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Methyl mercury concentrations in seafood collected from Zhoushan Islands, Zhejiang, China, and their potential health risk for the fishing community Capsule: Methyl mercury in seafood causes potential health risk

Xinwei Yu[a,b], Sardar Khan[c,d,e,*], Anwarzeb Khan[e,h], Yuting Tang[f], Luis M. Nunes[c,d,g], Jianbo Yan[b], Xingqian Ye[a,*], Gang Li[c,d,*]

[a] College of Biosystems Engineering and Food Science, Zhejiang University, Fuli Institute of Food Science, Zhejiang Key Laboratory for Agro-Food Processing, Zhejiang R & D Center for Food Technology and Equipment, Hangzhou 310058, China
[b] Key Laboratory of Health Risk Factors for Seafood of Zhejiang Province, Zhoushan Municipal Center For Disease Control and Prevention, Zhoushan 316021, China
[c] CAS Key Laboratory of Urban Environment and Health, Fujian Key Laboratory of Watershed Ecology, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
[d] Zhejiang Key Laboratory of Urban Environmental Processes and Pollution Control, Ningbo Urban Environment Observation and Research Station, Chinese Academy of Sciences, Ningbo 315800, China
[e] Department of Environmental Science, University of Peshawar, Peshawar 25120, Pakistan
[f] School of Geographical Sciences, Faculty of Science and Engineering, The University of Nottingham, Ningbo 315100, China
[g] Faculty of Sciences and Technology, Civil Engineering Research and Innovation for Sustainability Center, University of Algarve, Faro, Portugal
[h] Department of Environmental and Conservation Sciences, University of Swat, Swat 19130, Pakistan

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ABSTRACT

Seafood is an important exposure route for mercury, especially methyl mercury (MeHg). Therefore, we quantified MeHg concentrations in 69 species of seafood including fish, crustaceans and mollusks collected from Zhoushan Islands, China. MeHg concentrations ranged from < 0.0020–0.2098 μg/g and did not exceed the threshold limit of 1 μg/g in all sampled species, However, MeHg concentrations significantly differed among fish species (0.0085–0.2098 mg kg^{-1}), crustaceans (< 0.002–0.0221 mg kg^{-1}) and mollusks (< 0.002–0.1389 mg kg^{-1}). The trophic magnification factor (TMF) was determined on the basis of the trophic level (TL). The TL values for fish, crustaceans and mollusks were above 3 when the TMF values were > 1. The daily dietary intake and hazard quotient for MeHg were calculated to estimate exposure and health risk through seafood consumption by local inhabitants. The calculated HQ was lower than 1, thus indicating that the exposure was below the risk threshold of related chronic diseases. However, higher MeHg concentrations in fish species such as *Scoliodon sorrakowah* and *Auxis thazard* are concerning and may pose health risk through continuous consumption by local inhabitants.

1. Introduction

Mercury (Hg) is a well-known biological, chemical and geologically active element. It is one of the most toxic and harmful pollutants in the marine environment, and it can have deleterious effects on human health even at very low concentrations (Al-Ansari et al., 2017; Monastero et al., 2017).

Hg may present in the environment in the form of organic Hg, such as methyl Hg (MeHg) and dimethyl Hg (DMeHg), or inorganic Hg, including elemental Hg (Hg0) and oxidized Hg (HgII). MeHg is considered the most important form, owing to its toxicological properties and abundance in food (Sevillano-Morales et al., 2015), particularly in fish and other seafood. The methylated form of Hg is the main culprit of Hg bioaccumulation in the food chain (Campbell et al., 2005). Among different food sources, fish is the predominant source consumed by humans (Choi and Grandjean, 2012) and is the primary route of human exposure to MeHg along the trophic chains (Covelli et al., 2012; Mason et al., 2012).

Hg occurs naturally on the earth's crust (approximately 7 ng/g) and in marine environments (50–80 ng/g), as previously reported (Liang et al., 2017; Chi, 2004; Fujii, 1976). Hg can be released into the atmosphere and water through various anthropogenic activities including

* Corresponding authors at: CAS Key Laboratory of Urban Environment and Health, Fujian Key Laboratory of Watershed Ecology, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China (S. Khan and G. Li).
E-mail addresses: sardar.khan2008@yahoo.com (S. Khan), psu@zju.edu.cn (X. Ye), gli@iue.ac.cn (G. Li).

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industrial processes, fossil fuel combustion and coal burning (Chakraborty and Babu, 2015: Sadhu et al., 2015). In 2009, the United Nations Environment Programme (UNEP) and the Inter-Organization Programme for the Sound Management of Chemicals, in its 25th session of the Global Ministerial Environment Forum, acknowledged that Hg is a chemical of global concern, owing to its long-range atmospheric transport, its persistence in the environment, its ability to bioaccumulate in ecosystems and its highly negative effects on human health and the environment (UNEP, 2009). The parties consented to further international action to produce a legally binding instrument for Hg, including both enforceable and voluntary approaches, together with interim activities, to reduce risk to human health and the environment. The UNEP position was more recently reinforced at the First meeting of the Conference of the Parties to the Minamata Convention on Mercury (COP1) (UNEP, 2017). The most recent estimates indicate the total amount of anthropogenic Hg in the ocean globally to be 290 ± 80 million moles, almost two-thirds of which is in water shallower than 1000 m (Lamborg et al., 2014). In marine environments, MeHg is released through anaerobic or low oxygen decomposition of organic matter (Mason et al., 2012), whereas in freshwater ecosystems, divalent Hg^{+} is converted to MeHg by methylation, mainly through the action of sulfate reducing bacteria or iron reducing bacteria (Parks et al., 2013; Driscoll et al., 2013). Furthermore, microbial and photochemical decomposition may also result in demethylation (Kotnik et al., 2015: Horvat et al., 2003).

In the European Union, the environmental quality standards for Hg and its compounds in marine biota were determined by the EU Directive (2008/105/CE) to be 0.02 μg/g ww (wet weight); there is a maximum allowable level (total Hg) of 0.5 μg/g ww in fish products and 1.00 μg/g ww in the muscle meat of some fish according to EU directive (2008/105/CE). The FAO/WHO, in its Codex Alimentarius (CODEX, 2012), established maximum limits of 0.5 and 1.0 μg/g for MeHg for low trophic level fish and high trophic level predators, respectively. The FDA (1994, 1998) has suggested an action level of 1 μg/g for total Hg in seafood, which converts to 0.95 μg/g according to a ratio of MeHg/total Hg of 0.95 (detail is given in Supporting Information (SI)), recently an action level of 1 μg/g of MeHg is recommended under fish and fishery products hazards and controls guidance for all fish (FDA, 2019). The USEPA has suggested a more stringent criterion for tissue residues of 0.3 μg/g of MeHg in fish (USEPA, 2005).

