Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Annals of Biological Research, 2011, 2 (5) :125-134
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0976-1233
CODEN (USA): ABRNBW

Bioaccumulation of heavy metals in muscle tissue of fishes from selected aquaculture ponds in east Kolkata wetlands

Bhupander Kumar[1*], D. P. Mukherjee[2], Sanjay Kumar[1], Meenu Mishra[1], Dev Prakash[1], S. K. Singh[1] and C. S. Sharma

[1]*Central Pollution Control Board, East Arjun Nagar, Delhi, India*
[2]*Central Pollution Control Board, Rajdanga Road, Kolkata, India*

ABSTRACT

Heavy metals i.e. Copper (Cu), Zinc (Zn), Manganese (Mn), Iron (Fe), Cadmium (Cd), Nickel (Ni), Mercury (Hg) and Arsenic (As) were analyzed in 35 samples of the fish muscle, from East Kolkata Wetlands (EKW). The concentration of heavy metals was in order of, Fe > Zn > Cu > Mn > Ni > As > Hg > Cd. The essential metals, such as iron, zinc, copper and manganese were in higher concentrations and, the non-essential and toxic metals such as cadmium, nickel, mercury and arsenic were generally low. The bioaccumulation pattern of metals in different species of fishes was as: Hypophthalmichthys molitrix > Oreochromis nilotica > Labeo rohita > O. mossambica > Channa marulius > Catla catla > Punctius ticto. The Pearson's moment correlation coefficients among heavy metal were investigated and presented. This study revealed that fishes from the East Kolkata Wetland (EKW) may not be harmful to consumers since; levels of heavy metals were below the permissible limits issued by FAO/WHO. However, intensive study on fishes is a need to determine the bioaccumulation of heavy metals and other toxic pollutants i.e. pesticides, PCBs, PAHs, and dioxins in this area.

Keywords: Heavy metal, freshwater fishes, muscle tissue, India.

INTRODUCTION

Heavy metals in the aquatic environment can affect aquatic biota and pose a risk to fish consumers, such as humans and other wildlife. Heavy metals may enter aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills/dumpsites and atmospheric deposits [1]. Metals like iron and manganese are required for metabolic activities in organisms, but some other elements like arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc exhibit toxicity effects on aquatic organisms [2]. In aquatic ecosystem, heavy metals have received considerable attention due to their toxicity and accumulation in biota and fishes [3-4]. Accumulation of heavy metals in fishes leads to biomagnifications in the food chain.

Fishes are major part of the human diet due to high protein content, low saturated fat and sufficient omega fatty acids which are known to support good health therefore, various studies have been taken worldwide on the contamination of different fish species by heavy metals [5-9]. Fishes have been widely used as bio-indicators of pollution by metals. Muscle tissue of fish is ~~the~~ most frequently used for analysis because it is a major target tissue for metal storage and is the main edible part of the fish.

In this study we emphasized measurement and distribution of heavy metals i.e. Copper (Cu), Zinc (Zn), Manganese (Mn), Iron (Fe), Cadmium (Cd), Nickel (Ni), Mercury (Hg) and Arsenic (As) in the muscle tissues of fishes, from East Kolkata Wetlands (EKW), India. Further, the observed levels of heavy metal are compared with available certified safety guidelines proposed by FAO/WHO [10-11].

MATERIALS AND METHODS

Sampling

Sampling area was eastern part of Kolkata (formerly Calcutta), where series of ponds are located in a large wetland area known as East Kolkata Wetlands (EKW), spreading over an area of 12,500 ha. The boundaries of the wetland system are currently located between $22^0$25'' to $22^0$40'' N and $88^0$20'' to $88^0$35'' E.

These wetlands are well known in the world for their multiple uses and these are the largest sewage fed wetlands in the world as they were included in the Ramsar List of Best Practice Wetlands (RLBPW) since August 2002. In this region wastewater aquaculture ~~has~~ have been flourished since 1918 [12].

