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Mercury contamination status of rice cropping system in Pakistan and associated health risks☆

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ABSTRACT

Rice is a known bioaccumulator of methylmercury (MeHg). Rice consumption may be the primary pathway of MeHg exposure in certain mercury (Hg)-contaminated areas of the world. Pakistan is the 4th-largest rice exporter in the world after India, Thailand, and Vietnam. This study aimed to evaluate the Hg contamination status of rice from Pakistan and the health risks associated with Hg exposure through its consumption. 500 rice grain samples were collected from two major rice-growing provinces, Punjab and Sindh, which contain 92% of Pakistan's rice cultivation area. Analysis of polished rice showed mean total Hg (THg) concentration of 4.51 $ng.g^{-1}$, while MeHg concentrations of selected samples averaged 3.71 $ng.g^{-1}$. Only 2% of the samples exceeded the permissible limit of 20 $ng.g^{-1}$. Samples collected from Punjab showed higher Hg contents than those from Sindh, possibly due to higher rates of urbanization and industrialization. Rice samples collected from areas near brick-making kilns had the highest Hg concentrations due to emissions from the low-quality coal burned. THg and MeHg contents varied by up to five and fourfold, respectively, between point and non-point Hg pollution sites. Moreover, the %Hg as MeHg in rice did not differ significantly between point and non-point Hg sources. Health risk was assessed by calculating a mean probable daily intake, revealing that Hg intake through rice consumption is within the safe limits recommended by the World Health Organization. However, rice intake may be a substantive pathway of MeHg exposure because fish, which are another major source of Hg, are consumed in Pakistan at some of the world's lowest rates. This study provides fundamental data for further understanding of the global issue of Hg contamination of rice and its related health risks. Furthermore, the current study suggests there is a need to conduct further research in rice-growing areas at the regional level.

1. Introduction

Mercury (Hg) is a global contaminant and ubiquitous in the natural environment. It can exist in three oxidation states metallic (Hg^0), mercuric (HgII) and mercurous (HgI) due to its physico-chemical properties. Consequently, chemical speciation largely determines Hg's mobility and toxicity in the contamination of the air, land, biota and water through natural or anthropogenic emissions (Fitzgerald et al., 2007;Mergler et al., 2007; O'Connor et al., 2019; Ullrich et al., 2001). Although all forms of Hg are toxic, among organic species, methylmercury (MeHg) is studied extensively because it can cause neurological disorders in humans and has the potential for biomagnification (Clarkson and Magos, 2006). Fish and other marine mammals occupy the upper trophic levels of aquatic food webs, so can bioaccumulate and biomagnify MeHg to high concentrations (Kidd et al., 2012; Lavoie et al., 2013). Consumption of such fish is thought to be a primary MeHg exposure pathway in humans (Al-Mughairi et al., 2013; Li et al., 2014). Alternatively, previous studies have suggested that MeHg can also bioaccumulate in terrestrial food webs (Ackerman et al., 2013; Wang et al., 2020).

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Rice is grown across the globe and serves as the primary nutrition source for 3 billion humans (Khush, 2005). Contamination of rice paddy soil by anthropogenic activities such as Hg mining, coal burning, chloralkali production, and using Hg-contaminated water usage for irrigation has been reported (Feng and Qiu, 2008; Li et al., 2009). Several studies conducted in Hg mining and non-mining areas have demonstrated that rice grain has the highest MeHg bioaccumulation capability of all cereals (Meng et al., 2010; Qiu et al., 2008). A typical rice paddy behaves like a potential hotspot of MeHg production, where inorganic Hg is converted into a more toxic form (i.e. MeHg) by iron and sulfate-reducing bacteria, methanogens, and archea under flooded (anoxic) soil conditions (Fleming et al., 2006; Gilmour et al., 2013; Liu et al., 2014; Zhao et al., 2016a, Zhao et al., 2016b). Elevated MeHg concentrations have also been observed in rice, even in the absence of definite Hg point sources (Horvat et al., 2003; Zhang et al., 2010b). Rice forms a significant proportion of the diet of residents from inland Hg-polluted areas and is considered a vital pathway of MeHg exposure (Feng et al., 2008; Meng et al., 2014b). With international trade and globalization, rice has also contributed significantly to human MeHg exposure in areas distant from contamination sources in recent years (Liu et al., 2019). Thus, human exposure to MeHg through rice ingestion is an emerging threat worldwide that has drawn the attention of researchers.

Agriculture holds an important position in the national economy of Pakistan. Each year, it produces an average of 6.8 million tons of rice. The country is responsible for exporting 8.2% of the world's paddy rice. Most of these crops are grown in the fertile Sindh and Punjab regions, with millions of farmers relying on rice cultivation as their major source of income (PBS, 2014). Pakistan is a developing country with rapid growth in population, urbanization, and industrialization, which is causing environmental pollution (Azizullah et al., 2011). Localized sources like chloralkali plants, cement industries, lighting manufacturing industries, hospital waste, municipal incinerators, and different coal-burning activities are emitting Hg to the environmental matrices of Pakistan at rates of 10,800–36,900 kg year^{-1} (Ali et al., 2019; Eqani et al., 2016; Malkani, 2012). There are around 20,000 brick kilns in Pakistan where handmade bricks are baked using poor-quality coal with the fixed-chimney bulls trench kiln (FCBTK) technique. This process is hazardous and the sector is highly unregulated and uncoordinated (CCAC, 2018; Khan et al., 2019; Khattak et al., 2009). Hence, brick kilns enrich the surrounding environment with heavy metals like Pb, Zn, Cu, Hg, As, Cd, Se and Fe as byproducts of coal burning (Ahmed and Hossain, 2008).

The Minamata Convention on Hg has provided guidelines for coping with Hg pollution globally. However, knowledge of Hg exposure remains limited due to data unavailability in various regions and subpopulations (Basu et al., 2018). According to a recent model-based study, across the world, India (South Asia) produced the most total Hg (THg) in rice grain and residues (2.1 and 64 Mg, respectively, in 2016), due to its large-scale rice production and the relatively high THg concentrations (Al-Saleh and Abduljabbar, 2017; Brombach et al., 2017; Liu et al., 2019). Bangladesh had the highest THg production density (3.8 and 120 g.km^{-2} in rice grain and residues, respectively, in 2016), followed by India (0.80 and 25 g.km^{-2}), primarily due to the high population densities and the use of rice as a staple food in these countries (Liu et al., 2019). However, the Hg contamination status of the rice system in Pakistan remains unstudied. The present study aimed to assess the Hg contamination status of rice grown in all major rice-growing areas of Pakistan and the associated health risks of rice consumption to the general population. Hotspots of Hg contamination and associated sources in the study area are also highlighted. This study quantifies the extent of Hg pollution in Pakistan's rice system.