MeHg toxicity varies among age groups, and the adverse effects it produces depend upon exposure duration, frequency and susceptibility factors. The adverse effects associated with chronic low dose exposure include cardiovascular diseases, hindered brain development and neurological disorders with symptoms such as impaired vision, muscle movement disorders and ataxia (Sadhu et al., 2015; Karagas et al., 2012; National Research Council, 2000). Fetal neurological damage upon exposure to high doses of MeHg have been reported in Iraq and Minamata (Choi and Grandjean, 2012).

Unprecedented growth has been observed in the fish and seafood industries in China in recent years, owing to equally high consumption of these products (Xu and Wang, 2017; Liang et al., 2013; Liang et al., 2016a, 2016b). The high levels of MeHg may be an important route of exposure to Hg for the population. Hg contamination is of particular concern for China and Zhoushan Islands. The coastal regions receive Hg from both natural and anthropogenic sources including, seafloor erosion, coastal aquaculture, shipping activities, mining discharge, wastewater flow and atmospheric deposition over a particular period of time. Hg released to aquatic ecosystems through various anthropogenic sources are transformed to organic and inorganic Hg through biogeochemical reactions and mechanisms and making it a true environmental pollutant. The present study determined the MeHg concentrations in fish, crustaceans and mollusks collected near the Zhoushan Islands, Zhejiang, the largest fishing area in China and the fourth largest in the world. Sixty-nine species including 43 fish, 17 mollusks and 9 crustaceans were studied. A diet survey was simultaneously performed, including both men and women in different age groups, to assess daily intakes of the selected species. Exposure to MeHg and the resulting health risk was also estimated.

2. Materials and methods

Zhoushan City is located in northeast Zhejiang Province in eastern China. It covers a terrestrial area of 1440.12 km^2 and a marine area of 20,800 km^2. It produces 1163 species of seafood, mainly croceine croaker (*Pseudosciaena crocea*), small yellow croaker (*Larimichthys polyactis*), hairtail (*Trichiurus japonicus*), cuttlefish (*Metasepia pfefferi*), seerfish (*Scomberomorus niphonius*), conger pike (*Muraenesox cinereus*), chub mackerel (*Scomber japonicus*), filefish (*Navodon septentrionalis*), swimming crab (*Portunus triuberculatus*), and various shrimps and mollusks including *Bullacta exarata, Loligo chinensis* and *Ruditapes philippinarum*. The amounts produced vary by season.

2.1. Sampling and sample preparation

The sampling was performed between June and October in 2011 (not released before, owing to food security/safety policy). A total of 492 samples were collected, including both native and alien species of fish (n = 43), crustaceans (n = 9) and mollusks (n = 17). Sampling was performed with gillnets, electro fishing devices and angling inside the fishing area in Zhoushan, China. The collected samples were identified, measured, weighed, divided into replicates and immediately frozen and stored at −20 °C for further analyses. The species were identified with the taxonomic key of the Integrated Taxonomic Information System (IT IS: https://www.itis.gov/) and World Register of Marine Species (WoRM: http://www.marinespecies.org/index.php).

2.2. Methyl mercury extraction and analysis

For MeHg analysis, 1–2 g wet samples were weighed into 15 mL centrifuge tubes, and then 10 mL of 5 mol/L HCl was added. The samples were extracted for 30 min with an ultrasonic water bath at room temperature and shaken at intervals. After extraction, the samples were centrifuged for 15 min at 4 °C at a speed of 6000 rpm. Lipids contents were largely removed after the centrifugal samples were cooled in a refrigerator to 4 °C for 1 h. Then 2 mL supernatant was collected and adjusted to pH 2–8 with 50% (v/v) ammonia, and the adjusted solution was diluted to 4 mL with ultrapure water. The solution was filtered through a 0.22 μm membrane and then purified on a C18 SPE column (250 mg, 3 mL, Supelco Company, USA). Subsequently, 2 mL mobile phase was used to elute the filtrate, and then the collected effluent and eluent were combined and diluted to 10 mL with ultrapure water (Shang et al., 2011). The blank and samples were processed according to the same procedure.

The extracts were analyzed with liquid chromatography coupled with atomic fluorescence spectrometry (LC-AFS). The mobile phase was composed of 5% (v/v) acetonitrile, 1 g/L (L)-cysteine and 50 mmol/L ammonium acetate. The flow rate of the mobile phase was 0.8 mL/min, and an aliquot of 100 μL solution was injected. The post-column conditions included an ultraviolet source of 253 nm wavelength, 2 g/L oxidant of $K_2 S_2 O_8$ and a flow rate of 1.6 mL/m. A series of standards of MeHg were used to determine MeHg concentrations with at least two replicates.

2.3. Sample preparation and stable isotope analysis

In the laboratory the samples were washed with distilled water and then freeze dried. We collected the following: for fish, an appropriate amount of white muscle on the back; for shrimp, the abdominal muscles; and for shellfish, the muscles of the shell. Stable isotopes were analyzed according to standard procedures. The frozen samples were thawed, and an appropriate amount of back white muscle

(approximately 1–2 g) was dried at 60 °C for more than 48 h and then ground into fine powder. Then 1 M HCl was added for acidification treatment, and samples were dried and stored.

The samples were lyophilized in a freeze dryer at −80 °C, and ground and homogenized with a quartz mortar. The samples were measured for the stable isotope ratios of nitrogen. The stable isotope mass spectrometer was constructed by connecting a Finnigan Flash EA1112 elemental analyzer with a Finnigan DELT plus XP stable isotope mass spectrometer via Con Flo II. For each bioassay, three parallel samples were used. To ensure the accuracy of the test results, one standard sample was added after each test of five samples, and two or three retests were performed on individual samples. The nitrogen stable isotope ratio was relative to the form of the δ value of atmospheric nitrogen (Minagawa and Wada, 1984).

$$\delta^{15}N = \frac{R_{Sample}}{R_{Atmos}} - 1 \times 1000 \quad (1)$$

where $\delta^{15}N$ is the nitrogen isotope ratio of the organism, R_{Sample} is the nitrogen isotope ratio ($^{15}N/^{14}N$) of the sample, and R_{atmos} is the standard nitrogen isotope ratio of the atmosphere.