Thirty five samples of seven fish species, *Catla catla*, *Oreochromis nilotica*, *Oreochromis mossambica*, *Labeo rohita*, *Hypophthalmichthys molitrix*, *Punctius ticto* and *Channa marulius* were collected from selected aquaculture ponds of EKW (Figure 1). Fish samples were labeled, they were preserved using ice and transported to the main laboratory. All the samples were stored at -20^0C prior to pre-treatment and analysis.

Pre-treatment of Sample

Samples were thoroughly washed with Mili-Q water after removing the scales, and muscle portion, which was taken for further processing. Muscle tissue was oven dried at 110^0C, powdered with pestle and mortar and was stored until chemical analysis. Heavy metals (Cu, Zn, Mn, Fe, Cd, Hg and As) were analyzed after digesting the homogenized samples in a mixture of concentrated nitric and perchloric acid [13]. Digestion was carried out after 0.5 gm homogenized powdered sample was placed in a Teflon beaker and digested with a few drops of sodium chloride solution (30%) and a 10 ml mixture (1:5) of concentrate Nitric acid (65%) and concentrated Perchloric acid (70%). The free chlorine developed loosens the chemical bonds in organic compounds after gentle heating (at 70±5^{o}C) in a water bath for 12 hrs and destroy the organic matter in order to transfer the metals into the solution. The digested samples were centrifuged and the supernatant was analyzed.

