Research Article

Zinc, Copper, Lead and Cadmium levels in edible finfishes from lower gangetic delta

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ABSTRACT

The aim of this study is to determine the heavy metals (Zn, Cu, Pb and Cd) in edible finfish species (*Polynemus paradiseus*, *Tenualosa ilisha*, *Liza parsia*, *Liza tade* and *Stolephorus commersonii*) and compare the level of heavy metals determined in the Gangetic delta region earlier. Finfish species were sampled from 4 different stations. Levels of four selected heavy metals were determined in the muscle of edible finfish species in the Gangetic delta region using a Perkin-Elmer Sciex ELAN 5000 ICP mass spectrometer and expressed as mg kg^{-1} dry weight. To determine whether heavy metal concentrations varied significantly between sites and species, analysis of variance (ANOVA) was performed. In addition to, heavy metal concentrations were compared with WHO and FAO's permited levels. In finfish species the concentrations of Zn, Cu, Pb and Cd ranged from 12.00±0.66 – 119.66 ±1.53, 10.89±0.19–73.22±0.83, 2.33±0.09–17.88±0.52 and BDL–3.12±0.10 respectively. For Zn and Cu, accumulated metal concentrations in Stn. 4 were significantly higher than accumulated metal concentration in Stn 1 and Stn 2. For Pb, significantly stational difference between stations was not found. Between all studies fish species, lowest metal acculation values was found for *S. commersonii* ($p<0.05$). The selected heavy metals in finfish muscle (except Zn in *Liza parsia* in station 1) were also within the permissible limits for human consumption as indicated by the Food and Agricultural Organization.

INTRODUCTION

The River Ganga emerges from a glacier at Gangotri, about 7010 m above mean sea level in the Himalayas and flows down to the Bay of Bengal covering a distance of 2525 km. In this length, Ganga passes along 29 class-I cities (population over 1,00,000), 23 class-II cities (population between 50,000-1,00,000) and 48 towns having less than 50,000 population. About 50% of Indian population lives in the Ganga basin, and there are about 100 urban settlements with a total population of about 120 million on its banks (Bandopadhyay, 1990). The delta of Ganga may be said to start from Farakka in West Bengal. The river divides into two arms about 40 km south-east below Farakka at Khejurtala village in Murshidabad district. The right arm of the river (which was the original course of Ganga) continues to flow south in West Bengal in the name of the Bhagirathi (called Hooghly in its downstream stretch) which crosses 500 km to the sea (Bay of Bengal).

The Gangetic delta, at the apex of Bay of Bengal is recognized as one of the most diversified and productive ecosystems of the Tropics. The deltaic lobe is unique for its wilderness, mangrove gene pool and tiger habitat. However due to intense industrial activities in the upstream zone, and several anthropogenic factors, the western part of the deltaic complex is exposed to pollution from domestic sewage and industrial effluents leading to serious impacts on biota (Mitra and Choudhury, 1992). The presence of Haldia port-cum-industrial complex in the downstream region of the River Ganga (also known as the Hooghly River) has accelerated the pollution problem with a much greater dimension (Mitra, 1998). The organic and inorganic wastes released from these industries and urban units contain substantial concentrations of heavy metals. The central part of the delta (encompassing the surroundings of Matla River) is relatively less stressful in terms of industrial discharge. Due to siltation of the Bidyadhari channel the area does not receive any water supply from the Hooghly River in the western sector and is therefore tide-fed in nature receiving the tidal flux from the Bay of Bengal (average salinity = ~32 psu). 85 percent of the people in this area consume fishes that are caught from the Gangetic delta region. The present paper aims to highlight the concentration of selective heavy metals (Zn, Cu, Pb and Cd) in the muscle tissue of five common finfish species namely *Polynemus paradiseus*, *Tenualosa ilisha*, *Liza parsia*, *Liza tade* and *Stolephorus commersonii* from four stations distributed in two sectors (western and central Indian Sundarbans) of the lower Gangetic region.

MATERIALS AND METHODS

DESCRIPTION OF THE STUDY SITE

Two sampling sites were selected each in the western and central sectors of Indian Sundarbans, a Gangetic delta at the apex of the Bay of Bengal. The deltaic complex has an area of 9630 sq. Km and houses 102 islands. The western sector of the deltaic lobe receives the snowmelt water of mighty Himalayan glaciers after being regulated through several barrages on the way. The central sector on the other hand, is fully deprived from such supply due to heavy siltation and clogging of the Bidyadhari channel since the late 15th century (Chaudhuri & Choudhury, 1994). The western sector also receives wastes and effluents of complex nature from multifarious industries concentrated mainly in the upstream zone. On this background four sampling stations (two each in western and central sectors) were selected (Table 1) to analyze the concentrations of heavy metals in the common edible finfish and shellfish species inhabiting the zone.