2. Materials and methods

2.1. Study area description

The Islamic Republic of Pakistan is a country in South Asia situated between 24.35390 and 35.91869° N and 61.74681–75.16683° E (Fig. 1a). Rice is cultivated on 2.62 million ha in Pakistan, out of which a 1.76 million ha area in Punjab (which is the most populated province and leading rice producer) has a total rice production of 3.17 million tons per annum (61% of national production) (PBS, 2014). The annual production of basmati rice in Pakistan is 2.9 million tones. Rice crops are cultivated mostly in the northern irrigated plains, where rice-wheat crop rotation dominates. The following 16 districts of Punjab are included in this study (Fig. 1b): Lahore (LHR), Faisalabad (FSD), Sialkot (ST), Narowal (NRW), Mandi Bahhaudin (MBD), Sahiwal (SHL), Okara (OKR), Kasur (KSR), Sheikhupura (SHK), Hafizabad (HFZ), Gujrat (GT), Nankana Sahib (NKN), Gujranwala (GRW), Chiniot (CHN), Pakpattan (PKP) and Bahawalnagar (BNR). These districts are famous for producing a long-grain, aromatic, fine rice variety known as *basmati* (*bas* = aroma, *mati* = soil). Among all of these Lahore, Faisalabad, Kasur, Gujranwala, Sheikhupura, and Sialkot are also considered the industrial hubs of the country for having industries ranging from the large-scale to small cottage industries. The climate is sub-tropical and sub-humid. The maximum temperature reaches 43 °C in summer and the minimum can be 4 °C in winter, with a maximum rainfall of 800 mm/year. Almost 80% of the precipitation falls in the monsoon period (July–September). Rice planting starts in May–July and harvesting takes place in October–November.

Sindh province is located in the southern part of Pakistan. Being close to the Arabian Sea, it has a typical tropical climate in its lower plains and a sub-tropical climate in its upper plains. Summer is hot and humid, with temperatures of up to 50 °C, while winter is short and mild. Six rice-growing districts in Sindh, namely Hyderabad (HYD), Thatta (THA), Badin (BD), Dadu (DAD), Larkana (LRK), and Shikarpur (SKR) were chosen for sample collection (Fig. 1b). The rice-growing season starts and ends a little earlier in Sindh than in Punjab because of the different climatic conditions. Cultivation of various hybrid coarse grain rice varieties dominates in this part of the country.

2.2. Sample collection and preparation

A total of 500 ($n = 500$) rice grain samples were collected from rice fields in major rice-growing areas of Sindh ($n = 86$) and Punjab ($n = 414$) provinces, Pakistan, during the 2017 harvest season (Fig. 1b & c). Punjab and Sindh represent 92% of total area under rice cultivation in Pakistan (PBS, 2014). Both targeted and random locations were sampled due to the large extent of the study area and the lack of Hg contamination data (Table S1). All conventional Hg emission sources (brick kilns, wastewater effluent, chloralkali plants, industrial areas, coal-fired power plants, incinerators, etc.) were considered as target locations (Fig. 2a). Samples were collected at distances of 8–10 km to ensure uniform sampling densities across both target and random sites, except for two types of sites: chloralkali plant and coal-fired power plant. At each sampling location, rice grains were collected from 4 to 5 healthy plants within an area of 5–10 m^2 using a pair of scissors. They were homogenized thoroughly to form a representative composite sample. At brick kiln locations, samples were collected 100–120 m downwind from the brick kiln unit.

Samples were washed with drinking water and then deionized water to remove particulate matter. Samples were air-dried in plastic gauze bags for freeze-drying (Eyela, FDU 2110, Japan), husk removal (huller, JLGJ4.5, China) and polishing (rice mill, JNMJ3,

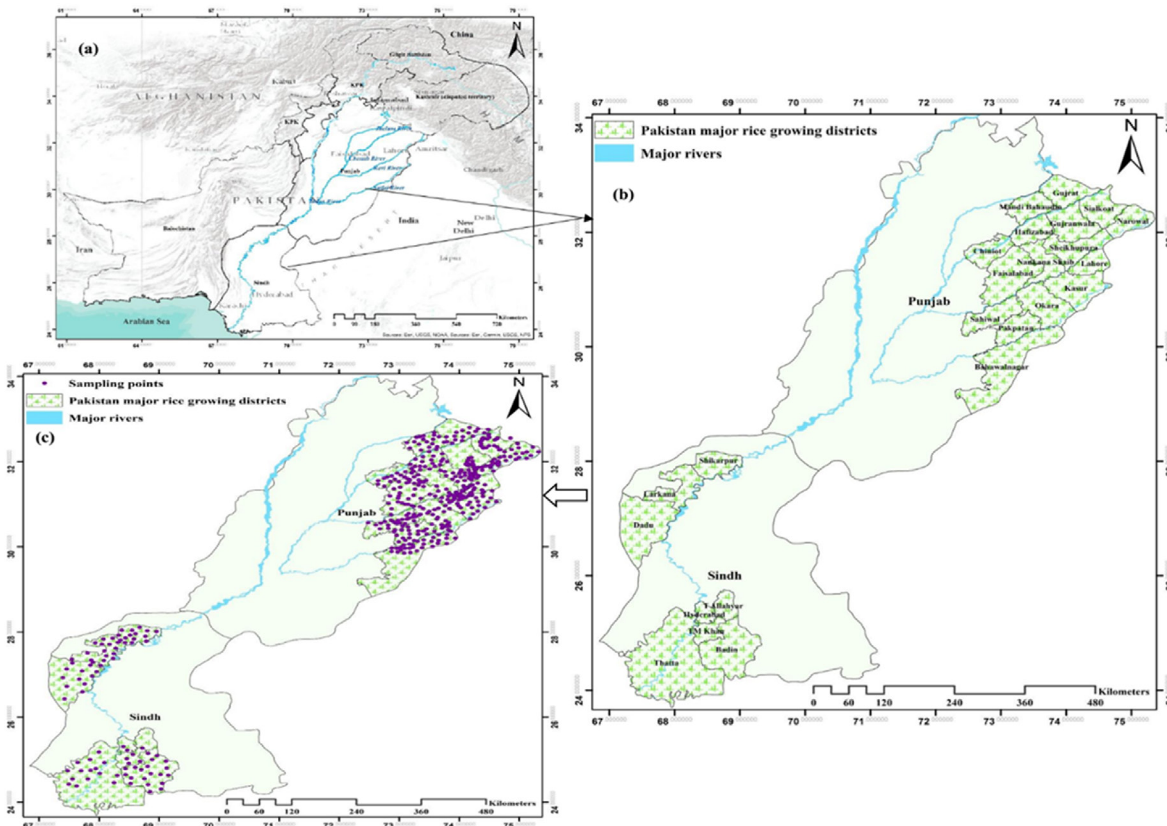

Fig. 1. Geographical map of Pakistan(a) Major rice-growing districts of Punjab and Sindh provinces (b) and sampling locations of the current study (c).

