RESEARCH ARTICLE



Assessment of Hg accumulation in fish and scalp hair in fishing communities along river Swat, Pakistan

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Abstract

Mercury (Hg) bioaccumulation in fish poses severe threats to the food safety and human health. This study was conducted to assess Hg bioaccumulation in fish (n = 24) and scalp hair (n = 77) of the fishing communities at up- and downstream of the river Swat, Pakistan. The mean Hg concentration in upstream fish *Salmo trutta fario* (Brown trout) and *Schizothorax plagiostomus* (Swati fish) species was $34.7\pm18 \ \mu g \ kg^{-1}$ and $29.4\pm15 \ \mu g \ kg^{-1}$, respectively. The mean Hg concentration in downstream Swati fish, *Crossocheilus diplochilus* (Spena deqa), and *Garra gotyla* (Tora deqa) was $65\pm21 \ \mu g \ kg^{-1}$, $123\pm33 \ \mu g \ kg^{-1}$, and $326\pm53 \ \mu g \ Kg^{-1}$, respectively. The mean Hg concentration in scalp hair of the up- and downstream fishing communities was $658\pm125 \ \mu g \ kg^{-1}$ and $3969\pm791 \ \mu g \ kg^{-1}$, respectively. Independent *T*-test showed significant difference (p < 0.001) in the mean Hg concentration in scalp hair of the up- and downstream fishing communities found in the fishing community were muscle pain, headache, visual impairment, arterial blood pressure, anemia, and kidney dysfunction. Multiple linear regression indicated that daily and weekly consumption of the fish significantly increase Hg accumulation in human scalp hair. Regular consumption of fruits and cruciferous and leafy vegetables were found to reduce Hg toxicity in the population. Further studies are recommended to identify the sources of Hg and welfare impact of fish contamination on the fishing community of river Swat.

Keywords Bioaccumulation · Fish contamination · Health risks · Mercury

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Introduction

Mercury (Hg) is a widespread heavy metal of great environmental concern (Hsu-Kim et al. 2018; Selin 2018; Tang et al. 2020). Hg exists in elemental, inorganic, and or organic form in nature and poses severe threats to the food safety and human health (Hsiao et al. 2011; Sun et al. 2013) due to its toxicity and bioaccumulation in food webs (Dus et al. 2005). The chemical form of Hg largely affects its mobility and toxicity (ATSDR 2013). For instance, the organic form of Hg such as methyl mercury (MeHg) is more toxic than the elemental or inorganic form (Hasegawa et al. 2005). The MeHg can be produced by the methylation of elemental and inorganic Hg by bacteria in anaerobic conditions, even in the intestine of fish (Ali and Khan 2018). The MeHg retention time in fish depends on the physiological response of the fish such as homeostasis and detoxification (Ali et al. 2019). The toxic effects of MeHg were first realized when serious Hg poisoning occurred in Japan near the Minamata Bay due to consumption of contaminated fish and sea food (Björkman et al. 2007; Karabedian et al. 2009), as the MeHg was released from an industrial plant into the coastal area of Minamata.

Hg contamination in aquatic ecosystems arise from both natural and anthropogenic origin such as mineral deposits, forest fires (Camargo 2002), agricultural runoffs, mining activities, combustion of Hg containing fuels, and municipal and industrial wastewater discharges (Moiseenko and Gashkina 2016; Pavlish et al. 2003; Wang et al. 2004; WHO 2005). During the recent decades, urbanization, industrialization, and population growth has intensified the contamination of freshwater ecosystems. The fish living in polluted waters tend to accumulate Hg in their tissues and transfer across the food chain (Moiseenko and Gashkina 2016). The Hg can enter the fish body through its skin or gills and this mechanism is called bio-concentration, whereas the entry of Hg in fish body through ingestion of contaminated food/prey is called dietary accumulation (Ali and Khan 2018).

Human exposure to Hg occurs mainly through ingestion of the contaminated food and fish (ATSDR 2013; Fakour et al. 2010; Shah et al. 2016) and may cause acute or chronic effects ranging from severe disruption of tissue, shock, cardiovascular collapse, autism, gastrointestinal damage, renal and neurological disturbances, dermatitis, fatigue, and respiratory problems to life-threatening toxicity (Bastos et al. 2006; Eisler 2006; Lee et al. 2003; Riaz et al. 2016; WHO 2005). The Hg, when combined with other heavy metals, can have more toxicity due to the synergistic effect of the heavy metals mixture (Ali and Khan 2019). The Codex Alimentarius guideline levels of MeHg for predatory and non-predatory fish are 1000 μ g kg⁻¹ and 500 μ gkg⁻¹ wet weight (w/w), respectively (WHO 2007). The concentration of Hg in scalp hair is a widely used biomarker to evaluate Hg toxicity and contamination particularly for MeHg exposure from dietary consumption of fish and other foods (Agusa et al. 2007; Anwar et al. 2007; Díez et al. 2008; Feingold et al. 2020; Nuttall 2006) because hair sequesters mercury during its formation. Once incorporated into hair, Hg remains stable for a longer period as it binds to cysteine and can provide a history of MeHg exposure (Nuttall 2006).