2.4. Trophic level calculation in the food chain

After determining the baseline biological and nitrogen nutrient enrichment of the system, we calculated the trophic level (TL) of each organism on the basis of the relative value of the stable isotope ratio of the biological nitrogen to the baseline. The TL was calculated with the following formula (Post, 2002):

$$TL = (\delta^{15}N_{Sample} - \delta^{15}N_0)/\delta^{15}N_C + 2.0 \quad (2)$$

where the $\delta^{15}N_{sample}$ is the δ value of the measured value of the sample (Eq. (1)); $\delta^{15}N_0$ is the baseline value of trophic level, and $\delta^{15}N_c$ is the nutrient level enrichment.

The trophic magnification factors (TMFs) were based on the MeHg concentrations in the food chain and TLs. The TMFs were derived from the slope of the log of the MeHg concentrations versus TL. TMF > 1 indicates MeHg biomagnification within the food chain (Fisk et al., 2001).

$$\mathrm{Ln[Concentrations]} = a + b(TL) \quad TMF = eb \quad (3)$$

2.5. Quality control

The method of standard addition of 2 μg/L and 5 μg/L of MeHg calibrations was used to validate the analysis and the recoveries were 83.69–126.60% (average 101.8%) and 77.82–122.75% (average 98.82%), respectively. Three standard Certified Reference Materials, tuna fish (CRM 436), NIST-2976 mussel tissue and TORT-2 lobster hepatopancreas, were used for quality control. The recoveries in this study were 97%, 99% and 98%, respectively. Furthermore, the reagent blanks were also run with each batch of samples to monitor the contamination of glassware or extraction and quantification processes.

2.6. Daily dietary intake of MeHg

The estimated daily intake of MeHg due to the consumption of seafood was determined with the following equation.

$$DIM_{ik} = C_i \times D_{ik}/BW \quad (4)$$

where DIM_{ik} is the dietary intake of MeHg (mg/kg-day) for species i in an individual of age group k, C_i (μg/g) is the measured average MeHg concentration in the aquatic species i; D_{ik} is the average intake of the aquatic species i by an individual of age group k (kg/day); BW (kg) is the average body weight for the sub-groups of the population surveyed: 25 kg, 64 kg and 76 kg for male children, adolescents and adults as well as 25 kg, 57 kg and 61 kg for female children, adolescents and adults, respectively (NAS, 2001; FAO/WHO, 2001).

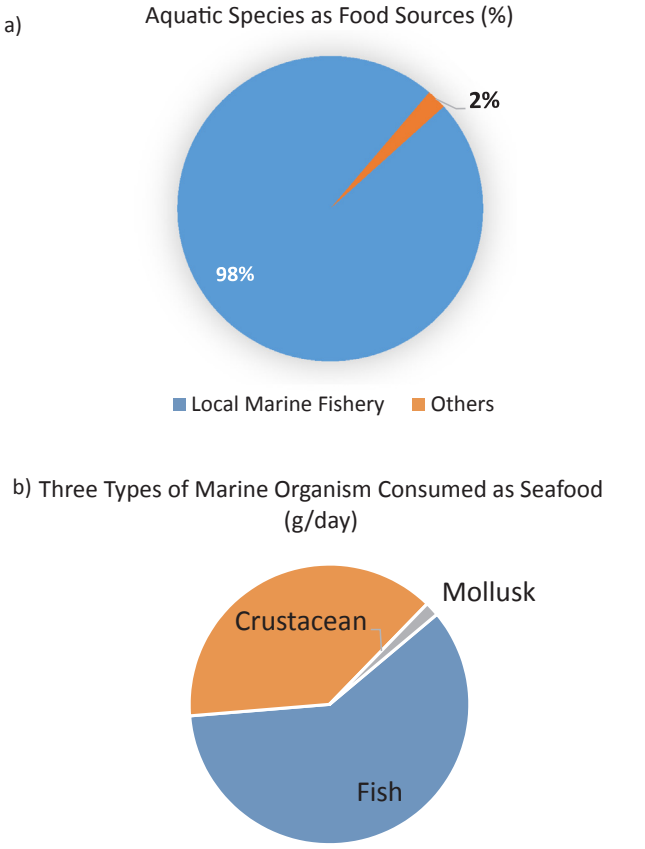

Fig. 1. The Consumption of Local Seafood by Households in Zhoushan Island (a) the percentages of local marine species and others (freshwater species or exotic species); (b) the total amound consumed by three villages during the day of survey (g/day) among three types of marine organism.

The daily food intake (D_{ik}) was obtained from a questionnaire given to a sample of the population (Table S1, Fig. 1a). In brief, the questionnaire was given between June and October in 2014 in three towns in Zhoushan, to 50 families randomly selected in each town. The total number of questionnaires was 952 including both male (49%) and female (51%) of different age groups (3 to 89 years). The data from the surveyed groups were divided into six subgroups on the basis of sex and age. The amounts and types (species) of seafood products consumed in the three main daily meals were quantified. On the basis of the survey data, the average daily MeHg intake per species *i* per individual was estimated for each age group k (DIM_{ik}), with the number of different seafood species *n*.

$$DIM_k(mg/kg - day) = \sum_{i=0}^{n} DIM_{ik} \quad (5)$$

The lifetime average intake of MeHg (mg/kg-day), DIM, was estimated on the basis of the assumption of an exposure frequency (EF) of 350 days/year. The exposure duration for children (ED_c), adolescents (ED_{ado}) and adults (ED_a) was 4 years, 14 years and 72 years (between 18 years and 90 years of age), respectively, on the basis of the survey definitions of children being between 0 and 14 years, adolescents being between 14 and 18 years, and adults being above 18 years. In the population surveyed, the oldest individual was 89 years old. Furthermore, the body weights of males and females were averaged for adults and adolescents when the intake based only on the age groups or lifetime was estimated:

