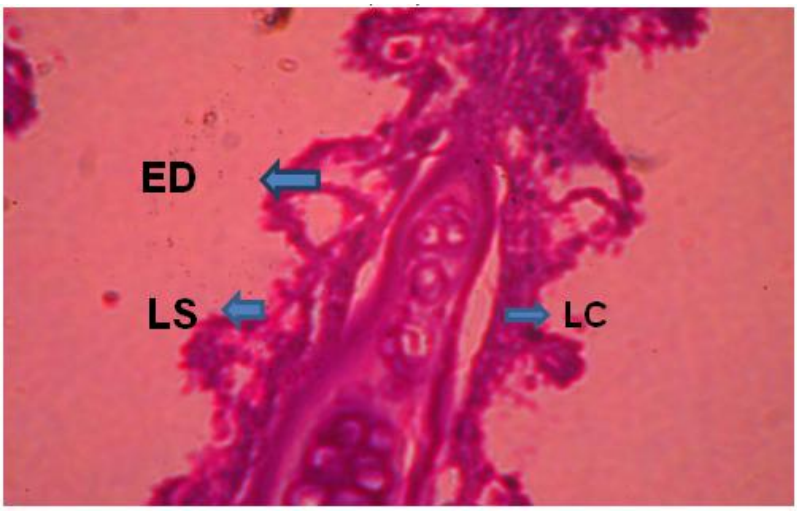


Plate 10: Photomicrograph of gills of *A. testudineus*from station I showing epithelial desquamation (ED), lamellar clubbing (LC) and lamellar shortening (LS) (40X)

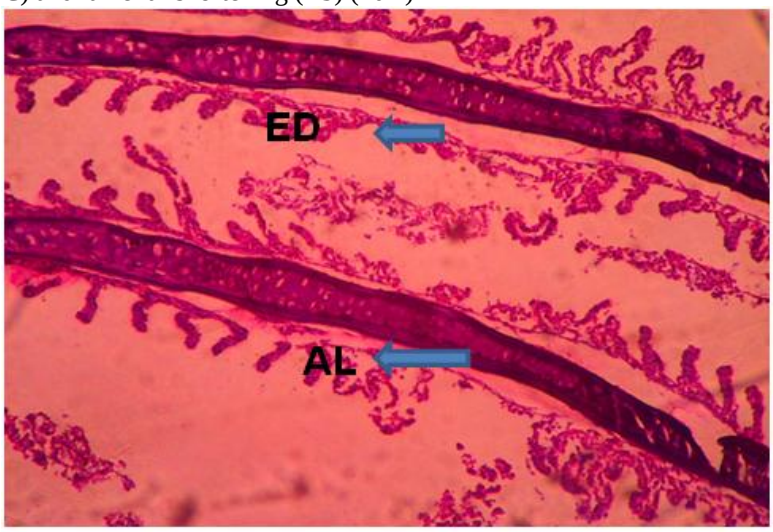


Plate 11: Photomicrograph of gills of *A. testudineus*from station II showing epithelial desquamation (ED), architectural loss (LS) (40X)


**Plate 12:** Photomicrograph of gills of *A. testudineus*after recovery from station Ishowing architectural loss (LS) (40X)

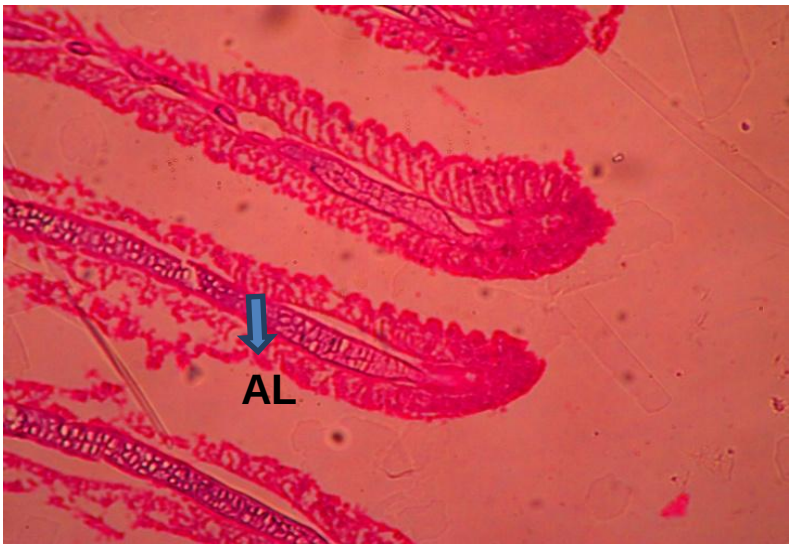


**Plate 13**: Photomicrograph of gills of A. testudineusafter recovery from station II showingarchitectural loss (LS) (40X)

