Heavy Metals Accumulation and Structural Alterations in Scales of Fish, *Labeo calbasu* (Hamilton-Buchanan) from Harike Wetland (Ramsar Site), India

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Abstract-The toxic and deleterious effects of heavy metals viz., lead (Pb), nickel (Ni), zinc (Zn), copper (Cu) and chromium (Cr) to aquatic organisms and ultimately to human beings are well known. The presence of these heavy metals in water creates a societal health risk in rivers that are otherwise useful for drinking water and fisheries. Fish play an important role in human nutrition and therefore need to be carefully and routinely screened. Scanning Electron Microscopy and Energy Dispersive X-ray microanalysis technique have been employed to determine the percentage deposition of Aluminum (0.1%), Chromium (0.32%), Copper (0.14%), Magnesium (0.76%), Nickel (0.04%), Lead (0.13%), Silicon (0.38%), Phosphorus (13.43%), Oxygen (48.13%), Carbon (17.18%), Calcium (18.4%) and Zinc (0.99%) in samples of fish *Labeo calbasu* from Harike wetland. It is evidenced from the results that bioaccumulation of heavy metals in the fish scales, therefore posing potential risk for inhabitants having dependency on the wetlands.

Index Terms-Harike wetland, *Labeo calbasu*, Scale, Lepidonts.

1 INTRODUCTION

Wetlands are very important ecosystems because they support valuable flora and fauna. Harike wetland possesses almost 70 fish species. Out of which 2 comes under Critically Endangered, 6 under Endangered, 13 under Vulnerable, 23 under LRnt and 5 under LRlc status of IUCN [1]. Harike wetland receives pollution from various quarters like industries, agricultural pollution and sewage from adjoining cities and villages. Pollution is causing loss of food, disturbs habitat and alters physic-chemical parameters, if proper conservation and management steps will not be initiated, the fish comes under LRnt and LRlc may slip into threatened categories. *Labeo calbasu* (Hamilton-Buchanan) has been selected for the present course of work because this fish is commercially important and generates revenue for the state and employment for the local youths.

Rapid industrialization and economic development in India has resulted in increased water pollution in the aquatic ecosystems. This issue has been become the focus of numerous studies these days. Pollutants deposited into water causes serious changes which in turn directly or indirectly affect the ecological balance of the environment, creating extensive damage and even mass mortality to the life and activities of aquatic organisms because of their high toxicity and accumulative behavior [2]. Heavy metal contamination of the aquatic environment continues to attract the attention of environmental researchers because of its increasing input to coastal waters, especially in developing countries. In fact, in recent decades, industrial and urban activities have contributed to the increase of heavy metal contamination in the water bodies and have directly influenced aquatic ecosystem [3,4].

Heavy metals can enter the food web through direct consumption of water or organisms or through uptake processes and these are potentially accumulated in different hard and soft tissues in edible fish [5]. These heavy metals may reach a toxic concentration level that can potentially destroy the ecological environment [6-8]. The rate at which this effect is pronounced greatly depends on the influx of industrialization level and the use of mechanized agricultural activities as well as on uncontrolled urbanization along the aquatic ecosystems [9]. Subsequently, these anthropogenic activities have increased the release of harmful heavy metals into the aquatic environment [10,11].

The deposition of these toxic metals in wetland areas has increased the uptake rate of these metals by aquatic organisms, such as fishes, consequently affecting humans through the food web [12,13]. The intake rate of these heavy metals by humans through the consumption of fish causes serious health hazards [14,15]. The increased accumulation of heavy metal levels in the aquatic environment is disastrous to aquatic organisms and humans alike [16,17] and has been progressing in a number of countries, including India [18]. As a result of the hazards associated with the consumption of heavy metals, their concentration in commercial fishes in India should be periodically examined to evaluate the possible risks associated with the consumption of contaminated fish.