The river Swat serves as a major fresh water body for a large portion of the Khyber Pakhtunkhwa province of Pakistan and has a significant role in fisheries and agricultural sector. The fish species found in river Swat provide significant contribution to the diet of the rural communities of Swat. However, river Swat receives most of the untreated wastewater (agricultural, industrial, and municipal) and a large proportion of solid wastes (hospital, industrial, and municipal) generated in the region. Emerald mining activities are also carried out in Swat and the local people take the mining debris to the bank of the river Swat for washing and search of emerald particles potentially contaminating the water body with different heavy metals. Furthermore, car batteries repairing shops and goldsmith shops present in the region are also likely to release heavy metals particularly Hg into the river Swat. As such due to the hazardous nature of Hg and its accumulation in food chain, it is imperative to monitor and quantify its levels in fish and fishing communities. This study is therefore conducted to (1) assess the Hg contamination in fish and scalp hair of the fishing community of river Swat, (2) assess the correlation between mercury accumulation in fish and human fish dietary patterns, and (3) assess the health effects of Hg exposure on the fishing community. To the best of our knowledge, no such study is conducted in the region previously.

Materials and methods

Study area

District Swat is mainly a mountainous terrain located between 34° 34' to 35° 55' N latitude and 72° 08' to 72° 50' E longitude in the Hindukush Himalaya range of Khyber Pakhtunkhwa Province Pakistan and covers a land area of 5337 km² (Bacha et al. 2018; Iqbal et al. 2018). According to the Government of Pakistan census (2017), the total population of district Swat was 2.31 million. The main source of income for majority of the population is agriculture, forestry, hunting, and fishing (Qasim et al. 2013). The river Swat originates from Hindukush mountains and extend from Mahodand and Gabral at about 3,000 m elevation and drains the entire watershed of Swat valley (DAWN 2007). Sampling was carried out in June–July 2019 at the up- and downstream of the river Swat (Fig. 1).

According to the Fisheries department in Swat, Government of Khyber Pakhtunkhwa, Pakistan, twenty-five different fish species are found in river Swat. The classification of fish based on their habitat/locality and feeding habit is given in supplementary information (Table S1). The fish species found in river Swat are largely site specific, for instance, Brown trout fish species found in upstream water of river Swat is scarcely found in downstream area. Similarly, the downstream fish species are rarely available in upstream area (Table S1).

Fish sample collection and preparation

The fish were randomly caught from up- and downstream of the river Swat with the help of local fishermen, and four juvenile fish specimens (n = 24) obtained in comparatively high number were included as study samples. The upstream fish included Brown Trout and Swati fish. Downstream fish included Swati fish, Spena deqa, and Tora deqa. Brown trout is carnivorous while Swati fish, Tora Deqa, and Spena Deqa are omnivorous. The fish species were measured for its length and weight, packed in clean polythene zip lock bags with identification code, and transferred to the laboratory in iceboxes.



Fig. 1. Sampling locations along the river Swat

The fish specimens were analyzed for mercury accumulation as individual samples on wet weight (w/w) basis.

In laboratory, the fish were washed with distilled water. Fish fillet [5 g (w/w)] excised from the pectoral region was thawed, washed with deionized water, and acid-wet digested (Ahmad et al. 2015). Briefly, the 5 g fish fillet were taken in Pyrex glass tube and added 2 mL mixture of analytical grade nitric acid manufactured by BDH AnalaR® and per chloric acid manufactured by Fischer® (HNO3-HClO4) with a ratio of 1:1, followed by 2 mL concentrated hydrochloric acid (HCl) manufactured by Sigma-Aldrich®, and the mixture was digested overnight in a digestion block at ambient temperature. Finally, 5 mL of concentrated sulfuric acid (H_2SO_4) manufactured by BDH AnalaR® was added and heated to 200 C° for 20 min until the digests were clear (Voegborlo and Adimado 2010). The extracts were cooled at room temperature, filtered into centrifuge tubes, diluted to 20 mL with double deionized water, and analyzed for T-Hg on the atomic absorption spectrometer (GBC 932 plus) via Hydride Generation technique.

