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Mercury in alpine fish from four rivers in the Tibetan Plateau

Junjuan Shao¹, Jianbo Shi^{1,2,*}, Bu Duo³, Chengbin Liu¹, Yan Gao¹, Jianjie Fu¹,
Ruiqiang Yang¹, Guibin Jiang¹

1. State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

2. College of Resources and Environment, University of Chinese Academy of Sciences, Beijing 100049, China

3. Science Faculty, Tibet University, Lhasa 850000, China

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ABSTRACT

As a global pollutant, high levels of mercury (Hg) have been found in remote ecosystem due to the long range atmospheric transport. In this study, a total of 60 fish samples were collected from four rivers across the Tibetan Plateau to study the accumulation of Hg in remote and high-altitude aquatic environment. The total Hg (THg) and methylmercury (MeHg) in fish muscles ranged from 11 to 2097 ng/g dry weight (dw) (average: 819 ng/g dw) and from 14 to 1960 ng/g dw (average: 756 ng/g dw), respectively. Significantly positive linear relationships were observed between the THg ($r = 0.591$, $p < 0.01$, $n = 36$) and MeHg concentrations ($r = 0.473$, $p < 0.01$, $n = 36$) with the trophic level of fish from Lhasa River, suggesting trophic transfer and biomagnification of Hg in this aquatic ecosystem. Moreover, the THg levels in fish had significantly positive correlations with the length ($r = 0.316$, $p < 0.05$, $n = 60$) and weight ($r = 0.271$, $p < 0.05$, $n = 60$) of fish. The high levels of Hg were attributed to the slow growth and long lifespan of the fish under this sterile and cold environment. Risk assessment revealed that the consumption of *Oxygymnocypris stewartii*, *Schizothorax macropogon*, *Schizothorax waltoni*, *Schizopygopsis younghusbandi* and *Schizothorax o'connori* would lead to a high exposure to MeHg.

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Introduction

Mercury (Hg) is a toxic and global pollutant. Hg in the environment majorly comes from two sources, natural sources and anthropogenic sources (Jiang et al., 2006). Natural emissions mainly originate from volcanoes (Zambardi et al., 2009), geothermal activity (Gustin et al., 2008) and emissions from soil and water. Mining, smelting, coal combustion and industrial usage of Hg in chlor-alkali production, batteries, fluorescent lamps, medical devices and so on comprise anthropogenic sources (Pirrone et al., 2010; Streets et al., 2011). Due to the long

lifetime of Hg in atmosphere and the ability of long range transport, Hg emitted from both natural sources and anthropogenic sources can be deposited into remote lakes and rivers, resulting in serious pollution in aquatic system (Fitzgerald et al., 1998; Miller et al., 2005; Poissant et al., 2008).

The Tibetan Plateau has an average elevation of 4000 m above sea level. The population in the Tibet Autonomous Region was 2.21 million in 1990 and increased to 3.00 million in 2010 (Yu et al., 2012). The total area of the Tibetan Plateau is more than 1.22 million square kilometers including 64.57% farmland, 0.05% construction land and 35.38% unused land

* Corresponding author. E-mail: jbshi@rcees.ac.cn (Jianbo Shi).

(Liao et al., 2008). The geological substrate in the Tibetan Plateau is mainly igneous rock (Ma, 1998). The vegetation on the plateau is the high-cold meadow, steppe and desert vegetation (Zhang, 1978). The Tibetan Plateau locates in Asia where the world's largest anthropogenic Hg emission source exists. In 2008, the global Hg emissions were 2000 tons and Asia was responsible for 64% of the total emissions (1280 tons) (Streets et al., 2011). The total anthropogenic Hg emissions in China were estimated at 609 tons in 2007 (Pirrone et al., 2010). Though local industrial activities in the Tibetan Plateau were rare, the long range atmospheric transport of Hg had resulted in Hg pollution in this plateau (Huang et al., 2012; Loewen et al., 2007; Yang et al., 2010). The backward air mass trajectory analysis suggested that dust storm containing particulate Hg originated in Iran and Afghanistan and finally reached Mount Nyainqentanglha in the Tibetan Plateau after traveling over thousands of kilometers (Loewen et al., 2007). Some trace metals (Cr, Ni, Cu, Zn, As) in aerosols from the Nam Co region, which is located in central Tibet and covering an area of 1980 km² at an elevation of 4718 m, might be from South Asia over the long range atmospheric transport (Cong et al., 2007).