China). Samples were finally ground to a powder using a grinder (IKA-A11 basic, IKA, Germany) and stored in polyethylene zip-lock bags, then transported in polythene zip bags to China for Hg analysis. Airtight vacuum bags were used to store samples in a refrigerator at 4 °C. The grinder and all the accessories involved in grinding were cleaned with ethanol and dried using a blow-drier before each operation. All possible measures were taken to avoid cross-contamination during the sample collection and processing.

2.3. Analytical methods

To analyze THg contents in rice samples, 0.3–0.5 g of each powdered rice grain (white rice) sample was digested after addition of 5 ml nitric acid (HNO_3), then heated in a water bath for 3 h at 95 °C. An appropriate volume of digested sample was taken for THg determination by Cold vapor atomic fluorescence spectrometer (CVAFS) (Brooks Rand Model III, Brooks Rand Laboratories, USA), following BrCl oxidation, $SnCl_2$ reduction, purging with N_2 and desorption of Hg at 450 °C (USEPA, 2002). All rice grain samples ($n = 500$) were analyzed for THg concentrations. For MeHg analysis, samples with THg concentrations >5 ng.g^{-1} ($n = 100$) and <5 ng.g^{-1} ($n = 24$) were selected at random. This sample selection process aimed to present the worst-case scenario of MeHg in all samples more than average THg concentration for the current study.

To quantify MeHg, an alkaline leaching and solvent extraction method was used. About 0.2–0.3 g of sample was weighed and digested with 25% KOH in an oven at 75–80 °C for up to 3 h. After cooling to room temperature, samples were acidified using concentrated HCl, then leached with dichloromethane (CH_2Cl_2) and back-extracted from the solvent to the water phase. The MeHg in samples was determined by CVAFS preceded by N_2 purging, Gas chromatography separation, and thermal decomposition to Hg^0 steps based on United States Environmental Protection Agency (USEPA) method 1630 (Liang et al., 1994, 1996; USEPA, 2001). Ultrapure-grade acids and analytical-grade reagents were used for analysis (Sinopharm Chemical Reagent Co, Ltd, China). The dichloromethane reagent was of chromatographic grade (Tedia Company, Inc., USA). All the glassware utilized in the analysis, including glass tubes, bubblers, and beakers, were washed with detergent and rinsed with deionized water (DI) and double-distilled water (DDW). After washing, the glassware was pre-heated in a muffle furnace at 500 °C for 2 h to ensure it had very low blanks for Hg.

2.4. Calculation of PDI for MeHg and IHg

To estimate the probable daily intake (*PDI*) of MeHg and IHg through rice consumption in the adult population of Pakistan, Equation (1) was employed:

$$PDI = \frac{C \times IR \times A}{bw} \tag{1}$$

PDI is expressed in micrograms per kilogram of body weight per day (μg.kg^{-1} bw.d^{-1}), $bw = 60$ kg (Iqbal et al., 2016); *C* is the concentration of MeHg or IHg in rice (ng g^{-1}); *IR* is the daily intake rate (0.047 kg.d^{-1}) (IRRI, 2013), and *A* is the Hg absorption rate of the human body, taken as 7% for IHg (THg minus MeHg) and 95% for MeHg (WHO, 1990). Human health risks caused by MeHg and IHg exposure according to a rice intake hazard quotient (HQ) were calculated using Equations (2) and (3). The HQ expresses the non-carcinogenic effects of a substance when the level of exposure is

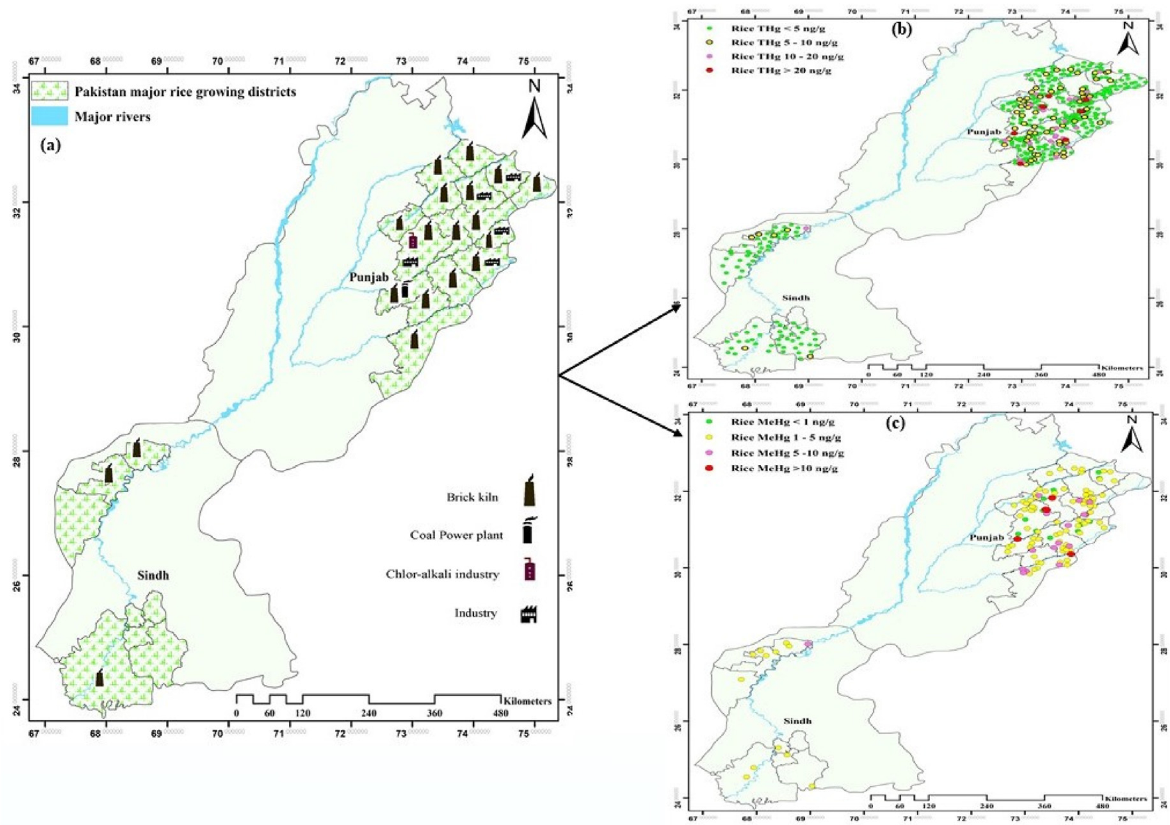

Fig. 2. Map showing the occurrence of different Hg sources encountered for the current study in rice-growing areas of Pakistan (a). THg concentrations measured in rice samples (b) and MeHg levels (c).