Hair sample collection and preparation

The local residents living along river Swat and having fishing license issued by District Swat Fisheries Department, Government of Khyber Pakhtunkhwa, were considered as the fishing communities. Record of the fishing licenses issued by the concerned fisheries department was obtained through which we identify the fishing communities. Scalp hair samples (n=77) (42 upstream and 35 downstream) were collected from the fishing communities including children (age ≤ 17 years) and adults (age ≥ 18 years) of both sexes with prior written consent. The number of upstream participants involved in each experimental class included female children (n=11), male children (n=6), female adults (n=9) and male adults (n=16). Likewise, the participants from downstream included female children (n=6), male children (n=6), female adults (n = 11) and male adults (n = 12). The research protocol for this study was approved by the Ethics Committee of the University of Peshawar (Approval No. 919/UOP/ERB/2018) and performed according to the 1964 Declaration of Helsinki (Association WM 2000). The hair was cut from the occipital part of the head closest to the scalp using stainless steel scissors (Ahmad et al. 2018; Ohno et al. 2007). Female enumerators were involved to collect hair samples from female participants following social and cultural values of the study area. The hair samples were packed in separate polyethylene ziplock bags and coded before transferring to laboratory for further analysis. In laboratory, the hair samples were thoroughly washed with distilled water and acetone manufactured by Sigma-Aldrich® using ultrasonic bath to remove the dirt and external contaminants (Ahmad et al. 2018; Al-Amodi et al.

2017). The clean hair samples were cut into small pieces and oven dried overnight at 50 C°. Dried hair samples (0.25 g) were weighed into clean Pyrex tubes, 2.0 mL of 1:1 HNO₃: HClO₄ was added, followed by the addition of 2 mL conc. HCl and the mixture was digested overnight in a digestion block at ambient temperature. Finally, 5 ml of concentrated H₂SO₄ was added and the tubes were heated at 200 °C for 20 minutes until the digests were clear (Koseoglu et al. 2017; Voegborlo and Adimado 2010). The digested solution was cooled at room temperature, filtered into centrifuge tubes, and diluted to 20 mL with double deionized water (Ahmad et al. 2018). The T-Hg concentration in prepared samples was assayed in triplicates using Atomic Absorption Spectrometer (GBC 932 plus) via hydride generation technique.

Household questionnaire survey

The household questionnaire survey comprised of contact and household information, economic status, dietary information, fish consumption habits, and health conditions including the prevalence of 21 acute and 10 chronic health effects of Hg toxicity reported in the literature. The inclusion criteria for the household questionnaire survey were interesting as only household involved in fishing were included. For the purpose, fishing villages were identified by computing the number of fishing licenses issued by the fisheries department Swat, Government of KPK, Pakistan. In upstream area, the villages selected for household survey and sampling were Koz Kalay, Tanqar, Shagai, and Jupin. Whereas in downstream area, the selected villages for sampling were Garho, Aligrama, and Maam Derai (Fig. 1). A total of 21 randomly selected households were surveyed among which 11 were from the upstream area and 10 from the downstream area. All the households were within 6 km distance from the river Swat.

Quality control

Rigorous quality control protocols were followed during sample preparation and analysis. The apparatus and glassware used were kept overnight in 5% HNO₃ (v/v) solution, thoroughly washed with deionized water and oven dried at 60 °C prior to use (Ahmad et al. 2019a). Reagent blanks were prepared and analyzed with each batch of samples to check the possible contamination of chemicals and glassware used (contribution of reagents and vessels) (Ahmad et al. 2019b). All the reagents used were of analytical grade. Aqueous control samples were also run along with prepared samples and all samples were analyzed in triplicates. The percent recovery for Hg in aqueous control sample was 96.2%.

Statistical data analysis

Statistical data analysis was carried out using multiple linear regression and group mean difference *t*-test. Descriptive and multivariate statistical analysis were performed using STATA and OriginPro 2018.

The regression model equation can be expressed as Eq. 1 (Gujarati 2009).

$$Y = \beta \mathbf{o} + \beta \mathbf{1}.X\mathbf{1} + \dots + \beta \mathbf{k}.X\mathbf{k} + \mu \mathbf{i} \tag{1}$$

where Y represents the dependent variable, X is independent variable with $k = 1, 2, ..., \beta_0$ is constant or intercept and β_1 is the slope variable or slope coefficient ($\frac{\Delta y}{\Delta x} = Slope$). Error in the regression is represented by μi .