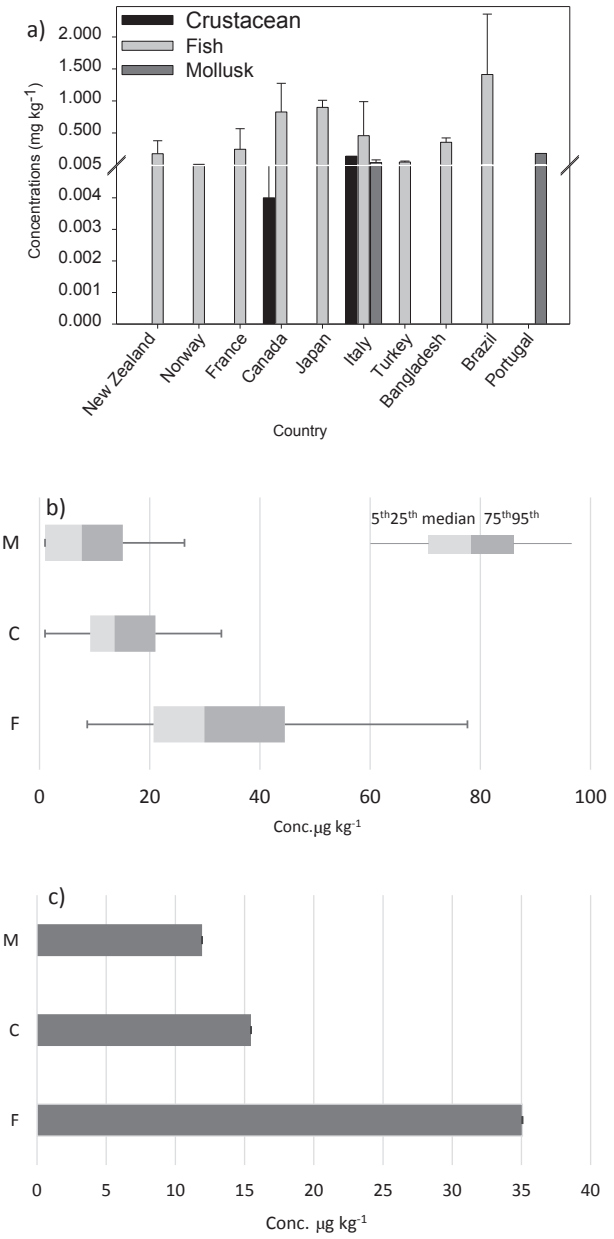

Fig. 2. (a) MeHg concentration in seafood from different regions of the world (reference: SI) (b) distribution of MeHg concentrations in the three types of seafood samples (M: mollusks; C: crustaceans; F: fish); (c) the mean concentration in the three types of seafood samples (M: mollusks; C: crustaceans; F: fish, error bars indicate 95% confidence interval of the mean).

$$\mathrm{DIM(mg/kg-day)} = \frac{(DIM_c \times ED_c + DIM_{ado} \times ED_{ado} + DIMa \times ED_a) \times EF}{AT \times 365} \quad (6)$$

2.7. Hazard quotient

The chronic health risk due to dietary exposure to MeHg in seafood was calculated with the hazard quotient (HQ):

$$HQ = DIM/RfD \quad (7)$$

The reference dose (RfD), value for MeHg was considered to be 1.00×10^{-4} mg/kg-day on the basis of the benchmark doses obtained from human epidemiological studies (USEPA, 2001). An HQ lower than 1 indicated that the DIM is lower than RfD; hence, the exposure of MeHg via consuming seafood in the area did not exceed the acceptable average daily allowable intake.

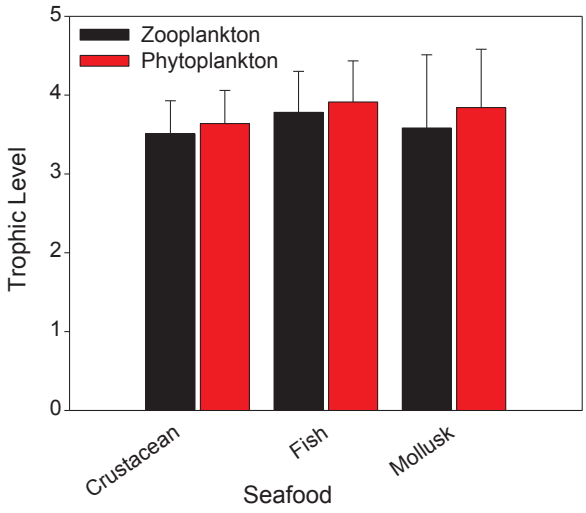

Fig. 3. Estimates of the Trophic level based on zooplanktons and phytoplanktons (mean ± SD).

Table 1
Trophic Magnification Factors (TMFs) and regression parameters (R^2, intercept, slope, and p-values) calculated for MeHg in marine food chain.

Sample	intercept	Slope	R^2	TMF	P
Zooplankton					
Mollusk	1.386	0.352	0.351	1.422	0.093
Crustacean	1.932	0.228	0.041	1.256	0.631
Fish	1.955	0.359	0.127	1.432	0.019
Seafood	1.734	0.377	0.140	1.458	0.003
Phytoplankton					
Mollusk	1.340	0.351	0.352	1.422	0.093
Crustacean	1.801	0.255	0.050	1.290	0.596
Fish	1.897	0.362	0.130	1.436	0.018
Seafood	1.671	0.380	0.143	1.462	0.003

2.8. Statistical analysis

ANOVA tests were performed to compare the mean concentrations of MeHg in the three major groups of marine species. When the results indicated significant differences ($p < 0.01$) in the mean values, *post-hoc* analyses were conducted to evaluate the difference between each of two paired groups. When MeHg concentrations were below detection limit, they were replaced by one half the detection limit (0.001 μg/g).

3. Results and discussion

3.1. Consumption of local seafood by villagers

The survey of the daily diet among local households revealed that the aquatic species consumed by the local villagers were mostly local and marine species (Fig. 1a). Among those local marine species, almost 60% were fish (average of 202 kg/day for all surveyed households), and 39% were crustaceans (130 kg/day); the consumption of mollusks was less than 2% (Fig. 1b). The average daily consumption of seafood for different age groups reported in the survey was 51.67, 65.87 and 67.17 g for male children, adolescent and adults, respectively, while for female children, adolescent and adults was 46.30, 69.30 and 57.33 g, respectively. This shows that the consumption of seafood reported in this survey was higher than the average rate of 23 g/person/day of marine products in China (Cheng et al., 2009).

