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RESEARCH ARTICLE

Biomonitoring of mercury in water, sediments, and fish (brown and rainbow trout) from remote alpine lakes located in the Himalayas, Pakistan

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Abstract

Mercury (Hg) contamination of aquatic ecological units and subsequent bioaccumulation are major environmental problems of international scope. Moreover, the biogeochemistry of Hg in the remote alpine lakes aquatic ecosystem in the Himalayas remains largely unexplored. The current study investigated Hg concentrations in different environmental compartments such as water, fish, and sediments in the remote alpine lakes (RALs) including Glacial-fed Lake, Ice melting-fed Lake, and Rain-fed Lake in northern areas of Pakistan. The mean concentration of Hg in Rain-fed Lake water was (1.07 μg L^{-1}), Ice melting-fed Lake (1.16 μg L^{-1}), and Glacial-fed Lake (1.95 μg L^{-1}). For fish muscle tissues, mean concentration of Hg was 1.02 mg kg^{-1} in the Rain-fed Lake, and 1.2 mg kg^{-1} for the Ice melting-fed Lake, and 1.51 mg kg^{-1} in the Glacial-fed Lake. Meanwhile, 0.27 mg kg^{-1} was observed for sediments in the Rain-fed Lake, 0.33 mg kg^{-1} for the Ice melting-fed Lake, and 0.38 mg kg^{-1} for the Glacial-fed Lake, respectively. Chronic daily intake (CDI) and potential health quotient (PHQ) for water showed high health risk in Glacial-fed Lake and low in Rain-fed Lake ($PHQ < 1$). The target hazard quotient (THQ) values for both the Brown and Rainbow trout in all the studied lakes water were less than 1, indicating no health risk. Furthermore, the Hg level showed high level of contamination in the sediments of all the studied lakes ($190 \leq RI < 380$). Overall, Glacial-fed Lake water was more polluted with Hg, as compared to Rain-fed Lake and Ice melting-fed Lake. In the light of the abovementioned results, further research work is urgently needed to shed light on the biological and geochemical monitoring of Hg in arid high-altitude ecosystems along with source identification, mercury speciation, and other potential pollutants.

Keywords Mercury · Alpine lakes · Bioaccumulation · Fish consumption · Human health risk

Introduction

Mercury (Hg) is considered to be a major environmental contaminant worldwide, because of its complex biogeochemistry in nature, high atmospheric deposition, strong tendency of bio-magnification, and high potential toxicity (Santos et al. 2021; Melnyk et al. 2021; Xu et al. 2019; Zupo et al. 2019; Gruszecka-Kosowska et al. 2018; Alves et al. 2017). The heavy metal Hg exists in three chemical forms such as inorganic form (IHg), elemental form (Hg^{o}), and organic form (Me-Hg and Et-Hg). The metallic Hg mostly occurs in white silver liquid form on the earth crust, and odorless at room temperature (Li et al. 2019; Beckers and Rinklebe 2017; Ullrich et al. 2001). These forms are capable of inter-convertible and create potential toxicity in the ecosystem (Wang et al. 2019). Hg is usually originating from natural sources such as weathering of rocks, volcanic eruptions, lixiviation, as

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well as erosion of soil particles, while anthropogenic sources include the use of fossil fuel, incineration, fungicides, catalyst processes, and industrial activities such as electric stuffs and asbestos fibers (Ponton et al. 2022; Tayemeh et al. 2020; UNEP 2018; Gerson et al. 2018; Jagtap and Maher 2015; Chiarelli and Roccheri 2014). Hg also originates from the mining activities on regional basis (Torres et al. 2018), and also release to the atmosphere by coal combustion process in the form of fly ash to the environment, and can adversely contaminate both the terrestrial and aquatic environments (Streets et al. 2018; Horowitz et al. 2017). As a result, it evaporates to the atmosphere and releases through precipitation to the earth surface, resulting from poor solubility of Hg compounds (Chiarelli and Roccheri 2014). Maximum proportion of water soluble (Hg^{+2}) releases to terrestrial environment and returns back to the atmosphere in elemental Hg form (Amos et al. 2015), and could be remained for 6 months (Horowitz et al. 2017). Hg can be deposited in the aquatic and terrestrial ecosystem by precipitation process in the atmosphere (UNEP 2008), and it can be transferred from one region to another region, depending on its chemical behavior and wind flow in the atmosphere (Pacyna et al. 2016; Cohen et al. 2004). Moreover, Hg can be transfer to remote areas due to less degradation and its volatile ability in nature (Lindberg et al. 2007). In Asia, Hg has been highly contributed about (67%) emissions through anthropogenic activities, followed by North America and Europe (EFSA 2012). In 1990s, the Asia including India and China were dominantly responsible for Hg emissions (Pacyna et al. 2010), and the coal-fired plants were primary source of Hg emissions in Asia (Driscoll et al. 2013).

Fish consumption was estimated about 154 million t in 2011 worldwide, produced (50%) by aqua-farming, which has significant impacts on countries economy (FAO 2012). Fish is enriched of essential nutrients such as proteins, vitamins, omega-3, fatty acids, and other minerals (Storelli 2008). Since, the exposure of Hg to humans and animals has been increased, due to consumption of fish through biomagnification process (Melnyk et al. 2021; Ki-Hyun et al. 2016). Hg has been listed as a high priority contaminant worldwide, recommended by the US EPA (2010). Due to the fact that Hg can be accumulated by fish in aquatic ecosystems through bio-magnification and thereby affects the human health via the food chain (Gruszecka-Kosowska et al., 2018). Previously, elevated Hg levels have been reported in fish species, and have posed adverse health issues in riparian people, due to the main source of fish diet (Lino et al. 2018; Salazar-Camacho et al. 2017). For instance, the first infected case was reported in 1965, caused by Me-Hg form in Minamata city, Japan (Ekino et al. 2007). Ingestion of Hg poses adverse effects to human health risk, resulting to impair and suffer the spoken and hearing ability, contributes to cause cancer, ataxia, and tremors in the body, disorders the sensory and vision features, and was briefly highlighted by Minamata Convention Treaty in 2013, and the main objectives were to protect and mitigate the Hg contamination in the environment (Bhattacharya 2020; Yang et al. 2020; Selin 2014). The majority of the studies have been conducted regarding Hg concentrations in field reservoirs and lakes water, sediments, and fish species. In recent studies, Ni et al. (2021) investigated elevated Hg concentrations in lakes water, sediments, and fish in microcosm experiments under the controlled conditions of water level fluctuations. Despite the significant progress in understanding of Hg contamination in different ecosystems, there are still considerable scientific research gaps of Hg contamination in alpine lakes of Himalayas region. Therefore, it is important to comprehensively investigate the abundance and composition of Hg in alpine lakes water, sediments, and in fish species in the Himalayas region.

