Heavy Metal Contamination and Health Risks Assessment in Soil-Rice System Irrigated with Wastewater

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The transfer of heavy metals (HMs) was investigated from wastewater used for irrigation to soil and subsequently to rice plants (*Oryza sativa* L.) in Northern Pakistan, a region where rice is widely consumed. The concentrations of cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) were measured in irrigation water, soil, and different parts of the rice plant (grain, shoot, and root) collected from various paddy fields. To assess potential health risks, daily metal intake (DIM) and the health risk index (HRI) were calculated. Heavy metal concentrations varied significantly across sampling locations. In the soil, Cd ranged from 0.1 to 0.49 mg/kg, Pb from 0.8 to 2.2 mg/kg, Cu from 4.2 to 18.2 mg/kg, and Zn from 7.0 to 25.1 mg/kg. The calculated DIM followed the order Zn < Cd < Cu < Pb, while the overall HRI for adults (0.612) and children (0.533) were below the threshold of 1. However, cadmium concentrations in the studied samples exceeded suggested permissible limits. Statistically significant differences ($P < 0.05$) revealed higher HM concentrations in the soil and rice crops from paddy fields irrigated with contaminated wastewater compared to a control site. The findings of this study indicate that the use of wastewater for irrigation leads to increased accumulation of HMs, particularly cadmium, in rice grains, potentially posing health risks to consumers in the region.

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INTRODUCTION

Heavy metal (HM) contamination poses a significant environmental and health concern, primarily stemming from anthropogenic activities such as mining waste, industrial effluents, agricultural practices, and construction processes (Chandra *et al.* 2017; Zhu *et al.* 2018). Improper waste disposal and the overuse of pesticides and other agricultural chemicals are major pathways through which HMs directly impact environmental health (Zhao *et al.* 2012; Chen *et al.* 2015). Notably, the use of wastewater for irrigation, often employed in water-scarce regions to enhance crop yields, has emerged as a principal source of agricultural soil contamination with HMs (Defarge *et al*., 2018; Raja *et al.* 2015; Verma *et al.* 2015). Industrial effluents, which are sometimes rich in toxic HMs, further contribute significantly to contamination in both soils and crops (Mahmood and Malik 2014; Meng *et al.* 2016; Singh *et al*., 2012). Due to its role as a primary sink for pollutants, soil is considered an excellent medium for assessing and monitoring HM pollution resulting from human activities (Pouyat *et al.* 2015; Govil and Krishna 2018). The transfer of HMs from soil to crop is critical because plants possess varying natural capacities to absorb and accumulate HMs in their different parts, with uptake and translocation rates differing among species and even cultivars (Hongjiang *et al.* 2014; Zhou *et al.* 2015). HMs are typically absorbed from the soil through the plant roots, as the surface soil layer receives contaminants from diverse anthropogenic activities. The edible portions of plants, such as rice grains, represent a direct route for HM transfer to humans, potentially leading to adverse health effects and toxicological endpoints. Consequently, HM pollution is a critical global issue due to the persistence, bioaccumulation, and biomagnification of these elements, as well as their severe eco-toxicological impacts on humans, plants, and animals (Clemens and Ma 2016; Shahid *et al.* 2017).

Human exposure to HMs primarily occurs through the consumption of contaminated food crops and water (Mahmood and Malik 2014; Keshavarzi *et al.* 2015; Jiang *et al*., 2017). The food chain is responsible for over 70% of dietary cadmium (Cd) intake alone (Wagner 1993; Zaza *et al.* 2015). Cd and lead (Pb) are classified as carcinogens and are associated with various chronic health problems, including cardiovascular, kidney, bone, blood, and nervous system disorders (Bonberg *et al.* 2017; Chung *et al.* 2017; Rietra *et al*., 2017). While copper (Cu) and zinc (Zn) are essential micronutrients, excessive intake can be toxic to both humans and animals. Consequently, these metals are frequently included in health risk assessment and environmental monitoring studies (Pandey and Madhuri 2014; Prashanth *et al.* 2015). Cultivating crops on HM-contaminated soil facilitates the uptake and accumulation of these contaminants in edible plant parts, posing a risk to human and animal health upon consumption (Verma *et al.* 2015; Clemens and Ma 2016).

