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Mercury Concentrations in Commercial Fish Species of Lake Phewa, Nepal

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Abstract Mercury (Hg) concentrations in four commercial fish species (Tilapia *Oreochromis niloticus*, Spiny Eel *Mastacembelus armatus*, African catfish *Clarias gariepinus*, and Sahar *Tor putitora*), were investigated in Lake Phewa, Nepal. Mean values of total mercury (THg mg kg^{-1}, ww) in these fishes were 0.02, 0.07, 0.05, and 0.12 respectively. Methylmercury contributed 82 % of THg. The lowest value was detected in *O. niloticus*, an exclusive plant feeder. The biomagnification rate of Hg through the fish community was 0.041 per $\delta^{15}N$ (‰). The present investigation produced an important baseline data of Hg pollution in the fish community in this region.

Keywords Mercury · Commercial fish · Biomagnification · Lake Phewa

A high correlation exists between atmospheric deposition rate and concentrations of mercury (Hg) in fish (Fjeld and Rognerud 1993). Even the biota from lakes far away from point sources can have high levels of Hg contamination (Chen et al. 2005) which indicates its high bioaccumulation and biomagnification properties. All the biota accumulates Hg mainly in the form of methylmercury (MeHg) and it has been shown that MeHg contributes up to 98 % of Hg in fishes (Carrasco et al. 2011). MeHg is highly neurotoxic (Bloom 1992) with high bioaccumulation and biomagnification properties (Wang et al. 2010); and fish is the main route of MeHg poisoning to humans.

Despite the fact that mercury pollution in fish has attracted considerable attention worldwide, studies related to accumulation of contaminants in biota and their trophic transfer are still lacking in Nepal where subsistence as well as commercial fisheries is gaining popularity. Therefore, the present work was carried out to investigate the concentration of Hg in Lake Phewa, Nepal focusing mainly on the fish community. The present findings not only provide baseline information of Hg contamination, but also shed light on the bioaccumulation properties of this pollutant in commercially important fish species of Lake Phewa, Nepal.

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Materials and Methods

Lake Phewa is situated at an elevation of 782 m approximately at the center of the country map, Nepal. The lake has a surface area of 4.35 km^2 and an average depth of 8.6 m (Gurung et al. 2010) with a maximum depth of 22.5 m. It is a small and warm monomictic lake near a sub-metropolitan city (Pokhara), Nepal.

A total of 122 samples belonging to four species of fish: Tilapia *Oreochromis niloticus*, Spiny Eel *Mastacembelus armatus*, African catfish *Clarias gariepinus* and Sahar *Tor putitora* were identified in situ and collected. Following the measurement of total length (TL, cm) and total weight (TW, g), fillets just behind the dorsal fin (axial muscles) was collected after removal of the skin for the analysis of Hg (both THg and MeHg species), $\delta^{15}N$ and $\delta^{13}C$. Stomach contents were collected and preserved in 70 % ethanol to characterize the dominant diets. The muscle samples were kept in a freezer until they were brought to the laboratory. Samples were analyzed in the laboratory of the Department of Biology, Hong Kong University of Science and Technology (HKUST) for the analysis of THg and MeHg. THg were also analyzed at Environmental Chemistry Section of the Department of Plant and Environmental Sciences, Norwegian University of Life Sciences (UMB).

At HKUST, fish muscle samples were freeze-dried and ground into fine powders. For the analysis of THg, all containers were vigorously cleaned with 4 N HCl. Approximately 0.2 g dried tissues were weighed and digested at 190°C with 'aqua regia' (2 mL HNO_3:6 mL HCl) in a microwave digestion system. An aquilot of digested samples was taken and diluted as appropriate. Bromine monochloride (0.5 % v/v) was added to the diluted sample until stable yellow color was obtained, after which the samples were pre-reduced by addition of $NH_2OH{\cdot}HCl$. THg was quantified using the single gold trap amalgamation technique by Cold Vapour Atomic Fluorescence Spectroscopy (CVAFS, QuickTrace® 8000, USA). At UMB, THg has been analyzed using Flow Injection Mercury System (Perkin-Elmer, model FIMS 400) (details in Sharma et al. 2008).