Data on Hg enrichment in natural aquatic lakes ecosystems are available, but it will be the first study to investigate Hg composition and its bioaccumulation in fish species in alpine lakes of Pakistan. The objectives of this study were to investigate Hg concentrations in water, sediments, and fish species in remote alpine lakes including Glacial-fed Lake, Ice melting-fed Lake, and Rain-fed Lake in northern areas of Pakistan. This study further evaluated the health risk associated with consumption of Hg through water and fish in lakes while the concerned study is the baseline for future studies in remote alpine lakes of Pakistan.

Materials and methods

Study area description

The study area of remote alpine lakes is located in Northern region of Himalayas in Pakistan, as shown in Fig. 1. Three natural lakes were selected for water, sediments, and fish sampling in remote Alpine region including Mahodand (Glacial-fed Lake), Satpara (Ice melting-fed Lake), and Kachura (Rain-fed Lake). The Mahodand Lake is located at 35.7139° N latitude and 72.6510° E longitude in Matiltan valley of upper Usho on a distance of 40 km away from Kalam district Swat, Khyber Pakhtunkhwa, Pakistan. The lake receives water from melting of glaciers and mainsprings of Hindu Kush mountain ranges, and exists with elevation of total 2865 m above (2 km in length). The Satpara Lake is situated at 35° 26′ 40″ N latitude and 75° 38′ 15″ E longitude in Northern area of Skardu, with total distance of 9 km in Gilgit Baltistan region. The Satpara Lake mainly receives water from the ice melting in the Deosai plains and water runoff flowing into the Skardu town from the lake. The lake receives 5.4-, and 7.4-in. annual precipitation on average in winter and summer seasons, respectively. The Kachura Lake is located at 35° 25′ 36″ N latitude and 75° 27′ 18″ E longitude with elevation of 2499 m at Kachura

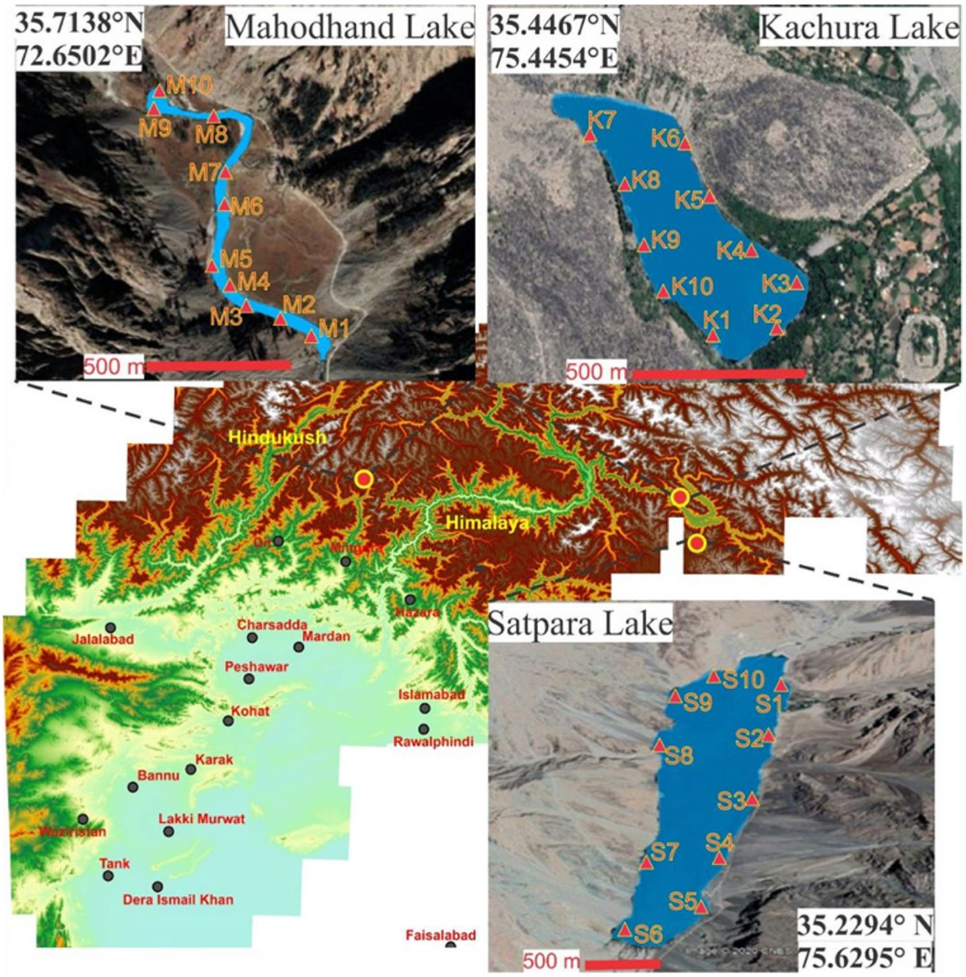

Fig. 1 Location map of the study area showing the remote alpine lakes of Pakistan

region. The source of this lake is mostly the rainfall, and lake receives monthly precipitation of about 4.4 in. with temperature ranges from − 6 to 20 °C. The temperature reaches to 15 °C in Kachura Lake during the summer season and gets frozen in the cold winter season. The lake environments are severely cold in winter season due to high elevation above the sea level with annual average of temperature of 0 °C. The alpine lakes are mostly covered with ice during December to April months, and the lakes contain high abundance of trout fishes in fishing grounds for tourist worldwide.

Samples collection and preparation

The water, sediments and fish samples were collected in April 2019, in aforementioned Northern remote alpine areas of Pakistan (Fig. 1). Total of 100 samples were randomly collected including water samples ($n = 40$), sediment samples ($n = 40$), and fish samples ($n = 20$) from selected lakes, respectively. For Hg sampling, one offshore surface water sample (10–15 cm) was collected at each lake site. Water samples were collected in 50-mL new polypropylene falcon tubes, and were acidified to 0.4% with ultrapure nitric acid (Sigma Aldrich) on site. Field blank samples were obtained by filling ultrapure water in situ for each sampling lake and were handled as samples. All samples were tightly sealed, double-packed in Ziploc bags and then stored in a cooler before transported back to the laboratory.

The surface sediment samples were collected by gravity corer at the bottom of the lakes, and sediments were pulled out by corer under vacuum pressure and were kept in polythene bags. All the water and sediment samples were transferred to laboratory for further analysis. The sediment samples were air dried on ground and sieved through a 74-µm stainless steel wire sieve, according to procedure adopted by Lino et al. (2019). Total of 20 fish samples were collected from selected lakes with the help of local fisherman. Fish species samples include Brown trout (*Salmo trutta*), belongs to Salmonidae family, collected from Glacial-fed Lake. While, Rainbow trout (*Oncorhynchus mykiss*) also belongs to Salmonidae family, collected from Ice melting-fed Lake and Rain-fed Lake. The fish samples were kept in plastic bags and stored at − 18 °C on site before transfer to laboratory. The fish species' heads, skin, their internal organs, and tails were removed because these parts cannot be generally consumed, and only the edible portions of muscles were considered for Hg analysis in the present study. The edible parts of all selected fish were cut into small pieces by stainless steel knife, and were three times washed with deionized water for further analysis. All the reagents and chemicals were used for analytical grade.