**3.3**The pollution induced variation in muscle histology of *Anabas testudineus*from station I and II and the respective recovery

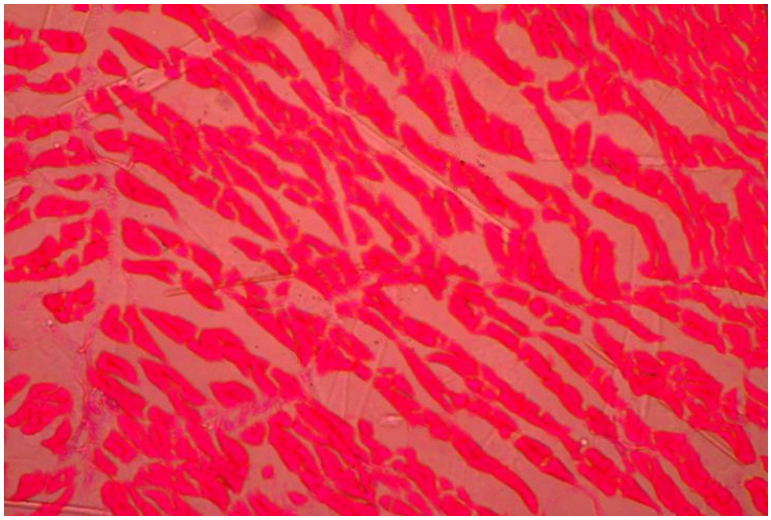

Plate 14: Photomicrograph of muscle of *A. testudineus* from control station showing normal characters (10X)

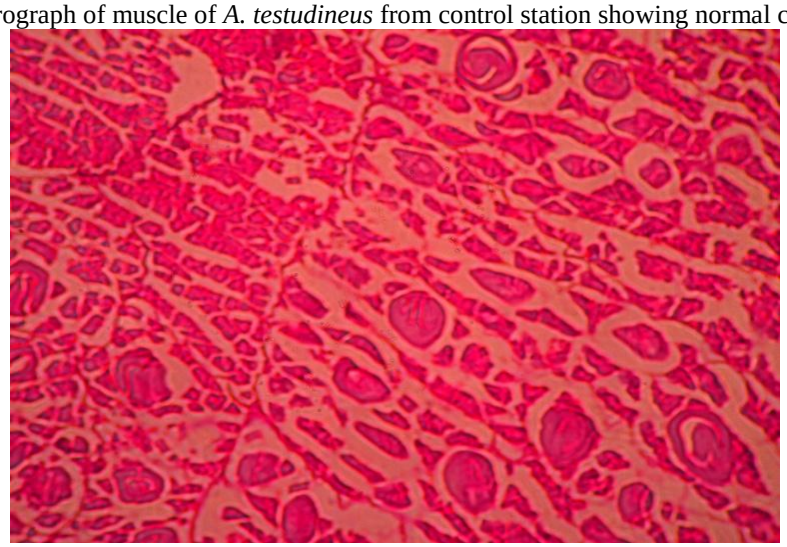

Plate 15: Photomicrograph of muscle of *A. testudineus*from Station I showing without any recognizable pathological changes (10X)

