

# Mercury bioaccumulation in relation to dietary habits in fishing communities along river Swat, Pakistan

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## Research Article

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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 were  $34.7 \pm 18 \mu\text{gKg}^{-1}$  and  $29.4 \pm 15 \mu\text{gKg}^{-1}$ . The mean Hg concentration in downstream Swati fish, *Crossocheilus diplochilus* (Spena dega) and *Garra gotyla gotyla* (Tora dega) were  $64.6 \pm 21 \mu\text{gKg}^{-1}$ ,  $113.3 \pm 33 \mu\text{gKg}^{-1}$  and  $326.3 \pm 53 \mu\text{gKg}^{-1}$  respectively. The mean Hg concentration in scalp hair of the up and downstream fishing communities were  $658 \pm 125 \mu\text{gKg}^{-1}$  and  $3969 \pm 791 \mu\text{gKg}^{-1}$ . Independent T-test showed significant difference ( $t = -3.37$ ) in the mean Hg concentration in scalp hair of the up and downstream communities.

The most prevalent health problems found in the fishing community were muscle pain, headache, visual impairment, arterial blood pressure, anemia, and kidney dysfunction. Multilinear regression indicated that daily and weekly consumption of the fish significantly increase Hg accumulation in human scalp hair. Regular consumption of fruits, 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.

## 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 methylmercury (MeHg) is more toxic than the elemental or inorganic form (Hasegawa et al. 2005). 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 (Barbosa et al. 2021, Camargo 2002), agricultural runoffs, mining activities, combustion of Hg containing fuels, municipal and industrial wastewater discharges (Moiseenko & 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 & Gashkina 2016).

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 Codex Alimentarius guideline levels of MeHg for predatory and non-predatory fish are  $1000 \mu\text{gKg}^{-1}$  and  $500 \mu\text{gKg}^{-1}$  wet weight respectively (WHO 2007). While, in Japan the established guideline limit for total Hg and MeHg in fish species is  $400 \mu\text{gKg}^{-1}$  and  $300 \mu\text{gKg}^{-1}$  respectively (WHO 2016). 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 serve as a major fresh water source for a large portion of the Khyber Pakhtunkhwa province of Pakistan and have a significant role in fisheries and agricultural sector. The variety of fish found in river Swat provides significant contribution to the diet of the rural communities of Swat. This study was 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 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 located between  $34^{\circ} 34'$  to  $35^{\circ} 55'$  N latitude and  $72^{\circ} 08'$  to  $72^{\circ} 50'$  E longitude. The river Swat originates from Hindukush mountains and extend from Mahodand and Gabral at about 3,000 meters elevation and passes through the valley of Swat (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, a large variety of fish are found in river Swat. The classification of fish based on their habitat/locality and mode of nutrition is given in supplementary information (Table. S1).

### Fish sample collection and preparation

The fish species (n=24) were caught from up and downstream of the river Swat with the help of local fishermen (Fig. 1). The upstream fish included *Salmo trutta fario* (Brown Trout) and *Schizothorax plagiostomus* (Swati fish). Downstream fish included Swati fish, *Crossocheilus diplochilus* (Spena dega) and *Garra gotyla gotyla* (Tora dega). Brown trout is carnivorous while Swati fish, Tora Dega, and Spena

Dega 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.

In laboratory, the fish were washed with distilled water. Fish fillet (5 g), 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 2 ml of  $\text{HNO}_3\text{-HClO}_4$  (1:1) was added, followed by 2 mL conc. HCl and the mixture was digested overnight in a digestion block at ambient temperature. Finally, 5 mL of conc.  $\text{H}_2\text{SO}_4$  was added and heated to  $200\text{ }^\circ\text{C}$  for 20 minutes until the digests were clear (Voegborlo & 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. All the samples were analyzed in triplicates.