Sample digestion and Hg analysis

For the analysis of Hg concentrations, 0.5 g of sediments was digested with 15 ml of sulfuric acid (H_2SO_4) and nitric acid (HNO_3) on ratio of (2:1 v:v) in water bath at 60 °C temperature. The clear suspension was made in the extraction process, and 6% potassium permanganate ($KMnO_4$) was added until the color changed to purple, and all the extracted samples were then kept in the ice bath. Hg concentrations were determined in all water samples by adding bromine monochloride (100 µl) to oxidized Hg forms, followed by reduction with stannous chloride (20% w/v) and hydroxylamine hydrochloride ($HONH_2{\cdot}HCl$) (30% w/v) (USEPA 2002). The pH of all the samples was recorded on-site using digital pH meter (W2015, Sinowell Company, Shanghai, China). Electrical conductivity (EC) and total dissolved solid (TDS) were measured using conductivity meter (Model HI 98,303) and TDS meter (Model S 518,877), respectively. Turbidity was determined on the field by electrochemical analyzer instrument (CONSORT C-931) which was calibrated before use. Fish muscle samples were extracted with 8 ml of HNO_3 and H_2SO_4 on the ratio of (2:1 v:v) for 3 h at 25 °C, and further digested for 5 h at 60 °C. In addition, (30%) of hydrogen peroxide (H_2O_2) was added to increase the sample to (0.5 mL) with time in extraction, following the protocol described by Azevedo et al. (2020). Temperature was further increased to 65 °C until the samples became colorless. After digestion, the digested mixture was filtered using a membrane filter paper (0.45 µm). The samples were then analyzed for Hg concentrations in water, sediments, and fish muscle samples through cold vapor atomic fluorescence spectrometry, by following USEPA Method 1631 (USEPA 2002).

Exposure risk assessments

In this study, the general exposure equations were followed to assess the potential risk assessments of water, sediments, and fish. These exposure equations were based on recommendations presented by various Canadian and American publications (Health Canada 2010; USEPA 1989, 1992, 2003).

Water exposure risk assessments

Hg was detected in each lake for the identification of risk assessments, while daily intake of Hg and health quotient were calculated for health risks assessment in water by the following Eqs. (1) and (2), respectively.

Chronic daily intake (CDI)

$$\mathrm{CDI} = \frac{\mathrm{C} \times \mathrm{DI}}{\mathrm{BW}} \tag{1}$$

Health quotient (HQ)

$$\mathrm{HQ} = \frac{\mathrm{CDI}}{\mathrm{RfD}} \tag{2}$$

where “C” represents Hg concentrations (µg L^{-1}), DI shows the daily intake of consumption of water whereas 2 L for adults and 1 L for children per day, respectively (USEPA 2011). The BW represents average body weight for children (32.7 kg) and adults (70 kg), respectively. The RfD shows the reference dose value for Hg (0.03 μg^{-1} kg^{-1} day^{-1}) described by (USEPA 1995). The observed values of HQ (<1) are considered as safe permissible level (Gul et al. 2016).

Exposure risk assessments of fish

Estimated daily intake (EDI)

EDI of Hg (mg kg^{-1}) via consumption of fish can be calculated by the following Eq. (3):

$$\mathrm{EDI} = \frac{\mathrm{DC} \times C}{\mathrm{ABW}} \tag{3}$$

where DC represents daily fish consumption rate in gm day^{-1} $person^{-1}$ (10 gm day^{-1} $person^{-1}$ for local people and 100 gm day^{-1} $person^{-1}$ for fishermen), C is the concentrations of Hg in fish samples, and ABW indicated average body weight for adult human 60 kg (Nawab et al. 2018). If the EDI values were observed in the range of 1×10^{-6} to 1×10^{-4} considered tolerable level (Chen et al. 2015).

Target hazard quotient (THQ)

THQ helps to evaluate human health risks through consumption of fish caused by Hg concentrations USEPA (2000). Thus, THQ was followed widely in some recent studies (Ahmad et al. 2015), expressed as follows:

$$\mathrm{THQ} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{FIR} \times C}{\mathrm{RfD} \times \mathrm{WAB} \times \mathrm{AT}} \times 10^{-3} \tag{4}$$

where EF represents exposure frequency of 365 days year^{-1}, ED is the exposure duration of 70 years for Hg concentrations of fish in mg kg^{-1}, RfD is the oral reference dose for Hg (0.004 µg gm^{-1} day^{-1} (USEPA 2010, 2000, 1997), and FIR shows the food ingestion rate 10 gm day^{-1} person^{-1} for local people and 100 gm day^{-1} person^{-1} for fishermen. WAB is the adult body weight approximately 60 kg and AT showed average time per year (Nawab et al. 2018). Although, some international agencies set limits for Hg concentration to protect against Hg toxicity to human health through consumption of fish and fish-related products. Moreover, the maximum allowable limit for Hg for fish consumption was 1 µg gm^{-1} wet wt suggested by US food and Drug Administration (FDA). Similarly, fish containing Hg (0.4 µg gm^{-1} wet wt) concentration has been considered toxicant for human health in Japan. Furthermore, MAL for Hg concertation was 0.5 µg gm^{-1} set by WHO/FAO (2003) and in Europe as well. Moreover, the concentrations of Hg were greater than MAL in some tropical countries (Burger et al. 2013; Nakagawa et al. 1997).

Ecological risk assessments of sediments

The pollution indices are useful tools to evaluate sediments contaminations. Therefore, several equations have been used in the study to assess pollution degree and ecological risks in the study area (Bourliva et al. 2018; Jafarabadi et al. 2017; Christophoridis et al. 2009).

Contamination factor (CF)

CF is used to calculate the Hg contamination level in the water of each lake, by the given Eq. (5):

$$CF = \frac{CMc}{CMb} \tag{5}$$

Here CM_c shows the Hg concentrations in contaminated sites, and CM_b is Hg (0.25 mg kg^{-1}) concentrations in the standard pre-industrial reference level (Hakanson 1980). However, CF categorized the values based on contamination levels as (i) $CF < 1$ is considered as low contamination, (ii) $1 \leq CF \leq 3$ represents the moderate contamination, and (iii) $3 \leq CF \leq 6$ shows the high contamination, while (iv) $CF > 6$ exhibits the significant contamination level (Mmolawa et al. 2010; Chen et al. 2005).