Station	Coordinates	Salient Features
Nayachar Island (Stn.1)	88° 15′ 24" E 21° 45′ 24" N	It is located in the Hooghly estuary in the western sector of the lower Gangetic delta and faces the Haldia port-cum-industrial complex that houses a variety of industrial units.
Sagar South (Stn.2)	88° 01′ 47" E 21° 39′ 04" N	Situated at the confluence of the River Hooghly and the Bay of Bengal in the western sector of Indian Sundarbans, the station is an important navigational channel for the major ports of the area.
Gosaba (Stn. 3)	88° 39′ 46" E 22° 15′ 45" N	Located in the Matla Riverine stretch in the central sector of Indian Sundarbans.
Annpur in Satjelia Island (Stn. 4)	88° 50′ 43" E 22° 11′ 52" N	Located in the central sector of Indian Sundarbans. Noted for its wilderness and mangrove diversity; selected as our control zone.

Table 1: Sampling stations with coordinates and salient features

SAMPLING OF SPECIMEN

Five commonly edible finfish species (*Polynemus paradiseus*, *Tenualosa ilisha*, *Liza parsia*, *Liza tade* and *Stoleophorus commersonii*) were collected during low tide condition from the selected stations (Table 1) during a rapid EIA study from 20th October to 30th October, 2013. The collected samples were stored in a container, preserved in crushed ice, and brought to the laboratory for further analysis. Similar sized specimens of each species were sorted out for analyzing the metal level in the muscle of finfish, shrimps, crabs and soft body parts of oyster.

ANALYSIS

Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) is now - a - day accepted as a fast, reliable means of multi-elemental analysis for a wide variety of sample types (Date & Gray, 1988). A Perkin-Elmer Sciex ELAN 5000 ICP mass spectrometer was used for the present analysis. A standard torch for this instrument was used with an outer argon gas flow rate of 15 L/min and an intermediate gas flow of 0.9 L/min. The applied power was 1.0 kW. The ion settings were standard settings recommended, when a conventional nebulizer/spray is used with a liquid sample uptake rate of 1.0 mL/min. A Moulinex Super Crousty microwave oven of 2450 MHz frequency magnetron and 1100 Watt maximum power

Polytetrafluoroethylene (PTFE) reactor of 115 ml volume, 1 cm wall thickness with hermetic screw caps, were used for the digestion of the collected biological samples. All reagents used were of high purity available and of analytical reagent grade. High purity water was obtained with a Barnstead Nanopure II water-purification system. All glasswares were soaked in 10% (v/v) nitric acid for 24 h and washed with deionised water prior to use.

The analyses were carried out on composite samples of 10 specimens of each species having uniform size. This is a measure to reduce possible variations in metal concentrations due to size and age. 20 mg composite sample from each species of finfish and shellfish were weighed and successively treated with 4 ml aqua regia, 1.5 mL HF and 3 ml H_2O_2 in a hermetically sealed PIFE reactor, inside a microwave oven, at power levels between 330-550 Watt, for 12 min to obtain a clear solution. The use of microwave-assisted digestion appears to be very relevant for sample dissolution, especially because it is very fast (Nadkarni, 1984; Matusiewicz & sturgeon, 1989; De la Guardia, 1990). After digestion, 4 ml H_2BO_3 was added and kept in a hot water bath for 10 min, diluted with distilled water to make up the volume to 50 ml. Taking distilled water in place of biological samples and following all the treatment steps described above the blank process was prepared. The final volume was made up to 50 ml. Finally, the samples and process blank solutions were analyzed by ICP-MS. All analyses were done in triplicate and the results were expressed with standard deviation.

The accuracy and precision of our results were checked by analyzing standard reference material (SRM, Dorm-2). The results indicated good agreement between the certified and the analytical values (Table 2).