higher than the reference dose; there may be some adverse but non-carcinogenic effects. The HQ for rice consumers in this study was estimated by comparing the PTWI for IHg and RfD for MeHg (Rothenberg et al., 2017; USEPA, 2000; Vieira et al., 2011). To estimate an additive effect, HQ values can be combined for IHg and MeHg to obtain a hazard index (HI; Eq. (4)) (Qian et al., 2010). A HI value > 1 depicts adverse non-carcinogenic effects, while HQ or HI values < 1 indicate no adverse health effects.

$$HQ_{IHg} = \frac{PDI \times 7}{PTWI} \tag{2}$$

$$HQ_{MeHg} = \frac{PDI}{RfD} \tag{3}$$

$$HI = HQ_{IHg} + HQ_{MeHg} \tag{4}$$

2.5. QA/QC, statistical analysis and geographical mapping

For each batch of THg and MeHg analysis, quality control was assured by triplicating every tenth sample along with matrix spikes, method blanks, and certified reference materials. The method detection limits (3∂) were observed as 0.005 $ng.g^{-1}$ for THg and 0.002 $ng.g^{-1}$ for MeHg analysis. For all analyzed triplicate samples, relative standard deviation percentages (RSD%) retained < 5.6% for THg and <7.2% in the case of MeHg analysis. Matrix spikes recoveries varied from 98–108% for THg and 92–106% for MeHg analysis. Various certified reference material (CRM) GSB-11 (citrus leaf), GBW-100359 (rice flour), TORT-2 (Lobster Hepatopancrease), and TORT-3 (Lobster Hepatopancrease) were analyzed in the present study and the results are listed in Table S2.

Dunn tests were applied to compare THg concentrations in rice samples collected from brickmaking locations, industrial sites, and other sites without known sources. Kruskal-Wallis one-way analysis of variance (ANOVA) tests on ranks were used to determine significant differences (Figure 5), between the THg concentrations of brick kiln samples and those of the other two groups. The latitudes and longitudes of sampling locations were recorded by GPS (Monterra, Garmin, USA) and the data were used to produce geographical maps in ArcGIS version 10.5 software (ESRI, USA). Data on THg and MeHg concentrations were subject to statistical analysis using Sigma Plot (Version 14, Systat Software Inc, USA). Measurements of Hg are presented as mean ± standard deviation (SD) and other descriptive statistics. Relationships among variables were determined by regression analysis. Significant differences between group means were also calculated by one-way ANOVA. Coefficients of correlation (r), regression (R^2) and significance of probabilities (p) were computed as well, using both linear and non-linear regression models.

3. Results and discussion

3.1. THg in rice grains

The THg concentrations measured in all collected samples are shown in Fig. 2b; they range from 0.44 to 157 $ng.g^{-1}$ with a mean ± SD of 4.51 ± 8.56 $ng.g^{-1}$. The highest mean THg concentration (6.75 $ng.g^{-1}$) was found in the Okara district of Punjab, which had a range of 1.28–21.00 ng.g^{-1}, followed by Bahawalnagar (mean = 6.47 ng.g^{-1}, range = 0.86–25.56 ng.g^{-1}). The THg concentrations from all districts of Punjab and Sindh provinces are shown in Fig. 3. ANOVA revealed significant differences in the THg values of districts in Punjab (Fig. 3a). On the other hand, there was no significant difference among the THg concentrations within Sindh province (p = 0.103; Fig. 3b), indicative of spatial homogeneity. Eighty percent (n = 400) of analyzed rice samples had THg < 5 ng.g^{-1}. Only 10 samples (2% of total samples) exceeded the permissible limit of 20 ng.g^{-1} (THg) in cereal grains recommended by the Chinese National Standard Agency (GB2762, 2017). Chinese standards for THg in cereal grains were considered as other national and international agencies do not provide THg limits. The maximum THg concentration (157 ng.g^{-1}) of all rice samples was from the immediate vicinity (100 m downwind) of a chloralkali plant in the Faisalabad district (Punjab), followed by a sample (75.96 ng.g^{-1}) collected from a rice paddy about 120 m away from sludge pile on the other side of the same source (Fig. 2b). This chemical industry started chloralkali production in 1985 with a capacity of 30 MT/day. Other chemicals, like ammonium chloride and bleaching powder, were also produced. Until 2005, chloralkali production was increased to 450 MT/day. In 2006, the old Hg chloralkali plant was disposed of and production was upgraded to 540 MT/day. Since 2010, the production of chloralkali has been 610 MT/day. Liquid carbon dioxide, calcium chloride, ammonium chloride, and agrochemical production also occur at the same facility. Hg usage in industrial processes like chloralkali production emitted 15.2 tons of Hg to the atmosphere in 2015 (AMAP/UNEP, 2019). These emissions can be in the form of elemental Hg, wastewater and brine sludge, and have the potential to enrich the atmosphere, soil, sediment, and water with Hg (Biester et al., 2002; Grangeon et al., 2012; Hissler and Probst, 2006; Kinsey et al., 2004; Ullrich et al., 2007; Xing et al., 2019a; Zhu et al., 2018b). The Hg cell chloralkali manufacturing plant was replaced in 2006. However, abandoned chloralkali plant sites can remain strong Hg contamination sources (Song et al., 2018), causing remission of Hg^0 from previous deposits in surrounding soils and hazardous waste like sludge or slurry (Wang et al., 2019; Zhu et al., 2018a). These continued Hg^0 emissions from legacy Hg deposition could be responsible for the maximum Hg contents in rice samples observed in this study.

Considerable work has been done to quantify Hg speciation in rice produced in different parts of the world (Table S3); however, there have been no studies on Pakistan. There have been a few studies conducted in India (Lenka et al., 1992; Sarkar et al., 2012; Srikumar, 1993), but they only quantified THg concentrations in rice. Recent research in China by Zhao and coworkers (Zhao et al., 2019), depicted the contributions of non-point Hg sources to THg concentrations in rice at several locations. The THg concentration averaged 4.74 ng.g^{-1}, which was used as a background level for comparison with our study. For ease of comparison, samples were segregated into two groups according to their THg concentrations. Samples of <5 ng.g^{-1} THg were considered to be from non-point Hg pollution sources (Zhao et al., 2019). The aim was to present data in a better way and allow comparison with previous studies regarding samples from contaminated and non-contaminated sites.

For non-point Hg-source sites in present study, the mean THg concentration of samples (n = 400) was 2.59 ng.g^{-1} (range: 0.44–4.91 ng.g^{-1}). Similar mean THg concentrations have been reported from non-contaminated sites in Guizhou Province, China (2.8 ng.g^{-1}, range = 1.0–5.5 ng.g^{-1}) and from similar sites in Hubei Province, China (3.7 ng.g^{-1}, range = 1.9–6.8 ng.g^{-1} and 3.3 ng.g^{-1}, range = 1.7–6.5 ng.g^{-1}) (Rothenberg and Feng, 2012; Rothenberg et al., 2011). Market-based surveys in Brazil reported a mean THg concentration of 3.1 ng.g^{-1} (2.1–4.4 ng.g^{-1}), while the reported means of 2.1 ng.g^{-1} (1.6–3.3 ng.g^{-1}; Spain), 2.6 ng.g^{-1} (1.3–3.7 ng.g^{-1}; Thailand) (da Silva et al., 2010), 3.04 ± 2.07 ng.g^{-1} in UK (Brombach et al., 2017) and 2.91 ± 0.86 in Republic of Korea (Eom et al., 2014) are highly consistent with the present study (Table S3).