In the agroecologically diverse Lower Dir district of Khyber Pakhtunkhwa, Pakistan, agriculture is the primary economic activity, with rice cultivation serving as a major livelihood and source of the region's staple food. The increasing water demand in this arid environment, coupled with the presence of a significant marble processing industry concentrated around tehsil Adenzai, has compelled local farmers to rely on untreated or partially treated marble factory wastewater for irrigation. The utilization of this specific, non-conventional irrigation source and the known presence of HMs from the marble cutting and polishing processes highlight a significant and understudied risk of HM accumulation in soil and rice, directly impacting food safety and potentially contaminating the Swat River (Jehan *et al.* 2020).

Rice (*Oryza sativa* L.) is a staple food in Asia and globally recognized as a significant pathway for dietary exposure toxic metals like Pb and Cd (Chen *et al.* 2017; Ndong *et al.* 2018). While the specific local context strongly suggests severe HM transfer in this area, there remains a critical gap in generating the empirical data required for effective local environmental management and policy decisions. Crucially, the lack of site-specific studies characterizing this effluent hinders the development of appropriate national effluent management guidelines for the marble industry and prevents informed policymaking regarding agricultural irrigation practices and food safety standards.

Therefore, this study addressed a critical research gap by comprehensively assessing the transfer of Cd, Pb, Cu, and Zn from marble factory wastewater used for irrigation to the soil and rice plants (grain, shoot, root) in tehsil Adenzai, Lower Dir, Khyber Pakhtunkhwa. Its unique contributions included providing recent empirical data on HM concentrations in irrigation water, soil, and rice specific to this agroecologically and socio-economically distinct region; evaluating potential health risks for adults and children from consuming wastewater-irrigated rice using daily metal intake (DIM) and health risk index (HRI); determining the extent to which HM levels exceed permissible limits compared to the groundwater-irrigated control site; and generating essential data to inform local environmental management and public health strategies focused on mitigating risks from rice, a staple food for communities relying on such irrigation practices.

EXPERIMENTAL

Study Area and Sampling Sites

This study was conducted during the 2015 summer cropping season in Lower Dir, Khyber Pakhtunkhwa, Pakistan, an area characterized by significant agricultural activity and proximity to the marble processing industry. Five distinct rice paddy fields were purposively selected to represent the dominant irrigation sources, capturing the variability in water quality and its impact on heavy metal (HM) accumulation. The rice fields in this region have a long history of cultivation (>20 years), utilize the Basmati-370 variety, and follow a summer monocropping pattern. Irrigation is typically sustained by flooding, with an estimated average seasonal water application of approximately 1,200 to 1,500 mm. The precise location of these sites in relation to the industrial discharge points is detailed in Fig. S1. The control site (S1), was irrigated exclusively with clean groundwater from a dedicated well, confirmed by preliminary water quality assessment to be free from industrial or agricultural contamination. S1 served as the baseline, located within the same geographical and climatic area as the experimental sites to isolate irrigation source as the key variable. The experimental sites (S2 to S5) were selected based on their irrigation with different types of wastewater known to be present in the Lower Dir region, particularly around the industrial and agricultural zones of the studied area. The S2 (Swat river water) site was selected to assess the impact of irrigation using surface water from the Swat river, a major water source in the region that may be subject to upstream agricultural and urban runoff. The S3 (Marble wastewater) site directly investigated the impact of the dominant marble processing industry wastewater prevalent in the vicinity of the studied region. Paddy fields irrigated with this type of effluent were specifically targeted due to the known use of abrasives and potential release of heavy metals during marble cutting and polishing processes. The S4 (Red and white stone wastewater) site was selected to evaluate the effect of wastewater from other stone processing activities (red and white stone), another industrial source in the area, on heavy metal levels in the soil and rice. The S5 (Sewage water) site was included to assess the impact of untreated or partially treated sewage water, a common non-industrial wastewater source in areas facing water scarcity. This selection aimed to provide a comprehensive understanding of heavy metal transfer from the most relevant wastewater sources in the region to the soil-rice system.