Group mean difference *t*-test can be expressed as Eq. 2.

$$t = \frac{difference \ between \ groups}{sampling \ variability} \tag{2}$$

Results and discussion

Hg concentration in fish

The mean Hg concentration observed in up- and downstream fish species is presented in Table 1. Generally, the concentration of Hg in downstream fish species was higher than upstream. The fish feeding habits, length, weight, and taxonomic considerations might have affected Hg accumulation in the studied fish in addition to the increasing input of the contaminant downstream in the river. Previously, many researchers found positive correlation between heavy metals accumulation with fish type, age, and weight (Ali and Khan 2018; Khan et al. 2012; Yousafzai et al. 2010). Likewise, high Hg concentration was observed in downstream fish species in Madre de Dios River (Peru) (Diringer et al. 2015). While the characteristics of Madre de Dios River are different from river Swat since Madre de Dior River flows in an area where artisanal and small scale gold mining is common, however, the results reflect site-specific contamination as high Hg concentration was observed in fish obtained from higher contaminated areas/sites. Conversely, some other researchers observed no significant variation of Hg concentration in up- and downstream fish (Michalak et al. 2014; Anwar et al. 2007). In present study, the high Hg concentration observed in downstream fish can be associated with anthropogenic inputs as our survey revealed that most of the agricultural, municipal, and industrial wastewater is directly discharged into the river Swat. Besides, a large proportion of the solid wastes (hospital, industrial, municipal) generated were also dumped into the river Swat.

Location	Fish Species		Feeding habit	Mean length (cm)	Mean weight (g)	Mean Hg conc. ($\mu g k g^{-1}$)
	Scientific name	Local name				
Up-stream	Salmo trutta fario (n=4)	Brown Trout	Carnivorous	28±8	240±55	34.7±18
	Schizothorax plagiostomus (n=6)	Swati fish	Omnivorous	28±12	193±40	29.4±15
Down-stream	Schizothorax plagiostomus (n=5)	Swati fish	Omnivorous	22±8	88±22	65±21
	Crossocheilus diplochilus (n=5)	Spena dega	Omnivorous	14±6	43±17	123±33
	Garra gotyla (n=4)	Tora dega	Omnivorous	13±4	26±11	326±53

Table 1. Fish characteristics and measured Hg concentrations from pectoral fish tissue (5 g) on wet weight basis (w/w)

The recommended guideline limits of Hg in predatory and non-predatory fish are 1000 μ g kg⁻¹ and 500 μ g kg⁻¹ (w/w), respectively (WHO 2007). In present study, the accumulation of Hg measured in the different fish species is below the limits allowed by law. However, higher Hg concentrations were observed for downstream fish species particularly *Garra gotyla*, and its excessive consumption must be limited by the local fishing communities to avoid excessive Hg body burdens.

Hg concentration in hair

The average Hg concentration in scalp hair of the up- and downstream fishing community was $658\pm125 \ \mu g \ kg^{-1}$ and $3969\pm791 \ \mu g \ kg^{-1}$, respectively. In upstream area, Hg concentration in scalp hair was above the WHO permissible limit of 1000 $\ \mu g \ kg^{-1}$ for 19% subjects. Likewise, in downstream area, Hg concentration in scalp hair was above the WHO permissible limit for 51% subjects. Age and gender wise mean Hg concentration in scalp hair of the children (female and male) and adults (female and male) for up- and downstream fishing communities is presented in Fig. 2.

The horizontal line indicates the WHO guideline limit of 1000 μ g kg⁻¹ Hg for scalp hair. The mean Hg concentrations in scalp hair of the upstream fishing community were below the WHO guideline limit of 1000 $\mu g kg^{-1}$ for children and adults (female and male). In contrast, the mean Hg concentrations in scalp hair of the downstream fishing community exceeded the WHO guideline limit for children and adults (Fig. 2). Independent T-test showed significant difference (p < 0.001) in the mean Hg concentration in scalp hair of the upand downstream communities. However, no significant difference was observed for the Hg concentration in scalp hair of the male and female. Furthermore, research studies from Karachi, Hyderabad, and Lahore, Pakistan, reported high Hg concentration in scalp hair of the female compared to male (Anwar et al. 2007; Shah et al. 2016). Conversely, a research study from Negro River Basin, Brazil, reported significantly low Hg concentration in scalp hair of the females than male (Barbosa

et al. 2001). In present study, no significant relationship was observed between Hg concentration in hair and age of the respondents. Similar findings were reported in some other studies (Karabedian et al. 2009; Michalak et al. 2014; Szynkowska and Pawlaczyk 2007). However, a positive correlation between Hg accumulation in hair and age of the respondents was reported by Wyatt et al. (2017) and Shah et al. (2016). The regression model indicated that literacy reduces the Hg accumulation in human hair; however, the result was not statistically significant, while Anwar et al. (2007) reported that individual having 11 or more years of education have relatively higher concentration of Hg in scalp hair.