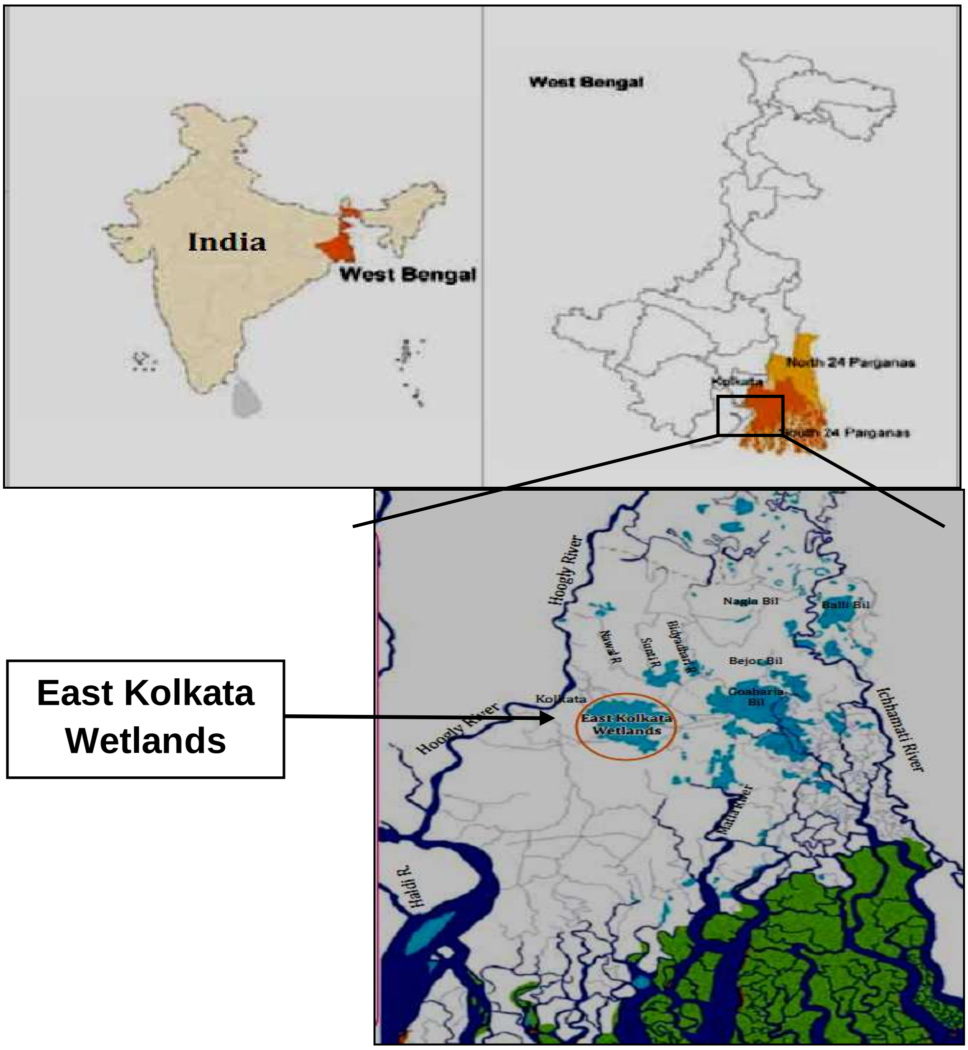

Figure 1: Maps showing East Kolkata Wetland (EKW)

Instrumental Analysis

Flame Atomic Absorption Spectrometry (FAAS, Thermo, UK) was used for determinations of copper, zinc, manganese, iron, cadmium and nickel. Hydride generator (HG) coupled to atomic absorption and spectrophotometer was used to analyze total mercury (cold vapor mode) and arsenic (heating mode). Background corrections were applied whenever required during the analysis and the method of standard additions was used to compensate for matrix effects.

Table1: Heavy metals concentration (µg g^{-1} dry wt.) in CRM-SW-8022)

Metals	Reference value	*Measured value	Recovery (%)	S D (±%)
Copper	71	73	103	2
Manganese	582	645	111	11
Zinc	289	312	108	8
Iron	13771	15431	112	7
Cadmium	173	172	100	1
Nickel	160	174	109	9
Mercury	26	27.2	104	4
Arsenic	14	13.7	97	3

*Note: * average of three replicate*

Performance of the instrument was checked by analyzing the standard reference material solutions (Merck NJ, USA) concurrently to check the precision of the instrument. After appropriate dilutions of stock standard solutions, a five level calibration curve was prepared. Samples were analyzed in triplicate. The values obtained from the sample were corrected for final digestion volume and sample weight was taken. The results were reported on dry weight basis as µg g^{-1} (dry wt.). The detection limit for Zn, Fe, Cu, Cd, Mn, Ni, Hg, and As was, 0.01, 0.06, 0.05, 0.01, 0.05, 0.02, 0.001, and 0.002 ppm, respectively. Duplicate method blanks were also processed and analyzed alongside the samples to check any loss or cross contamination. A certified reference material (SW 8022) was processed along with samples to determine the accuracy of the method and the results were compared to the acceptable limits (Table 1). The recovery of the studied heavy metals was ranged between 97±1 to 112±11 percent.

Statistical Analysis

The Pearson's correlation coefficient was used to measure the strength of the association between heavy metal concentrations in the muscle tissue of fishes [14]. The *p*-values of less than 0.1 and 0.05 were considered to indicate statistical significance.

RESULTS AND DISCUSSION

Concentrations of copper, zinc, manganese, iron, cadmium, nickel, mercury and arsenic in muscle tissue of fishes from East Kolkata Wetlands were presented in Table 2 and Table 3. The study of heavy metal concentrations in fishes was important with respect to human consumption of fish. Several studies shows heavy concentration in tissue of coastal fishes may vary considerably among the different species. This was possibly due to differences in metabolism and feeding patterns of the fishes. In the present study, the concentration of heavy metals in fishes was as:

$$Fe > Zn > Cu > Mn > Ni > As > Hg > Cd.$$

Copper

Copper is an essential metal in fish and is regulated in the muscle tissue with high molecular weight proteins (metallothionein-like). The copper concentration in muscle tissue varied from 2.12 µg g^{-1} to 27.94 µg g^{-1} and average value of 7.54±0.94 µg g^{-1} for fishes from EKW. The maximum concentration was observed for *H. molitrix* (21.1±6.1 µg g^{-1}) and minimum for *Punctius ticto* (2.6±0.5 µg g^{-1}). The observed values of our study were higher than fishes in

Nigeria, Turkey, Malaysia, Bhopal, and earlier report from India [15-23] but, lower than from EKW, India [8,24].