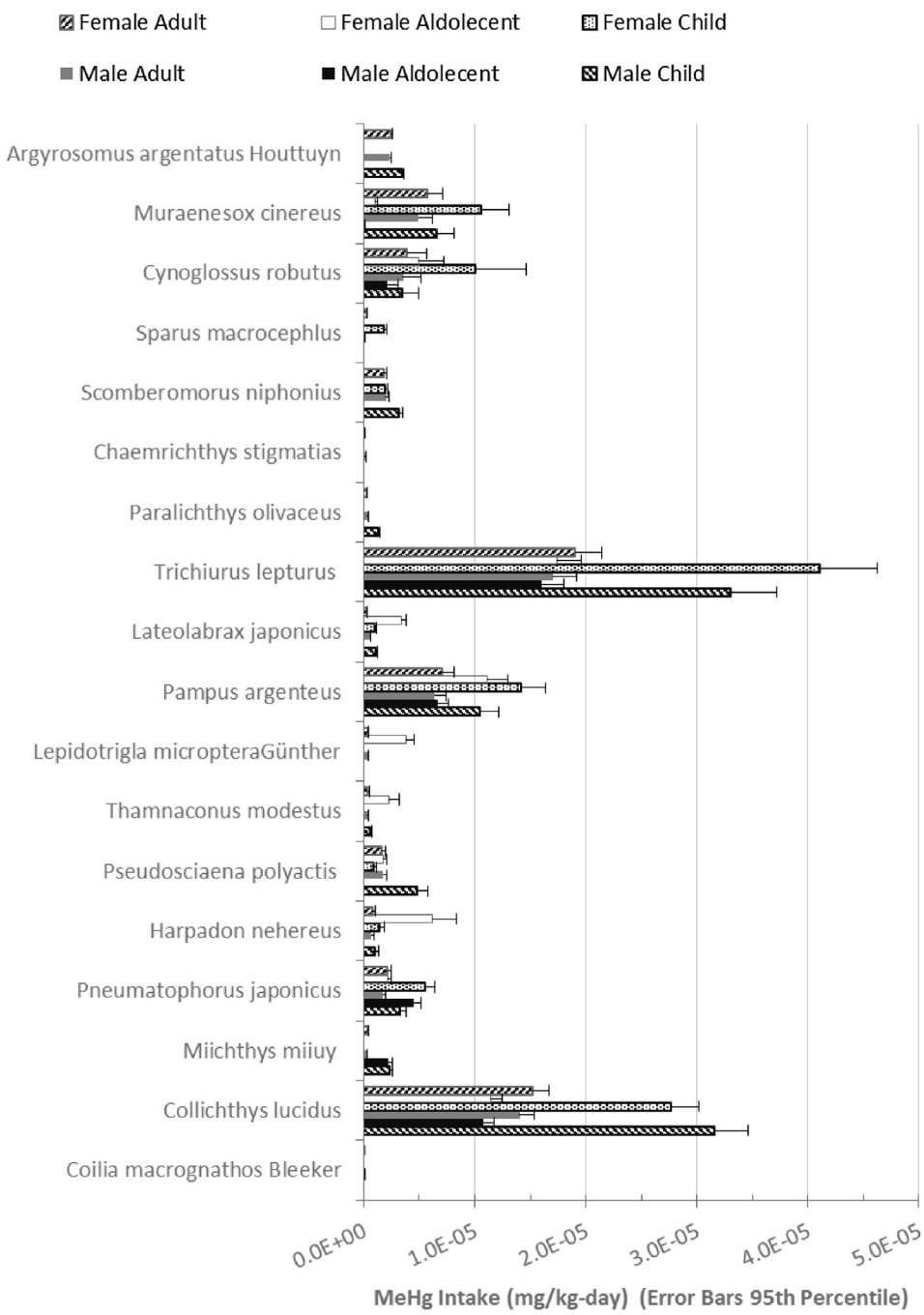

Fig. 4. Estimated Average MeHg Intake for Subgroups of Surveyed Population for Marine Fish Species (error bars indicates the intake calculated based on the 95th percentile of the MeHg concentration detected in the fish samples).

3.2. MeHg in seafood

MeHg concentrations in fish, crustaceans and mollusks are shown in Fig 2 and Tables S3–S5. The mean MeHg concentrations in fish species ranged from 0.009 to 0.210 μg/g. Similarly, in crustaceans and mollusks, the mean MeHg concentrations were < 0.002–0.033 μg/g and < 0.002–0.139 μg/g, respectively. Among the selected species, the highest mean MeHg concentration was found in fish. A one-way ANOVA indicated that the MeHg levels detected in fish (mean = 0.0351 μg/g, SD = 0.0392) were significantly higher than those in crustaceans (mean = 0.0155, SD = 0.0094) and mollusks (mean = 0.0119, SD = 0.0222) ($F_{(2,433)}$ = 22.03, p < 0.01). The species with the highest concentrations were the frigate tuna, Auxis *thazard*, (mean = 0.1228 ± 0.0048 μg/g ww), and the spadenose shark, *Scoliodon sorrakowah* (mean = 0.2098 ± 0.0899 μg/g). High concentrations of Hg in these species have been reported worldwide (Kumar, 2018; see also Table S2 in Supplementary Material). The higher MeHg concentrations in predator fish species were due to biomagnification. Long-lived predators at the top of the food chain are susceptible to concentrating large amounts of Hg in their tissues.

The MeHg concentrations found in our study were below the maximum allowable level of 1.00 μg/g ww for total Hg (0.95 μg/g MeHg); the MeHg concentrations surpassed the USEPA's 0.3 μg/g recommended value only in the spadenose shark, with a maximum concentration of 0.4005 μg/g.