### **Hair sample collection and preparation**

Scalp hair samples (n=77) (42 upstream and 35 downstream) were collected from the fishing communities including children (age  $\leq 17$  years) and adults (age  $\geq 18$  years) of both genders with prior written consent. 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 zip-lock bags and coded before transferring to laboratory for further analysis. In laboratory, the hair samples were thoroughly washed with distilled water and acetone solution 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  $50\text{ }^\circ\text{C}$ . Dried hair samples (0.25 g) were weighed into clean Pyrex tubes, 2.0 mL of 1:1  $\text{HNO}_3\text{: HClO}_4$  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  $\text{H}_2\text{SO}_4$  was added and the tubes were heated at  $200\text{ }^\circ\text{C}$  for 20 minutes until the digests were clear (Koseoglu et al. 2017, Voegborlo & 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 using Atomic Absorption Spectrometer (GBC 932 plus) via Hydride Generation technique.

### **Household Questionnaire Survey**

A questionnaire survey was conducted with prior approval from Ethical Research Committee, University of Peshawar. The 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 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, 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 up and 10 from the downstream area. All the households were within 6 Km distance from the river Swat.

### Statistical data analysis

Statistical data analysis was carried out using Multilinear regression and Group mean difference t-test. Descriptive and multivariate statistical analysis were performed using XLSTAT 2017 and OriginPro 2018.

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

$$Y = \beta_0 + \beta_1 \cdot X_1 + \dots + \beta_k \cdot X_k + \mu_i \dots \dots \dots \text{Eq. 1}$$

Where **Y** represents the dependent variable, **X** is independent variable with **k** = 1, 2 .....n. **β<sub>0</sub>** is constant or intercept and **β<sub>1</sub>** is the

slope variable or slope coefficient ( $\frac{\Delta y}{\Delta x} = \text{Slope}$ ). Error in the regression is represented by **μ<sub>i</sub>**.

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

$$t = \frac{\text{difference between groups}}{\text{sampling variability}} \dots \dots \dots \text{Eq. 2}$$

## Results And Discussion

### Hg concentration in fish

The mean Hg concentration in upstream fish *trutta fario* (Brown Trout) and *Schizothorax plagiostomus* (Swati fish) were  $34.7 \pm 18 \mu\text{gKg}^{-1}$  and  $29.4 \pm 15 \mu\text{gKg}^{-1}$  (Table. 1). The mean Hg concentration in downstream Swati fish, *Crossocheilus diplochilus* (Spena dega) and *Garra gotyla gotyla* (Tora dega) were  $64.6 \pm 21 \mu\text{gKg}^{-1}$ ,  $113.3 \pm 33 \mu\text{gKg}^{-1}$ , and  $326.3 \pm 53 \mu\text{gKg}^{-1}$  respectively (Table 1). Highest Hg concentration of  $336.73 \mu\text{gKg}^{-1}$  was found in Tora dega fish from downstream area. Generally, the concentration of Hg in downstream fish species was high than upstream. Previously, (Diringer et al. 2015) observed high Hg concentration in downstream fish species in Madre de Dios River. However, Michalak et al. (2014) and Anwar et al. (2017) observed no significant variation in up and downstream fish. In our study the high Hg concentration observed in downstream fish can be associated with anthropogenic inputs. 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.

Table 1. Native fish characteristics and measured Hg concentration

Location	Fish Species		Nutrition	Length (cm)	Weight (g)	Hg Conc. ( $\mu\text{gKg}^{-1}$ )
Up-stream	Salmo trutta fario (n=4)	Brown Trout	Carnivorous	28 $\pm$ 8	240 $\pm$ 55	34.7 $\pm$ 18
	Schizothorax plagiostomus (n=6)	Swati fish	Omnivorous	28 $\pm$ 12	193 $\pm$ 40	29.4 $\pm$ 15
Down-stream	Schizothorax plagiostomus (n=5)	Swati fish	Omnivorous	22 $\pm$ 8	88 $\pm$ 22	65 $\pm$ 21
	Crossocheilus diplochilus (n=5)	Spena dega	Omnivorous	14 $\pm$ 6	43 $\pm$ 17	123 $\pm$ 33
	Garra gotyla gotyla (n=4)	Tora dega	Omnivorous	13 $\pm$ 4	26 $\pm$ 11	326 $\pm$ 53