Sample Collection

Soil samples were collected following the methodology outlined by Wu *et al.* (2010). At each of the five sampling sites, five individual soil subsamples were collected to form a single composite sample. Specifically, four topsoil subsamples (0 to 20 cm depth, representing the plow layer) were collected using a stainless-steel auger from the four corners of each field, and one subsample was collected from the center. These five subsamples were then thoroughly homogenized in clean plastic bags to create one representative composite sample per site. These composite samples were then air-dried at room temperature, mechanically ground using a mortar and pestle, and sieved through a 2-mm stainless steel mesh. The processed soil samples were stored in clean Petri dishes until further analysis. At each sampling site, mature rice plants (*Oryza sativa* L.) of similar size and physiological age were carefully collected. The harvested plants were thoroughly washed, first with tap water to remove adhering soil and debris, followed by rinsing with deionized water to remove any residual tap water contaminants. The plants were then separated into grain (dehusked), shoot (straw and leaves), and root fractions. These sub-samples were oven-dried at 70 °C for 24 h to achieve a constant weight. The dried plant tissues were then weighed and ground into a fine powder using a laboratory blender. The powdered plant samples were stored in clean, labeled containers until digestion. Irrigation water samples were collected from the water source used to irrigate each of the five selected paddy fields. Samples were collected in pre-cleaned polyethylene bottles. Immediately after collection, the water samples were filtered through 0.45-µm membrane filters to remove particulate matter. The filtered water samples were acidified with nitric acid (HNO_3) to pH < 2 to prevent precipitation of metal ions and stored at 4 °C until heavy metal analysis.

Sample Digestion and Analysis

For heavy metal analysis, the powdered soil and plant tissue samples were digested using a tri-acid mixture (nitric acid:sulfuric acid:perchloric acid in a 5:1:0.5 ratio, v/v/v), following a modified method based on Liao *et al.* (2016). Briefly, 1.0 g of each dried and grounded sample was accurately weighed into a 50-mL borosilicate flask. A total of 10 mL of the tri-acid mixture was added to each flask. The flasks were placed on a hot plate in a fume hood and heated at 80 °C. The temperature was gradually increased to 120 °C until the organic matter was completely digested, indicated by the evolution of dense white fumes of perchloric acid and a clear or light-colored digestate. The digestion process was continued until the volume was reduced to approximately 5 mL. The flasks were then cooled to room temperature, and the digestate was diluted to 50 mL with deionized water. The resulting solutions were filtered through Whatman No. 42 filter paper to remove any undissolved residues.

The filtered and acidified water samples, as well as the digested soil and plant samples, were analyzed for the concentrations of cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) using an Atomic Absorption Spectrophotometer (Perkin Elmer USA, AAS-PEA-700) equipped with appropriate hollow cathode lamps for each element. Calibration curves were prepared using certified standard solutions of each metal. Quality control measures included running reagent blanks and certified reference materials (where available) to ensure the accuracy and precision of the analysis.

Bioaccumulation Factor

The Bioaccumulation Factor (BAF), calculated as the ratio of heavy metal concentration in rice grains to that in the corresponding soil (USEPA 2009; Cai *et al.* 2015), is a widely used index to quantify the uptake and transfer of heavy metals from the soil to the edible parts of plants. A BAF > 1 indicates that the plant efficiently accumulates the metal in its grains, posing a higher potential risk to human consumers.

$$\mathbf{BAF = [HM]_{grain} / [HM]_{soil}} \qquad (1)$$

In Eq. 1, $[HM]_{grain}$ and $[HM]_{soil}$ represent the concentrations of the specific heavy metal in the rice grain (mg/kg dry weight) and the soil (mg/kg dry weight), respectively.