In the present study, the Harike wetland was selected because of its economic and strategic factors. Economically, this wetland is a potential breeding ground for fishes; strategically, it receives pollution from many sources around this area, such as wastage of agriculture, urbanization, tannery, dying, paint, pesticides and insecticides industries.

Fish play an important role in human nutrition and therefore need to be carefully and routinely screened to ensure that there are no high levels of heavy metals being transferred to man through consumption. With the assumption that fish and water in Harike wetland poses a heavy risk of exposure of heavy metals and that the inhabitants relying on the wetland are threatened. During present studies, reporting of Pb, Ni, Zn, Cu and Cr in *Labeo calbasu* have been done. The results are geared towards providing baseline data on the current pollution status of this wetland.

2 MATERIALS AND METHODS

2.1 Study Area

The present study was carried out in internationally important manmade Harike wetland. This wetland ecosystem rich in acquire flora and fauna with a rich genetic pool spread into three districts Ferozpur, Tarn Taran and Kapurthala in Punjab India, was created in 1953 by the construction of a barrage at the confluence of the Sutlej and Beas rivers. Harike wetland was declared a wildlife sanctuary in 1982. Considered a wetland of international importance and it was included in the list of Ramsar sites in 1990. It is located between latitudes 31º13'N and longitudes 75º12'E and covers an area of 41 sq. km (Fig.1).

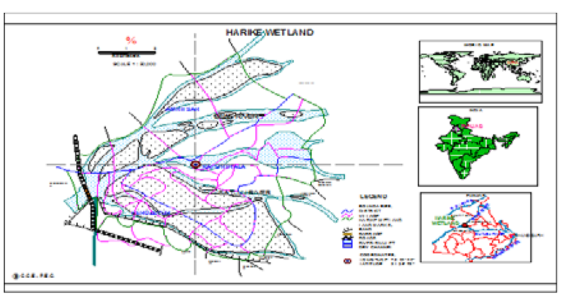

Fig. 1. Map showing the location of Harike wetland.

2.2 Laboratory Procedure

The scales of fish were removed with the help of forceps from the second row above the lateral line and below the dorsal fin on the spot from the collection site. The scales were cleaned with distilled water and mucous left on the scale is completely removed by rubbing between finger tips. The cleaned and air dried scales were mounted on the carbon stubs with the help of double adhesive tape, keeping the dorsal surface of scale upwards and ventral surface sticking to the tape. Scales were sputter coated with a thin layer of gold to make the surface of scale conductive in gold coating unit. The scale specimens were studied under vacuum using JEOL JSM-6610LV scanning electron microscope at an accelerating voltage of 20kv.

The quantitative analysis of the various elements presents in the scale of the control as well as affected fish was observed by using the energy dispersive X-ray microanalysis (EDX) technique. The elemental composition was determined by placing the scanner of "INCAx-act analyzer" on the area of interest on the scale. This scanner was attached to JEOL JSM-6610LV scanning electron microscope. The X-ray spectrum from the specimen was then processed and analyzed to calculate the composition of different elements in the scale sample. The quantitative results were obtained by extracting the net peak intensities.

3 RESULTS

The normal scale of fish *Labeo calbasu* consists of circuli which bear pointed tooth like lepidonts in the anterior part of scale. These lepidonts are regularly placed in deep sockets on the circuli (Figs.2,3).

Various alterations were observed in scale structure of fish taken from Harike wetland by employing Scanning Electron Microscopic technique. The pollutants present in water have adverse effect on scale structure resulted in uprooting of lepidonts from their point of attachment to circuli in rows (Fig.4). Circuli were broken and disorganized (Fig.5). Tips of some lepidonts were also broken (Fig.6) and this might have caused the loosening of the scale from the body of the fish. Dislocation and uprooting of lepidonts from the circuli leaving hole-like depressions at some places in circuli (Figs.7,8). Thus on the basis of present studies, it is demonstrated that due the damaged calcareous structure, fish scale can be considered as pollution indicator in the waters of Harike wetland.

