Ultrastructural Studies to Evaluate Fish Scales as Indicators of Heavy Metals in *Labeo rohita*

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Abstract

Environmental pollution by heavy metals has been of nagging concern globally. A preliminary study was undertaken to study structural alterations including lepidontal aberrations on the fish scales of *Labeo rohita* using Scanning Electron Microscopy (SEM). In addition, the atomic percentage of heavy metals on the scales were analysed by Energy Dispersive X-ray microanalysis (EDX). The scales were gathered during two seasons (winter and summer) from Ludhiana’s fish markets. Various heavy metals including Aluminium (Al), Chromium (Cr), Zinc (Zn), Nickel (Ni), Copper (Cu), Lead (Pb) and mercury (Hg) were detected on fish scales gathered from local fish markets compared to control groups. In addition, Silicon (Si) was also found on the scales gathered from markets. Heavy metal accumulation in the water source from which fish were gathered might be responsible for deposition of heavy metals on scale’s surface. Along with this, lepidontal anomalies on the scale’s dorsal surface were examined using EDX. In addition, structural aberrations such as broken, disorganized and eroded circuli, damaged margins of scales, alterations in focus and tubercles were also studied. The intensity of alterations was observed higher in scales of fish larger in size gathered from market compared to fish smaller in size, which might be due to longer duration of heavy metal exposure in the water source. The results clearly suggested that fish scales of *L. rohita* have a potential to be used as indicators of water quality.

Key words: Fish scales, Heavy metals, *Labeo rohita*, SEM-EDX, Water quality indicator

Introduction

Water plays an important role in any nation’s development (Vikrma and Sandhu, 2022). India has a range of freshwater assets of 10.55 million hectares (ha) area spread over various geo-climatic areas such as high altitude wetlands (0.12 million ha), cut-off and oxbow lakes meanders (0.10 million ha), riverine systems and streams (5.35 million ha), waterlogged wetlands (0.45 million ha), lakes (0.73 million ha), tanks and reservoirs (3.79 million ha) (Deepak et al., 2022). However, In India, 70% of the riverine water is contaminated with pollutants due to agricultural, chemical and industrial discharge coupled with poor waste disposal and management (Jindal and Sharma, 2011; Lau and Li, 2023). Water pollution induced by heavy metals and related risk to aquatic biota and human health has aroused wide concern globally due to their persistence, toxicity, bioaccumulation, ubiquitous nature and biomagnification in the food chain (Yang et al., 2023). Heavy metals are naturally occurring element having specific gravity higher than water and they get easily dissolved in water (Ali et al., 2019). Some heavy metals such as iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) are essential for organisms and play role as cofactors in many reactions while others are poisonous with no biological role such as mercury, lead and cadmium (Güngor and Kara, 2017).

Fish serve as suitable bio-indicator of heavy metal contamination as they explore freely among different trophic levels in the water resources (Abdel-Baki et al., 2011). Multiple factors such as seasonal changes, concentration of metals, physico-chemical properties of water, exposure period, metabolic rate and size of fish lead to variations in heavy metal accumulation (Shaikh, 2014). In addition, fish constitute a healthy protein source but the presence of toxic metals in muscles and other tissues could impair human health upon fish consumption. Numerous ecotoxicological studies focus on metal accumulation in various fish tissues such as liver, gills and muscle.
Scales as indicators of water quality (Squadrone et al., 2013). However, fish must be sacrificed in order to sample fish tissues for contamination studies which affect studied species and communities. On the other hand, non-lethal sampling provides an opportunity to gather larger sample size including specimens from endangered and rare species without resulting in death (Baker et al., 2004). Scales provide protection to skin from contamination in the environmental water to which the fish has been exposed due to their external location (Santana et al., 2016). *Labeo rohita* was chosen as model organism due to its sensitivity to heavy metals in water (Jindal and Verma, 2015). Studies have already been conducted on the effect of heavy metals on the structure of fish scales (Braich and Jangu, 2016; Dwivedi and Sehgal, 2017). However, to our knowledge, effect of different exposure periods and seasons on lepidontal alterations is scarce. Based on the hypothesis, the objective of the current investigation is to study the atomic percentage of heavy metals and surface alterations especially in lepidonts on scales of *L. rohita* of different sizes with respect to seasons.