Geo-accumulation index (I_{geo})

I_{geo} can be used to identify the extent of heavy metal concentrations in water sediments, developed by Muller and Geyer (1969), and I_{geo} classifies the quality of sediments (Nowrouzi and Pourhabbaz 2014).

$$I_{geo} = \log_2 \frac{CMc}{1.5CMb} \tag{6}$$

Here CM_c represents the Hg concentration in contaminated lakes site, and CM_b shows Hg concentrations in reference sediments level, and 1.5 used as constant value due to variation in metal concentration of background sediments (Nowrouzi and Pourhabbaz 2014). Although, I_{geo} categorized the contamination levels in sediments as (i) $I_{geo} < 0$ shows the low contamination, (ii) $0 \leq I_{geo} < 1$ exhibits moderate contamination, (iii) $2 \leq I_{geo} < 3$ presents moderate-high contamination, (iv) $3 \leq I_{geo} < 4$ shows high contamination, and (v) $4 \leq I_{geo} < 5$ indicates high-significant contamination, while (vi) $I_{geo} > 5$ represents the significant contamination level (Hu et al. 2019).

Enrichment factor (EF)

EF is used to evaluate the variation in metals concentration in contaminated sediments and in the background sites, described by Simex and Helz (1981). The EF can be estimated by the following Eq. (7):

$$EF = \frac{CMc/Fe_{sample}}{CMb/Fe_{background}} \tag{7}$$

where CM_c represents the Hg concentrations in each contaminated lake site, and "CM_b" is the Hg concentrations in the background site. EF classified the observed values as (i) $EF < 2$ shows low enrichment level, (ii) $2 \leq EF < 5$ is normal enrichments level, and (iii) $5 \leq EF < 20$ is high enrichments level, whereas (iv) $20 \leq EF < 40$ is significant enrichments level (Sutherland 2000). Although, iron (Fe) oxidizes to iron hydroxide [Fe $(OH)_3$] in aquatic environment, thereby tends to major sink for heavy metals. Even low level of [Fe $(OH)_3$] has effects on the distribution of metal in aquatic ecosystem (Forstner and Wittmann 1983). The EF values range (from 0.5 to 1.5) might be considered in the natural geogenic sources whereas above from ($EF < 1.5$) considered anthropogenic sources (Elias and Gbadegesin 2011). Some studies evaluated the HMs pollution, to their average shale for quantification of the concentrations and degree of Hg contaminations (Muller and Geyer 1969; Forester and Muller 1973).

Nemerow's pollution index (NPI)

NPI was used to determine the overall degree of metals contamination in contaminated sites, used by (Nemerow 1991). NPI can be identified by the given Eq. (8) as follows:

$$\mathrm{NPI} = \sqrt{\frac{\mathrm{CF}_{\mathrm{Ave}}^2 + \mathrm{CF}_{\mathrm{Max}}^2}{2}} \tag{8}$$

$\mathrm{CF}_{\mathrm{Ave}}$ represents the average contamination factor and $\mathrm{CF}_{\mathrm{Max}}$ shows the maximum contamination factor. The NPI classified degree of contamination as (i) $\mathrm{NPI} < 1$ is low contamination, (ii) $1 \leq \mathrm{NPI} < 2.5$ is low contamination, (iii) $2.5 \leq \mathrm{NPI} < 7$ is normal contamination, and (iv) $\mathrm{NPI} \geq 7$ is high contamination level (Yan et al. 2016).

Ecological risk index assessment

Ecological risk index (ERI) used to calculate the ecological risk degree related with metal concentrations (Hakanson 1980). ERI can be calculated by Eq. (9) below:

$$\mathrm{RI} = \sum_{\mathrm{i}=1}^{n} \mathrm{E}_{\mathrm{ri}} \tag{9}$$

where n shows the number of analyzed Hg in the ecological risk factor, which can be calculated by Eq. (10):

$$E_{\mathrm{ri}} = T_{\mathrm{ri}} \times \mathrm{CF} \tag{10}$$

T_{ri} represents the toxic response factor of T-Hg is 40, while CF is the contamination factor for analyzed Hg concentrations in sediments of each lake. Whereas, the ERI categorized ecological risk degrees as (i) $\mathrm{ERI} < 95$ shows low ecological risk, (ii) $95 \leq \mathrm{RI} < 190$ is moderate ecological risk, (iii) $190 \leq \mathrm{RI} < 380$ is high ecological risk, and (iv) $\mathrm{RI} > 380$ is significant ecological risk; and for E_{ri}, (i) $E_{\mathrm{ri}} < 40$ is low ecological risk, (ii) $40 \leq E_{\mathrm{ri}} < 80$ is moderate ecological risk, (iii) $160 < E_{\mathrm{ri}} \leq 320$ is high ecological risk, and (iv) $E_{\mathrm{ri}} > 320$ is significant ecological risk (Hu et al. 2019; Hakanson 1980).

Quality control and assurance

Quality control and assurance of the analysis were made through a combined measurement and assessment of replicate, field blanks, method blanks, and ongoing precision and certified reference materials. In this study, the method detection limit (MDL), defined as three times the standard deviation of ten replicate measurements of a blank solution, was less than 0.25 ng L^{-1}. During Hg determination, a method blank and standard of 5 ng L^{-1} for water and 100 ng Hg kg^{-1} for (fish muscles and sediments) were loaded with a batch of 10–15 samples to check instrument operation. Results showed that method and field blanks were below the MDL with only few of field blank samples show lower than 0.2 ng L^{-1} and 5 ng Hg kg^{-1}, representing insignificant pollution for the period of sampling, its transport, and analysis. The percentage recovery of OPR was 90–107% of the certified value for water, and 85–105% for fish muscles and sediments. The certified reference materials such as (SRM 1641e) for water, (ERM-CE464) for fish, and (CRM 580) for sediments were used for the recovery rate of Hg and verify the analytical procedure. The percentage recovery of reference materials was 90–107% of the certified value for water, and 85–105% for fish muscles, and 84–92% for lake sediments.

Data analysis

All the data were prepared in Microsoft Excel, and statistical software (SPSS 21) version was used for calculation of the data. The graphs and figures were prepared in Origin 9 (Origin Lab Corporation, USA). Arc-GIS was used for geographical map for the study area.

