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LEPIDOLOGICAL AND TOXICOLOGICAL STUDIES ON THE SCALE OF A COMMERCIALLY IMPORTANT FISH, *CIRRHINUS MRIGALA* (HAMILTON-BUCHANAN) UPON EXPOSURE TO POLLUTANTS IN HARIKE WETLAND

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ABSTRACT
Contamination of Harike wetland (Ramsar site) has become a current serious problem because of increased industrialization as well as sewage pollution through river Sutlej. Investigations on the accumulation of heavy metals toxicants in commercially important fish *Cirrhinus mrigala* in Harike wetland water were selected and analyzed for the heavy metal concentrations in the scales. Energy dispersive X-ray microanalysis (EDX) of the scales has shown that there are nine elements viz., calcium, phosphorus, oxygen, carbon, silicon, sulphur, chromium, nickel and lead present in the scale of fish from Harike wetland as compared to the control fish where only four main elements viz., oxygen, calcium, phosphorus and magnesium were analyzed, which means all these metal pollutants presents in the water of wetland in which the fish abode. The structural damage includes uprooting of lepidonts, cracks and disruption of circuli. Structural damage and alteration in elemental composition in the scales of fish indicated the presence of pollution in the wetland water.

KEY WORDS: *Cirrhinus mrigala*, EDX,Harike wetland, Lepidonts, Pollution, Scales.

INTRODUCTION

Wetland ecosystems in India are under severe threat of pollution from various quarters. The deteriorating water quality affects man, animals and plant life with far-reaching consequences. Due to tremendous urbanization and industrialization, the problem of water pollution has assumed an alarming situation and about 70% rivers in India are polluted. In the last few decades, deterioration of water quality of Indian rivers and wetlands has been increased. Now, it has been realized to lay greater emphasis on their study. Pollution comprises of liquid waste discharge from domestic residences, commercial properties, industries and agricultural runoff. Suspended solids, organic and inorganic matter, pathogens, nutrients, trace constituents, total dissolve solids are commonly found in untreated waste water (Singh and Nautiyal, 1990; Trivedy *et al.*, 1990; Kishore *et al.*, 1998; Asano *et al.*, 2007).

It has been estimated that 20% of the known range of biodiversity in India are supported by freshwater wetlands (Deepa and Ramachandra, 1999). The direct benefits of wetlands are the components such as fish, timber, recreation and water supply, and the indirect benefits arise from the functions occurring within the ecosystems such as flood control, ground water recharge, nutrient retention, water purification and storm protection (Ramachandra, 2001). Wetland provides critical habitat for a large number of flora and fauna and act as important repositories of aquatic biodiversity (Prasad *et al.*, 2002). Wetlands also provide vital ecosystem services for human populations worldwide by means of freshwater, food and biodiversity and most importantly regulate the climate change (Millennium Ecosystem Assessment, 2005).

Yet these ecosystems are particularly vulnerable to threats from human impact through increasing economic development and population growth. The Harike wetland situated at the confluence of two major rivers Sutlej and Beas. It is internationally known as an important wetland for migratory birds and was registered as a Ramsar site in 1990. The water quality of the wetland is deteriorating due to the inflow of domestic, agricultural and industrial wastewater from neighboring cities. Toxicants belong to undesirable substances in water environment. They are known for their ability of persistent accumulation in animal tissues (Pundir and Saxena, 1992; Mukherjee *et al.*, 1994). River Sutlej receives polluted water from different industries from Ludhiana town. The physic-chemical parameters where there is no polluted water have optimum level and drastically changed near the entry point of Budha Nallah which carries load of industrial effluents and ultimately reaches at Harike wetland (Jindal and Sharma, 2011). Water pollution affects the ecological conditions which usually affects the biological and physiological conditions of the fish (Santiago *et al.*, 1985). Heavy metal pollutants have tendency to accumulate in various organs of aquatic organisms, especially fish, which in turn may enter into human metabolism through consumption causing serious health hazards (Puel *et al.*, 1987). The effect of waste water exposure on scales and chromatophores on freshwater fish *Channa punctatus* was studied from international water channel Tung Dhab drain and observed significant alterations in scales as uprooting and damage in lepidonts and dispersal of chromatophors (Kaur and Dua, 2012). The metal industry contributes almost

50% of the total wastewater discharged. Other industrial plants constructed at Ludhiana city on the banks of the river Sutlej directly pour their effluents into the river without any treatment which ultimately reaches at Harike wetland. However, due to rapid industrialization and urbanization of the adjacent cities and towns, a negative impact is often exerted on this highly productive ecosystem. One freshwater fish which is presently being studied intensively as its response to heavy metal contamination is *Cirrhinus mrigala* (Hamilton-Buchanan) which is very significant from ecologically as well as economically point of view.