### Hg concentration in hair

The average Hg concentration in scalp hair of the up and downstream fishing community were 658 $\pm$ 125  $\mu\text{gKg}^{-1}$  and 3969 $\pm$ 791  $\mu\text{gKg}^{-1}$  respectively. In upstream area, Hg concentration in scalp hair was above the WHO permissible limit of 1000  $\mu\text{gKg}^{-1}$  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  $\mu\text{g Kg}^{-1}$  Hg for scalp hair. The mean Hg concentrations in scalp hair of the upstream fishing community were below the WHO guideline limit of 1000  $\mu\text{g Kg}^{-1}$  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 ( $t = -3.37$ ) in the mean Hg concentration in scalp hair of the up and downstream communities. However, no significant difference was observed for the Hg concentration in scalp hair of the male and female. Previously, (Anwar et al. 2007) and (Shah et al. 2016) observed high Hg concentration in scalp hair of the female compared to male. In contrast, (Barbosa et al. 2001) reported significantly low Hg concentration in scalp hair of the females than male.

No significant correlation was observed between Hg concentration in hair and age of the respondents. Similar findings were reported by (Szykowska & Pawlaczyk 2007), (Karabedian et al. 2009), and (Michalak et al. 2014). 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 by 12.98  $\mu\text{gKg}^{-1}$  from the constant,

however, the correlation was not significant. While Anwar et al. (2017) reported that individual having 11 or more years of education ( $p$  for trend = 0.013) have relatively higher concentration of Hg in scalp hair.

### **Effect of dietary habits on Hg concentration**

The multilinear regression indicated that in the upstream area consuming carnivorous fish (Brown trout) daily or 2-3 times a week significantly increases the Hg concentration in scalp hair by 3246 and 368  $\mu\text{gKg}^{-1}$  respectively from the constant value of 515.4 (confidence level 99%) (Table S-2). Similarly, consuming omnivorous fish (Swati fish) 2-3 times a week significantly increases the Hg concentration in scalp hair by 1981  $\mu\text{gKg}^{-1}$  from the constant value of 2734  $\mu\text{gKg}^{-1}$  (confidence level 90%). In the downstream area, consuming omnivorous fish (Swati fish, Tora dega and Spina dega) 2-3 times a week significantly increases the Hg concentration by 2605  $\mu\text{gKg}^{-1}$  from the constant value of 1474 (confidence level 95%) (Table S-2). 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  $\mu\text{gKg}^{-1}$ .

The variation of Hg accumulation in human scalp hair in relation to non-fish dietary habits is given in Table S-3. The Regression analysis showed that consuming dry fruits at least 1-2 times a month or 1-2 times a week significantly lowers the Hg accumulation in scalp hair by 5347 and 5077  $\mu\text{gKg}^{-1}$  respectively from the constant value of 8943 (confidence level 90%). 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 consumption.

## **Conclusion**

The concentration of Hg in up and downstream fish species were within the WHO permissible limits. 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 fishing community and exceeded the WHO guideline limit of 1000  $\mu\text{g Kg}^{-1}$  for both children and adults. The Hg concentration observed in different fishes of the river Swat is a serious public

health concern especially for the fishing community. Further studies are recommended to identify the sources of Hg and welfare impact of fish contamination on the fishing community of river Swat.

## 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

### 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.

### Competing interests

The authors declare no competing interests.