3.1 Elemental composition of the normal scale of *Labeo calbasu* or control fish

The elemental composition of the scales is directly related to the composition of water of aquatic body in which the fish inhabits. Keeping this fact in mind, the EDX of the normal scale of fish has indicated that there are four elements detected in the elemental composition of the scale (Fig.9) viz. Calcium (Ca-42.15%), Oxygen (O-33.93%), Phosphorus (P-22.98%) and Magnesium (Mg-0.94%). Amongst these elements, Ca and Mg comprise maximum and minimum percentage deposition in the scale respectively.

3.2 Elemental composition of affected scale of *Labeo calbasu*

The elemental composition of the scale of fish from Harike wetland has been altered due to the effect of pollutants. Various heavy metals were recorded in the composition of scale viz. Carbon (C-17.18%), Calcium (Ca-18.4%), Phosphorus (P-13.43%), Oxygen (O-48.13%), Zinc (Zn-0.99%), Aluminum (Al-0.1%), Magnesium (Mg-0.76%), Chromium (Cr-0.32%), Silicon (Si-0.38%), Nickel (Ni-0.04%), Copper (Cu-0.14%) and Lead (Pb-0.13%). The percentage composition of all the elements in the anterior part of the scale has been given in (Fig.10). Major elements in the composition of scale are Ca, P, and O whereas Si, Cr, S, Al, Ni, Cu, Mg and Pb make a small percentage.

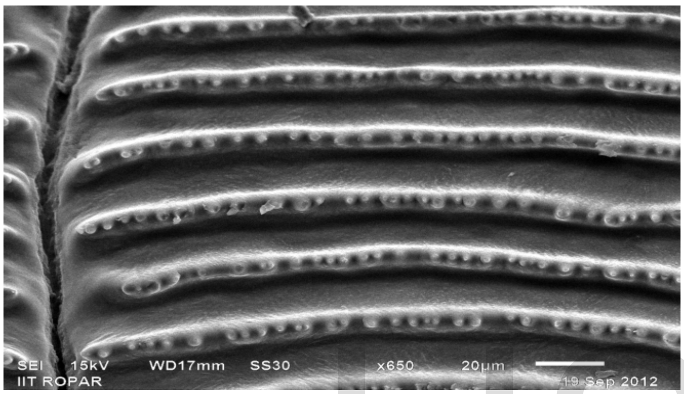

Fig. 2. Scanning electron micrograph of scale of *Labeo calbasu* taken as control showing regularly placed lepidonts on circuli.

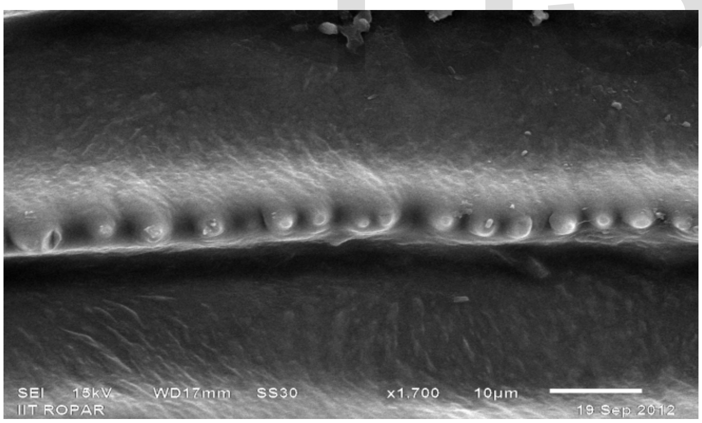

Fig. 3. Closely placed lepidonts on the anterior circuli in normal scale.