Table 2: Concentration mean and range (µg g^{-1}dry wt) of heavy metals in muscle tissues of fishes from East Kolkata Wetlands (n=35)

Metals	FAO/WHO guidelines	Mean	Range	
			Minimum	Maximum
Copper	30	7.54±0.94*	2.12	27.94
Zinc	100	47.80±3.29	12.30	92.70
Manganese	1.0	4.24±0.65	NT	12.97
Iron	100	58.66±6.34	16.52	186.03
Cadmium	1.0	0.31±0.12	NT	2.99
Nickel	10	4.03±0.55	NT	8.97
Mercury	-	0.41±0.04	0.09	0.77
Arsenic	-	0.45±0.07	NT	1.22

** SE= standard deviation /√n*

Zinc

Zinc was the second abundant metal followed by iron. Like copper zinc is also an essential element in our food. The average concentration of zinc in all species was 47.80±3.29 µg g^{-1}. The highest mean concentration of Zinc was in *Labeo rohita* (60.7±5.0 µg g^{-1}) and lowest in *Punctius ticto* (14.5±2.2 µg g^{-1}). It is generally believed that fish actively regulate zinc concentration in their muscle tissue. The observed values of Zn were higher than fishes from Afikpo, Nigeria, Turkey, Malaysia, Cameroon, Bhopal, India, river Ganges, West Bengal, India and earlier report from EKW [15,17-24] but, lower than fishes from lake Mugla, Turkey and river Ravi, Pakistan [7,26].

Manganese

Manganese is a metal with low toxicity but has a considerable biological significance and seems to accumulate in fish species. The manganese concentration in muscles of fish from EKW ranged between not traceable to 12.97 µg g^{-1} with the mean of 4.24±0.65 µg g^{-1}. The lowest concentration of manganese was 1.4±0.5 µg g^{-1} in *Punctius ticto* and highest was 13.0±0.1 µg g^{-1} in *O. mossambica*. Lower concentrations of Mn in fish tissue were reported by other workers from Nigeria, Malaysia and from EKW, India, respectively [15, 19, 23]. However, our concentrations were lower than fishes from lake Mugla, Turkey [7] and river Ravi, Pakistan [26].

Iron

Iron was found most abundant metal in muscle tissue of all the studied species from EKW. Fish is the major source for iron in adults and children and deficiency of it causes anemia. The concentration of Fe in fish muscles from this study varies between 16.52 to 186.03 µg g^{-1} (mean, 58.66±6.34 µg g^{-1}). The maximum concentration of iron was in *O. nilotica* (89.6±18.6 µg g^{-1}) while the minimum was in *Punctius ticto* (38.5±18.3 µg g^{-1}). The reported concentration of Fe was higher than reports from Turkey [16], Malaysia [19] and earlier reports from West Bengal [23] but, lower than fishes from Nigeria [15], other reports for EKW [24] and river Ravi, Pakistan [26].

Table 3: Heavy metal concentrations (range and mean) in muscle tissue of fishes (µg g^{-1}dry wt)