Results and discussion

Characteristics of physiochemical parameters

The description of pH, electric conductivity (EC µs/cm), total dissolved solid (TDS mg L^{-1}), and turbidity of water in each selected lake is summarized in Table 1. In the present study, the pH ranged from 7.4 to 7.6, 7.5 to 7.6, and 7.2 to 7.4, with mean values of 7.54, 7.56, and 7.32 observed for Rain-fed Lake, Ice melting-fed Lake, and Glacial-fed Lake, respectively. Similar pH values were recorded for the previous study of surface water of alpine streams in Khunjerab National Park of Gilgit, Pakistan (Ali et al. 2017). The pH

Table 1 Characteristics of physiochemical parameters of the studied lakes

Lakes and parameters	Units	Range	Mean	SD	NDL
Kachura (Rain-fed)					
pH		7.4–7.6	7.54	0.08	
EC		0.13–0.17	0.14	0.01	
TDS		0.06–0.8	0.07	0.007	
Turbidity			< 5		
Satpara (Ice melting-fed)					
pH		7.5–7.6	7.56	0.05	
EC		0.01–0.11	0.08	0.04	
TDS		0.4–0.5	0.04	0.004	
Turbidity			< 5		
Mahodand (Glacial-fed)					
pH	-	7.2–7.4	7.32	0.10	6.5–8.5
EC	µs cm^{-1}	0.1–0.3	0.02	0.01	
TDS	mg L^{-1}	0.01–0.02	0.01	0.004	
Turbidity	(NTU)		< 5		

SD standard deviation, *NDL* normal detection limit values in lakes were slightly neutral, while the maximum pH value was detected for Ice melting-fed Lake, which is associated with the increasing rate of algal productivity in lakes ecosystem, as demonstrated by high accumulation of algal bloom in lake sediments (Michelutti et al. 2005). Although, the surface runoff is attributed to slightly increase level in pH because runoff brings organic matter which can be decomposed in water, releasing carbon dioxide with the addition of carbonate and bicarbonate, which may lead to increase of alkalinity (Castrillon-Munoz et al. 2022). The turbidity for each Lake was (> 5), i.e., the turbidity of Glacial-fed Lake, Ice melting-fed Lake, and Rain-fed Lake was within maximum allowable limit (MAL) levels. The electric conductivity (EC) ranged from 0.13 to 0.17, 0.01 to 0.11, and 0.1 to 0.3 µs/cm, while mean values were 0.14, 0.08, and 0.02 µs/cm for Rain-fed Lake, Ice melting-fed Lake, and Glacial-fed Lake, respectively. The concentrations of Hg in lakes water were found in the decreasing order of Glacial-fed Lake < Ice melting-fed Lake < Rain-fed Lake. Moreover, the high EC in Rain-fed Lake is attributed to the precipitation and surface runoff from the mountains and high level of organic matters lead to increase in EC. The low TDS concentrations were detected in surface water ranged from 0.06 to 0.8, 0.4 to 0.5, and 0.01 to 0.02 mg L^{-1}, with mean values of 0.07, 0.04, and 0.01 mg L^{-1} for Rain-fed Lake, Ice melting-fed Lake, and Glacial-fed Lake, respectively. These TDS values were found in very low concentration in comparison with the previous study of drinking water sources and surface water in Malakand Agency, Pakistan (Nawab et al. 2016). However, TDS concentrations rely on ions in surface water (Baig et al. 2009), and the presence of TDS in surface water reflects the ions dissolution and the gradual deposition of minerals (Chabukdhara et al. 2017). Results showed that the physiochemical parameters of Rain-fed Lake, Ice melting-fed Lake, and Glacial-fed Lake were found within MAL of WHO. High turbidity indicated that lakes prone to surface runoff from the surrounding environment were due to population and other human activities which increased suspended solid leading to increase in turbidity (Sharma and Singh 2019).

Hg concentration level in surface water and sediments

The substantial variation has been observed in Hg concentrations detected in lakes surface water ranged from 0.5 to 1.8, 0.3 to 2.6, and 1.1 to 3.1 µg L^{-1}, with mean values of 1.07, 1.16, and 1.95 µg L^{-1} for the Rain-fed Lake, Ice melting-fed Lake, and the Glacial-fed Lake, respectively. The total Hg concentrations were found in the decreasing order of Glacial-fed Lake > Ice melting-fed Lake > Rain-fed Lake surface water, as shown in Table 2. However, the above results exceeded the MAL of (≤ 1 µg L^{-1}) set by Pak EPA (2008), WHO guideline (1 µg L^{-1}), and pre-industrial acceptable limit of EU and American Lakes (0.25 ppm) (Hakanson 1980). All the Hg values were exceeded than the previous study of 30 ng/L, conducted by (Babiarz et al. 2003), and apparently lower than the surface water study of Tapajós River basin in the Brazilian Amazon (Lino et al. 2019). However, surface water contaminated by Hg is attributed to the direct atmospheric depositions or originated from the sediments (Biber et al. 2015). Hence, the exposure of these Hg concentrations results in affecting the surface water quality and aquatic ecosystem and may pose harmful effects to human health (Zhong et al. 2018) and could ultimately cause disorders and death after exposure to elevated level of Hg. For instance, experimental study of Soni et al. (2012) demonstrated that high Hg concentration (27 mg m^{-3}) in gaseous state, led to the instant death of rats and rabbits after exposure to Hg for 2 h and 20 h, respectively. Other influencing factors of high Hg in lakes water might be related to geogenic sources of Hg, vegetation cover, local climate, and alteration in topography (Nasr et al. 2011). In addition, flooding of snowmelt in elevated alpine lake areas could be another reason of Hg important source, releasing to the lakes water. For instance, Chetelat et al. (2015) stated that snowmelt was an important source of Hg to lakes water in the Canadian Arctic Archipelago region. It has also been reported that concentrations of Hg and Me-Hg were highest in lakes water during spring season due to snowmelt inputs in Cornwallis Island, Canada (Loseto et al. 2004).

Table 2 Mercury concentrations such as range, mean, and standard deviation in the study area Lakes

Lakes	Statistics	Water (µg L^{-1})	Fish (mg kg^{-1})	Sediments (mg kg^{-1})	Permissible limits
Rain-fed Lake	Range	0.5–1.8	0.79–1.49	0.11–0.45	Pak-EPA (2008), ≤ 1 µg L^{-1}
	Mean	1.07 ± 0.66	1.02 ± 0.31	0.27 ± 0.14	Hakanson (1980), 0.25 pp
					WHO, 1 µg L^{-1}
Ice melting-fed Lake	Range	0.3–2.6	0.79–1.69	0.13–0.5	EU, 0.5 µg g^{-1}
	Mean	1.16 ± 1.25	1.2 ± 0.4	0.33 ± 0.15	FDA (2001) 0.5 µg g^{-1}
Glacial-fed Lake	Range	1.1–3.1	1.36–1.72	0.25–0.53	WHO/FAO (2009), 0.5 µg g^{-1}
	Mean	1.95 ± 1.04	1.51 ± 0.15	0.38 ± 0.12	