For analysis of MeHg, approximately 40 mg of tissues were digested with 25 % KOH in methanol at 60°C for 3 h. MeHg in the extract was measured with an automated MeHg analytical system (MERX, Brooks Rand). Briefly, 20–50 μL of extract was buffered with sodium acetate at pH 4.9, and ethylated by sodium tetraethyl borate in a 40 mL Teflon line borate glass bottle. The quantification of MeHg was automatically carried out by the MeHg analyzer with gas chromatographic separation and pyrolysis following atomic fluorescence detection. The recovery of certified reference material (IAEA-436, tuna fish) when used in conjunction with sample batches was between 87.3 % ± 4.2 % for MeHg and 90.5 % ± 3.6 % for THg.

For the analysis of $\delta^{15}N$, samples were burned under high temperature to convert into N_2 and measured for ^{15}N and ^{14}N using elemental analyzer in conjunction with Isotope-ratio mass spectrometry (IR-MS). Similarly, for $\delta^{13}C$, samples were burned under high temperature to convert into CO_2 and measured for ^{13}C and ^{12}C. The $\delta^{15}N$ was reported relative to atmospheric N_2 and $\delta^{13}C$ using global standard reference material (Pee Dee Belemnite or PDB). The accuracies of $\delta^{15}N$ and $\delta^{13}C$ analyses were ±<0.2 ‰ and ±<0.1 ‰, respectively. Details for the sample analysis at UMB for the analysis of $\delta^{15}N$ and $\delta^{13}C$ are described in Sharma et al. (2008).

The $\delta^{15}N$ and $\delta^{13}C$ are used to study the feeding relationships of organisms (McCutchan et al. 2003) where $\delta^{15}N$ indicates the trophic position and $\delta^{13}C$ indicates the carbon source. Stomach contents were analyzed in the Aquatic Ecology Centre at Kathmandu University under dissecting microscope. Mean volume percentages of prey items were measured in all fish species. 52 out of 105 analyzed fish had empty stomachs. A regression of log-transformed THg (logTHg) concentrations against $\delta^{15}N$ values across all fish species was carried out to see if this gives an idea of the quantitative measure of biomagnification rate (Kidd 1998).

Comparisons of Hg concentrations between different fish species were performed by ANCOVA (statistic F) using length as covariate. Spearman Rank Order test was performed to evaluate the correlations. All the analysis were considered statistically significant at $p < 0.05$ unless otherwise stated.

Results and Discussion

Mercury concentrations in all fish samples were <0.31 mg kg^{-1} in the present study (Table 1). Most African freshwater systems sharing at least some fish species with Lake Phewa (e.g., *C. gariepinus, O. niloticus*) also showed low Hg concentrations (Desta et al. 2007; Tadiso et al. 2011). The concentrations of Hg in *C. gariepinus* and *O. niloticus* in Lake Phewa were comparable to the respective species (same length classes) in Lake Awassa (Desta et al. 2007) and Lake Ziway (Tadiso et al. 2011) in Africa. The reason for such a low concentrations of Hg in Lake Phewa could be the absence of local sources of Hg to the lake as also described by Tadiso et al. (2011) for Lake Ziway.

