Scale of murrel: A non-invasive approach to monitor heavy metals in freshwater

Satyam Dwivedi and Neeta Sehgal*

Department of Zoology, University of Delhi, New Delhi-7, India

*Corresponding author: neetasehgal.du@gmail.com

ABSTRACT

Indian freshwater murrel *Channa punctata* were maintained in a floating cage in river Yamuna at Canal Colony, Kalindi Kunj, Delhi during the summer, monsoon and winter seasons in a year. Incidence of heavy metals (Pb, Hg, Fe, Cu, As) on fish scales were investigated by employing Scanning Electron Microscopy (SEM) and Energy Disruptive X-Ray (EDX) analyser. Fish scales revealed altered elemental composition and uprooted lepidonts. The present study indicated that structural integrity of the scale is compromised under stress of heavy metals and pollutants.

**Introduction**

The river Yamuna has been treated as a convenient dumping site for all types of waste generated in Delhi NCR which is discharged into the river by 22 drains, making it the most heavily polluted and degraded river stretches in the country (Babu et al. 2013; Paul et al. 2014). The course of River Yamuna in Delhi has two segments viz., the upper segment (Pre-Wazirabad barrage section) and Delhi segment (Wazirabad-Okhla barrage section) (Fig. 1). The upper segment receives its water from Hathnikund barrage. The flow of water in the river Yamuna’s Delhi segment is mainly dependent on the quality of Monsoon and operations of Wazirabad and Okhla barrages. During the non-monsoon period, river virtually stagnates specifically at Canal Colony, Kalindi Kunj because very little amount of water is allowed to flow downstream of Wazirabad barrage (Misra 2010; Babu et al. 2013).

The river carries with itself a variety of pollutants including heavy metals discharged into the water from various industrial sources located upstream to the Okhla barrage (Dhillon 2013). In addition, domestic waste and sewage are regularly discharged into the river due to its location within a densely populated area. Heavy metals are non-biodegradable and get accumulated by the aquatic fauna including fish (Javed and Hayat 1999; Forombi et al. 2007). Scales of fish directly come in contact with water and accumulate heavy metals.
The present investigation was undertaken to study the change in the structure and elemental composition of scales of *Channa punctata*. Canal Colony, Kalindi Kunj was selected as an experimental station wherein murrel were caged in a floating type ‘hapa’ cage and exposed to riverine water conditions during different seasons in a year.

**Material and Methods**

Specimens of Indian freshwater murrel, *Channa punctata* (Hamilton, 1822), were procured from the backwaters of river Yamuna around Delhi (28°35’ N. 77°12’ E) during the first week of May (Summer season), September (Monsoon season) and December (Winter season), in the year 2014. Fish were acclimatized to laboratory conditions (25±1°C; L: D: 12: 12) at least 7 days prior to the start of experiment (Sehgal and Goswami, 1994). Thereafter, fish were divided in two groups, ‘A’ and ‘B’ in every season. Specimens of group ‘A’ were maintained in river Yamuna at Kalindi Kunj, Delhi, whereas specimens of group ‘B’ were maintained under laboratory conditions in aquarium containing dechlorinated water.

For *in situ* study, a floating type hapa cage was set up at the station (Fig 2). Acclimatized fish were transferred to hapa or aquaria and after 55 days, scales were collected from four specimens of group ‘A’ as well as of group ‘B’. Scales were collected from second row below the lateral line under the dorsal fin with the help of forceps.
Fig 2: Floating type cage (hapa) installed at Kalindi Kunj, Delhi during the course of experiment.

Scales were wiped, dried with a fine brush and stored at room temperature in labelled, airtight containers. Cleaned and dried scales were mounted on aluminium stubs with dorsal side facing upwards. Scales were coated with a thin gold coating (100 Å thickness) and scanned under Scanning Electron Microscope (JEOL-6610LV). Micrographs of the scales were obtained and recorded on the computer for further analysis.

The elemental composition of the scales was determined by using Energy Disruptive X-ray (EDX) analyser. EDX analyser, attached to the microscope, was focused on the area of interest and data was recorded on the computer. The experiment was repeated in every season.