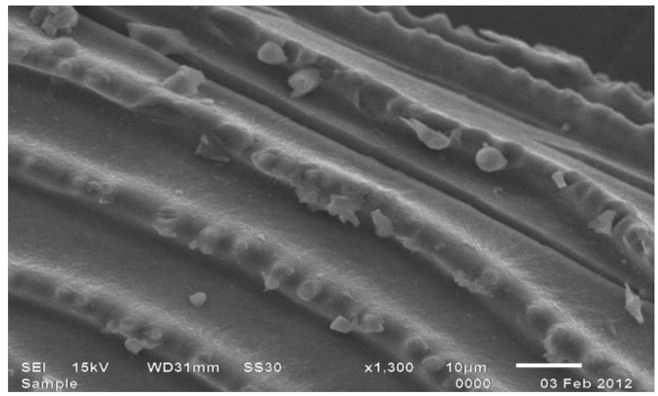

Fig. 4. Scanning electron micrograph of scale of *Labeo calbasu* showing uprooted lepidonts from circuli.

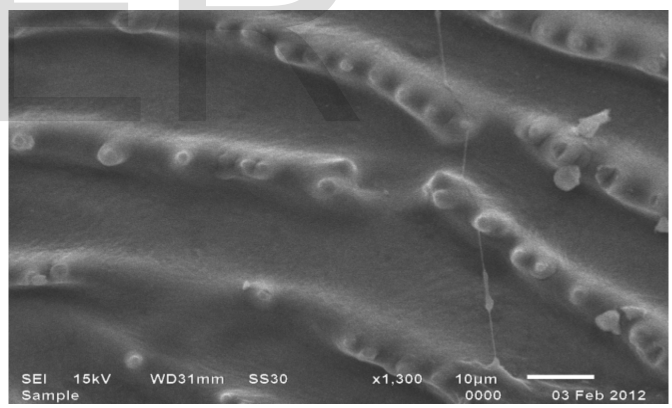

Fig. 5. Breakage and disorganized circuli in the anterior portion of scale of *Labeo calbasu*.

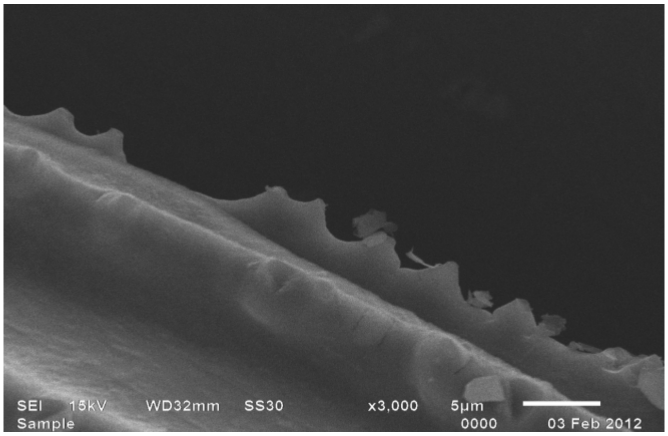

Fig. 6. Broken lepidonts in the anterior part of scale and with broken tip position.

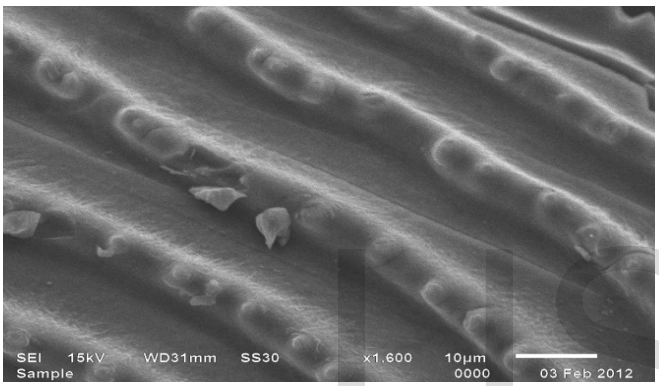

Fig. 7. Broken lepidonts and depressions made by displacements of lepidonts.