Regarding Hg speciation, earlier studies (e.g., Bloom 1992) indicated that most of the Hg in fish muscles from higher trophic levels is in the form of MeHg. The concentrations of MeHg followed the same trend as THg (Fig. 1a) because there was a significant positive correlation between THg and MeHg in fish muscles ($r^2 = 0.92$, $p < 0.001$; Fig. 1b). In the present study, an average of

Table 1 Mean (± SD) and range of length (cm), weight (g), T-Hg (mg kg^{-1}, ww), Me-Hg (mg kg^{-1}, ww), δ^{15}N (‰), and δ^{13}C (‰) of four commercial fish species from Lake Phewa

Species	n	Length (cm)		Weight (g)		T-Hg (mg kg^{-1}, ww)		Me-Hg (mg kg^{-1}, ww)[a]		δ^{15}N (‰)		δ^{13}C (‰)	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
O. niloticus	30	25.9 ± 5.9	9.5–40	392 ± 258.5	20–1,300	0.02 ± 0.01	0.01–0.08	0.02 ± 0.01	0.01–0.04	6.8 ± 2.1	1.4–11.1	−21.5 ± 2.5	−27.4–(−17.4)
M. armatus	39	32.9 ± 6.8	21–54	83.1 ± 48.3	34.4–290	0.07 ± 0.04	0.02–0.22	0.05 ± 0.02	0.02–0.08	9.9 ± 1.5	6.9–13.4	−21.8 ± 1.98	−29.6–(−17)
C. gariepinus	39	30.1 ± 16.3	17–95	511.1 ± 1,373.9	30–6,500	0.05 ± 0.05	0.01–0.31	0.03 ± 0.01	0.01–0.08	8 ± 1.6	5.6–12.3	−24.9 ± 2.8	−31.8–(−18.4)
T. putitora	14	42 ± 12	30–72	769.3 ± 770.6	200–3,000	0.12 ± 0.05	0.05–0.21	–	–	7.4 ± 1	5.5–9.4	−23.9 ± 2.9	−29.6–(−17.6)

[a] The sample sizes for MeHg analysis were different for different species: *O. niloticus* (n = 20), *M. armatus* (n = 19), *C. gariepinus* (n = 19)

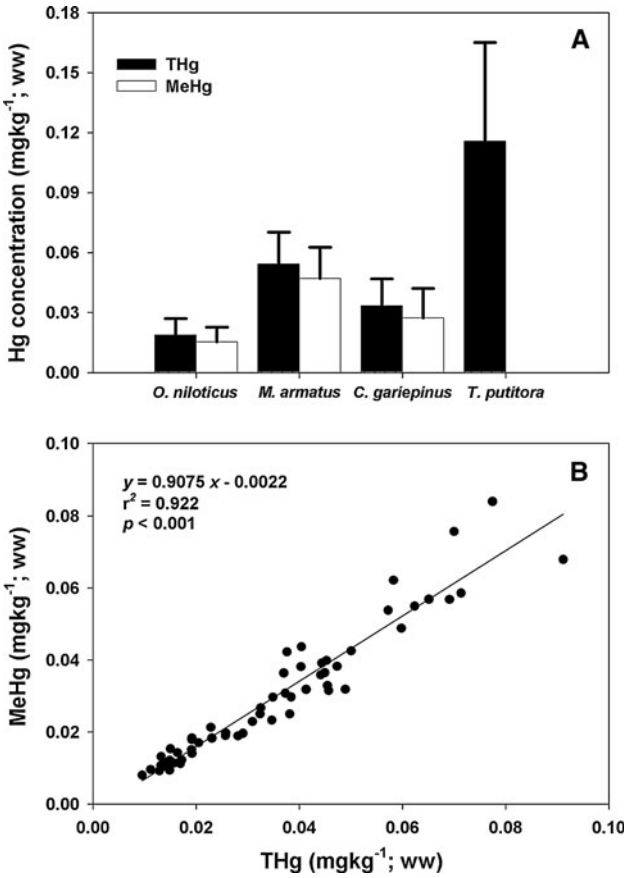

Fig. 1 **a** Comparison of Hg concentrations (both THg and MeHg; mg kg^{-1}, ww) in four species of fish (both in *O. niloticus*, *M. armatus, C. gariepinus* and only THg in *T. putitora*) from Lake Phewa; **b** regression between THg and MeHg in the fish community shows their relationships

82 % of THg was found in the form of MeHg in fish fillets. Some recent studies indicated varied ranges (50 %–98 %) of MeHg in different fish species (e.g., Carrasco et al. 2011).