The mean ± SD THg concentration of the remaining samples (n = 100) was 12.20 ± 16.97 ng.g^{-1} (range = 4.99–157 ng.g^{-1}), which might represent Hg-contaminated sites. The THg ranges reported in the present study were dynamic and are largely comparable with the results of other studies conducted in Hg-polluted areas. Rice samples exceeding the permissible limits could be attributed to Hg pollution point-sources like industrial effluent run-off (Cao et al., 2010), chemical plants (Cheng J et al., 2013), industry (Haiyan and Stuanes, 2003), Hg mining sites (Feng et al., 2008; Horvat et al., 2003; Meng et al., 2014b; Zhang et al., 2010a), chloralkali plant (Lenka et al., 1992) and coal-fired power plant (Xu et al., 2017) (Table S3).

Brick kilns are the most common Hg sources in the study area, where low-grade coal, along with other materials, are used for baking bricks. During the sampling campaign, rice samples (n = 46) from locations close to brick kilns showed THg concentrations

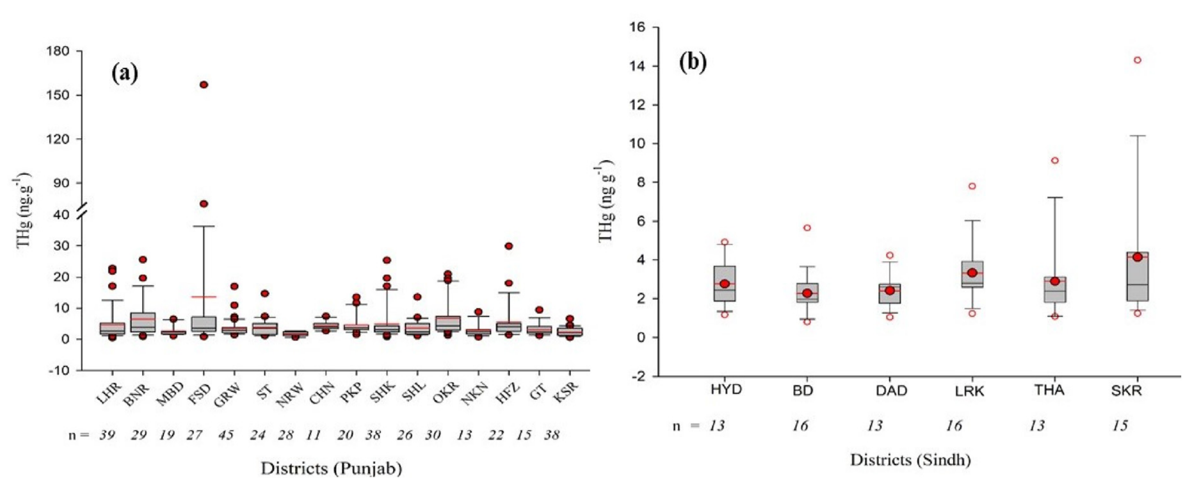

Fig. 3. Boxplots showing THg concentration comparisons in rice samples among different districts of two provinces of Pakistan Punjab(a) Sindh(b); Each box is representative of interquartile range (25th and 75th percentiles), while the band appearing in the (mid of the box is 50th percentile (the median), and whisker extends from 10th to 90th quartiles. Open squares represent mean values.

(mean ± SD = 8.62 ± 6.95 $ng.g^{-1}$, range = 1.62–29.88 $ng.g^{-1}$) that were higher than the overall mean obtained in this study. Five samples even exceeded the permissible limit of 20 $ng.g^{-1}$. The least significant differences were calculated among the THg concentrations of the three groups (Fig. 5; $p < 0.001$), which indicated that brick kilns were the most prominent Hg-emission sources related to rice in the study area. Several studies have shown the effects of brick kiln pollution on surrounding ecosystems, such as elevated hydrogen fluoride accumulation in apricot, plum and mango leaves (Ahmad et al., 2012). Soil and plant samples, including wheat (*Triticum aestivum*), kikar (*Acacia nilotica*), deodar (*Euphorbia helioscopia*) and grass (*Cenchrus ciliaris*) from locations near operational brick kilns in Pakistan were measured to have toxic amounts of fluoride. Health impacts due to elevated fluoride concentrations have been reported in kiln workers and neighboring populations (Khalid and Mansab, 2015). Similarly, the deposition and accumulation in high concentrations of heavy metals like Zn, Cu, Cd, Pb, Ni, Mn, Se, and Fe have been reported as being higher in soil and vegetation adjacent to brick kilns than at control sites (Achakzai et al., 2017; Sikder et al., 2016). Hg accumulation in rice has not been reported before in brick kiln surroundings. Note that these brick kiln-related samples represent point-source Hg pollution from each individual kiln.

Coal combustion has been highlighted as a concern for worldwide public health due to its Hg contents (Pacyna et al., 2010). In particular, the usage of coal in power plants is a major source of anthropogenic Hg release to the atmosphere. Gaseous elemental Hg (GEM) emissions from coal combustion for power generation can be deposited in the form of oxidized and particulate Hg, which severely impacts the locality of the facility (Carpi and Lindberg, 1997; Flues et al., 2002; Keeler et al., 2006). Pakistan is rich in indigenous coal resources with estimated reserves of 185 billion tons, of which 175 billion tons (95%) occur in the Thar coalfield of Sindh Province. However, 97% of Pakistan's coal reserves are classified as lignite (Ali et al., 2015; Malkani, 2012), which is the poorest of all forms (including anthracite, bituminous and sub-bituminous) due to its low energy, high moisture, low carbon, and high Hg, sulfur, and nitrogen contents; hence, its use is of increasing environmental concern (Dong, 2011). Higher proportions of gaseous elemental Hg (Hg^0) are emitted relative to gaseous oxidized Hg (HgII) during the combustion of lignite coal compared with bituminous coals (Dong, 2011). (The devastating effects of Hg in coal are more concerning while referring to public health (Pavlish et al., 2004·Vejahati et al., 2010). Ali et al. (2016) determined the Hg contents of coal samples from the Thar coalfield of Pakistan and found that the THg contents (mean = 1120 $\mu g.kg^{-1}$, range = 863–1460 $\mu g.kg^{-1}$) were 10-fold higher than the global mean of 100 $\mu g.kg^{-1}$ Hg for lignite coals (Ketris and Yudovich, 2009; Yudovich and Ketris, 2005a, b). This locally-produced coal is used extensively for firing bricks, with Pakistan's brick kiln industry being the largest consumer of this low-grade coal (Rauf et al., 2015; Rehman et al., 2017). Thus, elevated gaseous elemental Hg levels near operational brick kilns during rice growing season result increased Hg contents in rice grain. This emitted Hg might also be deposited in paddy soil, then readily methylated and accumulated in rice grain, as freshly-deposited Hg is more susceptible to methylation (Meng et al., 2011; Meng et al., 2014b; Zhao et al., 2016a). The extent of Hg pollution caused by individual brick kiln units away from the source was not studied in this work. However, it is imperative to conduct further in-depth investigation of brick kilns due to the long-range atmospheric transportation of Hg that originates from them.