Fish Species (no of samples)	Cu	Zn	Mn	Fe	Cd	Ni	Hg	As
Catla catla (4)	3.0-11.0 (5.5±1.9)	22.9-41.0 (29.2±4.2)	NT-3.0 (1.5±0.7)	43.6-71.3 (55.9±6.4)	NT	2.6-4.5 (3.8±0.5)	0.1-0.5 (0.3±0.1)	0.8-1.2 (1.1±0.1)
Oreochromis nilotica (8)	5.0-10.0 (7.1±0.7)	34.0-72.0 (51.2±3.9)	NT-11.0 (4.9±1.3)	27.4-186.0 (89.6±18.6)	NT	NT-7.5 (2.6±1.1)	0.1-0.7 (0.5±0.1)	NT-1.0 (0.5±0.2)
Labeo rohita (14)	4.0-14.0 (6.7±0.8)	31.7-92.7 (60.7±5.0)	NT-11.7 (3.2±0.9)	16.5-126.0 (42.3±7.0)	0-1.2 (0.2±0.1)	NT-8.5 (4.0±1.0)	0.1-0.8 (0.4±0.1)	NT-1.0 (0.4±0.1)
Hypophthalmichthys molitrix (3)	9.0-27.9 (21.1±6.1)	40.9-50.6 (45.1±2.9)	4.0-6.0 (4.9±0.6)	52.9-110.1 (83.2±16.6)	0-1.1 (0.7±0.3)	5.0-8.5 (6.4±1.1)	0.1-0.5 (0.3±0.1)	NT-0.2 (0.1±0.1)
Oreochromis mossambica (2)	4.0-6.0 (5.0±1.0)	30.9-46.9 (38.9±8.0)	12.9-13.0 (13.0±0.1)	30.9-51.9 (41.4±10.5)	2.0-3.0 (2.5±0.5)	5.0-9.0 (7.0±2.0)	NT-0.1 (0.1±0.1)	NT.7-0.8 (0.8±0.1)
Punctius ticto (2)	2.1-3.0 (2.6±0.5)	12.3-16.7 (14.5±2.2)	0.9-1.9 (1.4±0.5)	20.2-56.8 (38.5±18.3)	NT	NT	0.6-0.8 (0.7±0.1)	0.1-0.2 (0.1±0.1)
Channa marulius (2)	5.2-7.7 (6.5±1.2)	22.3-33.1 (27.7±5.4)	6.4-9.5 (8.0±1.6)	27.6-83.8 (55.7±28.2)	NT	NT	0.4-0.5 (0.4±0.1)	NT-0.1 (0.1±0.1)

Cadmium

Cadmium is a non-essential toxic metal, and may accumulate in humans from food chain magnification. Cadmium could be readily bioaccumulated in lower portion of food chain and bio-concentrate in multiple organs of fish. Cadmium values in this study were ranged from not traceable to 2.99 µg g^{-1} with an average of 0.31±0.12 µg g^{-1}. Higher concentration of Cd was observed in *O. mossambica* (2.0-3.0 µg g^{-1}), and other species shows low or negligible accumulation (NT-1.2 µg g^{-1}). The observed values were comparable with fishes from Saudi Arabia [27] however, lower than levels from river Ravi, Pakistan, lake Mugla, Bhopal, Cameroon and fishes from Tamilnadu, India [6,7,20,25,28,29] but, comparatively higher than fish species from Turkey and Malaysia [16-19].

Nickel

Aquatic environments generally have low concentration of nickel. The concentration of nickel in the muscle tissue of fishes from EKW was ranged between not traceable to 8.97 µg g^{-1} with the mean of 4.03±0.55 µg g^{-1}. Maximum concentration was observed for *O. mossambica* (7.0±2.0 µg g^{-1}) and minimum for *Channa* and *Punctius* sp (not traceable). The observed values were comparatively higher than fishes from Turkey, India, Malaysia, Cameroon and river Ravi, Pakistan [16, 19, 25, 26].

Mercury

Mercury is recognized as a highly toxic metal and stringently regulated by waste discharges. Movement of Hg (II) into aquatic ecosystem and its bioaccumulation as methylmercury in higher trophic levels are strongly influenced by the uptake of bioavailable forms of Hg (II). Fish obtained methylated mercury through dietary uptake, which could be influenced by size, diet, ecological and environmental factors. The concentration of mercury in muscle tissues of different fish species from EKW varied from 0.09 to 0.77 µg g^{-1} (mean 0.41±0.04 µg g^{-1}). The highest mean concentration was observed in *Punctius ticto* (0.7±0.1 µg g^{-1}), and lowest was in *O. mossambica* (0.1±0.1 µg g^{-1}). Our result of mercury are in agreement with earlier report on fish muscle from EKW [5] and fishes from Bangladesh [30].The average concentration of Hg in fishes from EKW were higher than Turkey [17], Bhopal, India [20] and Niger Delta, Nigeria [31].