Some studies have investigated the Hg contamination in the Tibetan Plateau. The results showed that the Hg concentrations ranged from 1.46 to 4.99 ng/L in water from Yarlung Zangbo River (Zheng et al., 2010). The average of total gaseous mercury concentrations in ambient air in the eastern slope of Mt. Gongga, south-eastern fringe of the Tibetan Plateau, was 3.98 ng/m³ (from 0.52 to 21.03 ng/m³) (Fu et al., 2008). Surprisingly, the maximum concentrations of total mercury (THg) and methylmercury (MeHg) in fish from lakes of the Tibetan Plateau reached 2384 and 1610 ng/g, respectively (Yang et al., 2011). However, there are still some questions remaining unclear about the bioaccumulation and biomagnification of Hg in the aquatic ecosystem in the Tibetan Plateau.

The toxicity, environmental behavior and bioavailability of Hg depend not only on its total concentration but also its species. As one of the organic mercurial species, MeHg is the most toxic. Due to its lipophilicity, MeHg can penetrate the blood-brain barrier and finally harm central nervous system (Cheng et al., 2005, 2015; Clarkson and Magos, 2006; Winship, 1986). MeHg constitutes more than 80% of THg in fish muscle (Akagi et al., 1995; Bloom, 1992; Campbell et al., 2005; Guentzel et al., 2007; Mohan et al., 2012). Consumption of fish with elevated MeHg is the principal pathway of human exposure to Hg (Liang et al., 2013; Tang et al., 2015). Hence, health risk assessment on MeHg through the consumption of fish is imperative and important.

The aim of this study was to investigate the pollution status of THg and MeHg in fish samples from rivers in the Tibetan Plateau, study the trophic transfer behavior of Hg in remote alpine aquatic ecosystem and further evaluate potential health risk posed via fish consumption.

1. Materials and methods

1.1. Sample collection

The map of the sampling area and the locations of sampling sites are shown in Fig. 1. A total of 60 fish samples were collected from four rivers (Yarlung Zangbo River (YZR), Lhasa

Fig. 1 – The map of the study area.

River, Salween and Niyang River) in the Tibetan Plateau in August of 2010, 2011 and 2012. YZR, the longest alpine river in China, is regarded as the mother river by Tibetan. As the largest branch of the YZR, the Lhasa River originates from Nyainqentanglha Mountain on the Tibetan Plateau and flows through Lhasa city, the provincial capital of the Tibet Autonomous Region. Salween originates from Tanggula Mountains. The Niyang River is also one branch of the YZR, which originates from the Mila Mountain and finally goes into the YZR in Nyingchi.

All fish species are unique in the Tibetan Plateau and these species belong to the family of Cyprinidae and subfamily of Schizothoracinae. Precautions were taken in order to avoid any contamination or loss during sampling. After collected, the fish samples were carried back to the laboratory as soon as possible in polyethylene zippered bags in boxes with ice. They were stored at –20°C before dissection. The dorsal muscles (50 g) were dissected from each fish using clean stainless steel scalpel after the length and weight were measured. The fish samples were freeze-dried, grounded and then stored at –20°C until analyzing.

1.2. Determination of THg, MeHg and trophic level

The concentrations of THg in fish samples were determined directly with a Hydra II-C atomic absorption spectrometry (Leeman Labs, Hudson, USA). Briefly, about 0.03 g of grounded fish samples was weighed in a nickel boat. A certain amount of diatomite was covered on the sample to prevent the lipid from entering the instrument. The sample was burned at high temperature and all forms of mercury were reduced to elemental mercury. Then, the elemental mercury was brought and trapped onto a gold amalgam. After decomposition from the gold amalgam, the concentrations of THg were determined.