**Materials and Methods**

Fish scales of *L. rohita* (Hamilton, 1822) of two sizes i.e. ≤ 40 cm (age <1 year) and >40 cm (age >1 year) were collected from local fish markets of Ludhiana (Punjab) from December 2017 to February 2018 (winter) and April 2018 to June 2018 (summer). 5 scales were gathered from fish in triplicate (n=3) during each month to study ultrastructural alterations. According to fish vendors, fish were captured from Sutlej River and various districts of Punjab, India. Scales of fish gathered from a private fish farm located in the village of Krodian (Tehsil Payal), Ludhiana (long. 76°00 E and lat. 30°70 N) were considered as control. Similarly, 5 scales of control fish were also removed from each fish (n=3) during each month. The farm used all safe fish farming practices. Using forceps, scales were extracted from the second row above the lateral line on the left side of the body of each fish. A fine brush was then used to clean the scales with de-ionized water and gently rubbed between fingers to remove mucous. Ascending ethanol series (30-70%) was utilized to dehydrate scales followed by drying on filter paper. Use of 100% ethanol was discouraged to avoid wilting of scale samples. Double adhesive carbon tape was utilised to mount dehydrated scales on aluminum (Al) stubs. The dorsal surface of mounted scales was kept upwards, sputter coated (to make the scales conductive) using sputter coater and ultrastructural alterations were studied at different magnifications using Scanning Electron Microscopy (SEM, Hitachi S-3400N) facility in Electron Microscope and Nanoscience (EMN) Laboratory, Punjab Agricultural University (PAU), Punjab (India), at accelerating voltage of 15 kV at low probe current (Dwivedi and Sehgal, 2017). The fish scales were compared for ultrastructural alterations such as damaged tubercles, circuli, lepidonts, focus and surface of scale. The atomic percentage (%) of various elements was further determined using Energy Dispersive X-ray microanalysis (EDX, Thermo Noran System SIX, United States of America). The qualitative analysis of heavy metals adsorbed on the surface of scales was performed by placing the scanner attached to SEM on the area of interest followed by extracting peak intensities. To avoid repetition, scales of fish gathered from fish farm and markets during two seasons were abbreviated as follows: Control fish d”40 cm during winter (CSW), control d”40 cm during summer (CSS), control fish >40 cm during winter (CLW), control fish >40 cm during summer (CLS), fish ≤40 cm collected from market during winter (MSW), fish ≤40 cm collected from market during summer (MSS), fish >40 cm collected from market during winter (MLW) and fish >40 cm collected from market during summer (MLS).

**Results and Discussion**

In the present study, scanning electron micrographs of lateral region of scales of CSW and CLW displayed normal arrangement of circuli, lepidonts and smooth surface at 1.00k SE at 15 kV voltage (low probe current) (Fig. 1a, b). Similarly, scanning electron micrographs of CSS and CLS showed normal arrangement of circuli, lepidonts and smooth surface at 1.00k SE (Fig. 1c, d). In contrast, scanning electron micrographs of scales of MSW, MLW, MSS and MLS displayed damaged lepidonts, broken lepidonts, distorted pattern of circuli, eroded surface, impaired
lepidonts, sloughed off circuli and distorted circuli pattern, eroded circuli, broken surface and broken lepidonts at 1.00k SE (Fig. 1e, f, g, h). Lepidonts are teeth like structures which help scales to anchor to the fish body and prevent loosening of scales from the skin surface. During winter season, scales of CSW and CLW depicted normal focus in focal area (Fig. 2a, b), normal tubercles (Fig. 3a, b) and smooth surface margin at 100 SE (Fig. 4a, b). Normal focus (Fig. 2c, d), normal tubercles (Fig. 3c, d) and smooth surface margin (Fig. 4c, d) were also observed in scales of CSS and CLS. In contrast, scales of MSW and MLW showed damaged focus (Fig. 2e, f), damaged tubercles (Fig. 3e, f) and torn edges (Fig. 4e, f). Similar patterns were observed in focal region (Fig. 2g, h), posterior region (Fig. 3g, h) and margin of scales (Fig. 4g, h) of MSS and MLS. Ultrastructural alterations in scales gathered from markets might be attributed to larger amount of pollutants in the water source from which fish were collected. The higher severity of damage in summer might be due to elevation in accumulation of heavy metals and metabolic rate of fish with increase in temperature leading to more chances of metal uptake and binding and our observation in this line of Negi and Maurya (2015).