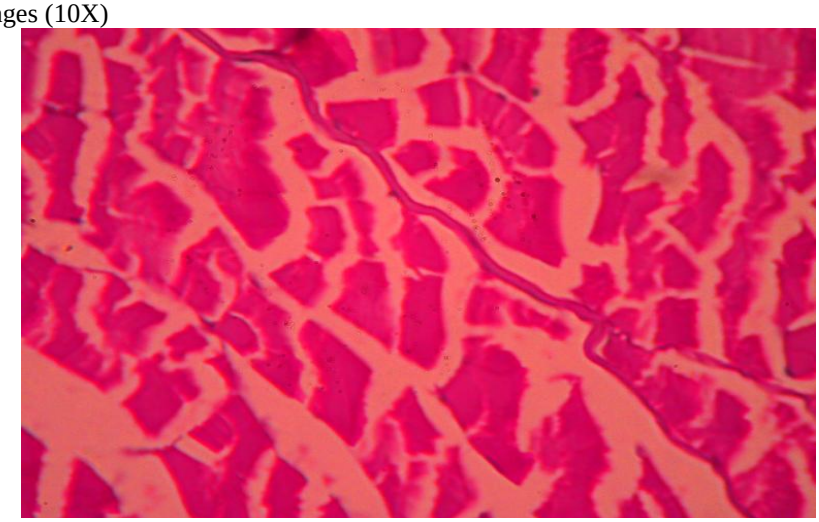

**Plate 16:** Photomicrograph of muscle of *A. testudineus*from Station II showing without any recognizable pathological changes (10X)

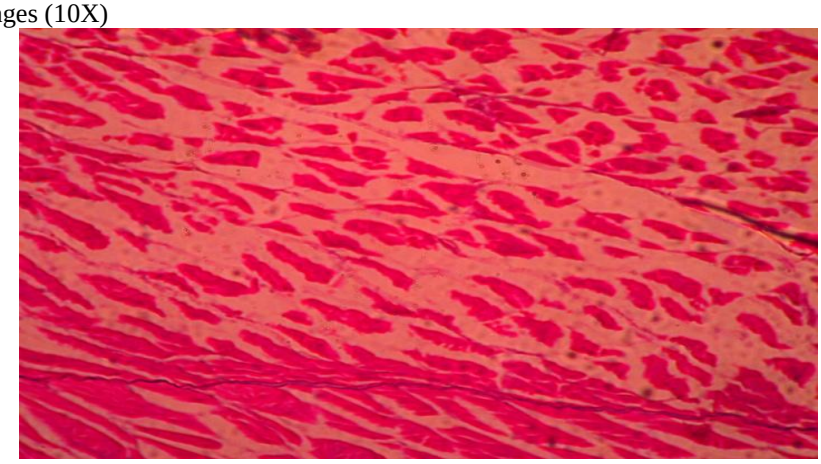

**Plate 17**: Photomicrograph of muscle of *A. testudineus*after recovery from Station I showing normal like appearance(10X)

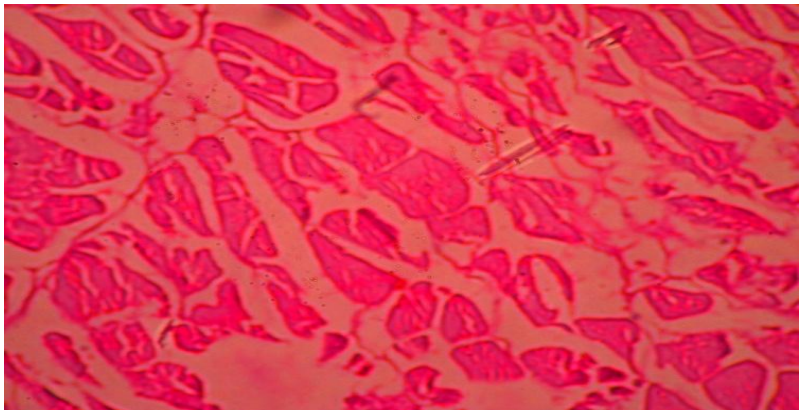

Plate 18: Photomicrograph of muscle of *A. testudineus*after recovery from Station II showing without any recognizable pathological changes (10X)

## V.CONCLUSION

Histological studies in fish got its relevance because fishes are in direct contact with the polluted habitat and the organs in fish are having some features; like gills have got large surface area making it exposes more to toxicant at first and at its extreme level, and its epithelium is very thin to render easy entry of toxicants and its detoxification system is poor compared to other organs making it a blameless candidate structure for histopathological analysis. Liver as the most important organ of metabolism and detoxification by

biotransformation of xenobiotics, histopathological studies using liver is also worthwhile. Muscle, the organ of storage is also necessary as its toxicant bioaccumulation behaviour and the most valuable part as far as human consumption are concerned. The histopathological study of muscle gives a warning sign of quality for consumption of fish from a particular habitat.

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