For MeHg analysis, alkaline extraction was applied (Meng et al., 2014, 2015). About 0.1 g of sample was weighed into an 8-mL brown glass bottle and 4 mL 25% (M/V) KOH/CH₃OH solution was added. The bottle was then mechanically shaken for 4 hr at 37°C to achieve complete extraction. The solution was centrifuged at 3000 r/min for 10 min after cooling down. Before being analyzed, each sample was diluted tenfold. Then, 30 µL of diluents was injected into the auto sampler vial and ethylated by sodium tetraethylborate (NaBEt₄). The contents of MeHg were determined by purge and trap gas chromatography–atomic fluorescence spectrometry (GC–AFS) method

Table 1 – Results of total mercury (THg) and methylmercury (MeHg) in certified reference materials (CRMs) (n = 5).

CRMs	THg (ng/g)		MeHg (ng/g)	
	Certified	Determined	Certified	Determined
TORT-2	270 ± 60	276 ± 6	152 ± 13	170 ± 4
DORM-4	410 ± 55	412 ± 31	354 ± 31	356 ± 1

with a MERX Automatic Methylmercury System (Brooks Rand Laboratories, USA).

Stable nitrogen isotope ratio (R, the isotopic abundance ratio of ^{15}N and ^{14}N) was determined by a DELTA V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, USA). Then, the isotopic composition ($\delta^{15}\text{N}$, ‰) was calculated using the following formula:

$$\delta^{15}\text{N} = ((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000\text{‰} \quad (1)$$

The trophic levels (TLs) of fish were calculated based on the nitrogen stable isotope analysis using the following formula (Fisk et al., 2001; Yuan et al., 2012):

$$\text{TL} = (\delta^{15}\text{N}_{\text{fish}} - \delta^{15}\text{N}_{\text{zooplankton}}) / 3.8 + 2 \quad (2)$$

The range of TLs was 2.60–4.12 and the average of the TLs was 3.22.

1.3. Quality assurance/quality control

Reagent blanks, certified reference materials (CRMs) and sample replicates were randomly applied in the analysis for the quality control. The determined concentrations of THg and MeHg in two CRMs (TORT-2 and DORM-4) were in good agreement with the certified values, indicating that the method was accurate and reliable (Table 1).

1.4. Statistical analysis

Multiple statistical analysis methods were used with SPSS 20.0 to find the distribution patterns of THg and MeHg in fish from the Tibetan Plateau. One-way ANOVA analysis was

applied to compare the THg concentrations in different fish species from the Lhasa River. Correlation Analysis was applied to find the correlations among the THg and MeHg contents and the length, weight and TLs of the fish.

2. Results and discussion

2.1. Concentrations of THg and MeHg in fish

Concentrations of THg and MeHg and related information of the samples are listed in Table 2. The THg concentrations in fish collected in this work ranged from 11 to 2097 ng/g (dw) with an average of 819 ng/g (Table 3). The lowest and the highest THg concentrations were found in the *Silurus asotus* from Salween and *Oxygymnocypris stewartii* from YZR, respectively. The concentrations of MeHg ranged from 14 to 1960 ng/g dw with an average of 756 ng/g dw. The mean ratio of MeHg in THg was 95.6%. Therefore, MeHg showed a significantly positive correlation ($r = 0.979$, $p < 0.01$, $n = 60$) with THg.

The concentrations of THg and MeHg in fish in this work were comparable with those values in previous studies (Yang et al., 2011, 2013; Zhang et al., 2014) conducted in the Tibetan Plateau. The THg concentrations in fish from French Pyrénées mountain lakes were also in the same order of magnitude with those in the Tibetan Plateau (Blais et al., 2006). Surprisingly, the average value of THg in fish from this pristine Tibetan Plateau was even higher than that in Hg-contaminated reservoir in Guizhou, China, where the concentrations of total Hg in fish tissue were in the range of 20–1270 ng/g dry weight (with an average of 265 ng/g) (Liu et al., 2012).

2.2. Accumulation of Hg in fish

THg and MeHg can biomagnify in fish through the food chain. The trophic levels (TLs) of the fish were calculated based on the nitrogen stable isotope analysis. The calculated TLs for all fish in the present study were in the range 2.60–4.12. In order to explore the biomagnification of Hg in fish from the Tibetan Plateau, the correlation analysis between Hg concentration (including THg

Table 2 – Fish sample information and average concentrations of THg and MeHg in fish muscles from rivers of Tibetan Plateau.