Mercury concentrations and body length showed significant positive correlations in *C. gariepinus* ($r^2 = 0.74$; $p < 0.001$) and *M. armatus* ($r^2 = 0.64$; $p < 0.001$) but not in other two species (Table 2). In general, there are significant positive relations between fish size and Hg concentrations in their muscle tissues (Gewurtz et al. 2011). Increasing trend (some significant and some non-significant) of Hg concentrations with body size in *C. gariepinus* have been reported in African lakes (Desta et al. 2007; Tadiso et al. 2011). In their studies, the normal diet of large size-classes of *C. gariepinus* included organisms from higher trophic levels, e.g. fish. The Hg concentrations in *C. gariepinus* in the present study showed a significantly increasing trend (Table 2) with the body size (TL). The diet composition of the larger individuals of this species was also dominated by the organisms from higher trophic

Table 2 The relationship between mercury concentrations (mg kg^{-1}, ww) and stable isotope ratio of nitrogen ($\delta^{15}N$) for all fishes from Lake Phewa was calculated

Species	Regression	n	Intercept	Slope	r^2	*p* value
All	LogT-Hg versus $\delta^{15}N$	119	−1.71	0.041	0.07	**0.004**
O. niloticus	LogT-Hg versus TL	30	−1.87	0.01	0.03	0.383
	LogT-Hg versus TW	30	−1.78	0.00	0.042	0.278
	LogT-Hg versus $\delta^{15}N$	30	−1.73	0.003	0.00	0.877
	$\delta^{15}N$ versus TL	30	5.11	0.065	0.034	0.33
	$\delta^{13}C$ versus TL	30	−22.53	−0.038	0.01	0.644
M. armatus	LogT-Hg versus TL	39	−1.8	0.017	0.34	**<0.001**
	LogT-Hg versus TW	39	−1.4	0.003	0.36	**<0.001**
	LogT-Hg versus $\delta^{15}N$	36	−1.4	−0.021	0.02	0.388
	$\delta^{15}N$ versus TL	36	7.57	0.07	0.095	0.068
	$\delta^{13}C$ versus TL	36	−22.3	0.01	0.002	0.808
C. gariepinus	LogT-Hg versus TL	39	−1.65	0.01	0.34	**<0.001**
	LogT-Hg versus TW	39	−1.43	0.001	0.38	**<0.001**
	LogT-Hg versus $\delta^{15}N$	39	−1.4	0.004	0.001	0.885
	$\delta^{15}N$ versus TL	39	8.52	−0.016	0.028	0.308
	$\delta^{13}C$ versus TL	39	−22.1	−0.094	0.29	**<0.001**
Tor putitora	LogT-Hg versus TL	14	−1.24	0.006	0.16	0.163
	LogT-Hg versus TW	14	−1.05	0.00	0.18	0.136
	LogT-Hg versus $\delta^{15}N$	14	−0.58	−0.054	0.08	0.333
	$\delta^{15}N$ versus TL	14	8.47	−0.026	0.097	0.278
	$\delta^{13}C$ versus TL	14	−25.29	0.033	0.02	0.645

Regression of mercury concentrations against total length (TL, cm), total weight (TW, g), and $\delta^{15}N$ were analyzed for individual fish species. Also included in the table are regressions of $\delta^{15}N$ and $\delta^{13}C$ against total length of individual species from Lake Phewa. The sample size (n), intercept, slope, r^2, and *p* values are given for each regression

Significant results at $p < 0.05$ are written in bold

positions (Fig. 2a). It has been recorded that the proportions of prey-fish in the diets of *C. gariepinus* increased as they grew in size (Fig. 2a, b) as also indicated by Desta et al. (2007) in African lakes. This phenomenon further justifies that the diet plays an important role in the increment of the mercury in fish flesh.