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 31°13'N and longitudes 75°12'E and covers an area of 41 sq. km (Fig.1). The ecological situation of the wetland is so favourable that help to attract good number and variety of migratory as well as domiciled birds. Harike wetland is facing many problems which includes silting and shrinking of water body, water hyacinth infestation, encroachment, illegal fishing, deforestation and water pollution (Jain *et al.*, 2008).

MATERIALS AND METHODS

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. 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.

RESULTS

The normal scale of control fish consists of circuli in the anterior portion which bear pointed and teeth like structures the lepidonts (Fig.2). The lepidonts are properly arranged on circuli which helps the scale to attach with the body of the fish (Fig.3). While various alterations were observed in the scale structure of fish taken from Harike wetland. Upon exposure to different pollutants, loosening of the scale from the body of the fish was observed. The lepidonts developed breaks near the point of attachment to the circuli (Fig.4) and uprooting of the lepidonts were observed (Fig.5). Whereas at some places, whole row of lepidonts were sloughed off from the circuli (Fig.6). Disruptions and cracks in the circuli were also observed (Fig.7).

Elemental composition of the normal scale of *Cirrhinus mrigala* 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.8) viz. Calcium (Ca-40.15%), oxygen (O-35.93%), Phosphorus (P-22.98%) and Magnesium (Mg-0.94%). Amongst these elements, Ca comprises the maximum percentage while Mg forms the least of the elements recorded in the scale.

Elemental composition of affected scale of *Cirrhinus mrigala*

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 (Fig.9) viz. Oxygen (O-53.67%), Carbon (C-18.16%), Phosphorus (P-8.69%), Calcium (Ca-12.88%), Sulphur (S-0.05%), Chromium (Cr-1.67%), Lead (Pb-3.16%), Silicon (Si-0.07%) and Nickel (Ni-1.65%). The occurrence of all these elements in the scale composition clearly announces the presence of pollution in the wetland water which degraded the quality of water. As already pointed out that the nature

of elements and their percentage composition has direct relation to the chemicals present in water in which the fish abode. The increasing pollution in Harike wetland affects the biodiversity of wetland. The bioaccumulation of heavy metals in the body of fish is a serious concern. It can affect the life of migratory birds as well as human being which are feeding on them.

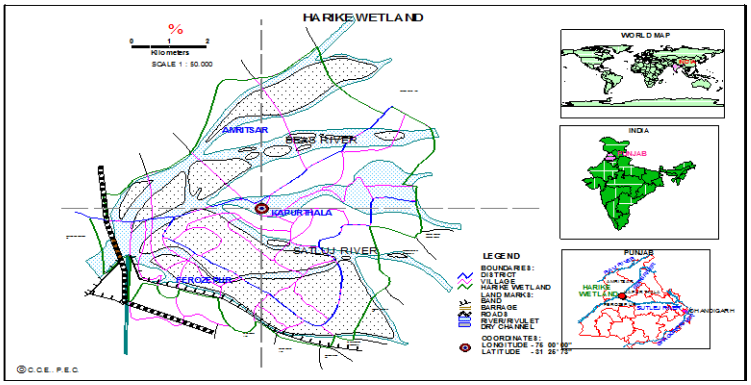

Figure 1. Map showing the location of Harike wetland

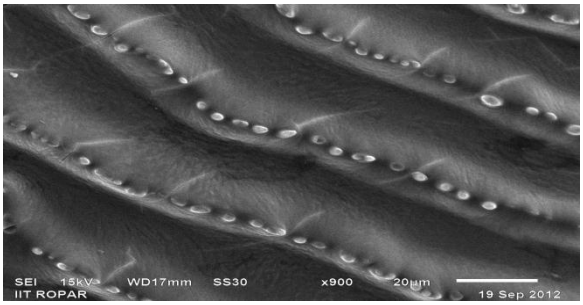

Figure 2.Scanning electron micrograph of scale of *Cirrhinus mrigala* taken as control showing regularly placed lepidonts on circuli.