The present study was designed on the basis of the work of Braich and Jangu (2016). As per the hypothesis of authors, the elemental constitution of fish scales depends upon the water quality. In the current investigation, the atomic percentage of the dorsal surface of scales removed from fish gathered from markets and fish farm of both sizes during two seasons (Winter and summer) is presented in Table 1. The elemental composition of scales of CSW was Magnesium (Mg) (0.73%), Oxygen (O) (61.59%), Phosphorous (P) (14.81%) and Calcium (Ca) (22.87%). Atomic percentage of CLW was observed as follows: Ca (19.81%), P (13.89%), O (65.41%) and Mg (0.89%). In contrast, elemental composition of scales of MSW was C (56.66%), Mg (0.28%), O (27.60%), Al (0.06%), Silicon (Si) (0.05), P (5.59%), Nickel (Ni) (0.01%), Ca (9.53%), Cu (0.01%) and Lead (Pb) (0.21%). Similarly, scales of MLW showed atomic percentage of C (38.55%), O (38.73%), Mg (0.48%), P (8.46%), Ca (13.09%), chromium (Cr) (0.06%), Mercury (Hg) (0.15%) and Pb (0.48%). However, O (65.01%), P (14.57%), Mg (0.61%) and Ca (19.81%) were detected on the scales of CSS. Similarly, elemental composition of CLS was as follows: O (62.49%), Ca (22.58%), P (13.96%) and
Mg (0.97%). In contrary, scales gathered from MSS indicated the presence of C (58.83%), O (24.58%), Cu (0.09%), Mg (0.33%), Al (0.08%), P (5.63%), Si (0.16%), Ca (10.05%), Ni (0.08%), Zn (0.01%) and Hg (0.16%). Likewise, C (34.26%), P (8.69%), O (42.89%), Mg (0.72%), Al (0.10%), Ca (13.19%), Ni (0.03%), Cr (0.02%) and Zn (0.10%) were observed on the surface of MLS. The atomic percentage of fish scales gathered from market during two seasons clearly indicated the adsorption of heavy metals on their surface, which might be due to heavy metals in the source from where fish were collected. In addition, the atomic percentage of Ca declined in the fish scales gathered from markets compared to control groups. Earlier, accumulation of heavy
Table 1. Atomic percentage (%) of dorsal surface of fish scales of smaller and larger fish collected from market and fish farm during winter and summer seasons

<table>
<thead>
<tr>
<th>Seasons</th>
<th>Fish type</th>
<th>Atomic percentage (%) on the dorsal surface of fish scales</th>
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<tr>
<td></td>
<td></td>
<td>C</td>
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<tr>
<td>Winter</td>
<td>CSW</td>
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<td></td>
<td>MSW</td>
<td>56.66</td>
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<td></td>
<td>MLW</td>
<td>38.55</td>
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<tr>
<td>Summer</td>
<td>CSS</td>
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<tr>
<td></td>
<td>CLS</td>
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<tr>
<td></td>
<td>MSS</td>
<td>58.83</td>
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<td></td>
<td>MLS</td>
<td>34.26</td>
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metals during two seasons in scales of fish gathered from market were also analysed by inductively coupled argon plasma atomic emission spectrophotometer (ICAP-AES) (Vaid and Hundal, 2019). The results showed duration- and size-dependent heavy metal accumulation in scales. Kaur et al. (2018) investigated the accumulation and histological effects of heavy metals on different organs viz. kidney, muscle and liver of *L. rohita* gathered from local market in Ludhiana (India). The results indicated that the elevation in the levels of heavy metals including Cadmium (Cd), Arsenic (As), Zn, Cu, Ni, Cr and Pb in comparison to control. In addition, the values of heavy metals were more than the permissible levels set by WHO/FAO. Furthermore, histological anomalies in studied organs were also recorded. The study concluded accumulation of heavy metals in the fish collected from markets of Ludhiana.