Enrichment Factor

The Enrichment Factor (EF) is used to assess the degree of soil contamination and heavy metal accumulation in both plants and soil relative to a background level or a control site (Sutherland 2000; Izah *et al.* 2017). In this study, given the establishment of a control site (S1) irrigated with clean groundwater, the EF for heavy metals in the experimental sites' soil and plant tissues was calculated using the average concentration of the respective metal in the control site as the background reference:

$$\mathbf{EF} = \frac{[\textbf{Metals concentrations (soil) at contaminated site}]}{[\textbf{Metals concentrations (soil) at uncontaminated site}]} \qquad (2)$$

An EF value close to 1 suggests that the metal is primarily derived from natural sources or background levels, while values greater than 1 indicate anthropogenic enrichment. The use of the control site as a local background reference is relevant for assessing the extent of contamination specifically due to the different irrigation water sources within the study area.

Health Risk Assessment

Health risks associated with heavy metal exposure through the consumption of rice grown in the study area were evaluated using the Daily Intake of Metals (DIM) and the Health Risk Index (HRI). Separate assessments were conducted for adults and children due to differences in body weight and food consumption patterns.

Daily Intake of Metals

The DIM for each heavy metal through rice consumption was calculated using the Eq. 3 (Hang *et al.* 2009; Likuku and Obuseng 2015),

$$\mathbf{DIM} = \boldsymbol{C}_{\mathbf{metal}} \times \boldsymbol{C}_{\mathbf{factor}} \times \boldsymbol{D}_{\mathbf{food\ intake}} / \boldsymbol{B}_{\mathbf{average\ weight}} \qquad (3)$$

where C_{metal} represents HMs in the food source (rice, mg/kg), D_{food} represents a daily intake of the food source (rice, g/person/day), while C_{factor} and $B_{average\ weight}$ represent conversion factor (fresh weight to dry weight, respectively).

Health Risk Index

The potential non-carcinogenic health risk associated with the intake of each heavy metal through rice consumption was estimated by the HRI. The HRI is the ratio of the estimated DIM to the corresponding oral reference dose (ORD) (Hang *et al.* 2009; Tepanosyan *et al.* 2017):

$$\mathbf{HRI} = \mathrm{DIM} / \mathrm{ORD} \tag{4}$$

The ORD values (mg/kg of body weight/day) used in this study were: 0.001 for Cd, 0.0035 for Pb, 0.04 for Cu, and 0.30 for Zn (USEPA, 2009). An HRI value less than 1 is generally considered to indicate no significant risk of adverse health effects, while a value equal to or greater than 1 suggests a potential health risk. The total HRI for each individual (adult or child) was calculated by summing the HRIs for all the considered heavy metals.

The use of the DIM and the HRI is a well-established methodology for assessing non-carcinogenic health risks associated with exposure to heavy metals through food consumption (Hang *et al.* 2009; USEPA 2009; Likuku and Obuseng 2015). The DIM estimates the amount of metal ingested daily based on consumption rates and metal concentrations in food. The HRI then normalizes this intake to the ORD, a threshold below which adverse health effects are not expected. This approach, recommended by regulatory agencies like the US Environmental Protection Agency (EPA) (2009), provides a standardized and widely accepted method for evaluating potential health risks from contaminated food.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism software (Version 6.01). Heavy metal concentrations in water, soil, and plant tissues across the different sampling sites were analyzed using one-way analysis of variance (ANOVA) to determine if there were statistically significant differences between the means. Where significant differences were found ($p < 0.05$), Tukey's honestly significant difference (HSD) *post-hoc* test was applied to identify which specific sites differed significantly from each other. The results were presented as mean ± standard deviation (Mean ± S.D).

RESULTS

Irrigation Water Quality

Irrigation water quality varied significantly across the studied sites (Table S1). Notably, wastewater sources exhibited elevated pH (S3) and EC (S5) compared to the groundwater control (S1). Heavy metal analysis (Table 1) revealed that Cd concentrations in all wastewater-irrigated sites (S2 to S5) significantly exceeded established permissible limits for irrigation water ($p < 0.05$). The highest Cd levels were observed in municipal wastewater (S5). Similarly, Pb, Cu, and Zn concentrations were also highest in specific wastewater sources (Pb in S4, Cu and Zn in S5), with statistically significant differences across sites ($p < 0.05$).

