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Bioaccumulation of total mercury and methyl mercury in soft tissue of the freshwater Carnivorous fish species

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Abstract : Mercury (Hg) is one of the most malignant metals in the environment and one of the organic forms of Hg; methyl mercury (MeHg) is internationally recognized as toxic contaminants and elevated methyl mercury (MeHg) concentrations in fish is a world-wide environmental concern. This study investigated the presence of total mercury and organic mercury levels in various Carnivorous fish species captured from river. The results of mercury analysis in various specimens indicated that the some fish muscles tend to accumulate high levels of Hg and approximately 50–84% of that Hg is organic mercury. *A. bengalensis bengalensis* and *W. attu* possessed highest amount of organic mercury in their muscle tissues and contamination in *M. armatus* (0.45 ± 0.46), *C. chitala* (0.25 ± 0.18), *R. rita* (0.34 ± 0.15), *L. calcarifer* (0.25 ± 0.02) and *O. pabda* (0.26 ± 0.04) were also above the 0.25 μg Hg/g of wet weight, the limit set by PFA with the maximum level for consumption of fish exposed to MeHg. Though in *P. pangasius* (0.12 ± 0.16), *B. bagarius* (0.12 ± 0.01) and *C. garua* (0.1 ± 0.01), concentration was below the recommended level but in *M. aor* (0.23 ± 0.1) it was threatening. Interestingly, a low concentration of Hg was found in post-monsoon samples of all the species.

Keywords : Bioaccumulation, mercury, methyl mercury, carnivorous fish.

Introduction

Mercury (Hg) is the most malignant metals in the environment present in food, soil and water and usually released in the environment from both natural and man-made sources and continuously leached into aquatic ecosystems[1]. From the atmosphere, mercury cycles from rainwater into lakes and oceans where it is converted by microbial activity into its most toxic forms, organic methyl mercury[2] (MeHg) which normally constitutes at least 90% of the total mercury (THg) burden in fish[3]. Methyl mercury is very persistent and arguably the most dramatic and the best documented example of high bioaccumulation, especially in aquatic food chains[4]. The general populations are exposed to MeHg through the diet; the main source is consumption of aquatic subjects. Organic mercurial is almost completely absorbed from gastrointestinal tract (90–95%) and remain stable in its initial form. Its lipid soluble property allows easy passage through the gastrointestinal tract into stream blood and red blood cells and thus accumulates in brain, kidney, liver, hair and skin. Exposure to this element, especially in its organic form, MeHg, may cause damage to the nervous[5], immune[6] and cardiovascular systems[7] and the kidney[8]. It may also induce genetic damage[9,10]. It easily crosses the blood brain barrier and causes tremor, depression and behavioral disturbances[11]. MeHg has been a public health concern of governments all over the world since mass poisoning in Minamata and Niigata in 1950's.

Literature also showed that the content of this volatile organic pollutant in fish and other aquatic subjects varies with certain factors; viz. species, age, source and food habit. Among all the variables, food habit plays the most important role in assessing the probable contamination[12]. Since MeHg biomagnifies through food webs, concentrations in top predator fish can be three orders of magnitude higher than these in the ambient water[13]. Hg absorbed to phytoplankton particles, as partially described by Mason *et al.*[14], may well be the major source of Hg intake at the planktivores. But the fate of piscivores, who usually consume the planktivores, is not known. Therefore carnivorous fish species are likely to accumulate various pollutants, discharged in the aquatic environment, and represent a potential risk not only to these fishes, but also to fish consumers, particularly humans.

The purpose of the present study was to determine the accumulation of THg and MeHg in various fresh water carnivorous fish species available in different location of West Bengal to know the pattern of mercury bioaccumulation of these species. The study is also comprised of data on seasonal variation of mercury bioaccumulation.

Results and discussion

Total 11 commonly available carnivorous fish species were collected from different location of West Bengal and analyzed. The results of the analysis of total mercury (THg) and methyl mercury (MeHg) in fish muscle tissues are summarized in Table 1. The current study showed that the accumulation pattern of THg and MeHg varies between species with maximum value of both THg and MeHg accumulation in *A. bengalensis bengalensis* (Eel) (2.61 ± 1.02 and 1.94 ± 2.23 μg Hg/g of wet weight respectively) followed by *W. attu* (Boal) (1.48 ± 1.01 μg/g of wet weight and 1.1 ± 0.60 μg Hg/g of wet weight). MeHg contamination in *M. armatus* (0.45 ± 0.46), *C. chitala* (Chital) (0.25 ± 0.18), *R. rita* (Rita) (0.34 ± 0.15), *L. calcarifer* (Vetki) (0.25 ± 0.02) and *O. pabda* (Pabda) (0.26 ± 0.04) were also above the 0.25 μg Hg/g of wet weight, the limit set by PFA with the maximum level for consumption of fish exposed to MeHg[15]. Though in *P. pangasius* (0.12 ± 0.16), *B. bagarius* (0.12 ± 0.01) and *C. garua* (0.1 ± 0.01), MeHg concentration was below the recommended level but in *Mystus aor* (0.23 ± 0.1) it was threatening. Higher level of MeHg content of *A. bengalensis bengalensis* and *Mastacembelus armatus* may because they are long-lived