Arsenic

Arsenic is released in the environment through natural processes such as weathering, and may circulate in natural ecosystems for long time. A well recognized arsenic problem of groundwater in West Bengal was first reported in the late 1980s. The muscle tissue of fishes from EKW shows low contamination levels and was ranged from not traceable to 1.22 µg g^{-1} with the mean of 0.45±0.07 µg g^{-1}. Comparatively higher concentration of arsenic accumulation was observed in *Catla and Oreochromis sp*, however lower concentrations were accumulated by other studied species. The observed concentrations of arsenic in muscle tissue of fishes from EKW were higher than fishes from Turkey [17].

The essential metals, such as iron, zinc, copper and manganese are in higher concentrations, presumably due to their function as co-factors for the activation of a number of enzymes and regulated to maintain a certain homeostatic status in fish. On the other hand, the non-essential metals such as cadmium, nickel, mercury and arsenic have no biological function or requirement and their concentrations in fishes are generally low. In this study the metal concentrations varied significantly among seven species of fishes (Figure 2). The bioaccumulation pattern of heavy metals in muscle tissue of different species of fishes from EKW was observed as: *H. molitrix* > *O. nilotica* > *L. rohita* > *O. mossambica* > *C. marulius* > *C. catla* > *P. ticto*. So, *Hypophthalmichthys molitrix* was the most contaminated fish with heavy metals and *Punctius ticto* was the least contaminated in East Kolkata Wetland (EKW).

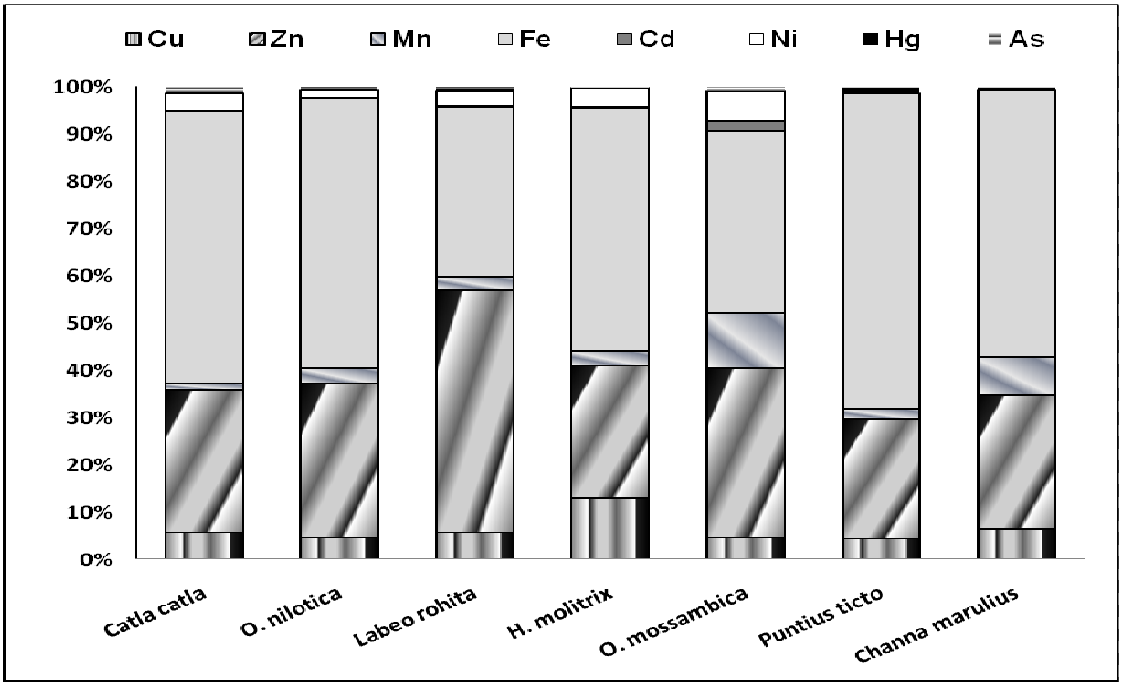

Figure 2: Percent distribution of heavy metals in muscle tissue of fishes

Accumulations of metals were generally found to be species specific and may be related to their feeding habits and the bio-concentration capacity [32-34]. It is well recognized that heavy metal uptake occurs mainly from water, food and sediments. However, the efficiency 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 salinity and temperature [35].