Soil Heavy Metal Accumulation

Soil analysis demonstrated that wastewater irrigation led to a significant increase in soil pH and EC ($p < 0.05$) (Table S2). Heavy metal concentrations in soil followed the order Zn > Cu > Pb > Cd across all sites, with the highest levels of Cd (S4), Pb, Cu, and Zn (S5) found in wastewater-irrigated soils ($p < 0.05$ compared to S1) (Table 2). Comparison with regulatory limits (Fig. 1) revealed that Cd concentrations in soils from wastewater-irrigated sites significantly exceeded EU (2002) and SEPA (2005) standards (p

< 0.05). Correlation analysis (Table S3) indicated significant positive associations between Cu-Cd and Pb-Zn, suggesting potential co-occurrence or similar accumulation pathways in the soil.

Table 1. Concentration (µg/L) of HMs in the Irrigation Water Used in the Specified Sampling Sites

HM	Metric	S1	S2	S3	S4	S5
Zn	Range	59.5-110.8	90.5-170.3	130.4-201.9	171.0-245.8	177.0-271.6
	Mean ± S.D.	90.6 ± 10.5^{d}	140.4 ± 28.7^{c}	190 ± 11.9^{b}	211.5 ± 12.8^{a}	231.5 ± 20.8^{a}
Cu	Range	38.8-54.9	8.91-15.30	58.4-97.5	150.5-240.4	169.0-280.4
	Mean ± S.D.	40.5 ± 15.8^{d}	12.8 ± 6.7^{e}	80.3 ± 22.7^{c}	210 ± 10.6^{b}	260.3 ± 15.6^{a}
Pb	Range	20.3-41.7	50.3-90.1	79.3-138.8	120.6-200.4	20.9-58.6
	Mean ± S.D.	30.2 ± 6.7^{e}	75.1 ± 20.6^{c}	110 ± 30.2^{b}	171.2 ± 23.8^{a}	40.4 ± 5.3^{d}
Cd	Range	6.71-10.4	8.4-16.3	11.4-21.7	14.3-25.2	27.2-41.5
	Mean ± S.D.	8.0 ± 4.5^{d}	13 ± 5.5^{c}	15.3 ± 6.2^{c}	17.7 ± 10.3^{b}	34.2 ± 12.3^{a}

Data is shown as Mean ± S.D.; Different letters in rows show significant difference at P < 0.05

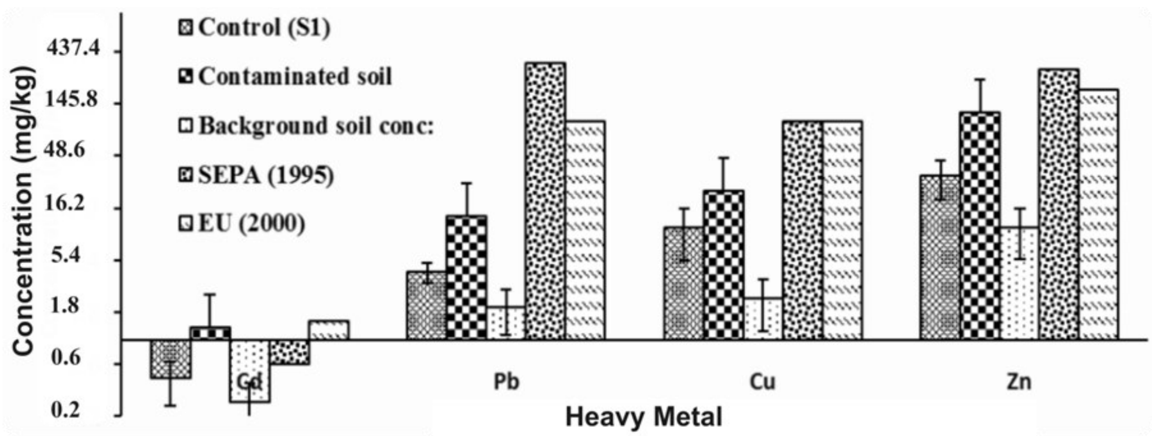

Fig. 1. Comparison of the studied HMs for the soil samples against the permissible limits set by EU (2000) and SEPA (2005); Data are shown as Mean ± S.D; n = 3.