Less variation has been observed in total Hg concentrations in selected lakes of sediments, ranged from 0.11 to 0.45, 0.13 to 0.5, and 0.25 to 0.53 mg kg^{-1}, with mean concentrations of 0.27, 0.33, and 0.38 mg kg^{-1} in Rain-fed Lake, Ice melting-fed Lake, and Glacial-fed Lake, respectively. The detected mean concentrations in lake sediments were found in the order of Glacial-fed Lake > Ice melting-fed Lake > Rain-fed Lake presented in Table 2. Although, the maximum concentrations in Glacial-fed Lake may be due to anthropogenic activities involved in residential area around it and runoff during high precipitations in Rain-fed Lake region. Hence, Hg deposits in the bottom of sediments, thereby act as a reservoir in waterbodies, and additionally the carbon contents also control and distribute Hg level in sediments (Soto-Jimenez and Paez-Osuna 2001). In addition, moderate pH levels have been observed in the present study. Therefore, Hg can also release from the sediments when pH becomes low (Duarte et al. 1991; Schindler et al. 1980). Elevated level of Hg in sediments accumulates to aquatic ecosystem, resulting to prone ecological toxicity by its spontaneous source of high contamination (Shen et al. 2017). Whereas, low level of Hg contamination in Ice melting-fed Lake sediments is attributed to its high elevations (2636 m) that is situated between the sidewise of high mountains. Moreover, low Hg in sediments can also be associated with resuspension process that transfers Hg to overlying surface water column from the sediments (Gosnell et al. 2016). Fujji (1976) reported that the worldwide background level of Hg was recorded to be 50, 100–300, and 50–80 µg/kg in river, lake, and sea sediments, which has been higher than the present study. The mean concentrations of Hg in Glacial-fed Lake, Rain-fed Lake, and Ice melting-fed Lake were lower than the previous studies conducted by Zhang et al. (2018), Engels et al. (2018), and Tong et al. (2013), but were exceeded than the studies of El-Kady et al. (2019) and Salem et al. (2014).

Hg concentration level in fish species

In the present study, the Hg concentrations in fish species ranged from 0.79 to 1.49, 0.79 to 1.69, and 1.36 to 1.72 mg kg^{-1}, with average concentrations of 1.02, 1.2, and 1.51 mg kg^{-1} in Rain-fed Lake, Ice melting-fed Lake, and Glacial-fed Lake, respectively. The total concentrations of Hg in fish were observed in the order of Glacial-fed Lake > Ice melting-fed Lake > Rain-fed Lake as shown in Table 2. The maximum concentrations of Hg were observed in *S. trutta*, while minimum concentrations were detected in *O. mykiss* species. The maximum level of Hg in *S. trutta* fish could be associated with the high exposure level or having the efficiency of strong physiological system to accumulate high Hg in its body organs. Besides, all the values were exceeded from MAL (0.5 µg/g) set by WHO/FAO (2009), and EU (2001), respectively. Additionally, the present results of Hg concentrations in fish species were similar with the previous study of southeast Peru in Amazon rainforest (Martinez et al. 2018) and were also compared with the other studies conducted in Lakes (Table 3), respectively. In the current study, the Hg levels in fish may pose potential disorders in humans via consumption, such as the previous study of Barregard et al. (1994) who reported that high level of Hg concentration in blood resulted to disrupt the male reproductive system and hampered infertility status. High Hg exposure to animals may defect and stimulate sperm abnormalities, as previously reported by Choy et al. (2002) and Homma-Takeda et al. (2001). It has also been identified in experimental studies that Hg concentrations were adversely affected the female reproductive system (Bloom et al. 2010).

However, similar results were found in the previous study of same fish species in freshwater lake ecosystems, and their bioaccumulation factor was associated with atmospheric Hg deposition in Canadian Arctic (Chetelat et al. 2015). Hence, the Hg was moderately enriched in the present study of *S. trutta* species at northern area of Glacial-fed Lake (Mahodand Lake) as compared to other fish species. This demonstrates that Hg ultimately releases to the lakes ecosystem by atmospheric deposition (Meili et al. 2003), and transporting factor of Hg in lakes water is highly complex before being bio-accumulated to fish, indicating the weak relationships between the degree of Hg concentrations in lakes water and fish (Sorensen et al. 1990).

Water contamination assessment

The CDI values of Hg via surface water consumption for adults were 0.030, 0.033, and 0.055, while CDI values for children were 0.032, 0.035, and 0.059 in Rain-fed Lake, Ice melting-fed Lake,

Table 3 Mercury level in the studied regions around the world, number of lakes, number of fish, and average (mg $kg^{-}.^{1}$) levels

Regions	Lakes	Fish	Average (mg kg^{-1})	References
Sweden	356	34,694	0.4	Braaten et al. (2017)
Finland	899	14,878	0.3	Fjeld and Rognerud (2009)
Norway	155	4792	0.3	
Kola Peninsula	17	199	0.1	
Norwegian Institute For Water Research	–	264	0.4	

and Glacial-fed Lake water samples, respectively as shown in (Fig. 2). Whereas, PHQ values were calculated for adults as (1.0, 0.09, and 1.8) and (1.07, 1.16, and 1.96) for children via surface water consumption in Rain-fed Lake, Ice melting-fed Lake, and Glacial-fed Lake respectively (Fig. 3). The maximum PHQ values for both the adults and children were observed in Glacial-fed Lake, while low PHQ value was indicated for Rain-fed Lake. The PHQ values were recorded higher than the recommended acceptable value of (HQ < 1), indicating the water nearly contaminated with Hg as presented in (Fig. 3). While in comparison, the results of CDI and PHQ values were consistent in the previous studies (Riaz et al. 2019; Shakir et al. 2016; Liu et al. 2012). Since high Hg concentrations have been reported in the present study, as a result, CDI and PHQ values were also high for the adults and children. Whereas, high level of Hg interferes in the body and disturbs the nervous system, hinders the thyroid and testosterone production, and disrupts the neurotransmitter production (Fatoki and Awofolu 2003), and also causes chronic effect, irritates respiratory tract, and lung inflammation (Gary 2012). Furthermore, high level of Hg exposure may hinder children growth and cause health problems such as cognitive impairments, kidney diseases, cough and sputum, neurasthenia, tremor disease, headache and tiredness, skin allergies, chest pain, hyporeflexia, poor vision of nyctalopia, and other sensory problems (Khan et al. 2012). For instance, night blindness of nyctalopia has been reported in biological samples due to exposure to elevated level of Hg in Pakistan (Afridi et al. 2015).