The MeHg concentration detected was within the range of reported values worldwide: those for fish were between 0.009 (Norway: Jæger et al., 2009) and 1.41 (Brazil: Sebrenski Silva, 2007; Dorea et al., 2006)

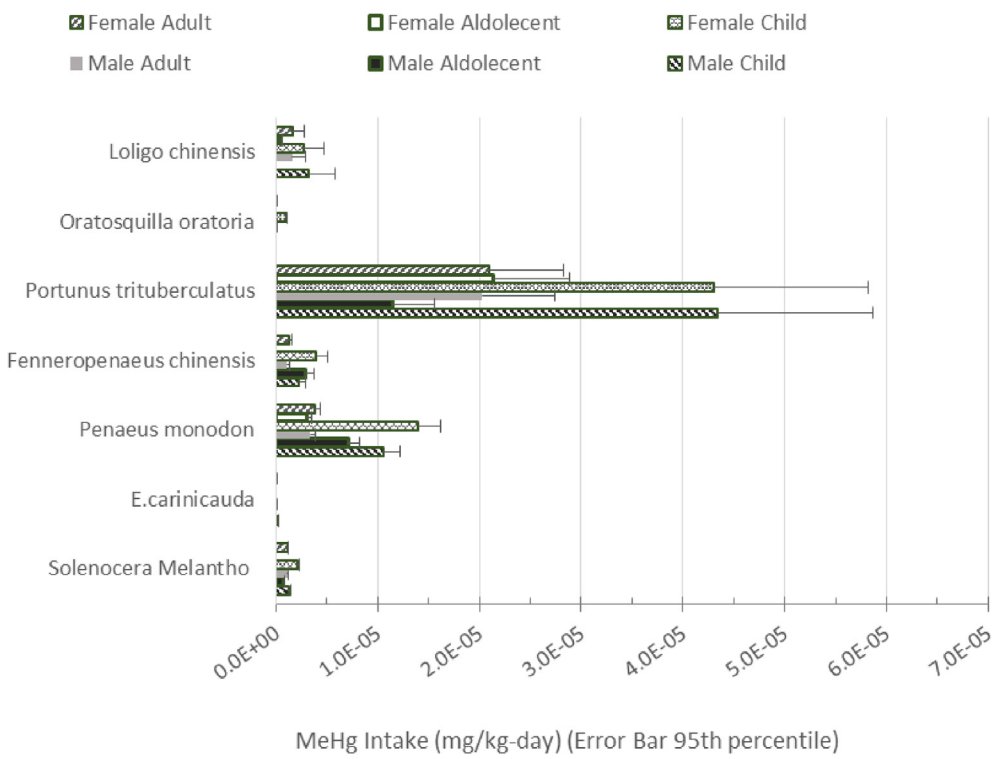

Fig. 5. Estimated Average MeHg Intake for Subgroups of Surveyed Population from Marine Crustaceans Species (error bars indicates the intake calculated based on the 95th percentile of the MeHg concentration detected in the crustaceans samples).

μg/g (Table S2); those for crustaceans ranged from 0.004 (Canada: Campbell et al., 2005) to 0.137 (Italy: Brambilla et al., 2013) μg/g; and those for mollusks were between 0.04 (Italy: Brambilla et al., 2013) and 0.18 (Portugal: Raimundo et al., 2010) μg/g.

MeHg readily bioaccumulates and biomagnifies in aquatic food chains (Kidd et al., 2011), as also supported by our results, in which MeHg levels decreased from fish (many of which are at the top of the food chain) to crustaceans (which are usually first level carnivorous consumers) and then to mollusks (which are usually herbivorous consumers).

Ingestion of contaminated food, particularly fish, is the main source of contamination and human exposure to MeHg. Therefore, the USEPA has suggested that MeHg concentration in fish is the key indicator of its toxicity to humans (USEPA, 2005). In the present study, the highest MeHg concentration was also found in fish species, thus indicating health concerns in the study area which is the major fishing ground. Although the mean MeHg concentrations in fish species were below the recommended value set by the USEPA (0.3 μg/g), continued consumption of contaminated fish may pose health risk to the consuming population.

3.3. Trophic transfer of MeHg

The stable nitrogen isotope ratio ($\delta^{15}N$) is an effective way to assess the trophic position of marine organisms in an aquatic food chain (Layman et al., 2012). The TLs were determined for selected seafood species on the basis of stable isotopes of nitrogen (Fig. 3 and Table S6). The data of trophic levels for different organisms were compared with the levels reported in Fishbase (www.fishbase.org). The TLs for fish, crustaceans and mollusks ranged from 2.84 to 5.28, 3.02–4.22 and 1.54–5.92, with mean values of 3.78 ± 0.52, 3.51 ± 0.42 and 3.58 ± 0.93, respectively. The TLs calculated were higher in the data reported by Campbell et al. (2005) and Jæger et al. (2009) in the arctic region, whereas the results of TL were in agreement with the findings reported by Brambilla et al. (2013) in Mediterranean Sea. TL determination based on phytoplankton showed nearly the same results as TL determination based on zooplankton, with few exceptions. For TL based on phytoplankton, the minimum TL value for molluscs was reported for *R. venosa* (3.14). Comparison of TL based on zooplankton with TL based on phytoplankton showed that phytoplankton had higher TL values.

The trophic transfer of MeHg in the entire marine food chain was measured on the basis of TMFs (Table 1). All marine species were included in the calculations of TMF to provide a clear picture of contaminant transfer. The results indicated that the TMF value was greater than 1 for fish, crustaceans and molluscs. Overall, the TMFs for crustaceans, fish and molluscs were 1.26, 1.43 and 1.42, respectively, based on zooplankton, and 1.29, 1.44 and 1.42, respectively, based on phytoplankton. The TMF values greater than 1 indicated statistically significant ($P < 0.01$) increases in MeHg concentrations, thus indicating biomagnification in the food chain. Fish, crustaceans and molluscs the types of highly consumed seafood in China, had higher TMF values, thus indicating a risk of MeHg transfer to humans, as well as associated health risk. Previous studies have also reported high TMF values and revealed that MeHg concentrations in the various strata of marine habitats influence MeHg transfer and TMF values (McMeans et al., 2015; Van der Velden et al., 2013).