Table 2. Concentration (µg/L) of HMs in the Soil Samples Collected from the Specified Sites

HM	S1	S2	S3	S4	S5	Background concentration
Zn	32.0 ± 10.5^{d}	84.0 ± 18.5^{c}	93.0 ± 13.7^{c}	127.3 ± 14.5^{b}	189.4 ± 21^{a}	10.7 ± 3.2^{e}
Cu	10.6 ± 4.3^{c}	14.8 ± 4.5^{c}	25.8 ± 8.3^{b}	32.8 ± 10.8^{a}	20.6 ± 7.4^{b}	2.4 ± 1.2^{d}
Pb	4.2 ± 0.9^{c}	8.4 ± 2.3^{b}	12.8 ± 2.2^{b}	14.6 ± 4.4^{b}	18.8 ± 3.2^{a}	2.0 ± 0.5^{c}
Cd	0.44 ± 0.19^{d}	0.9 ± 0.14^{d}	1.4 ± 0.32^{b}	1.73 ± 0.7^{a}	1.2 ± 0.6^{c}	0.27 ± 0.12^{e}

Data are shown as Mean ± S.D.; Different letters in rows show significant difference at P < 0.05.

Heavy Metal Uptake by Rice Plants

Heavy metal concentrations in rice roots and shoots followed the order Zn > Cu > Pb > Cd, with significantly higher levels observed in plants from wastewater-irrigated sites compared to the control ($p < 0.05$) (Table 3). In rice grains (Table 3), the same order prevailed, with the highest concentrations found in grains from contaminated sites ($p < 0.05$). Comparison with food safety standards (Table 4) showed that Cd in grains from marble, stone, and municipal wastewater-irrigated sites exceeded SEPA (2005) limits. Pb in grains from all wastewater-irrigated sites surpassed FAO/WHO (2001) limits. Notably, Zn concentrations in grains from all wastewater-irrigated sites exceeded the permissible limits of SEPA (2005), FAO/WHO (2001), and Indian Standards (Awasthi 2000). Correlation analysis in grains (Table S4) revealed significant positive correlations between Cu-Cd and Zn-Pb, indicating potential co-uptake mechanisms.

Table 3. Concentration (µg/g dry weight) of Heavy Metals (HMs) in the Roots, Shoots, and Grains of Rice Samples across Sampling Sites