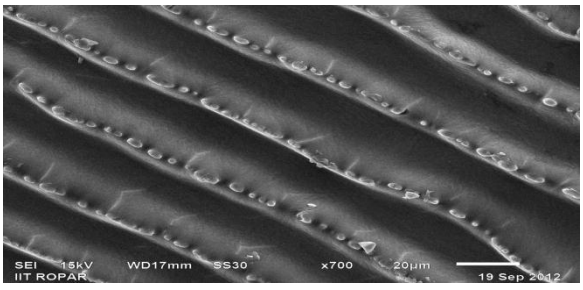

Figure 3. Scanning electron micrograph of scale of control fish showing arrangment of closely placed lepidonts on circuli.

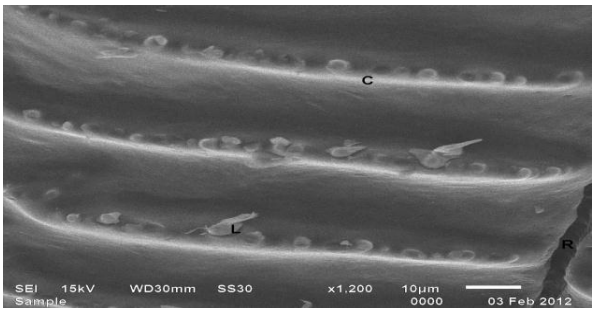

Figure 4. Scanning electron micrograph of scale of *Cirrhinus mrigala* showing displacement of lepidonts (L) from their point of attachment.

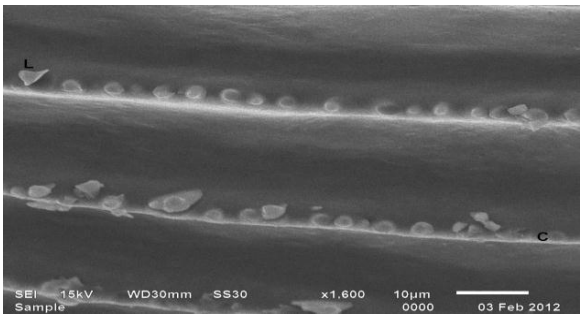

Figure 5. Individual displacement of lepidonts (L) and whole row slougged off in the scale of *Cirrhinus mrigala.*

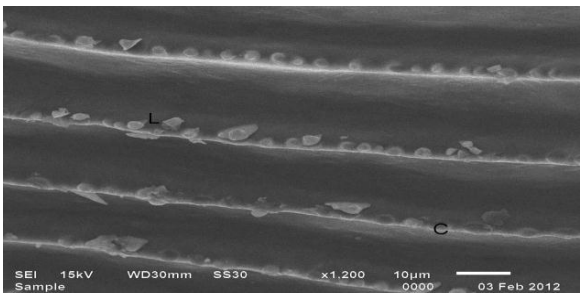

Figure 6. Scanning electron micrograph of scale of *Cirrhinus mrigala* showing uprooted lepidonts (L) from the basal region of circuli (C).

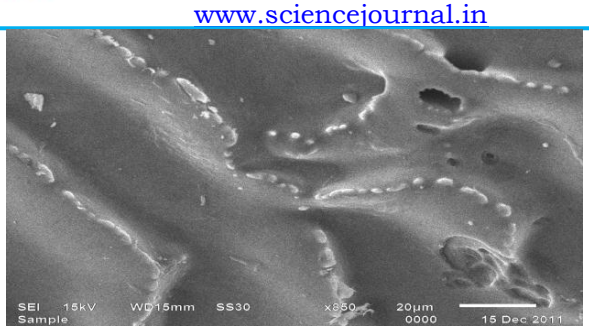

Figure7. Disruption of circuli in the anterior portion of scale of *Cirrhinus mrigala.*