STATISTICAL ANALYSIS:

A logarithmic transformation was done on the data to improve normality. Analysis of variance (ANOVA) was performed to assess whether heavy metal concentrations varied significantly between sites and species; possibilities less than 0.05 ($p < 0.05$) were considered statistically significant. All statistical calculations were performed with SPSS 21.0 for Windows. Superscripts were used to show the statistically significant difference between stations and species

RESULTS

The species-wise variation was not uniform for all the metals. Zn accumulated as per the order *Liza parsia* > *Liza tade* > *Tenualosa ilisha* > *Polynemus paradiseus* > *Stolephorus commersonii* (Table 2). Cu accumulated as per the order *Liza parsia* > *Tenualosa ilisha* > *Polynemus paradiseus* > *Liza tade* > *Stolephorus commersonii* (Table 2). Pb accumulated as per the order *Liza parsia* > *Liza tade* > *Tenualosa ilisha* > *Polynemus paradiseus* > *Stolephorus commersonii* (Table 3). Cd was BDL in all the stations except station 1, where the order was *Liza parsia* > *Liza tade* > *Tenualosa ilisha* (Table 3). For Zn and Cu, accumulated metal concentration in Stn. 4 was significantly higher than accumulated metal concentrations in Stn 1 and Stn 2. For Pb, significantly difference between stations was not found. Between all studied fish species, lowest metal accumulation values was found for *S. commersonii* ($p<0.05$)

Species	Stn. 1	Stn. 2	Stn. 3	Stn. 4
Polynemus paradiseus	73.45 ± 1.40	63.61 ± 1.09	37.23 ± 1.34	11.90 ± 0.88
Tenualosa ilisha	89.22 ± 1.44	74.89 ± 1.67	42.14 ± 1.87	14.00 ± 0.98
Liza parsia	119.66 ± 1.53	90.55 ± 1.54	56.22 ± 1.51	23.67 ± 1.22
Liza tade	99.45 ± 1.38	87.77 ± 1.88	49.89 ± 1.49	21.54 ± 1.21
Stolephorus commersonii	41.10 ± 1.09	21.33 ± 1.09	15.67 ± 1.29	12.00 ± 0.66
WHO (1989) level for Zn in food		100 ppm		
FAO (1992)level for Zn in fish		30 – 100 ppm		

Table 2: Zn concentrations (in ppm dry wt.) in finfish muscles

Species	Stn.1	Stn.2	Stn.3	Stn.4
Polynemus paradiseus	60.21 ± 0.74	41.00 ± 1.45	19.18 ± 0.39	12.43 ± 0.41
Tenualosa ilisha	63.09 ± 0.66	44.65 ± 1.58	23.75 ± 0.30	15.89 ± 0.30
Liza parsia	73.22 ± 0.83	52.44 ± 1.44	26.11 ± 0.55	19.10 ± 0.30
Liza tade	41.22 ± 0.50	47.38 ± 1.29	25.99 ± 0.57	15.56 ± 0.44
Stolephorus commersonii	13.17 ± 0.19	12.09 ± 0.29	11.19± 0.38	10.89 ± 0.19
WHO (1989) level for Cu in food		30 ppm		
FAO (1992)level for Cu in fish		10 – 100 ppm		

Table 3: Cu concentrations (in ppm dry wt.) in finfish muscles

Species	Stn.1	Stn.2	Stn.3	Stn. 4
Polynemus paradiseus	9.99± 0.45	8.17 ± 0.55	7.04 ± 0.40	6.02 ± 0.45
Tenualosa ilisha	12.70 ± 0.51	11.22 ± 0.78	10.03 ± 0.50	8.93 ± 0.56
Liza parsia	18.88 ± 0.45	14.61 ± 0.50	13.58 ± 0.66	11.29 ± 0.47
Liza tade	14.77 ± 0.54	12.50± 0.44	14.77 ± 0.99	10.56 ± 0.41
Stolephorus commersonii	3.66 ± 0.40	2.99± 0.99	3.99 ± 0.41	2.33 ± 0.09
WHO (1989) level for Pb in food		2 ppm		
FAO (1992)level for Pb in fish		0.5 – 6.0 ppm		

Table 4: Pb concentrations (in ppm dry wt.) in finfish muscles (BDL means below detectable level)

Species	Stn.1	Stn.2	Stn.3	Stn. 4
Polynemus paradiseus	BDL	BDL	BDL	BDL
Tenualosa ilisha	1.21 ± 0.28	BDL	BDL	BDL
Liza parsia	3.12 ± 0.10	BDL	BDL	BDL
Liza tade	1.76 ± 0.31	BDL	BDL	BDL
Stolephorus commersonii	BDL	BDL	BDL	BDL
WHO (1989) level for Cd in food		1 ppm		
FAO (1992) level for Cd in fish		0.05 – 5.5 ppm		

Table: 5 Cd concentrations (in ppm dry wt.) in finfish muscles (BDL means below detectable level)