Sites	Species	Number	Length (cm)	Weight (g)	Trophic Level	Lipid (%)	THg (ng/g, dw)	MeHg (ng/g, dw)
Lhasa River	<i>Oxygymnocypris stewartii</i>	13	36.7	433.8	3.65	13.64	1136	1031
	<i>Schizothorax macropogon</i>	6	29.5	260.9	3.01	9.65	941	929
	<i>Schizothorax o'connori</i>	6	34.5	361.1	2.95	13.98	316	281
	<i>Schizopygopsis younghusbandi</i>	5	31.8	269.9	2.75	14.01	416	390
	<i>Schizothorax waltoni</i>	6	33.4	333.4	3.29	15.64	1461	1331
Salween	<i>Silurus asotus</i>	4	18.8	91.1	2.80	–	20	22
Niyang River	<i>Schizothorax o'connori</i>	4	40.3	594.1	3.20	7.62	416	364
	<i>Schizothorax waltoni</i>	3	22.7	157.2	3.03	12.54	279	276
	<i>Schizothorax macropogon</i>	2	25.5	274.3	3.13	6.97	662	649
	<i>Oxygymnocypris stewartii</i>	1	34.0	401.0	3.82	11.43	1344	1158
Yarlung Zangbo	<i>Schizopygopsis younghusbandi</i>	5	29.4	222.0	3.42	–	1150	1033
	<i>Schizothorax macropogon</i>	2	30.5	348.4	3.13	14.40	877	877
	<i>Oxygymnocypris stewartii</i>	1	32.0	365.8	3.65	15.55	1826	1961
	<i>Schizothorax o'connori</i>	1	28.0	378.4	3.22	6.96	227	258
	<i>Schizothorax waltoni</i>	1	32.0	396.1	3.33	15.44	1189	1367

Table 3 – Comparison of THg concentrations in fish muscle from other remote areas.

Study sites	THg (ng/g, dw)	Number	Years	Reference
Rivers and lakes, Tibetan Plateau	502.5 (125.5–6090) ^a	166	2009, 2010	Zhang et al. (2014)
Niyang river, Tibetan Plateau	129 (85–217)	8	2007	Yang et al. (2013)
Lakes, Tibetan Plateau	145 (243–2384)	62	2006–2007	Yang et al. (2011)
Stewart lake, sub-Arctic	350–2950 ^a	244	1999	Power et al. (2002)
Lakes in Pyrénées, French	1515 ± 935 ^a	37	2001	Blais et al. (2006)
Lancaster sound, Arctic	190–240	13	1992	Atwell et al. (1998)
Admiralty bay, Antarctica	16.0–16.3	11	2004	Dos et al. (2006)
Svalbard, Arctic	n.d. ^b –100 ^a	30	2006	Jæger et al. (2009)
Terra Nova bay, Antarctica	92.6–907.3	6	2006	Maggi et al. (2009)
Rivers, Tibetan Plateau	819 (11–2097)	60	2010–2012	This study

^a The concentrations (ng/g, wet weight) were converted to (ng/g, dry weight) based on the water content in muscle of 80%.

^b Not detected.

and MeHg) and TLs were performed. The correlation analysis was only performed among the data from Lhasa River, due to the limited number of samples and fish species in other three rivers. Significantly positive linear relationships were found between THg and TL ($r = 0.591$, $p < 0.01$, $n = 36$), MeHg and TL ($r = 0.473$, $p < 0.01$, $n = 36$) (Fig. 2), suggesting trophic transfer and biomagnification of Hg in fish from Lhasa River. Similar results were also observed for Hg in Arctic marine food chain (Atwell et al., 1998; Campbell et al., 2005), sub-Arctic lake (Power et al., 2002) and in Lake Murray, Papua New Guinea (Bowles et al., 2001). Contents of THg and MeHg in fish from Lhasa River decreased on the descending order: *Schizothorax waltoni* > *Schizothorax macropogon* and *O. stewartii* > *Schizopygopsis younghusbandi* and *Schizothorax o'connori* (one-way ANOVA analysis, $p < 0.05$) (Fig. 3). Overall, the contents of THg and MeHg in the muscles increased along with the trophic levels.