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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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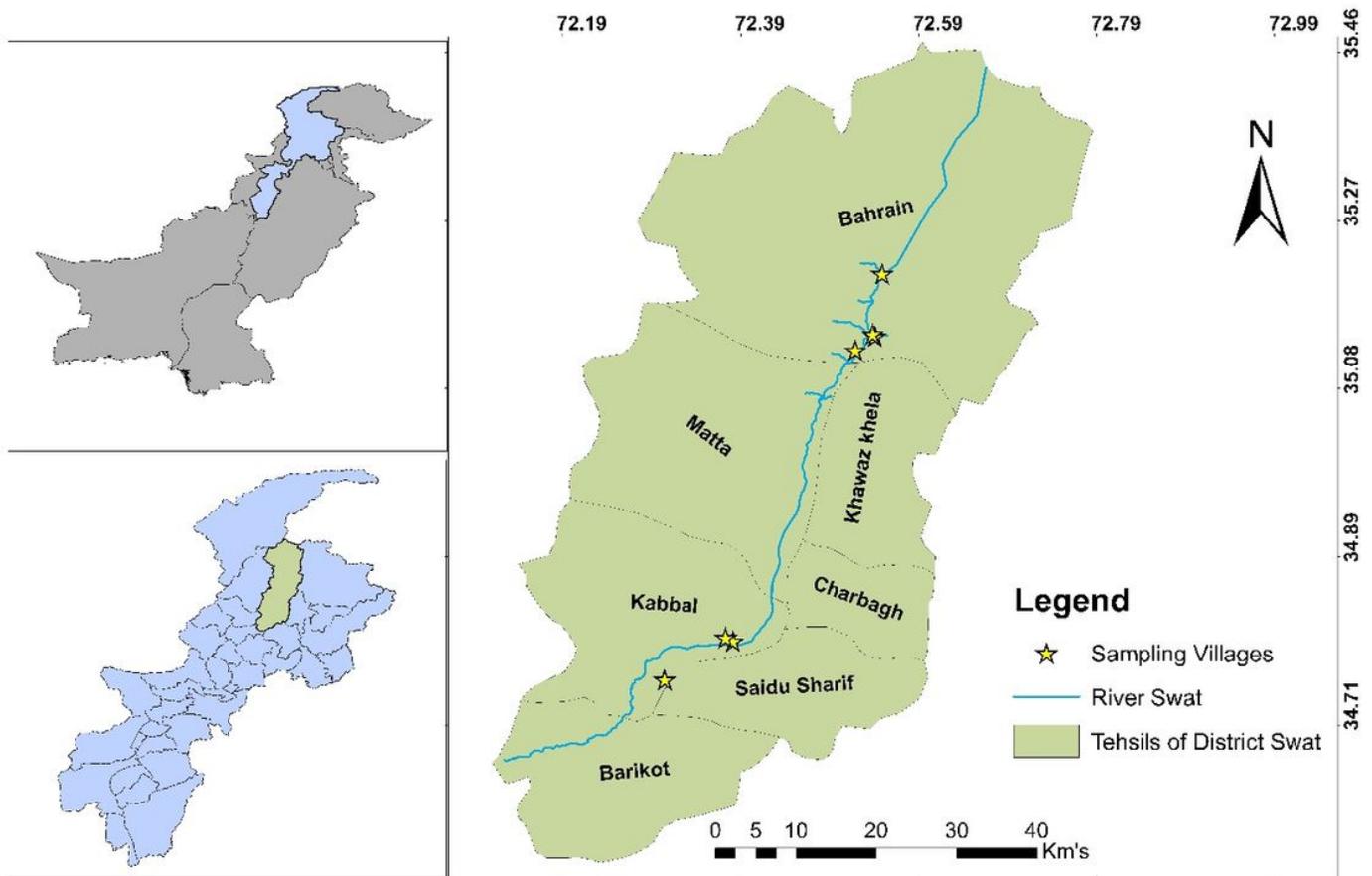
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## Figures



**Figure 1**

Sampling locations along the river Swat. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