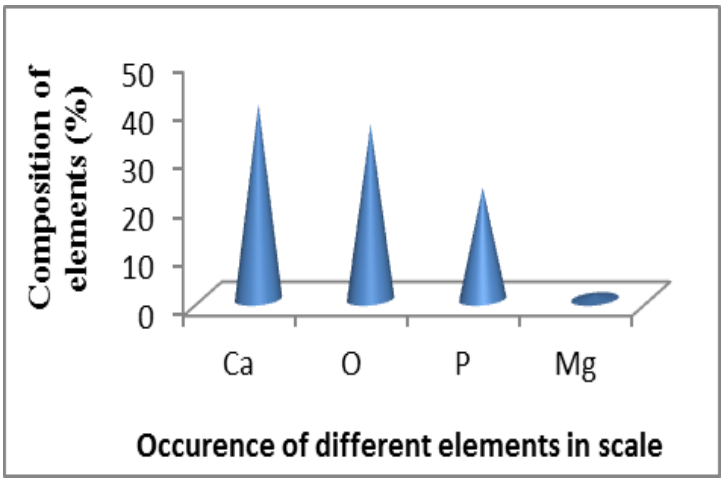

Figure 8. Elemental composition in the scale of control *Cirrhinus mrigala* fish.

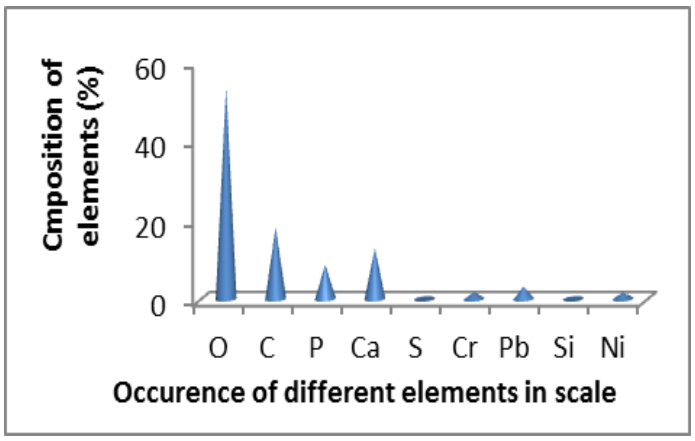

Figure 9.Elemental composition in the affected scale of *Cirrhinus mrigala.*

DISCUSSION

The alterations in structural and elemental composition of the scales due to the effect of pollutants has been supported by many workers who reported the presence of various elements like Ca, Al, Si, Mg, F, Li, Cu, Ba, and Na in the scales of bony fishes (Lanzing and Higginbotham, 1974; Tandon and Johal, 1993; Johal and Dua, 1994; Javed and Hayat, 1999; Rauf *et al.*, 2009). The variation in elemental composition of the scales and presence of various elements in the scales of fishes clearly

indicate that scales are excellent pollution indicator and presence of pollution in the water of Harike Wetland. During the present investigations the commercial fish *Cirrhinus mrigala* shows high concentration of various pollutants which are ultimately consumed by human being and it is a matter of great concern from health point of view.

The research conducted on the water of river Sutlej in Himachal Pradesh and observed that various toxicants like S, Cl, Ti, V, Cr, Fe, Mn. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraj, 2005; Farombi *et al.*, 2007; Singh and Saharan, 2010). Contamination of aquatic ecosystems with heavy metals has seriously increased worldwide attention (Wagner and Boman, 2003; Yilmaz *et al.*, 2007). Scales of fishes Labeo rohita and *Puntius sarana* were used to determine deteriorating water quality of river Ganga (Khanna et al., 2007). Alterations in the scale structure of fish *Cyprinus carpio* upon exposure to pollutants was conducted (Brraich and Jangu, 2012). In the present study, it is observed that wetland water is contaminated with different kinds of heavy metals, which were obviously high and appeared to be harmful to fishes. The alterations in architectural pattern of the scales, uprooted and damaged lepidonts and the altered elemental composition strongly suggest that fish scales can be successfully employed as indicators of pollution in Harike wetland.

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