Fish scales are hard calciferous structures having type I collagen fibrils and minerals primarily calcium-deficient hydroxyapatite (HAp) (Gil-duran et al., 2016). HAp can be utilized as adsorbent and have an excellent ion exchange capability leading to substitution of Ca with various metal ions (Vila et al., 2012). The substitution of Ca with heavy metals present in the water body might be the reason for decrease
in the atomic percentage of Ca in market vis-a-vis control fish. The current investigation is corroboration with the findings of Pala (2018). The author observed anomalies such as broken lepidonts, displacement of lepidonts and damaged circuli in *Cyprinus carpio* gathered from government sponsored fish farms located in Meghalaya, India, which had various pollution sources. Based on alterations observed, the author suggested that structural aberrations might be due to greater susceptibility of fish scales to external stressors and their ability to respond to lower levels of contaminants making scales sensitive indicators of water quality. Adsorption of Pb, Fe, Hg, arsenic (As) and Cu on the surface of scales of *Channa punctatus* gathered from river Yamuna, Delhi was testified by Dwivedi and Sehgal (2017) using EDX. The scientists observed higher concentration of Pb and Hg during summer which might be due to stagnant water conditions.

Similarly, effect of Fe and Al on the surface of scales of caged fish, *Oreochromis mossabicus* was studied by Hidayati et al. (2014) using SEM. The results showed decline in the number of spherules and ridges whereas the number of pits increased. Khanna et al. (2007) also investigated ultrastructural alterations in scales of *Puntius sarana* and *L. rohita* gathered from Ganga using SEM. The results showed higher level of heavy metals such as Fe, Ca and Mg during the monsoon season which might be responsible for lepidontal damage and loss of single and complete row of lepidonts. In addition, seasonal variability in heavy metal accumulation was also conducted by the authors. The results showed higher level of heavy metals such as Fe, Ca and Mg during the monsoon season which might be responsible for lepidontal damage. Similarly, Coban et al. (2013) also investigated the effect of different concentrations of Cr (7.5, 15, 30 and 60 µg L⁻¹) on scales of *Cyprinus carpio* procured from State Hydraulic Works (Turkey) for the period of 21 days. Alterations were observed in the order: Focal region > anterior region > posterior region. The results revealed that alterations in focus and circuli depend on Cr concentration in water. Tandon and Johal (1993) also indicated that Al is responsible for torn edges in the scales of *Tor putitora.*

Kaur and Dua (2015) studied the impact of sublethal concentrations of municipal wastewater (17.7, 26.6 and 35.4%) over a range of time periods- 15, 30 and 60 days on fish scale structure of commercially important fish *L. rohita* procured from Rajasansi, Amritsar. Using SEM, the authors observed time- and concentration-dependent alterations on the scale surface such as lepidontal uprooting, damaged tubercles, radii, circuli and focus. Deposition of heavy metals (Zn, Ni, Cr, Al, Fe, Cd, Cu and Pb) on the scales of fishes (*Catla catla, L. calbasu, C. carpio, L. rohita* and *Cirrhinus mrigala*) gathered from Harike wetland (India) was studied by Braich and Jangu (2016) using EDX. In addition, structural alterations viz. uprooting of lepidonts, damaged circuli and empty pockets were observed on the scale's surface. The results concluded that atomic percentage of heavy metals acts as indicator of heavy metal accumulation and pollution in Harike wetland. Similarly, the elemental composition of scales of *L. calbasu* gathered from Harike wetland depicted the presence of P (13.43%), Al (0.10%), Zn (0.99%), Mg (0.76%), C (17.18%), Cr (0.32%), Ni (0.04%), Ca (18.4%), Cu (0.14%), O (48.13%), Pb (0.13%) and Si (0.38%) on the scale surface (Jangu and Brраich, 2014).

**Conclusions**

The study indicated destructive impact of heavy metal pollution on the fish scales especially lepidonts including lepidontal damage and broken lepidonts. In addition, damage in circuli, focus, tubercles and surface margin was also observed. Higher severity of damage in microstructure of scales was observed during summer season than winter season. The intensity of ultrastructural alterations was observed higher in scales of MLS and MLW, which might be due to longer duration of exposure to heavy metal pollution. Different heavy metals including Ni, Cu, Zn, Pb, Al, Cr and Hg were found on the scales gathered from local fish markets when compared to control groups. In addition, Si was also detected on the scales gathered from markets. Higher atomic percentage of metals in scales gathered from market due to higher heavy metal levels in the water source from which fish were collected. Based on above observations, it is suggested that lepidontal alterations and other anomalies on the scales are useful indicators of heavy metal pollution.
However, the scales were gathered from fish markets for the present preliminary study. Therefore, more in situ studies are required to confirm that the deformities are due to heavy metal pollution to eliminate the influence of external parameters. For this, further work can be done to evaluate the effects of individual heavy metals on the surface of scales and at organ level in controlled conditions to know target region of each metal.

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References


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