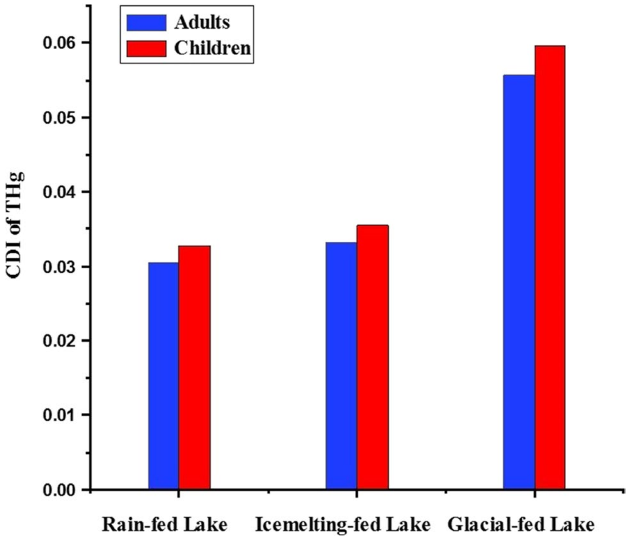

Fig. 2 Chronic daily intake (CDI) of mercury in the studied lakes water

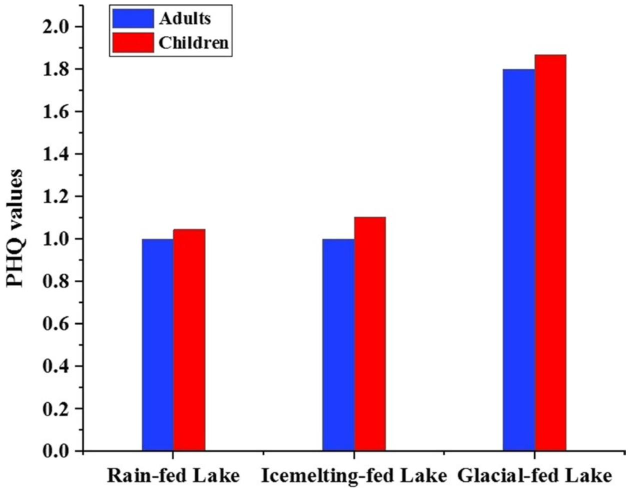

Fig. 3 Potential health quotient (PHQ) of mercury in the studied lakes water

Sediments contamination assessment

Several international guidelines have been developed for the sediments quality assessment. Hg concentrations of sediments in selected lakes were compared with the background values of USEPA (1997). However, Hg concentrations in sediments of Rain-fed Lake, Ice melting-fed Lake, and Glacial-fed Lake have been compared with the sediments quality guidelines in Table 5.

Numerous indices were used comprehensively to understand the Hg contamination in the alpine lake sediments. The indices such as CF, I_{geo}, EF, NPI, Eri, and RI were applied to assess the sediments quality in Rain-fed, Ice melting-fed, and Glacial-fed Lakes. The CF mean values were 1.39, 1.65, and 1.95 in Rain-fed, Ice melting-fed, and Glacial-fed Lakes as shown in Table 4. The results revealed moderate level of Hg contamination according to CF classifications (Hakanson 1980). The observed CF values were higher than the other previous studies (El-Kady et al. 2019; Mills et al. 2009; Wiener et al. 2006). Likewise, the I_{geo} values were 0.73, 0.99, and 1.23 for the same lakes. The I_{geo} values were found below the acceptable limit of ($I_{\text{geo}} < 1$) indicating, low level of Hg contamination in the studied lakes except Glacial-fed Lake. The Glacial-fed Lake indicating nearly high contamination. Comparatively, other studies also reported that I_{geo} values of Hg were below zero, i.e., $I_{\text{geo}} < 0$ and showed low level of contamination (El-Kady et al. 2019; Tripathee et al. 2018, 2016; Sheng et al. 2012).

On the other hand, EF values of Hg were as 0.57, 1.03, and 1.66 for Rain-fed, Ice melting-fed, and Glacial-fed Lakes, respectively. Although, Rain-fed and Ice melting-fed Lakes values were between 0.5 and 1.5 indicating natural origin while Glacial-fed Lake showed above from 1.5 suggested that Hg source has more likely as anthropogenic origin. Low enrichments of sediments for Hg were identified and showed low enrichments than guideline value of (EF < 2), according to classifications of Sutherland (2000). Moreover, the NPI values of Hg were 1.05, 1.19, and 1.31 for Rain-fed, Ice

Table 4 Risk assessment for mercury in sediments of the remote alpine lakes in the Himalayas, Pakistan

Lakes	CF	I_{geo}	EF	NPI	E_{ri}	RI
Rain-fed Lake	1.39	0.73	0.57	1.05	44.3	215
Ice melting-fed Lake	1.65	0.99	1.03	1.19	52.8	
Glacial-fed Lake	1.95	1.23	1.66	1.31	62.0	

CF, contamination factor; I_{geo}, geo-accumulation index; *EF*, enrichment factor; *NPI*, Nomerov's pollution index; E_{ri}, ecological risk factor; *RI*, potential ecological index

melting-fed, and Glacial-fed Lakes and were found consistent with CF by with the order of Glacial-fed Lake > Ice melting-fed Lake > Rain-fed Lake, respectively. The NPI values for Hg were found ($1 \leq NPI < 2.5$), indicating that overall degree of Hg contamination was showed low level contaminations. At the same time, the EF and PI values were less than for Himalayas, demonstrating that low Hg concentrations are related to atmospheric deposition (Tripathee et al. 2018) and naturally originated from regional parent bed rocks materials (Raut et al. 2017). The ecological risk factors (E_{ri}) for Hg were 44.3, 52.8, and 62.0 at Rain-fed, Ice melting-fed, and Glacial-fed Lakes (Table 4). The E_{ri} values were between ($40 \leq E_{ri} < 80$), showing moderate ecological risk factor. Furthermore, the potential ecological risk index (RI) was 215 for the studied lakes ($190 \leq RI < 380$) which is high ecological risk, hence, poses high potential risk to the lakes aquatic ecosystem in the study area. The potential ERI values for Rain-fed, Ice melting-fed, and Glacial-fed Lake values were greater than the past study conducted by Tripathee et al. (2018). The natural low degree of Hg contamination could be associated with the transportation of aerosol particles to the atmosphere by domestic emissions, which mostly contain black carbon contents release over the Himalayas hills regions during spring season (Lau et al. 2010).

In addition, burning of high African biomass and combustion of fossil fuel on Eastern sites may possibly transfer the atmospheric aerosols to the high Himalayas regions (Kopacz et al. 2011) and surrounding regions of Indian subcontinent also contributed to release aerosols in the atmosphere, as demonstrated by previous observations of Lau and Kim (2006). Consequently, the present study suggested that natural processes such as atmospheric depositions, long-range atmospheric transport, particulate matters, and other less human intrusions contributed to release Hg in the study area of alpine lakes in the Himalayas region of Pakistan.

Fish species contamination assessment

EDI and THQ for Hg were calculated via consumption of fish for adults and children. EDI results were also compared with the tolerable daily intake (TDI) in the studied Lakes, as shown in (Fig. 4). The EDI's of Hg for local people were 0.17, 0.20, and 0.25 μg/kg day^{-1}, and 1.7, 2.0, and 2.5 μg/kg day^{-1} for fishermen in Rain-fed, Ice melting-fed, and Glacial-fed Lakes, respectively. High EDI values were found for fish in Glacial-fed Lake, while lowest EDI values were observed for Rain-fed Lake fish. EDI's were found lower than the tolerable daily intake (0.71 μg/kg day^{-1} and 40 μg/kg day^{-1}), set by WHO (1990) and Ostapczuk et al. (1987), while also lower than the previous study of Cheng et al. (2009).