The results also show the contribution of industrial activities to Hg contents in rice in the Lahore, Gujranwala, Sialkot, Sheikhupura, Sahiwal, and Faisalabad districts. The chloralkali industry in the Faisalabad district has been highlighted and discussed for Hg contamination above. Similarly, samples collected ($n = 5$) from rice fields around the recently established Qadirabad coal-fired power plant (1300 MW capacity) in Sahiwal district had THg mean contents of 4.48 ± 2.36 $ng.g^{-1}$ (range = 1.66–7.06 $ng.g^{-1}$), which is not high. This power plant is equipped with supercritical technology to lower emissions, which could be the reason for the low THg concentrations observed. Three samples from Khudpur village in Lahore were high in THg (mean = 20.56 ± 6.40 $ng.g^{-1}$, range = 17.00–22.79 $ng.g^{-1}$). The probable source may be a nearby industrial zone, as an industrial waste effluent drain passed through the locale. The higher Hg concentrations in rice could be related to higher gaseous elemental Hg deposition from ambient industrial effluents near industrial sites (Xu et al., 2017). Describing and assessing other sampled industrial sites, including the nature of their emissions, were beyond the scope of this study; however, it is imperative to conduct research focusing on the industrial zones of the study area.

3.2. MeHg in rice grains

The MeHg contents of all analyzed rice samples ($n = 124$) ranged from 0.16 to 67.85 $ng.g^{-1}$ with an arithmetic mean ± SD of 3.71 ± 6.69 $ng.g^{-1}$ (Fig. 2c). The highest concentration of MeHg (67.85 $ng.g^{-1}$) was recorded in a rice sample collected from a site near a chloralkali plant in Faisalabad district, which also had the highest THg concentration, with 43% of the THg being recorded as MeHg. The %Hg as MeHg in all samples ranged from 3 to 60% with a mean ± SD of 32 ± 11%. A significant correlation was found between THg and MeHg concentrations in rice ($r = 0.89$, $p < 0.001$; Fig. 4a) but not between the ratio of %Hg as MeHg and THg concentration ($r = 0.27$, $p = 0.07$; Fig. 4b), suggesting there are different enrichment mechanisms for IHg and MeHg in rice (Li et al., 2008; Meng et al., 2014a, 2010; 2011). ANOVA of MeHg values revealed non-significant differences among samples from different districts (Fig. 6; $p = 0.213$), implying that there were similar MeHg levels in rice paddy soil across the widely distributed sampling sites.

The MeHg results obtained by the current study were segmented into two groups for better comparison of point and non-point Hg sources. Rice samples with THg concentrations <5 $ng.g^{-1}$ ($n = 24$) measured a mean ± SD MeHg concentration of 1.25 ± 0.51 $ng.g^{-1}$ (range = 0.24–2.05 $ng.g^{-1}$). The mean ± SD %Hg as MeHg obtained for this segment was 28 ± 11% (range = 6–49%). This value is comparable with those of previous studies on MeHg in rice from non-point Hg sources from different regions, including 15 Chinese provinces (0.682 $ng.g^{-1}$, range = 0.03–8.71 $ng.g^{-1}$) (Zhao et al., 2019), Cambodia (1.44 $ng.g^{-1}$, range = 1.17–1.96 $ng.g^{-1}$) (Cheng et al., 2013), and Madagascar (0.120 $ng.g^{-1}$; 0.0150–1.10 $ng.g^{-1}$) (Rothenberg et al., 2015), It is also comparable with market-based studies from the UK (1.91 ± 1.07 $ng.g^{-1}$, range = 0.110–6.45 $ng.g^{-1}$) (Brombach et al., 2017) and China (1.37 ± 1.18 $ng.g^{-1}$, range = 0.0200–19.0 $ng.g^{-1}$; Table S3) (Xu et al., 2020). MeHg analysis of the remaining samples from Hg point sources ($n = 100$) had a mean ± SD concentration of 4.37 ± 7.39 $ng.g^{-1}$ (range = 0.16–67.84 $ng.g^{-1}$) and %Hg as MeHg of 33 ± 11% (range = 3–60%). Only two rice samples collected around chloralkali plants had MeHg concentrations comparable with those of highly contaminated sites (67.84 $ng.g^{-1}$ and 29.81 $ng.g^{-1}$). Our MeHg findings are more or less consistent with work conducted at Hg point-source sites; e.g., a coal-fired power plant (Xu et al., 2017), Hg mining sites (Feng et al., 2008; Rothenberg et al., 2013), and historical gold mining sites (Windham-Myers et al., 2014) (Table S3).

The overall %Hg as MeHg evaluated by this study (mean = 32 ± 11%, range = 3–60%) is consistent with the findings

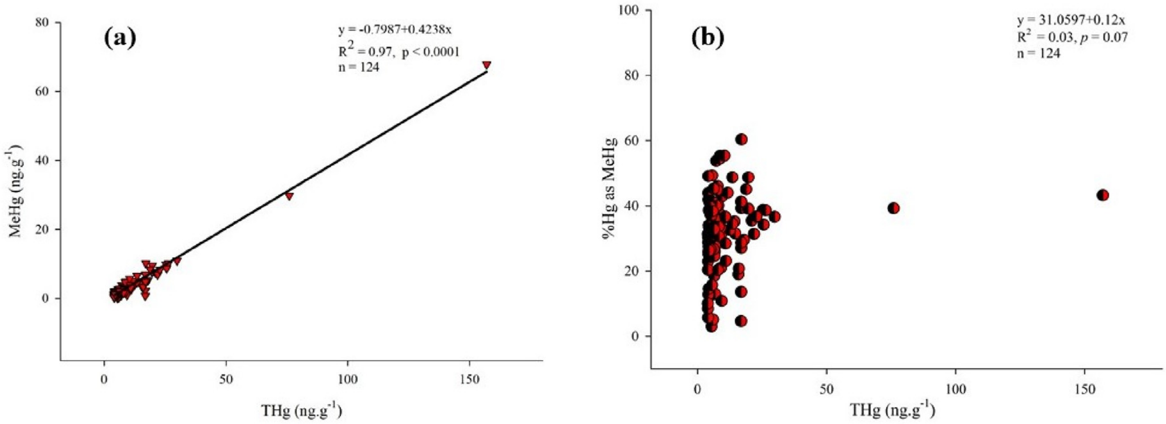

Fig. 4. Relationship between THg and MeHg concentrations in rice from Pakistan(a). Relationship obtained between %Hg as MeHg and THg in rice from the study area(b).