HM	Plant Part	S1	S2	S3	S4	S5
Cd	**Root**	0.32 ± 0.8^{d}	0.5 ± 0.21^{c}	1.02 ± 0.7^{b}	1.29 ± 0.5^{a}	0.9 ± 0.48^{c}
	Shoot	0.23 ± 0.11^{c}	0.38 ± 0.11^{b}	0.78 ± 0.51^{a}	0.94 ± 0.41^{a}	0.46 ± 0.13^{b}
	Grain	0.1 ± 0.03^{d}	0.18 ± 0.09^{c}	0.25 ± 0.2^{b}	0.49 ± 0.03^{a}	0.29 ± 0.05^{b}
Pb	**Root**	3.4 ± 2.1^{c}	6.8 ± 2.1^{b}	8.6 ± 3.8^{b}	10.4 ± 2.1^{b}	15.4 ± 3.1^{a}
	Shoot	2.62 ± 1.8^{c}	4.6 ± 1.5^{b}	6.2 ± 0.9^{a}	7.4 ± 1.8^{a}	10.4 ± 2.2^{a}
	Grain	0.8 ± 0.12^{e}	1.4 ± 0.7^{c}	1.83 ± 0.3^{b}	1.3 ± 0.8^{c}	2.2 ± 1.1^{a}
Cu	**Root**	7.2 ± 1.9^{d}	12.6 ± 1.3^{c}	21.4 ± 6.7^{b}	23.2 ± 2.1^{b}	17.7 ± 4.7^{a}
	Shoot	5.9 ± 2.2^{c}	9.7 ± 3.2^{c}	17.6 ± 1.5^{a}	21.2 ± 4.2^{a}	14.8 ± 2.1^{b}
	Grain	4.2 ± 0.12^{c}	8.9 ± 3.6^{b}	14.2 ± 3.86^{b}	18.25 ± 2.1^{a}	10.76 ± 4.4^{f}
Zn	**Root**	21.08 ± 8.2^{d}	55 ± 10.3^{c}	39.28 ± 11^{c}	79.89 ± 12.8^{b}	128.34 ± 15.3^{a}
	Shoot	19.23 ± 4.2^{d}	40.39 ± 8.9^{c}	23.92 ± 5.1^{d}	59.94 ± 11.8^{b}	88.3 ± 16.4^{a}
	Grain	7.03 ± 1.08^{b}	15.92 ± 3.19^{a}	18.89 ± 6.5^{a}	22.04 ± 4.8^{a}	25.1 ± 3.2^{a}

Data are shown as Mean ± S.D; n = 3. Different letters in rows show significant difference at $P < 0.05$.

Table 4. Comparison of the Studied HMs for the Soil Samples against the Permissible Limits

HM	Sampling Sites					Standards		
	S1	**S2**	**S3**	**S4**	**S5**	**SEPA[a]**	**Indian Std.[c]**	**WHO[b]**
Zn	7.03	15.9	18.9	22.1	25.1	100	50	9.4
Cu	4.2	8.9	14.2	18.2	10.8	20	30	73.3
Pb	0.8	1.4	1.8	1.3	2.2	9	2.5	0.3
Cd	0.1	0.18	0.25	0.49	0.29	0.1-0.2	1.5	NA[d]

[a] SEPA (2005); [c] Indian standards (Awashti 2000); [b] FAO/WHO (2001); [d] NA = not available

Bioaccumulation and Enrichment

The BAFs from soil to rice grains (Fig. 2) were generally higher in contaminated sites, with Cu exhibiting the highest transfer efficiency, followed by Cd. The BAF values followed the order Pb < Zn < Cd < Cu, with significant differences across irrigation sites ($p < 0.05$).