3.4. Daily dietary intake and human health risk of MeHg

Figs. 4–6 show the daily dietary intake values of MeHg among subgroups of the population from three types of marine species of fish, crustaceans and mollusks, and the cumulative intake is shown in Fig. 7. The values of DIM were based on average daily consumption of seafood by children, adolescents and adults. The consumption of fish species provided more MeHg intake (on average among the six subgroups, ranging from 1.2E−4 to 4.2E−5 mg/kg-day) than crustaceans (6.7E−5 to 2.2E−5 mg/kg-day) and mollusks (3.8E−6 to 4.7E-− mg/kg-day). Among fish species, the highest MeHg intake was reported for ***Trichiurus lepturus* (4.1E−05 mg/kg-day)** in female adolescents, and

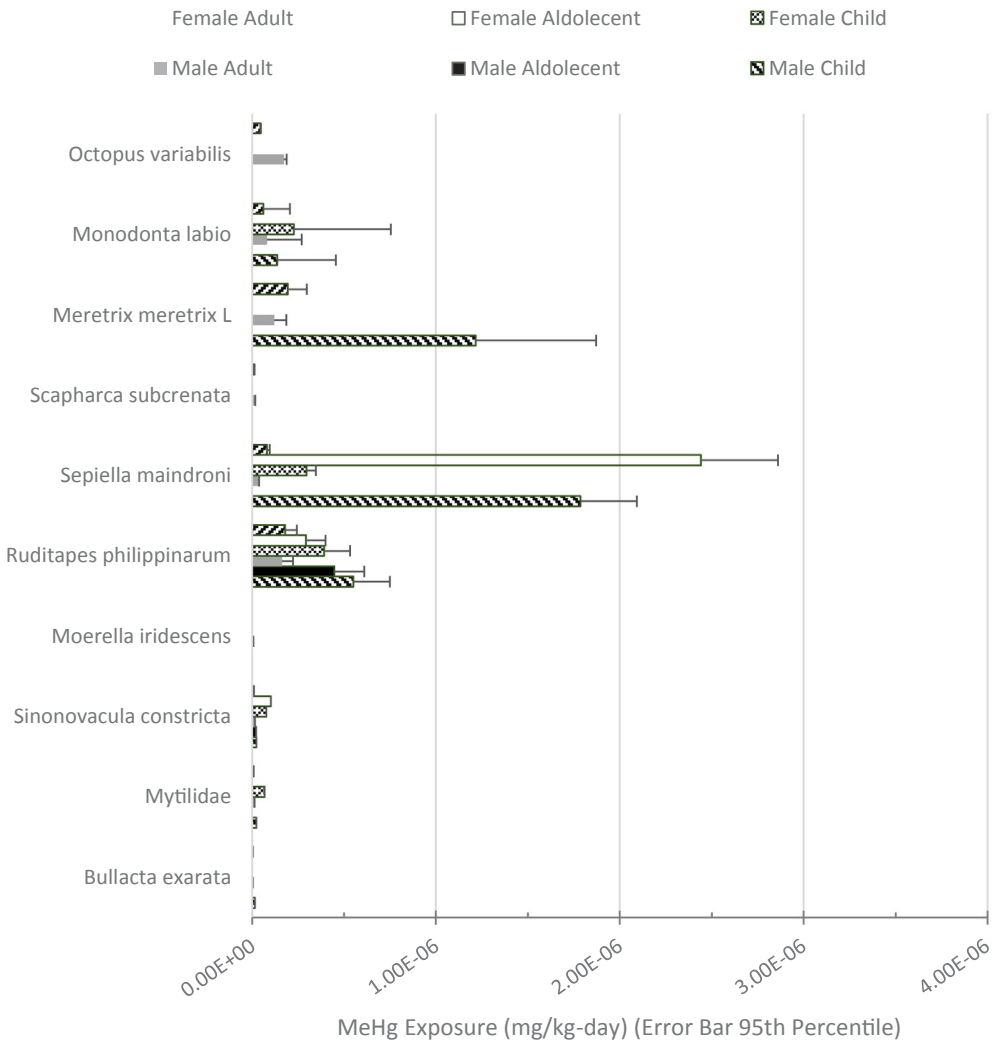

Fig. 6. Estimated Average MeHg Intake for Subgroups of Surveyed Population from Marine Mollusks Species (error bars indicates the intake calculated based on the 95th percentile of the MeHg concentration detected in the mollusks samples).

the lowest non-zero intake was reported for *Coiliamacrognathos bleeker* (2.2E−8 mg/kg-day) in female adults (Fig. 4). Interestingly, the consumption of *Trichiurus lepturus* in the surveyed area was only the second highest (52 kg/day for all surveyed households), followed by *Collichthys lucidus* (75 kg/day for all surveyed households). Thus, the high MeHg intake was partly a result of the higher MeHg concentrations detected in *Trichiurus lepturus*. The highest intake for crustaceans and mollusks was noted for ***Portunus trituberculatus*** (4.3E−05 mg/kg-day) in female adolescents and for ***Sepiella maindroni*** **(2.4 E−06 mg/kg-day)** in male adolescents. Similarly, the lowest non-zero intake was found for *E. carinicauda* (5.3E−08 mg/kg-day) and *Scapharca subcrenata* (1.0E−08 mg-kg-day) in female adults (Figs. 5 and 6). On average, via consuming the three types of the marine organisms, children exhibited higher MeHg intake than adults and adolescents (Fig. 7a). Children were more susceptible to MeHg toxicity; notably, solely because of fish consumption, the average exposure doses for both male and female children exceed the threshold suggested by the USEPA (1E−4 mg/kg-day). The variation in MeHg uptake by individuals among age groups was due to the variation in consumption of seafood, because individuals had different daily consumption rates of fish, crustaceans and mollusks (Figs. 4–6). Additionally, the sample sizes for the children and adolescent groups were relatively small (Fig. 4). Thus, the results for these two groups (children and adolescents) were more susceptible to variations in the type of fish consumed on the day of the survey.

In accordance with Eqs. (6) and (7) for calculating the DIM and HQ, the exposure risk resulting from the local seafood intake was proportional to the estimated MeHg DIM. The cumulative HQ values for different seafood groups were in the order fish > crustaceans > mollusks, and children were the most exposed age group (Fig. 7b). Although the consumption of individual species of fish did not exceed the reference dose of 1E-4 mg/kg-day suggested by the USEPA (2001) (Fig. 4), collectively, the consumption of fish posed an unacceptable level of risk of MeHg exposure for children and adolescents in the surveyed population.

According to FDA assessments, people who consume more than 100 g of fish per day on a regular basis and who fishes the same water bodies are more susceptible to MeHg toxicity. They are considered as high-end consumers. The FDA recommends that people who consume fish with MeHg concentrations of approximately 1.0 and 0.5 mg/kg should limit their consumption to approximately 28 and 57 g per day, respectively (ATSDR-US, 1999; FDA, 1998). In the present study, during the questionnaire survey, we observed that the local community used the same territory for fishing, most of the individuals had consumption rates below 100 g per day, and the MeHg concentrations in the sampled fish species were below 0.5 μg/g.