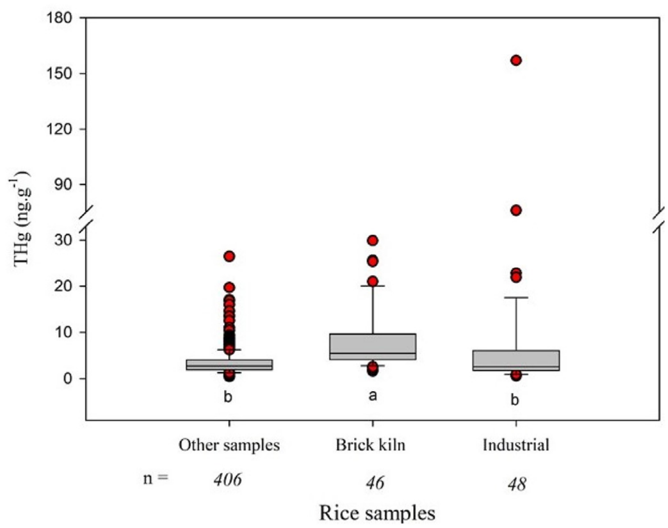

Fig. 5. Comparison of THg concentrations among collected rice samples from vicinities of brick kilns, industrial zones, and all other samples from Pakistan. Each box is representative of the interquartile range (25th and 75th percentiles), while the band is appearing in the (mid of the box is 50th percentile (the median), and whisker extends from 10th to 90th quartiles. Open squares represent mean values.

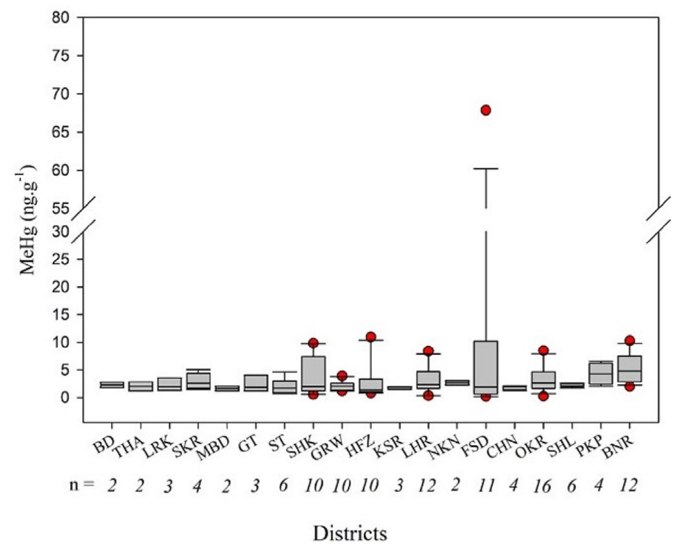

Fig. 6. Boxplots showing MeHg concentration comparisons in rice samples among different districts of two provinces of Pakistan (Punjab Sindh); Each box is representative of interquartile range (25th and 75th percentiles), while the band appearing in the (mid of the box is 50th percentile (the median), and whisker extends from 10th to 90th quartiles. Open squares represent mean values.

of Feng et al. and Rothenberg et al. (Feng et al., 2008; Rothenberg et al., 2011) (Table S3). However, %Hg as MeHg did not differ significantly between polluted and non-polluted sites in the present study, implying there were similar methylation rates in the paddy soils sampled across these extensive locations (Rothenberg et al., 2014). Furthermore, there are other factors that can influence MeHg%, like rice variety, microbial activity, and soil properties (N, S, organic matter and pH) (Beckers and Rinklebe, 2017; Rothenberg et al., 2012; Salman et al., 2019; Xing et al., 2019b).

3.2.1. Probable factors influencing rice MeHg contents

Each ecosystem has its own unique combination of environmental factors, making it difficult to accurately predict net Hg methylation rates in different environments (Ma et al., 2019). Similarly, the mechanisms of Hg methylation or demethylation in rice paddies, MeHg accumulation in rice plants, and their controlling factors are complex and far from being fully understood (Zhao et al., 2020). Hg methylation rates in soil are considered to be a function of the interaction between various geochemical factors (e.g., temperature, anoxia, pH, organic carbon, sulfur, iron speciation), which are further influenced by rice paddy cultural and management practices (Benoit et al., 2003; Marvin-DiPasquale et al., 2003, Marvin-DiPasquale et al., 2014; Rothenberg et al., 2014; Ullrich et al., 2001). These factors may vary across the globe's geographical regions, which may explain the wide range of MeHg concentrations in rice grain observed in the current study. The factors relevant to the study area are discussed below.

In the study area, the wetland rice production system is practiced. Lowland rice is primarily cultivated on clayey soils under puddled soil conditions (Jehangir et al., 2007). Puddling is an ordinary tillage operation used to create a soft soil bed by tilling paddy soil at field capacity. This land preparation method aims to control weed infestation, nutrient leaching, and water percolation, thereby promoting a flooded environment favorable to rice growth (Fang et al., 2019). It also degrades soil aggregation and porosity, leading to the formation of an impermeable hardpan or plow layer (Zhang et al., 2016). Puddled soil may retain more Hg(II) than unpuddled soil (as it does other nutrients), which could elevate rice MeHg contents.

The conventional rice cultivation irrigation regime used in Pakistan is continuous flooding in bundled units with ponding depths of 50–75 mm, which is maintained by 15–25 irrigation events over the 100–150 day growth period (Ahmad et al., 2007; Jehangir et al., 2007). These over-supplemented irrigation practices ensure complete anoxic conditions in the root zone. Fields are drained only 14–20 days before harvest. Mid-season drainage is not practiced in Pakistan, which is similar to alternate wetting and drying (AWD) irrigation methods.

Weather data for the rice-growing season (June–September) over 1981–2010 showed a mean maximum temperature range of 34–38 °C and mean minimum temperature range of 22–26.5 °C in the rice cultivation regions of the country. Agriculture in Pakistan is vulnerable to the potential impacts of climate change; by 2050, there is predicted to be more precipitation and a mean maximum temperature increase of 2–2.5 °C during the rice-growing season (Ahmad et al., 2015; Bokhari et al., 2017). Temperature has been reported to contribute 30% of the variation in net Hg(II) methylation rates, with an optimum temperature of 35 °C observed in surficial river sediments (Ullrich et al., 2001). The relatively high MeHg concentrations found in rice samples from Southern China may be due to high soil MeHg contents. There are probably elevated soil Hg and temperatures in this region (Xu et al., 2020). While, microbial Hg methylation rates being accelerated by the high temperature (Loseto et al., 2004; Ma et al., 2019).