Inter-Metal Relationship in fishes

Inter-metal correlations of fish species were assessed and presented in Table 4. The correlations between the different metals may result from the similar accumulation behavior of the metals in the fishes and their interactions [36]. Noted significant correlations among metals may reflect a common source of occurrence and indicative of similar biogeochemical pathways for subsequent accumulation in the muscle tissue of fishes. In the present study, manganese is strongly correlated with cadmium and arsenic. Nickel, similar to manganese showed high correlation with cadmium and arsenic. No other significant correlation was observed between studied heavy metals.

Table 4: Pearson's moment correlation coefficients between the heavy metals

	Zn	Mn	Fe	Cd	Ni	Hg	As
Cu	0.103	-0.026	0.150	0.138	0.131	-0.167	-0.265
Zn		-0.084	-0.034	-0.069	-0.164	0.136	-0.083
Mn			-0.059	**0.501**^{a,b}	0.197	-0.136	**0.257**^{a}
Fe				-0.170	-0.347	0.132	-0.219
Cd					**0.380**^{a,b}	-0.266	0.123
Ni						-0.559	**0.369**^{a,b}
Hg							-0.396

Note: significant correlations at $p<0.01$ are mark as ^{a}, and at $p<0.05$ are mark as ^{b}

CONCLUSION

When considering the heavy metals concentrations in fish species, the most important aspect is their toxicity to humans suitable for human consumption. The results of this study revealed that consuming fish from the East Kolkata Wetland (EKW), India may not be harmful to consumers because observed values of heavy metals were below the permissible limits issued by FAO/WHO for human consumption. However, Mn is higher than certified level so, it is a matter of concern in fish accumulation. More intensive study is needed in order to determine the bioaccumulation of heavy metals in fishes from the study area. Further study on accumulation of organochlorine pesticides, PCBs, PAHs, and dioxins in fish tissues should be undertaken due to usage of these chemicals in India.

Acknowledgements

The authors are thankful to the chairman and member secretary of the central pollution control board for their constant guidance and interest in conducting the study. Sh. B. R. Naidu, then Incharge central pollution control board zonal office Kolkata is also acknowledged for providing facilities.

REFERENCES

[1] U. Forstner and G. T. W. Wittmann. Metal pollution in aquatic environment. Berlin, Springer-Verlag., **1983**, pp: 30-61

[2] C. F. Mason. Biology of Freshwater Fishes. **1991,** Longman Science and Technology, New York.