There was a significantly lower concentration of THg detected in *O. niloticus* compared to *T. tor* ($F = 9.974$; $df = 1,117$; $p < 0.001$), whereas no statistically significant difference was detected between *C. gariepinus* and *M. armatus* (Fig. 1a). The predator fish species in Lake Phewa, e.g. *T. putitora*, *M. armatus* and *C. gariepinus* had higher concentrations of Hg in only one individual from each species. The individuals of *M. armatus* showed significant correlations between Hg concentrations and body size ($r^2 = 0.34$; $p < 0.001$; Table 2). Both small and large size classes of this species had prey-fish in their stomach contents (Fig. 2a, b). It has been demonstrated that the dominant pathway of overall mercury accumulation in *O. niloticus* is its dietary exposure (Wang et al. 2010). Major proportion of diets from the lower trophic levels, such as plant parts, is one of the major factors for such a low concentrations of mercury in *C. gariepinus* and *O. niloticus* (Tadiso et al. 2011). The diet of *T. putitora* contained the highest percentage of fish followed by *M. armatus* (Fig. 2a, b). The aquatic plants were the only diet of large *O. niloticus*, whereas small individuals had a large proportion of insect larvae. *C. gariepinus* mainly fed on insect larvae irrespective of the size classes (Fig. 2a, b).

The relative trophic position, based on $\delta^{15}N$, indicated that *M. armatus* occupied the higher position whereas *O. niloticus* the lower (Fig. 3). The lowest $\delta^{15}N$ was found for *O. niloticus* (1.35) and the highest in *M. armatus* (11.93). This indicates a food chain of about 3–4 trophic levels. All the individuals from the lowest trophic position (as indicated by the stomach contents as well as the $\delta^{15}N$ signatures), *O. niloticus*, had plants as their dominant diet. The stomach contents of *M. armatus*, the species at the highest trophic position based on $\delta^{15}N$ signatures, were dominated by animal diets (e.g., insects and prey-fish). In addition, $\delta^{13}C$ signature of *M. armatus* did not indicate a shift in food source as they grow in size (Table 2). *T. putitora*, having the highest Hg concentrations, occupy the intermediate trophic position among the four species. This

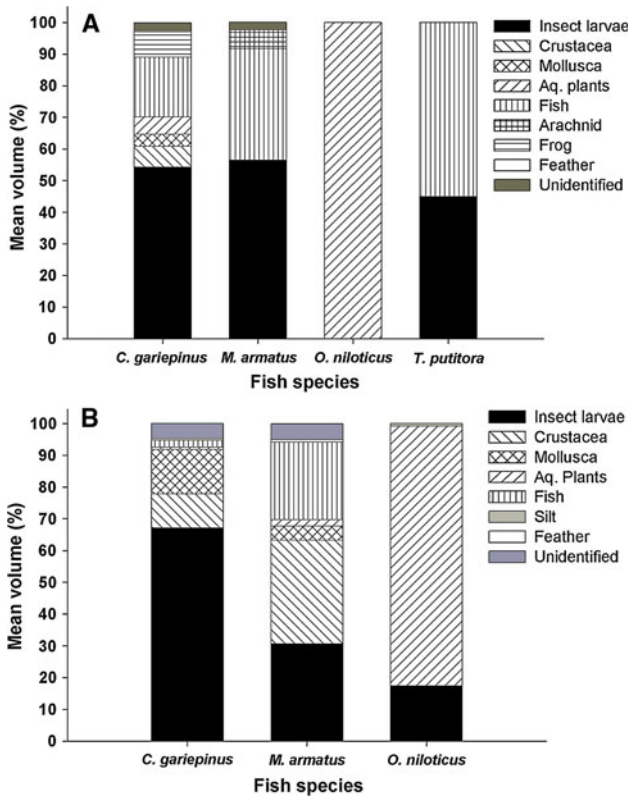

Fig. 2 **a** Mean volume (%) of diet composition (stomach contents) of large-size class of *C. gariepinus, M. armatus, O. niloticus*, and *T. putitora*. **b** Mean volume (%) of diet composition (stomach contents) of small size-class of *C. gariepinus, M. armatus,* and *O. niloticus*