DISCUSSION

Heavy metals are stable and persistent environmental contaminants of aquatic environments. They occur in the environment both as a result of natural processes and as pollutants from human activities (Garcia-Montelongo et al., 1994; Jordao et al., 2002). Some metals like Zn and Cu, which are required for metabolic activity in organisms, lie in the narrow "window" between their essentiality and toxicity. Other heavy metals like Cd and Pb, may exhibit extreme toxicity even at low levels under certain conditions, thus necessitating regular monitoring of sensitive aquatic environments (Cohen et al., 2001; Fergusson, 1990; Peerzada et al., 1990). From an environmental point of view, coastal zones can be considered as the geographic space of interaction between terrestrial and marine ecosystems that is of great importance for the survival of a large variety of plants, animals and marine species (Castro *et al*., 1999). The coastal zone receives a large amount of metal pollution from agricultural and industrial activity (Usero *et al.,* 2005). Adverse anthropogenic effects on the coastal environment include eutrophication, heavy metals, organic and microbial pollution and oil spills (Boudouresque & Verlaque, 2002). The discharge of these wastes without adequate treatment often contaminate the estuarine and coastal water with conservative pollutants (like heavy metals), many of which accumulate in the tissues of resident organisms like fishes, oysters, crabs, shrimps, seaweeds *etc*. In many parts of the world, especially in coastal areas and on smaller islands, fish is a major part of food, which supplies all essential elements required for life processes in a balanced manner (Iyengar, 1991). Hence, it is important to investigate the levels of heavy metals in these organisms to assess whether the concentration is within the permissible level and will not pose any hazard to the consumers (Krishnamurti & Nair 1999).

Heavy metal contamination of the environment has been occurring for centuries, but its extent has increased markedly in the last fifty years due to technological developments and increased consumer use of materials containing these metals. Pollution by heavy metals is a serious problem due to their toxicity and ability to accumulate in the biota (Islam & Tanaka, 2004). There is still a general concern about the impact of metals in the aquatic environment (Grosell & Brix, 2005). Heavy metals have contaminated the aquatic environment in the present century due to intense industrialization and urbanization. The Gangetic delta is no exception to this usual trend. The rapid industrialization and urbanization of the city of Kolkata (formerly known as Calcutta), Howrah and the newly emerging Haldia complex in the maritime state of West Bengal has caused considerable ecological imbalance in the adjacent coastal zone (Mitra & Choudhury, 1992; Mitra, 1998). The Hooghly estuary, situated on the western sector of the Gangetic delta receives drainage from these adjacent cities, which have sewage outlets into the estuarine system. The chain of factories and industries situated on the western bank of the Hooghly estuary is a major cause behind the gradual transformation of this beautiful ecotone into stinking cesspools of the megapolis (Mitra & Choudhury, 1992). The lower part of the estuary has multifarious industries such as paper, textiles, chemicals, pharmaceuticals, plastic, shellac, food, leather, jute, tyres and cycle rims (UNEP, 1982). These units are point sources of heavy metals in the estuarine water. Due to toxic nature of certain heavy metals, these chemical constituents interfere with the ecology of a particular environment and on entering into the food chain they cause potential health hazards, mainly to human beings. It was reported by several workers that the discharge of heavy metals into the sea through rivers and streams results in the accumulation of pollutants in the marine environment especially within fishes (Yusof *et al*., 1994). Thus fishes can be used for monitoring potential risk to humans because these are directly consumed by a large population (Subramanian & Sukumar, 1988).

Romeo *et al*., (1999) pointed out that the affiance of metal uptake from contaminated water and food may differ in relation to ecological needs, metabolism, and the contamination gradients of water, food and sediment, as well as other factors, such a salinity, temperature and interacting agents. The selected finfish species in the present study have different food preference and different behavioral pattern e.g., *Liza parsia*, *Liza tade*, *Polynemus paradiseus*, and *Stolephorus commersonii* are resident fish species in the study area, while *Tenualosa ilisha* exhibit migration from coastal region (~ salinity = 20 psu) to freshwater system in the upstream zone of the River Ganga for breeding. These factors may be attributed to species-wise variation of heavy metals in the study zone. The spatial variation of bioaccumulation followed the order station 1 > station 2 > station 3 > station 4, which may be related to different degree of contamination in different locations. All the heavy metals (except Zn in *Liza parsia* in station 1) were found to be lower than the recommended maximum level allowed in food as prescribed by the World Health Organization (WHO, 1989). Furthermore the selected heavy metals in finfish muscle (except Zn in *Liza parsia* in station 1) were also within the permissible limits for human consumption as indicated by the Food and Agricultural Organization (FAO, 1992).