THQ of Hg were found varied for studied fish species in Rain-fed, Ice melting-fed, and Glacial-fed Lakes (Fig. 5). The THQ values for local people were 0.07, 0.34, and 0.64, and fishermen were 0.06, 0.30, and 0.57 in Rain-fed, Ice melting-fed, and Glacial-fed Lakes, respectively. Comparatively, THQ values for ingested fish species in the present study were found lower than the previous study of Dongting Lake, China (Torres et al. 2018). Maximum THQ values were recorded for BT in Glacial-fed Lake, whereas the lowest THQ values were observed for RT in Rain-fed Lake. The THQ values were found less than the acceptable range of (THQ < 1). If the EDI values were in the range of 1×10^{-6} to 1×10^{-4} considered tolerable level (Chen et al. 2015). The low concentration of Hg in BT and RT in the studied lakes may be due to the low atmospheric transport to the elevated height of approximate 2500 to 3000 m. The other possible reason may be the Indian monsoon, which cannot reach to the northern parts of Pakistan due to height of Himalayas which changes the direction of Indian monsoon. So the Hg

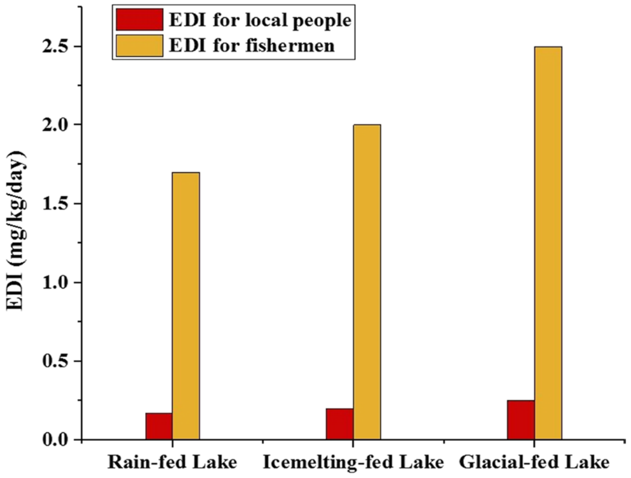

Fig. 4 Estimated daily intake (EDI) of mercury in the studied lakes fish species

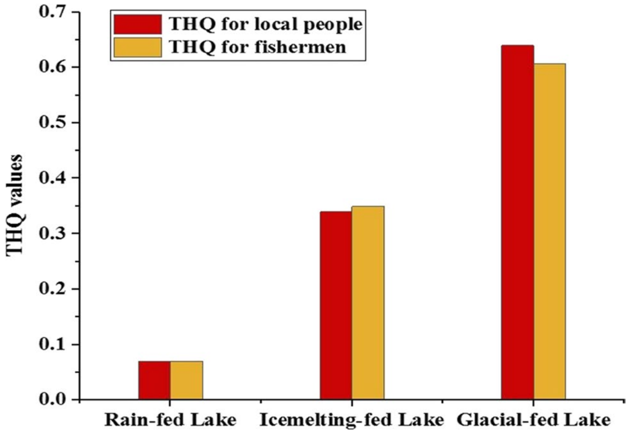

Fig. 5 Target hazard quotient (THQ) of mercury in the studied lakes fish species

concentration in these remote alpine lakes may be due to the local sources as well as transportation of Hg through valleys to the colder regions.

Conclusion

The extent of Hg contamination in water, sediments, and fish species (*S. trutta* and *O. mykiss*) was investigated in remote alpine lakes of Glacial-fed Lake, Rain-fed Lake, and Ice melting-fed Lake along with the potential risk assessment. This report is the first one in the northern areas of Pakistan related to Hg concentration in water, sediments, and their bioaccumulation in *S. trutta* and *O mykiss* species. Moderate levels of Hg were above the MAL of Pak-EPA, WHO, and EU acceptable limits in water and fish species in the present study. The HQ results indicated that Glacial-fed Lake showed higher level of contamination as compared to the other studied Lakes. Several indices results showed the moderate level of pollution in all the lake sediments as compared with other previous studies. Moreover, all the indices observed values were also compared with the sediments quality guidelines (SQGs), and consequently exceeded the SQGs acceptable limits (Table 5). In addition, the present study also represents a useful dataset for Hg compositions in water, sediments, and fish species and their risk assessment

Table 5 Sediments quality guidelines (SQGs) for mercury globally

SQGs	Hg (mg kg^{-1})	Reference
TEL	0.17	Macdonald et al. (2000) Smith et al. (1996) ANZECC (1997) Swartz (1999) Chapman et al. (1999) Shiga Prefecture (2001) Classification of Illinois Stream Sediments Laybauer (2002)
ERL	0.15	
LEL	0.2	
MET	0.2	
CB TEC	0.18	
EC-TEL	0.13	
ANZECC ERL	0.15	
ANZECC ISQG-low	0.15	
SQAV TEL-HA28	-	
SQO Netherlands target	0.3	
Hong Kong ISQG-low	0.15	
Hong Kong ISQV-low	0.28	
Flanders RV X	35	
EQS human health items (Lake Biwa)	0.0005	
Slightly elevated stream sediments	0.07	
RV	0.06 (μg g^{-1})	
BG	0.03 (μg g^{-1})	
ISQG	0.17 (μg g^{-1})	

SGQ sediments quality guidelines, *TEL* threshold effect level, *ERL* effect range low, *LEL* lowest effect level, *MET* minimal effect threshold, *CB-TEC* consensus based-threshold effect concentration, *EC-TEL*, Environment Canada Threshold Effect Level, -, *ANZECC-ERL* Australian and New Zealand Environment and Conservation Council-Effects Range Low, *ANZECC-ISQG-low* Australian and New Zealand environment and conservation council-interim sediments quality guidelines, *SQAV-TEL-HA28* sediments quality advisory value-threshold effect level, Hong Kong *ISQV-low*, *SQO* Netherland Target, interim sediments quality guidelines, Flanders *RVX* reference value, EQS-human health items; Lake Biwa, *EQS* environmental quality standard, slightly elevated stream sediments, *RV* reference values, *BG* background values, *ISQG* interim sediments quality guidelines as well as background level for future studies in northern areas of Pakistan. Nevertheless, future studies are necessary for shedding further light on the influence of Hg transport to higher altitude areas such as forest soil, trees barks, melting glaciers, and pastures and also the tropic transfer such as bio-magnification mechanisms.

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Author contribution Javed Nawab, Sardar Khan, Muhammad Idress, and Ali Asghar collected the samples from remote alpine lakes, and analyzed and interpreted the data for Hg. Javed Nawab, Junaid Ghani, and Muhammad Idress helped in Hg data analysis and reviewed the manuscript. Muhammad Idress, Junaid Ghani, and Ali Asghar performed the Hg analysis and calculated the values. Javed Nawab, Syed Aziz Ur Rehman, Muhammad Luqman, Sardar Khan, and Ziaur Rahman play important role in writing, revising, and polishing the manuscript. All the authors carefully read and approved the final manuscript.

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Data availability The analyzed datasets used are available on reasonable request from the corresponding author during the current study.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication The authors are aware of this publication.

Competing interests The authors declare no competing interests.

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