Table 1. Mercury in freshwater carnivorous fish of West Bengal : synopsis

Species		*n*	Weight (kg)	Total mercury (μg Hg/g of wet tissue)	MeHg (μg Hg/g of wet tissue)	Amount of MeHg in THg (% w/w)
Scientific name	Local name					
Pangasius pangasius	Pangas	8	3.42 ± 2.47	0.16 ± 0.21	0.12 ± 0.16 min : 0.00; max : 0.41	75.56 ± 10
Wallagu attu	Boal	20	0.93 ± 0.08	1.48 ± 1.01	1.1 ± 0.60 min : 0.15; max : 2.17	62.84 ± 12
Mystus aor	Aor	14	1.19 ± 1.04	0.37 ± 0.20	0.23 ± 0.10 min : 0.14; max : 0.36	51.0 ± 10
Rita rita	Rita	6	0.89 ± 0.25	0.45 ± 0.05	0.34 ± 0.15 min : 0.07; max : 0.47	75.56 ± 4
Ompok pabda	Pabda	6	0.28 ± 0.03	nd[a]	0.26 ± 0.04 min : 0.24; max : 0.29	nd[a]
Clupisoma garua	Gherua	6	0.17 ± 0.18	0.22 ± 0.0	0.10 ± 0.07 min : 0.00; max : 0.1.3	54.54 ± 9
Bagarius bagarius	Bagar	6	0.15 ± 0.07	0.20 ± 0.01	0.12 ± 0.03 min : 0.09; max : 0.14	50.00 ± 10
Chitala chitala	Chital	6	3.32 ± 0.85	0.45 ± 0.33	0.25 ± 0.18 min : 0.05; max : 0.56	55.56 ± 10
Anguilla bengalensis bengalensis	Eel	10	0.40 ± 0.23	2.61 ± 1.02	1.94 ± 2.23 min : 0.15; max : 5.38	74.29 ± 15
Mastacembelus armatus	Baam	9	0.29 ± 0.14	0.54 ± 0.54	0.45 ± 0.46 min : 0.15; max : 1.35	83.33 ± 10
Lates calcarifer	Vetki	6	0.60 ± 0.1	0.35 ± 0.07	0.25 ± 0.02 min : 0.23; max : 0.28	65.71 ± 10

[a]nd– not detected.

and inhabit in a small range for a long time; they may accumulate a lot of toxic substances in even less polluted waters, especially mercury (Hg) and MeHg. Higher level of MeHg in muscle of eel is also sppported by the study of Le *et al.*[16]. The elevated levels of Hg may also relate to the high accumulation rate of Hg from eel's prey due to the energy-storing phase of young eel.

Fig. 1 represents the comparison of methyl mercury concentrations in six randomly selected samples of each fish species. Fig. 1 clearly indicates that in maximum sample of these species cross the safety level of MeHg. Excluding *A. bengalensis bengalensis* and *W. attu*, all the cies through the food chain and then was excreted slowly; hence these species are susceptible to the bioaccumulation and biomagnifications of Hg. The elevated THg concentrations in *W. attu* deserve a closer examination, and are likely due to the catfish's high trophic position, its larger size (suggesting older fish) and its highly piscivorous diet. *C. garua* is essentially an omnivore on the basis of its gut content. Gut analysis include 43.3% of plant matter consisting mainly of the macrophytes and filament algae[18]. Hence bioaccumulation of MeHg is expected to be low which supports the authors result but the lower concentration in *P. pangasius*, *B. bagarius* suggest that not all