Of the four metals studied in the present work, Zn and Cu are essential elements, while Pb and Cd are non-essential elements for most of the living organisms. The concentrations of Zn and Cu in all the finfish and shellfish species were relatively higher, compared to the concentration of other metals in same samples. It can be explained because these metals (Cu and Zn) are essential elements required by animals for metabolic process. Zn and Cu appear to diffuse passively (probably as a soluble complex) the gradients created by adsorption of membrane surfaces and are found in blood proteins metallothioneins. Carbonell and Tarazona (1994) concluded that different tissues of aquatic animals provide and/or synthesize nonexchangeable binding sites resulting in different accumulation levels. The primary sources of Zn in the present geographical locale are the galvanization units, paint manufacturing units and pharmaceutical processes, which are mainly concentrated in the Haldia industrial sector (opposite to station 1). Reports of high concentrations of Zn were also highlighted in the same environment by earlier workers (Mitra and Choudhury, 1992; Mitra and Choudhury, 1993; Mitra, 1998).

The main sources of Cu in the coastal waters are antifouling paints (Goldberg, 1975), particular type of algaecides used in different aquaculture farms, paint manufacturing units, pipe line corrosion and oil sludges (32 to 120 ppm). Ship bottom paint has been found to produce very high concentration of Cu is sea water and sediment in harbours of Great Britain and southern California (Bellinger & Benham, 1978; Young et al., 1979). In the present study area, the major source of Cu is the antifouling paints used for conditioning fishing vessels and trawlers apart from industrial discharges (that is predominant around station 1). This is the reason why Cu was detected in the fish samples of stations 3 and 4, even there are no existence of industries. The complete siltation of the Bidyadhari River also does not permit the industrial effluents released in the Hooghly River to mix with the rivers in the central sector of the deltaic complex (location zone of stations 3 and 4).

Pb is a toxic heavy metal, which finds its way in coastal waters through the discharge of industrial waste waters, such as from painting, dyeing, battery manufacturing units and oil refineries (Mitra, 1998). Antifouling paints used to prevent growth of marine organisms at the bottom of the boats and trawlers also contain Pb as an important component. These paints are designed to constantly leach toxic metals into the water to kill organisms that may attach to bottom of the boats, which ultimately is transported to the sediment and aquatic compartments. Lead also enters the oceans and coastal waters both from terrestrial sources and atmosphere and the atmospheric input of lead aerosols can be substantial. Station 1 is exposed to all these activities being proximal to the highly urbanized city of Kolkata, Howrah and the newly emerging Haldia port - cum - industrial complex, which may be attributed to high Pb concentrations in the finfish species.

The main sources of Cd in the present geographical locale are electroplating, manufacturing of Cd alloys, production of Ni-Cd batteries and welding (Mitra, 1998). No trace of Cd was recorded in the fish muscle from stations 3 and 4, which are located almost in industry-free zone surrounded by mangrove vegetation.

Apart from industrial discharges, significant difference in metal concentrations in biological samples between stations ($p < 0.01$) may also be related to contrasting physico-chemical characteristics between the western and central part of the Gangetic delta. The western part of the Gangetic delta is connected to Himalayan glacier through Bhagirathi River. Researchers pointed out that the glaciers in the Himalayan range are melting at the rate of 23 m/yr (Hasnain, 1999; 2000; 2002). This along with Farakka discharge has resulted in gradual freshening of the system (Mitra et al., 2009), which has role in elevation of dissolved metal level in the aquatic phase by way of lowering the salinity, pH and enhancing the process of dissolution of metallic species (Mitra, 1998). The central sector on contrary is deprived from freshwater supply of Ganga-Bhagirathi system, and the Matla River is now tide fed with an increasing trend of salinity. This results in the precipitation of dissolved metals on the sediment bed (an event of compartmentation due to variation in metallic species) making the availability of metal low to the tissue of fish species thriving in the system (Mitra and Choudhury, 1992; Mitra and Choudhury, 1993; Mitra, 1998).

The International official regulatory agencies have set limits for heavy metal concentrations above which the fish is considered unsuitable for human consumption. However in the Indian sub-continent there is no safety level of heavy metals in fish tissue (recommended by the Pollution control authority or any other regulatory body) although the Indian population is the major fish consumer in the tropics with a weekly average consumption of 1050 g and an annual rate of 55 kg per person. The presence of high concentrations of Zn, Cu, Pb and Cd in fish muscle of station 1, in combination with the fact that fish is consumed by 85% of the people in the study area, call for effective preventive measures to safeguard public health.

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