Crop residue burning after harvest is a matter of concern as it degrades air quality, not only in Pakistan and India but also across all of South Asia (Bijay-Singh et al., 2008; Irwin, 2014; Singh and Kaskaoutis, 2014). Incineration of biomass, including rice crop residues, contributed 24% of global atmospheric Hg emissions in 2006 (Streets et al., 2009). Elemental Hg (Hg^0) is one of the dominant forms of Hg released by biomass burning. Moreover, it potentially increases soil MeHg levels and methylation rates in response to elevated inorganic Hg(II) availability (Caldwell et al., 2000), due to the fact that newly deposited Hg is more easily methylated into MeHg in paddy soils (Meng et al., 2010, 2011; Zhao et al., 2016b). The incorporation of rice plant residues into paddy soils is another potential cause of enhanced Hg(II) methylation rates, owing to readily available carbon being released upon residue degradation (Kalbitz et al., 2013; Liu et al., 2016; Rothenberg et al., 2014; Tang et al., 2018). In Pakistan, black carbon and organic carbon emissions caused by crop residue burning increased by 63% from 2000 to 2014 (Azhar et al., 2019). According to Edmondson et al. (2015), black carbon accounts for 28–39% of total organic carbon stocks, depending upon soil texture.

Hg methylation rates seemed to be influenced by the above-mentioned factors in the rice cropping system of Pakistan, which sufficiently reasoned for obtained MeHg concentrations in selected samples of the current study. Thus, the elevated MeHg concentrations observed indicate that there are active Hg pollution sources in some areas. Moreover, MeHg in rice is more likely a function of proximity to a current Hg pollution source (Qiu et al., 2013; Zhang et al., 2010a). Likewise, newly deposited Hg is more prone to methylation (Meng et al., 2010). Furthermore, thorough investigation is required to validate the interactions of these factors with other intrinsic soil parameters as related to MeHg contamination in rice.

3.3. Chronic MeHg exposure through rice intake in Pakistan

In Pakistan, wheat is a staple food for the population and rice is not a primary source of carbohydrates. The per capita rice consumption of Pakistan and other countries of South and Southeast Asia are shown in Table S4. It is known that IHg and MeHg have different exposure pathways, metabolism, and uptake in the human body, with MeHg being more toxic than IHg to humans. Therefore, we considered MeHg and IHg data for rice separately to assess the health risk. The mean PDI for MeHg calculated in this exploratory study was 0.0028 $\mu g.kg^{-1}$ $bw.d^{-1}$.bw with a range of 0.0001–0.0505 $\mu g.kg^{-1}$ $bw.d^{-1}$, which is 35 times lower value than the limit of 0.10 $\mu g.kg^{-1}$ $bw.d^{-1}$ prescribed by the World Health Organization (JECFA, 2003) and 80 times less than the 0.23 $\mu g.kg^{-1}$ $bw.d^{-1}$ limit of the USEPA (USEPA, 2001b).

Similarly, the mean PDI for IHg intake through rice ingestion in the country is 0.0004 $\mu g.kg^{-1}$ $bw.d^{-1}$ with a range of 0.0001–0.0049 $\mu g.kg^{-1}$ $bw.d^{-1}$. This is 1400 times lower than the permissible limit of 0.57 $\mu g.kg^{-1}$ $bw.d^{-1}$. This limit was established based on a provisional tolerable weekly intake (PTWI) of 4 $\mu g.kg^{-1}$ $bw.week^{-1}$ for IHg rather than the previous dose of 5 $\mu g.kg^{-1}$ $bw.week^{-1}$ for THg due to the different absorption rates of inorganic and organic Hg by humans (JECFA, 2010). HQs were estimated for both IHg (0.0049; Eq. (2)) and MeHg (0.0280; Eq. (3)), with the HI (0.0330; Eq. (4)) for the combined pathway having values far less than 1. Several previous studies have predicted HQ or HI values less than 1. The HQ and HI values of our work are lower than or consistent with those studies (Han et al., 2019; Li et al., 2015; Qian et al., 2010; Wang et al., 2017; Xu et al., 2020). Conclusively, rice from the study area is safe to consume. It should be noted that the health risk assessment undertaken in this study was only based on the 124 samples for which MeHg was determined, which also had relatively high concentrations of THg. Lower PDI values were obtained here as less rice is consumed in Pakistan than wheat. In different areas of the world, aquatic food is the primary source of Hg exposure to humans, accounting for 95% of MeHg exposure (Houserova et al., 2007). In Pakistan, fish is not the primary source of protein and its consumption is only 1.9 kg per person per year (Table S2). This intake rate is much lower than that of the rest of the world (Food and Agriculture Organization of the United Nations, 2013; Laghari, 2018). A lack of studies on MeHg concentrations in fish from Pakistan prevents us from estimating the contribution of MeHg to overall PDI (i.e., in combination with rice). Although MeHg intake through rice is within safe limits in Pakistan, chronic low-level MeHg exposure is still detrimental to humans and can lead to neurotoxicity (NRC, 2000).

4. Conclusions and recommendations

The results of this large-scale exploratory study on Hg contamination in the rice cropping system of Pakistan revealed very low levels of Hg contamination. The general population of Pakistan is at low risk of MeHg and IHg exposure through rice consumption. Significantly higher Hg levels were observed in rice samples collected in proximity to brick kilns than in those from other areas. These higher Hg contents can be attributed to the burning of poor-quality coal for baking bricks. Further work is urgently needed to ascertain the relative contributions of the various Hg sources in the study area. Relatively high MeHg values in rice from certain areas raises a concern that should be dealt with according to an integrated approach that considers all possible factors responsible for such contamination. The Hg contamination status of paddy soils will be analyzed further to highlight the behavior of Hg species according to local environmental and geochemical conditions, with an emphasis on Hg origins. It is imperative to conduct further work to quantify MeHg contributions from fish and other foodstuffs to PDI. There is a need for national organizations to determine ingestion rates at the district and provincial levels, which will help to provide a more detailed picture of the intake of Hg and other pollutants by residents of specific areas of the country. Although the PDIs of Hg through rice intake were found to be safe both for IHg and MeHg (due to the general preference for wheat over rice in Pakistan) any shift to a preference for rice, either at the individual or general levels, could be cause for concern.

CRediT authorship contribution statement

Muhammad Wajahat Aslam: Conceptualization, Formal analysis, Investigation, Methodology, Writing - original draft, Visualization, Data curation. **Waqar Ali:** Investigation, Formal analysis. **Bo Meng:** Supervision, Project administration, Funding acquisition, Conceptualization, Methodology, Validation, Visualization, Writing - review & editing, Resources. **Muhammad Mohsin Abrar:** Data curation, Formal analysis. **Benqi Lu:** Investigation. **Chongyang Qin:** Investigation, Resources. **Lei Zhao:** Visualization, Writing - review & editing. **Xinbin Feng:** Supervision, Conceptualization, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114625.

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