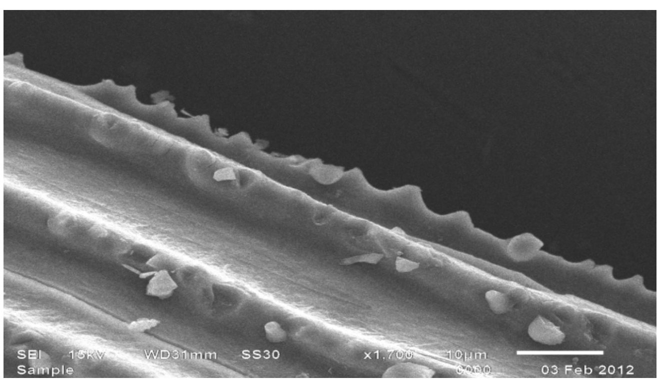

Fig. 8. Individual displacement of lepidonts from circuli and hole-like depression formed by uprooted lepidonts in scale of *Labeo calbasu.*

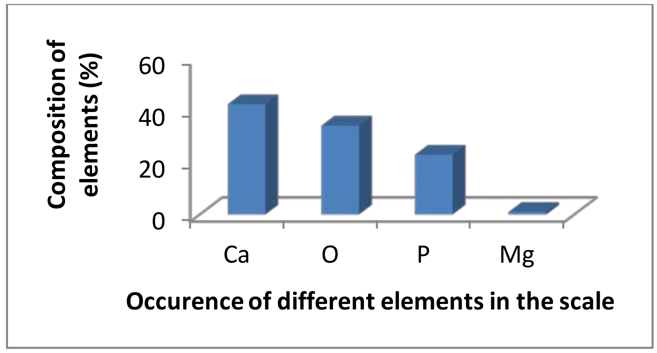

Fig. 9. Elemental composition in the normal scale of *Labeo calbasu.*

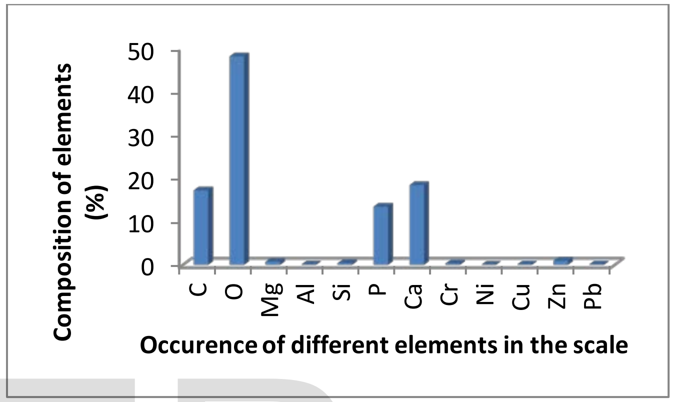

Fig. 10. Elemental composition in the affected scale of *Labeo calbasu.*

4 DISCUSSION

Fishes posses an excellent ability to concentrate heavy metals in their tissues. The concentration of any pollutant in any given tissue depends on its rate of absorption and the dynamic processes associated with its elimination by the fish.

The level of different metals in the present study was the capacity to accumulate these metals in the scales of fish *Labeo calbasu*. Preferential accumulation of metals in the liver, kidney, muscles, scales and gills has also been reported [19-28]. Different organs in the body are known to accumulate a particular metal to a high level while others do not accumulate the metal though present in the medium [29,30].

It is well understood that metal ions taken up by a fish through any route are not totally accumulated because fish can regulate metal concentrations to a certain extent, after which accumulation occurs. Therefore, the ability of each tissue to regulate or accumulate metal ions can be directly related to the total amount of metal uptake in that specific tissue.

Results of the present study emphasized that metals has a high potential to be accumulated in the scales of fishes. In conclusion, bio-monitoring of trace metal pollution in a Harike wetland is necessary. Since, the toxic effects of metals have been recognized. Periodic monitoring of heavy metals in fishes to ensure continuous safety of people in the area is recommended.

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