Effect of dietary habits on Hg concentration

Swati fish was the most consumed fish species by the communities for being abundantly available in both up- and downstream areas of river Swat. Multiple linear regression results showed that in upstream area, daily and 2–3 times consumption of carnivorous fish (Brown trout) significantly (p < 0.01)



Fig. 2. Age and gender wise Hg concentration in scalp hair of the fishing communities

increases the Hg concentration in scalp hair (Table S2). Similarly, consuming omnivorous fish (Swati fish) 2-3 times a week significantly (p < 0.1) increase the Hg concentration in scalp hair. In the downstream area, consuming omnivorous fish (Swati fish, Tora dega, and Spina dega) 2–3 times a week significantly (p < 0.5) increase the Hg concentration in scalp hair (Table S2). Previously, Feingold et al. (2020) and Díez et al. (2008) found a positive correlation between fish consumption and Hg level in hair, while Fakour et al. (2010) observed that 76.4% of the fish consumers have Hg levels exceeding the USEPA recommended level of 1000 μ g kg⁻¹. The variation of Hg accumulation in human scalp hair in relation to non-fish dietary habits is given in Table S3. The moderating effects of some of the non-fish dietary habits were also found (Table S3). The regression analysis showed that consuming dry fruits at least 1-2 times a month and/or 1-2 times a week lowers the Hg accumulation in scalp hair (p <0.1). Previously, Wyatt et al. (2017) observed a decrease in hair Hg levels with consumption of grains and fruits. In contrast, Feingold et al. (2020) did not find any significant trend of hair Hg levels in relation to rice and fruits consumption.

Disease prevalence

The prevalence of acute and chronic diseases caused by Hg toxicity were assessed in the fishing community through the questionnaire survey (Fig. 3).

The most prevalent health problems recorded for the fishing community of up- and downstream were muscle pain (14.3%), depression (6.9%), headache (8.6%), nausea (4.8%), irritability (4.8%), low vision (4.8%), loss of hearing (5.7%), blood pressure (20.1%), anemia (9.5%), and nephropathy (7.1%). Previously, Shah et al. (2016) found back pain, headache, irritability, and loss of hearing as the common symptoms resulting from MeHg exposure due to fish



Fig. 3. Prevalence of diseases among the fishing community

consumption. We conducted literature review to identify the most common ailments caused by Hg toxicity and on the basis of dietary pattern associated the observed ailments to Hg toxicity since the respondents were fish consumers on regular basis. However, the observed ailments might have been caused by a multitude of other factors.

Conclusion

The concentration of Hg in up- and downstream fish species was within the WHO guideline limits of 1000 μ g kg⁻¹ and 500 μ g kg⁻¹ (w/w) for predatory and non-predatory fish, respectively. However, the mean Hg concentration in downstream fish species was 5 times higher than upstream fish. Likewise, the mean Hg concentration in scalp hair of the downstream fishing community was 6 times higher than upstream community and exceeded the WHO guideline limit of 1000 μ g kg⁻¹ for both children and adults. A positive correlation was found between fish consumption and Hg accumulation in scalp hair. The feeding habits, length, and weight as well taxonomic considerations might have affected Hg accumulation in the studied fish in addition to the increasing anthropogenic input of the contaminant downstream in the river. Various ailments associated with Hg toxicity were observed among the fishing communities. The higher Hg concentrations observed in river Swat fish species particularly in Brown trout indicated that its consumption must be limited to avoid excessive Hg ailments among the community. Further studies are recommended to identify the sources of Hg and welfare impact of fish contamination on the fishing community of river Swat.

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Author contribution Conceptualization: BK, IAM, IA, MAM. Statistical analysis: IA, MR, MAM. Investigation: BK, MAM, SS, KN. Chemical analysis: SS, KN. Project administration: BK, IAM. Field expedition supervision: BK, IAM, MAM. Data interpretation: IA, BK, MR, MAM. Writing—review and editing: IA, BK, IAM, MAM, MR. All authors read and approved the final manuscript.

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Availability of data and materials Summary of the data generated or analyzed during this study is included in the article. While detailed data of this study are available upon request.

Declarations

Ethical approval and consent to participate This study was conducted with prior approval from Ethical Research Committee, University of Peshawar. Written consent was obtained from all the sampling population involved in this study.

Consent for publication Not applicable

Competing interests The authors declare no competing interests.

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