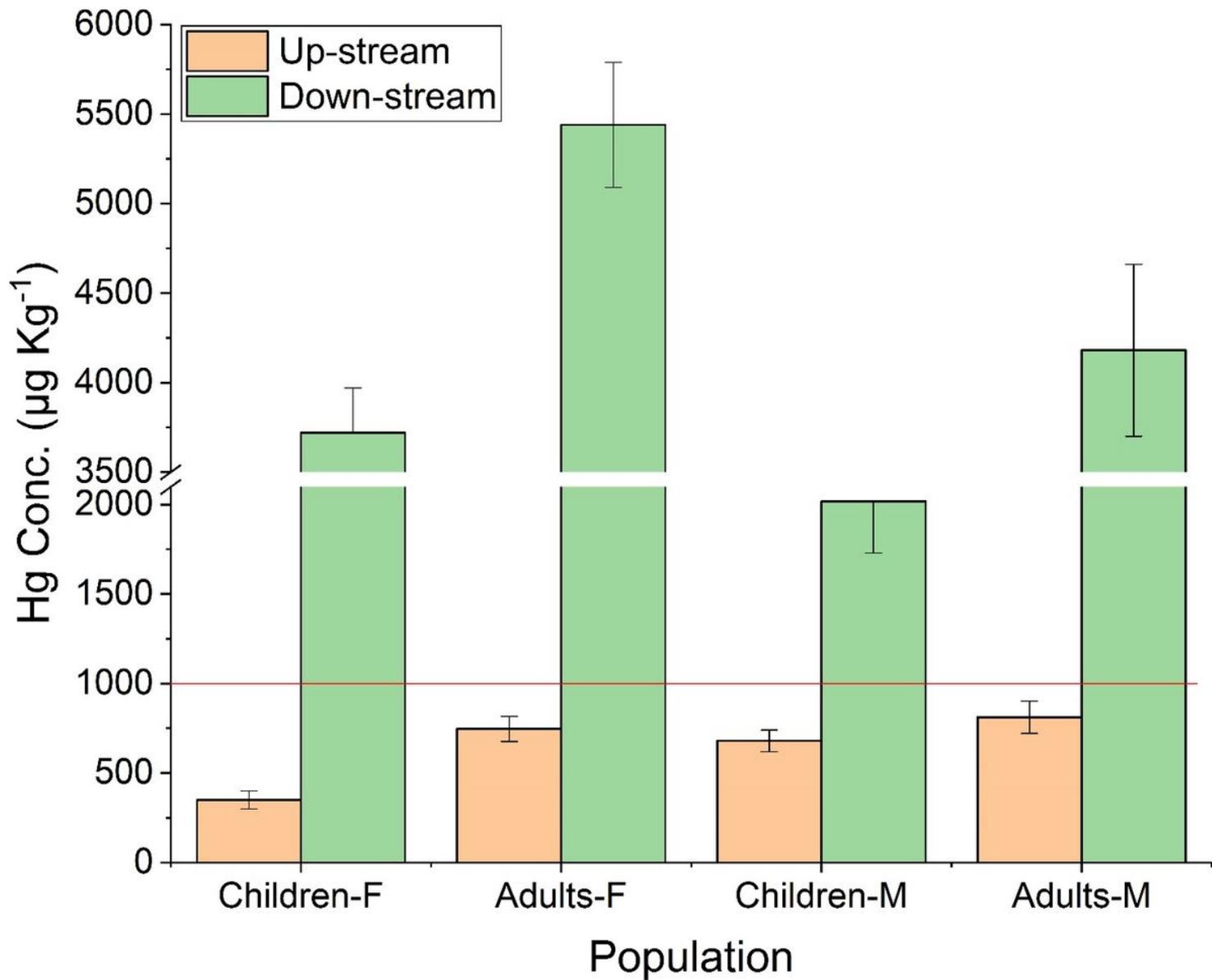
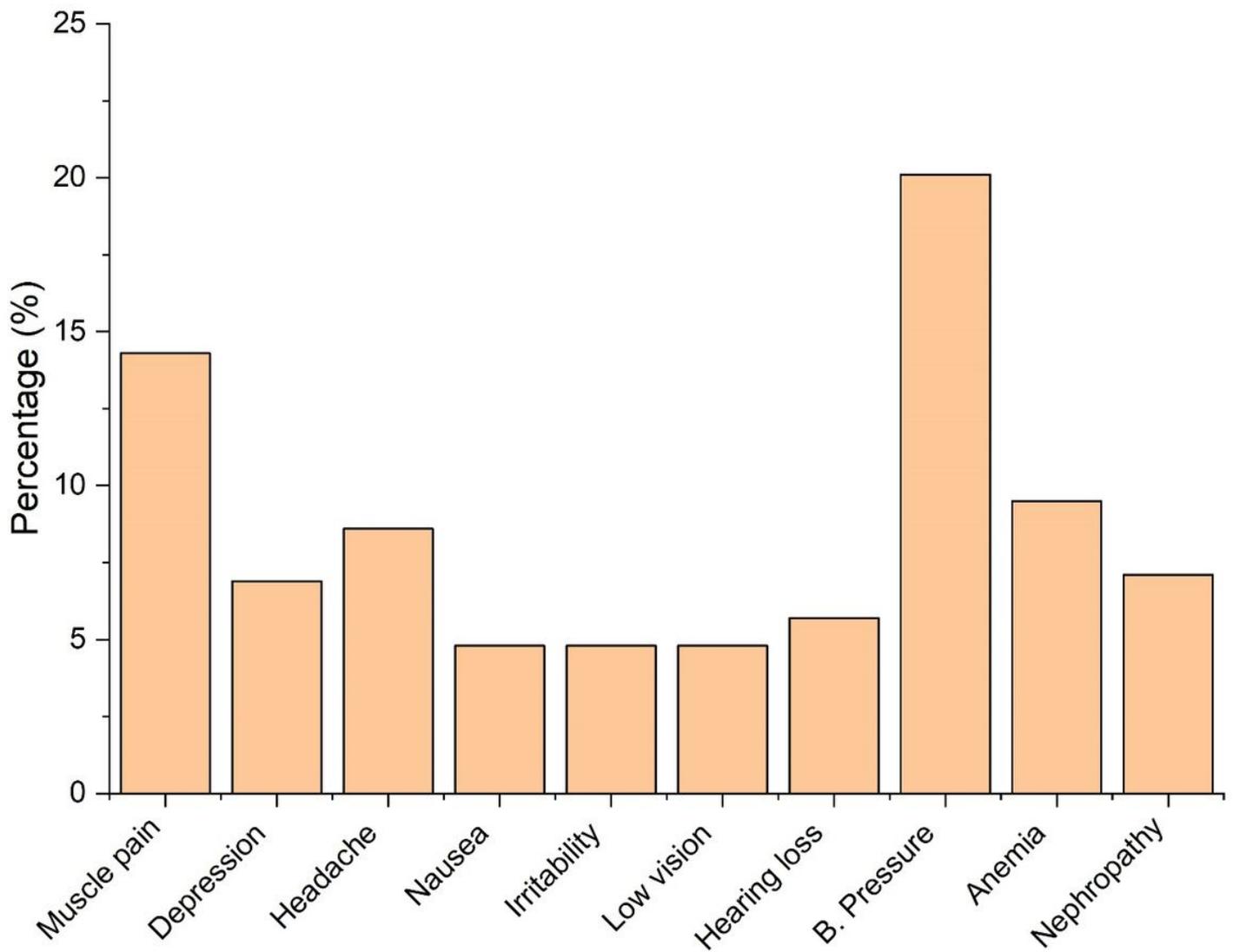


Figure 2

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



**Figure 3**

Prevalence of diseases among the fishing community.

## Supplementary Files

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