**Results**

Photo electromicrographs of the scales revealed the general structure of the scale as depicted in Figure 3. Scales collected from fishes maintained in riverine condition (Group ‘A’) revealed alterations in the arrangement of lepidonts, as compared to those scales collected from group ‘B’ fishes kept in laboratory conditions (Fig. 4).

Density of the lepidonts was less, with clearly visible gaps among the rows in scales collected from group ‘A’ fishes. On the contrary scales collected from fish maintained in laboratory (Group ‘B’) showed well preserved lepidonts. Morphological changes in the scales in fishes kept under riverine conditions, were observed during all three seasons.

The elemental composition of scales collected from fish of both groups was also compared and data presented in Figure 5. EDX analysis confirmed the presence of metals viz., lead (Pb), copper (Cu), iron (Fe) and mercury (Hg) and absence of Arsenic (As) in all the scales of specimens irrespective of their maintenance under riverine/laboratory conditions. The atomic percentage of heavy metals was significantly high in fishes kept in riverine water (Group ‘A’). The highest values for atomic percentage of Lead (2.17%) and Mercury (1.52%) was recorded during summer in the scales of specimens of Group ‘A’ and the lowest value for both elements Pb and Hg (0.02% and 0.13% ) was recorded during monsoon in the scales of fishes maintained under laboratory conditions (Group ‘B’). The results presented in Figure 5 depicts that the atomic percentage of Pb, Hg and Fe was highest during summer season and lowest during monsoon in scales of fishes maintained either in riverine or in laboratory conditions (group ‘A’ & ‘B’).
Fig 3: Scanning Electro Photomicrographs of scale of *Channa punctatus* showing:

a) Anterior margin which consists of circuli and radii; Focus and Basal margin from where scale is attached to body.

b) Enlarged view of Anterior margin depicting Circuli which are shaped like discs and Radii which run between two Circuli separating them.

Fig 4: Scanning electron micrograph of scales of *Channa punctatus* in Group ‘A’ and Group ‘B’ during summer (s), monsoon (m), and winter (w) showing- I: Early damage to lepidonts in the scales group of fish kept in the river Yamuna during summer (As), monsoon (Am) and winter (Aw), as indicated by the arrows in the micrograph. II: Rows of lepidonts were well preserved in the scales of group of fish which were kept under laboratory conditions during summer (Bs), monsoon (Bm) and winter (Bw).
Discussion
The river Yamuna has been overexploited in Delhi-NCR (Misra 2010; Babu et al. 2013). Several drains containing industrial and domestic waste release the contaminated water in river every day. The situation worsens during non-monsoon seasons because limited amount of water is released from the source at Hathnikund barrage (situated 230 Km.), upstream at Yamuna Nagar, Haryana (CPCB 2006; CWC 2009). In addition, a significant amount of water is abstracted at the start of Delhi segment of river Yamuna at Wazirabad barrage for domestic supply of city that leaves less amount of water in the river. Lesser amount of water in river affects the flow of water, reduces the dilution capacity (Babu et al. 2003; Sharma and Kansal 2011; Soni 2013) and increases the concentration of heavy metals in the river Yamuna (CPCB 2006; Sehgal 2012). Regular rainfall during monsoon season and release of higher quantity of water by Hathnikund barrage adds freshwater to the stagnating river. Additional water increases flow and results in flushing of pollutants and dilution of heavy metals in the river.
**Fig 5:** EDX analysis of scales of *Channa punctatus* in **Group ‘A’** and **Group ‘B’** during summer (s), monsoon (m), and winter (w) showing: **I:** Atomic percentage of heavy metals in group of fish maintained in river Yamuna (**Group ‘A’**) is highest in summer (**As**), followed by winter (**Aw**) and is found to be lowest in monsoon (**Am**). **II:** Atomic percentage of heavy metals in group of fish maintained in laboratory conditions (**Group ‘B’**) is highest in summer (**Bs**), followed by lower levels in monsoon (**Bm**) and winter (**Bw**). The values of atomic percentage of heavy metals were found to be higher in scales obtained from **Group ‘A’** compared to **Group ‘B’** as depicted in the figure.