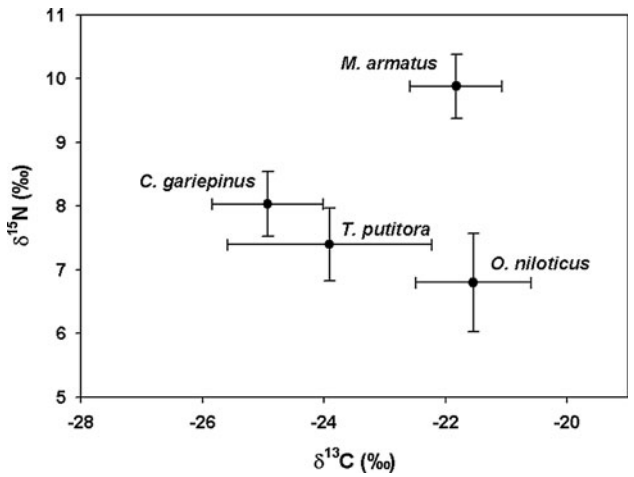

Fig. 3 Simple food web structure based on the relationships between $\delta^{15}N$ and $\delta^{13}C$ in four fish species (*O. niloticus*, *M. armatus, C. gariepinus* and *T. putitora*) from Lake Phewa. Ranges of *error bars* indicate 95 % confidence interval from the mean; *vertical bars* for $\delta^{15}N$ and *horizontal bars* for $\delta^{13}C$ values

could be mainly due to the fact that only large individuals were represented in the present investigation. Further study is necessary for this species to find out the dynamics of Hg in all size-classes.

The Hg levels in *O. niloticus* were found to decline with the increase in size in some African lakes (Desta et al. 2007; Tadiso et al. 2011) possibly due to shift in their diet; younger individuals feeding on zooplankton and switching to plant materials when they grew larger (Desta et al. 2007). The present investigation also found that there was a shift in diets in this species from ⟨mixture of insect larvae and plants⟩ to ⟨plants only⟩ when they grew in size (Fig. 2a ,b). This species did not show an increasing tendency of Hg concentrations with body size in the present investigation. This could mainly be due to the fact that *O. niloticus* fed mainly on plant materials as also indicated in the study conducted by Zengeya et al. (2011).

An effort to regress the logTHg versus $\delta^{15}N$ produced an equation with slope 0.041 per ‰ $\delta^{15}N$ ($p < 0.001$; Table 2), however the food chain seems to have no direct feeding relationship among the fish community (Fig. 3). The resource partitioning was observed in this fish community with depletion of carbon in *C. gariepinus* and *T. putitora* compared to *O. niloticus* and *M. armatus* (Fig. 3). Our study also showed that *T. putitora*, although had higher concentration of Hg and fish contents in the diet, occupied the lower position in the food chain. This warrants the investigation of more fish that can act as a bridge between the investigated fish community. Hence, the slope of this regression should not be interpreted as the bio magnification rate of the fish community in this case.

$$\log\mathrm{THg}(\mathrm{mg\,kg^{-1}}) = -1.702 + 0.041\delta^{15}\mathrm{N}(‰).$$

Fishes of Lake Phewa have shown low concentrations of Hg although water and sediments contain comparable Hg concentrations (own unpublished data) to other uncontaminated freshwaters around the globe (Ullrich et al. 2001). Here, diets of these individual could explain the Hg burden in muscle tissues (Ullrich et al. 2001), and warrants a detailed study including the entire food web.

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