For toxicological assessment of contaminants to a particular organism, the level of exposure through various pathways is a key component (Caussy et al., 2003). To assess the health risk of environmental pollutants (MeHg), exposure level estimation and route tracing of the pollutant is crucial. Among different exposure pathways, food chain

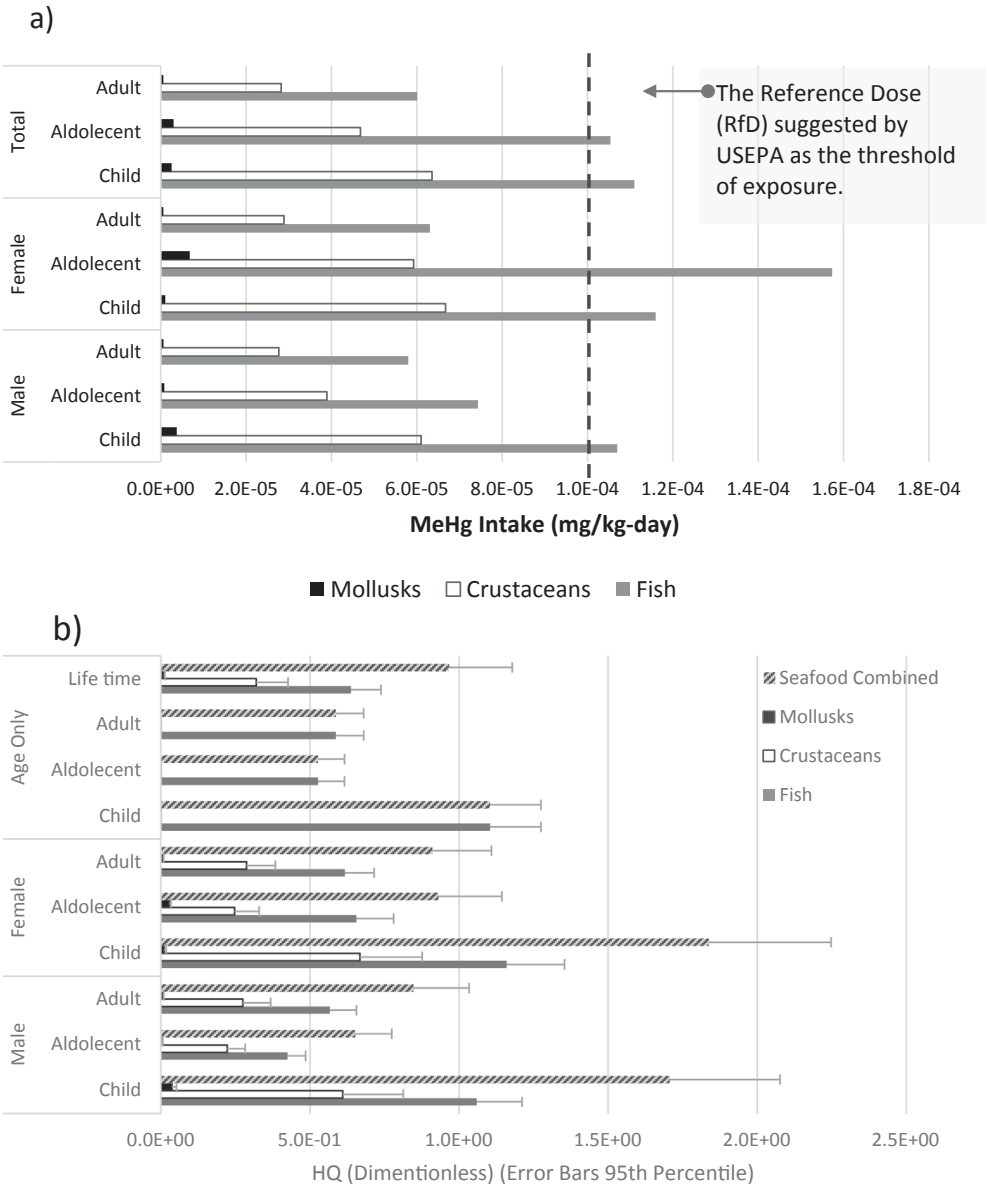

Fig. 7. (a) Cumulative MeHg DIM_k (mg/kg-day) among Subgroups of Surveyed Population in Relation to the Reference Dose (RfD) and (b) The hazard quotient among subgroups of surveyed population and the based on the average life-time exposure.

contamination is the most important (Amin et al., 2013). Like other toxic metals, MeHg has several exposure pathways depending on environmental conditions; however, ingestion of contaminated seafood, particularly fish, is the main exposure pathway for humans. The HQ values of a particular metal depend on the oral RfD values suggested by different organizations and DIM. In this study, the DIM values for crustaceans, fish and mollusks were lower than the USEPA recommended RfD value (1.00E − 04).

4. Conclusion

MeHg concentrations were examined in different seafood species. The MeHg concentrations reported in selected sample species were below the recommended levels set by the FDA and USEPA. Among different seafood species, excess MeHg was found mostly in fish species. Similarly, crustaceans and mollusks showed high variations in MeHg concentrations. The variations in MeHg concentrations in sampled seafood may have been due to the position of those marine species in the trophic level, because biomagnification is an important factor in the aquatic food chain. The average TL and TMF values based on zooplankton and phytoplankton were > 3 and > 1, respectively, thus indicating biomagnification in the food chain. The DIM values showed that the consumption of MeHg contaminated seafood substantially contributes to food chain contamination. The DIM was highest for fish species. These results were expected because fish species were the highest consumed species in the study area. The HQ values reported were < 1. Although the HRI values were below the recommended value set by the USEPA, continuous and consistent consumption of contaminated food may pose a great risk to the consuming population. From the present study, we conclude that the seafood samples collected were contaminated with MeHg, and long term and continued consumption may cause serious health problems in the consuming population. Therefore, daily consumption of fish species must be reduced.

Author contribution

Xinwei Yu: Investigation, Data collection. **Sardar Khan:** Supervision, Conceptualization, Methodology. **Anwarzeb Khan:**

Software, Writing - original draft. **Yuting Tang:** Writing- Reviewing and Editing. **Luis M. Nunes:** Writing - Reviewing and Editing. **Luis M. Nunes:** Software, Data curation. **Jianbo Yan:** Software, Visualization. **Xingqian Ye:** Writing - Reviewing and Editing. **Gang Li:** Project administration, Funding.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105420.

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