Presence of Pb and Hg is more common in industrial effluents and domestic sewage as compared to Fe and Cu. Perusal of literature suggests that levels of these pollutants are higher than their toxic concentration in the environment. These heavy metals are transferred to larger water bodies and pose a serious threat to aquatic flora and fauna (Karrari et al. 2012). Mercury (Hg) gets accumulated in the fish, directly from the water or via food chain (Stokes and Wren 1987; Hall et al. 1997; Ward et al. 2011). Fish have been reported to accumulate heavy metals in their scales and visceral tissues by absorption along the skin, gills and through the gut surface (McGeer et al. 2000; Francis et al. 2004; Brraich and Jangu 2016).
Fish are directly exposed to heavy metals present in water. Therefore, fish scales have been used to study the effect of heavy metals (Dua and Johal 1996; Jangu and Braich 2014; Sultana et al. 2016). This non-invasive approach causes minimum harm to the fish and is an excellent method for monitoring heavy metal pollution in water bodies. A typical scale of *Channa punctatus* consists of focus, circuli and radii. Circuli are located above the focus in the anterior part of the scale (Fig. 3). Circuli contain teeth like structures called lepidonts which are arranged in numerous rows on its outer edge (Fig. 3). The size, arrangement and position of lepidonts facilitate contact between a scale and body. These lepidonts help the scale in anchoring to the body, preventing its detachment and displacement from its position (Lanzing and Higginbotham 1974). Damage to the lepidonts as well as uprooting of lepidonts observed in the present study can be attributed to stress experienced by fish due to presence of various pollutants and heavy metals in the water. Similar observations have been reported by Johal and Dua (1994) and Braich and Jangu (2016) which support potential loosening of the hold of scales on body.

Atomic percentage of heavy metals in scales were consistently higher in scales of fish maintained in river as compared to those kept in dechlorinated tap water. Heavy metals are known to bioaccumulate in marine and fresh water fish (Van Oosten 1957; Tandon and Johal, 1993; Dua and Johal 1996; Jangu and Braich 2014). Higher atomic percentage of heavy metals in scales indicates presence of heavy metals in the river Yamuna, at Kalindi Kunj, Delhi. Similar set of fish maintained under laboratory condition, showed lower percentage of heavy metals. Since the stock of fish was collected from backwaters of the river Yamuna, the specimens were already exposed to various pollutants and heavy metals prior to commencement of experiment. However, due to absence of heavy metals in the tap water and possible leaching of heavy metals from fish, the levels were low in scales by the end of experiment. Specimens exposed to river water showed higher levels of mercury and lead in scales. Absorption of metals such as Lead via surface, has been reported by Tao et al (2000). It binds to the surface mucous layer of the fish, in the form of ions and gets accumulated since it has a high persistence and therefore, degrades slowly. Murrel, *Channa punctatus* obtains its nutrition by consuming the smaller fishes available in the surrounding water. Studies have shown higher levels of mercury in the tissues of piscivorous fish, compared to herbivorous, (EPA 1996; Ward et al. 2011; Pouilly et al. 2013) because the amount of mercury keeps augmenting at each trophic level after being metabolized.

Variations in the atomic percentage of elements deposited in scale of murrel during different seasons, suggest that the concentrations of heavy metals in the river Yamuna vary with season. Concentration of these metals is higher in scales during summer and winter as compared to monsoon season. This could be assigned to dumping of industrial pollutants and domestic waste on a daily basis in addition to stagnant water conditions in the river Yamuna during non-monsoon seasons.

**Conclusion**

All the examined fishes showed presence of heavy metals in their scales, however atomic percentage of heavy metals was found to be significantly higher in scales of fishes maintained in River Yamuna at Kalindi Kunj, Delhi than those maintained in dechlorinated tap water. Deposition of heavy metal showed a seasonal variation with values recorded at their highest in summer season and lowest in monsoon. Water pollution in river Yamuna at Delhi-NCR has been a cause of serious concern. Lack of adequate flow and indiscriminate dumping of industrial and domestic waste in river stand out as primary reasons for present state of the river. Strict preventive measures must be taken to revive the river which is ill suited for sustaining aquatic life in present state.
Conflict of interest
There are no conflict of interest of any kind regarding this manuscript.

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