Trophic magnification factors (TMFs) have been applied to determine the biomagnification of pollutant and the extent of biomagnification, which were calculated as the antilogarithm of the slope of the linear regression of the log-transformed concentration on TLs (Foster et al., 2012; Jæger et al., 2009). Normally, TMF higher than 1 was considered occurring in the biomagnification. In this work, the TMF for THg was 2.64 and for MeHg was 2.72, suggesting that both THg and MeHg could be biomagnified in fish from the Lhasa River. The TMFs for

THg and MeHg in this study were lower than those in an Arctic marine ecosystem in Svalbard (Jæger et al., 2009). Moreover, the TMF for MeHg was higher than that for THg, indicating that Hg was biomagnified mainly in the form of MeHg.

Fish length has been suggested as an important factor influencing fish Hg levels (Kamman et al., 2005; Matthew et al., 2010). In this study, fish length had a significantly positive correlation ($r = 0.316$, $p < 0.05$, $n = 60$) with THg concentrations in fish muscle tissues. This result was consistent with the previous reports of other studies ($r = 0.48$, $p < 0.05$, $n = 23$, Farias et al., 2005; $r = 0.8459$, $p < 0.0001$, $n = 58$, Farkas et al., 2003). Moreover, significantly positive linear relationships ($r = 0.271$, $p < 0.05$, $n = 60$) were also found between THg concentrations and fish weight, indicating that weight partly influenced THg contents in fish.

2.3. Risk of MeHg in fish

The Joint FAO/WHO Expert Committee on Food Additives established provisional tolerable weekly intakes (PTWIs) for MeHg at 1.6 µg/kg body weight (WHO, 2010).

The consumption rate was 24.8 g/day according to the surveying results of the national diet and nutrition in China in 2002 (Zhai and Yang, 2006). The body weight for adults was assumed to be 60 kg. The following formula (USEPA, United

Fig. 2 – Correlations between THg and MeHg concentrations and trophic level (TL) for fish from Lhasa River.

Fig. 3 – Boxplots of THg and MeHg in different fish species from Lhasa River.

State Environmental Protection Agency, 2000) was used to estimate the daily intakes of MeHg via the consumption of fish:

$$\text{Estimated daily intake (EDI)} = \text{fish consumption (g/day)} \times \text{MeHg concentrations}(\mu\text{g/g})/\text{average body weight (kg)} \quad (3)$$

The estimated weekly intake (EWI) was calculated from the formula:

$$\text{EWI} = \text{EDI} \times 7 \quad (4)$$

The results of the EWIs of MeHg are shown in Fig. 4. All EWIs of the *O. stewartii* and *S. macropogon* exceeded the PTWI of MeHg.

Fig. 4 – Estimated weekly intakes (EWIs) of MeHg through consumption of fish by adults. PTWI: provisional tolerable weekly intakes for MeHg (1.6 $\mu\text{g/kg}$ body weight by the Joint FAO/WHO Expert Committee on Food Additives).

Consumption of 70% of the *S. waltoni* and *S. younghusbandi*, 9% of the *S. o'connori* would result in the EWI of MeHg exceeding the PTWI (1.6 $\mu\text{g/kg}$ body weight) for adults. The EWIs of MeHg for adults for all *S. asotus* were lower than the PTWI value. The results suggested that consumption of some species of fish from the cold and high-altitude Tibetan Plateau would lead to a high exposure to MeHg.

3. Conclusion

The bioaccumulation of Hg in remote and high altitude aquatic ecosystem in the Tibetan Plateau was studied. The concentrations of THg and MeHg in fish from rivers in the Tibetan Plateau were relatively high. The MeHg concentrations were significantly positively correlated with trophic levels (TLs), while THg concentrations were significantly positively correlated with TLs, the length and weight of fish. The results of this study indicated that the consumption of *O. stewartii*, *S. macropogon*, *S. waltoni*, *S. younghusbandi* and *S. o'connori* in this area might impose health risk.

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