[3] K. Senthilkumar, K.S. Sajwan, J. P. Richardson and K. Kannan. *Mar. Poll. Bull.,* **2007,** 56:136-149
[4] K. S. Sajwan, K. Senthilkumar, S. Paramasivam, S.S. Compton and J.P. Richardson. *Archives Environ. Conta. Toxicol.,* **2008**, 54:245-258
[5] S. Bhattacharyya, P. Chaudhuri, S. Dutta and S. C. Santra. *Bull. Environ. Cont. Toxicol.,* **2010**, 84 (5): 618-622.
[6] A. Raouf, M. Javed and M. Ubaidullah. *Pakistan Vet. J.,* **2009**, 29 (1): 24-26.
[7] F. Yilmaz. *Turkish J. Sci. & Tech.,* **2009,** 4(1):7-15
[8] S. Raychaudhuri, M. Mishra, S. Salodkar, M. Sudarshan and A.R. Thakur. *Am. J. Environ. Sci.,* **2008,** 4(2):140-144.
[9] P. Sivaperumal, T. V. Sankar and N. Viswanathan. *Food Chemistry,* **2007,** 102:612-620
[10] FAO (Food and Agriculture Organization). *FAO fishery circular,* **1983,** 464: 5-100.
[11] WHO (World Health Organization). *Environ. Health Criteria*. **1989,** No. 85. Geneva, Switzerland.
[12] EKW (East Kolkata Wetlands). Published by: East Kolkata Wetlands Management Authority and Wetlands International- South Asia, **2010,** Vol. 1.
[13] K. Honda, R. Tatsukawa and T. Fujiyama. *Agri. Biol. Chem*., **1982,** 46: 3011-3021
[14] A. Pentecost. Analyzing environmental data. **1999,** Pearson Education limited, England.
[15] C. D. Nwani, D. A. Nwachi, O. I. Okogwu, E. F. Ude and G. E. Odoh. *J. Environ. Biol.,* **2010,** 31 (5): 595-601.
[16] M. Ozturk, G. Ozozen, O. Minarcci, E. Minarcci. *Iranian J. Environ. Health Sci. Eng.,* **2009,** 6 (2): 73-80.
[17] S. Mol, O. Ozden and S. A. Oymak. *Turkish J. Fish. Aqua. Sci.,* **2010,** 10: 209-213.
[18] A. K. Ahmad and M. Shuhaimi-Othman. *J. Biol. Sci.,* **2010,** 10 (2): 93-100.
[19] M. B. Mokhtar, A. Z. Aris, V. Munusamy and S. M. Praveena. *European J. Scient. Res*., **2009,** 30 (3): 348-360.
[20] N. Malik, A. K. Biswas, T. A. Quereshi, K. Borana and R. Virha. *Environ. Monit. Assess.,* **2010,**160: 267-276.
[21] A. K. Bhattacharya, S. N. Mandal and S. K. Das. *Tr. Appl. Sci. Res.,* **2007,** 2 (6): 492-499.
[22] A. K. Bhattacharya, S. N. Mandal and S. K. Das. *Tr. Appl. Sci. Res.,* **2008,** 3 (1): 61-68.
[23] S. Chatterjee, B. Chattopadhyay and S. K. Mukhopadhyay. *Acta Icthyologica et Piscatoria*. **2006,** 36 (2): 119-125.
[24] B. Kumar, K. Senthilkumar, M. Priya, D. Mukhopadhyay and R. Shah. *Toxicol. Environ. Chem.,* **2010,** 92 (2): 243-260.
[25] A. Ali, D. Ahmadou, B. Adji, C. Saidou and D. Tenin. *J. Fish. Int.,* **2010,** 5 (3): 54-57.
[26] M. Javed. *Pakistan J. Biol. Sci.,* **2005,** 8 (10): 1337-1341.
[27] A. Mohammed Al-Kahtani. *Am. J. Appl. Sci.,* **2009,** 6 (12): 2024-2029.
[28] R. Vinodhini and M. Narayanan. *Int. J. Environ. Sci. Tech.,* **2008,** 5 (2): 179-182.
[29] G. Ambedkar and M. Muniyan. *Archives Appl. Sci. Res.,* **2011,** 3 (3): 261-264.
[30] A. K. M. Sharif, M. Alamgir, Stanly A. Bajue and Rashid Alam. *J. Nat. Ocean. Atm. Mar. Inst.,* **2008,** 25 (2): 1-7.
[31] N. U. Benson, J. P. Essien, A. B. Williams and D. E. Bassey. *CURRENT SCIENCE,* **2007,** 92 (6): 781-785.
[32] Z. Friba, T. Hossein, A.R. Siamak, M. Saeed, A. Aziz and R. Mohammad. *J. Animal Vet. Adv.,* **2009,** 8 (7): 1288-2009
[33]A. H Abu Hilal, and Naim S Ismail. *Jordan J. Biol. Sci.,* **2008**, 1(1):13-18

[34] W.B. Huang *J. Food and Drug Analysis,* **2003**,11(4):324-330
[35] G. K. Pagenkopf. *Environ. Sci. Technol.,* **1983,** 17 (6): 342-347.
[36] G. Rejomon, M. Nair and T. Joseph. *Environ. Monit. Assess.,* **2010,** 167: (1-4): 243-255.