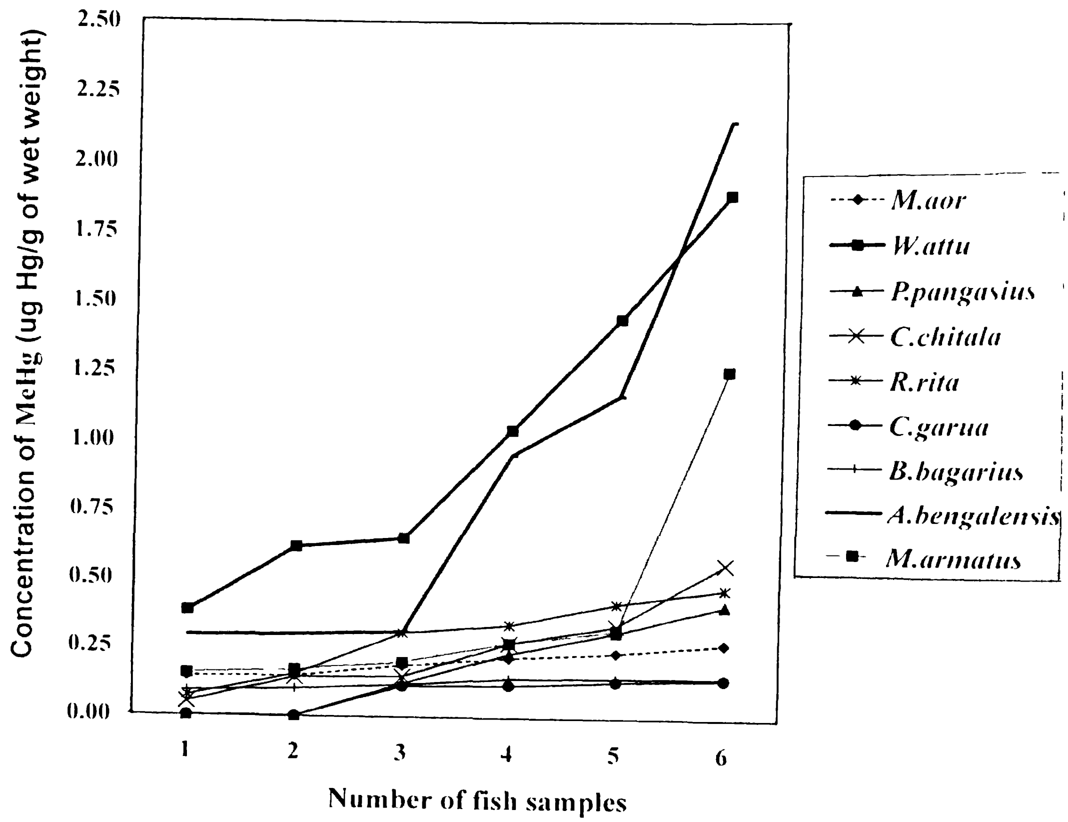

Fig. 1. Comparison of methyl mercury concentrations with international standard (0.25 μg Hg/g of wet weight)[15] for consumption of different carnivorous fish samples contaminated by methyl mercury.

carnivorous fish species examined had total mercury content below the regulatory limit/permissible level of 0.50 μg/g (wet weight of fish) for human consumption recommended by World Health Organization[17]. All the carnivorous fish examined in our study had higher mercury level, thus, our findings supported that the THg as well as MeHg was accumulated in muscle of carnivorous species will have predictable patterns of mercury distribution and bioaccumulation. Percent of MeHg was varied from 50 to 84% of total mercury. Highest value of MeHg in respect to total mercury (in % w/w) percent was observed in *M. armatus* (baan) (8.33 ± 3%) and lowest value of that was shown by *B. bagarius* (bagar) (50.00 ± 10%).

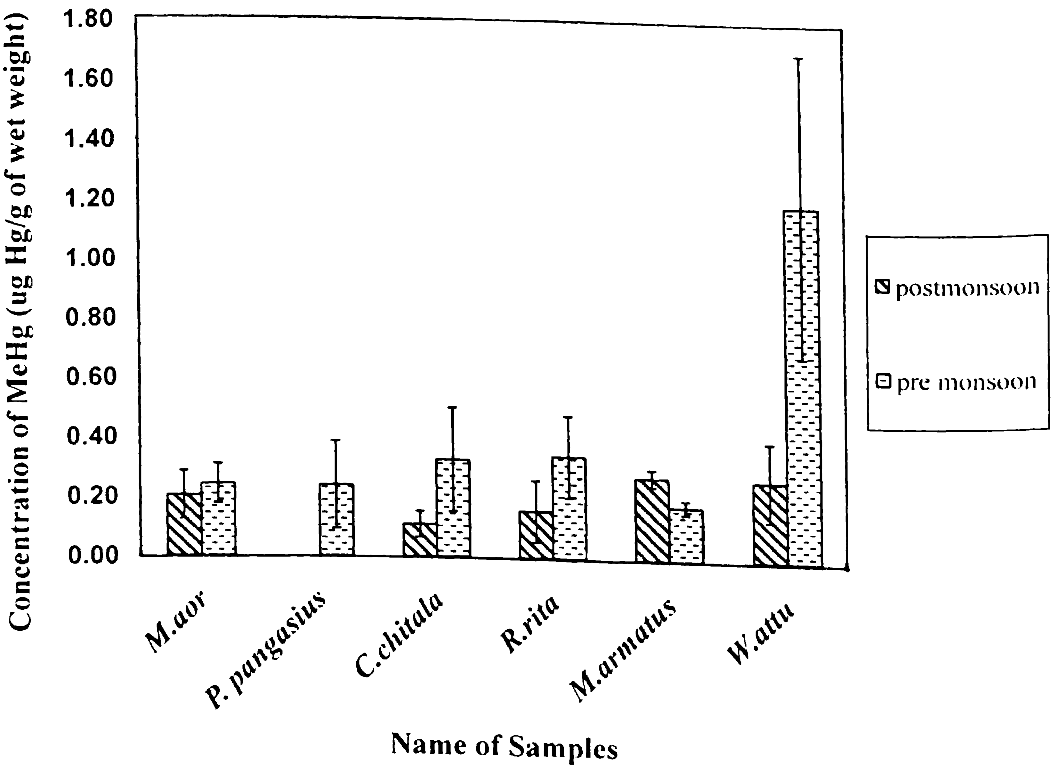

Fig. 2. Seasonal variation of methyl mercury concentration (μg Hg/g of wet weight) in the carnivorous fish species available in both seasons.

Fig. 2 represents the seasonal variation of MeHg contamination among the fish species available in both post- and pre-monsoon reasons. All tested samples accumulated more methyl mercury in pre-monsoon than the post-monsoon season. This result was similar to the finding of Bhattacharyya *et al.*[19] in other fish species. The highest seasonal variation was observed in *P. pangasius* followed by *W. attu*, *R. rita* and *C. chital*. Monsoon is the breeding seasons for all the tropical and sub-tropical fish species[20]. Hence in the monsoon a part of the MeHg accepted by these fish species is transported to the eggs because MeHg is highly lipid soluble and is able to cross the placental barrier and has been detected in cord blood in concentrations greater than those found in maternal blood. It has also been detected in the fetal brain at concentrations 5–7-fold higher than that found in the maternal blood[21]. Monsoon is the breeding season of most of the fish species examine in this study. May be due to this, after laying eggs, in the post-monsoon season the concentration of MeHg was lower than that of pre-monsoon season. Though female duail also exhibits excretion of mercury through egg[22], detailed studies on fish eggs are required to establish this concept. The current study may provide a baseline data for river carnivorous fish. Though till now there had been no incident of mercury toxicity reported from the areas from where the samples wer collected but consumption of all the collected carnivorous fish samples was vulnerable for pregnant women, individuals under 15 years or frequent fish consumers.

Experimental

Sampling :

Chitala chitala (Chital), *Pangasius pangasius* (Pangas), *Rita rita* (Rita), *Anguilla bengalensis bengalensis* (Eel), *Mastacembelus armatus* (Baan), *Lates calcarifer* (Vetki), *Ompok pabda* (Pabda), *Clupisoma garua* (Gherua), *Bagarius bagarius* (Bagar), *Wallagu attu* (Boal) and *Mystus aor* (Aor) fish species were collected from different location of West Bengal, India during the month of March-April, July-August and December-January for 2008-2009 by cooperation with game fishermen. The number of fish species and the number of specimens sampled for each species were limited by availability of fish during the sampling campaign. All the fishes were visually inspected for fin and body deformations to avoid farmed fish in the samples. No stocking programs were known in these systems. The collected fish were packed in ice and brought to laboratory within 2-3 h of catching. Samples were dissected immediately, muscle tissue was collected and soaked on filter paper to remove moisture and weighed and stored at -20 °C for further analysis.

All the reagents and used was procured from Merck India Ltd., Mumbai, India. The standard methyl mercury chloride was procured from Sigma-Aldrich Chemicals Co., St. Luis, USA.

Methods :

Methyl mercury estimation :

The sample preparation was based on acidic digestion followed by the extraction with toluene[23] which was designed for the packed column determination. The combined extracts were left for 1 h in a freezer at 4 °C, and then they were used for MeHg determination on a gas chromatograph (Agilent Technologies, 6890N). A capillary column DB5 (30 m, 0.32 mm) from J &W Scientific and an electron capture detector (ECD) were used. The evaluation was made using HP 3365 Chem Station Series II software. The following heating pattern was used : 2 min 140 °C; gradient 4 °C/min to 160 °C/2 min at 160 °C; Injector temperature 250 °C, detector temperature 250 °C. The sample volume of 2 μL was injected on the column in split less condition. The external standard was used for the calibration.

Total mercury estimation :

The Association of Official Analytical Chemists (AOAC) method[24] was used for digestion of tissues for total mercury analysis. The digested samples were analyzed by the Atomic Absorption Spectrophotometer (A Analyst700+ coupled with FIAS100, Perkin-Elmer) equipped with a cold vapor generation assembly for total mercury estimation.

Statistical analysis :

Results are expressed as mean ±SD. All analyses were carried out in triplicate.

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