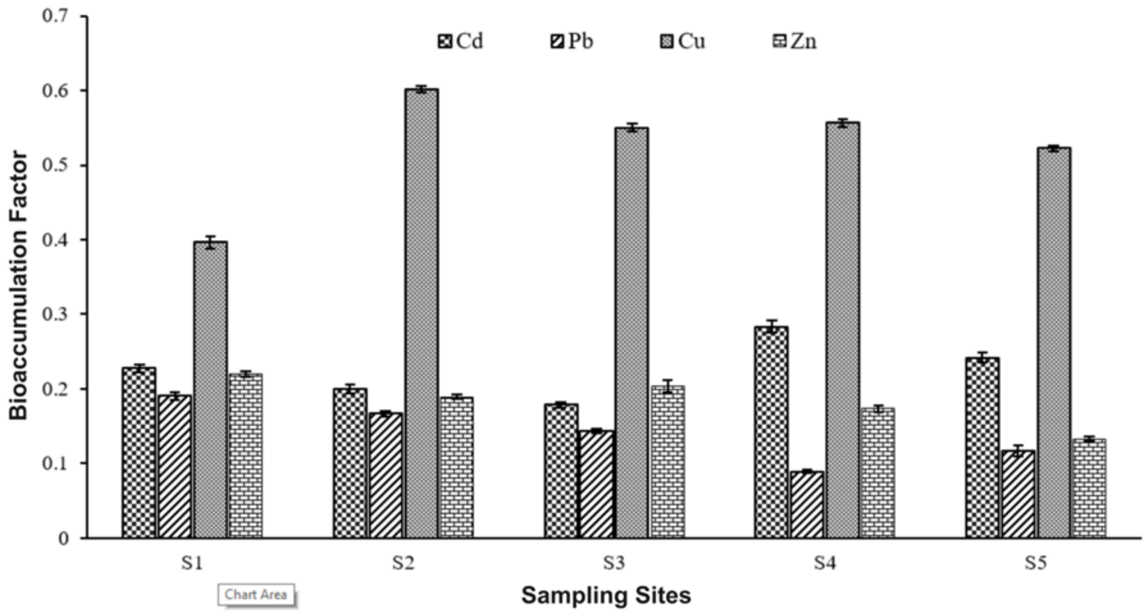

Fig. 2. BAFs for the studied HMs across the sampling sites which calculate as the ratio of heavy metal concentration in rice grains to that in the corresponding soil; Data are shown as Mean ± S.D.

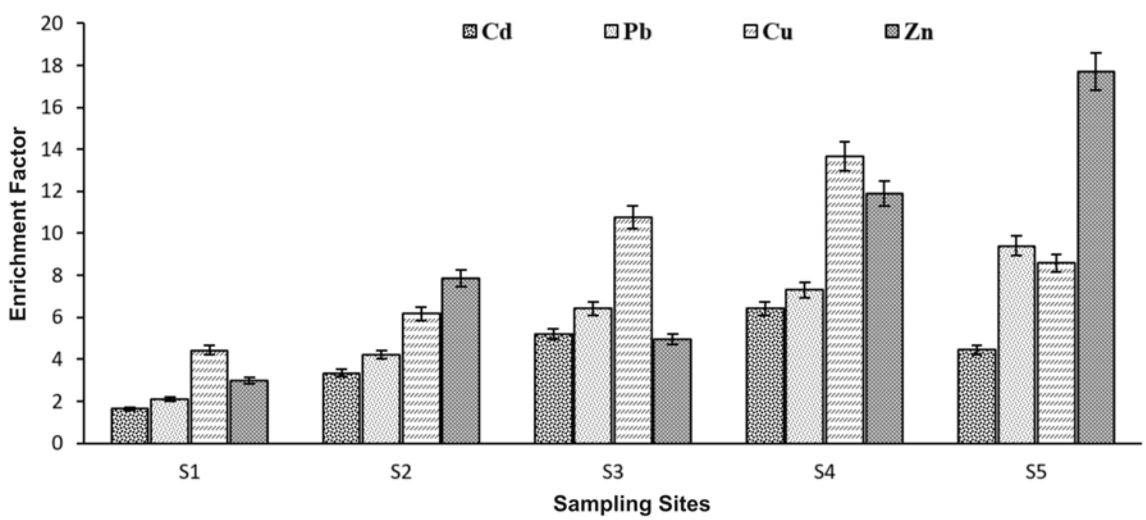

Fig. 3. Enrichment factor is used to assess the degree of soil contamination and heavy metal accumulation in soil; Data are shown as Mean ± S.D.

The EFs in soil (Fig. 3) were significantly elevated in wastewater-irrigated sites compared to the control ($p < 0.05$), indicating substantial accumulation, with the order being Cd < Pb < Cu < Zn. The highest EFs for Zn and Pb were in municipal wastewater-irrigated soil (S5), while Cu and Cd EFs were highest in stone wastewater-irrigated soil (S4).

Potential Human Health Risks

The DIM through rice consumption was significantly higher for both adults and children in wastewater-irrigated areas ($p < 0.05$) (Table S5), with Zn exhibiting the highest DIM. While individual HRI values for each metal remained below the US EPA's threshold of 1 (Table S6), the total HRI (THRI) (Fig. 4) was significantly elevated in consumers of rice from contaminated sites compared to the control ($p < 0.05$). Lead was the primary contributor to THRI across all sites. Although THRI values were below 1, they approached this threshold in sites irrigated with stone and municipal wastewater, indicating a potential for non-carcinogenic health risks with prolonged exposure.

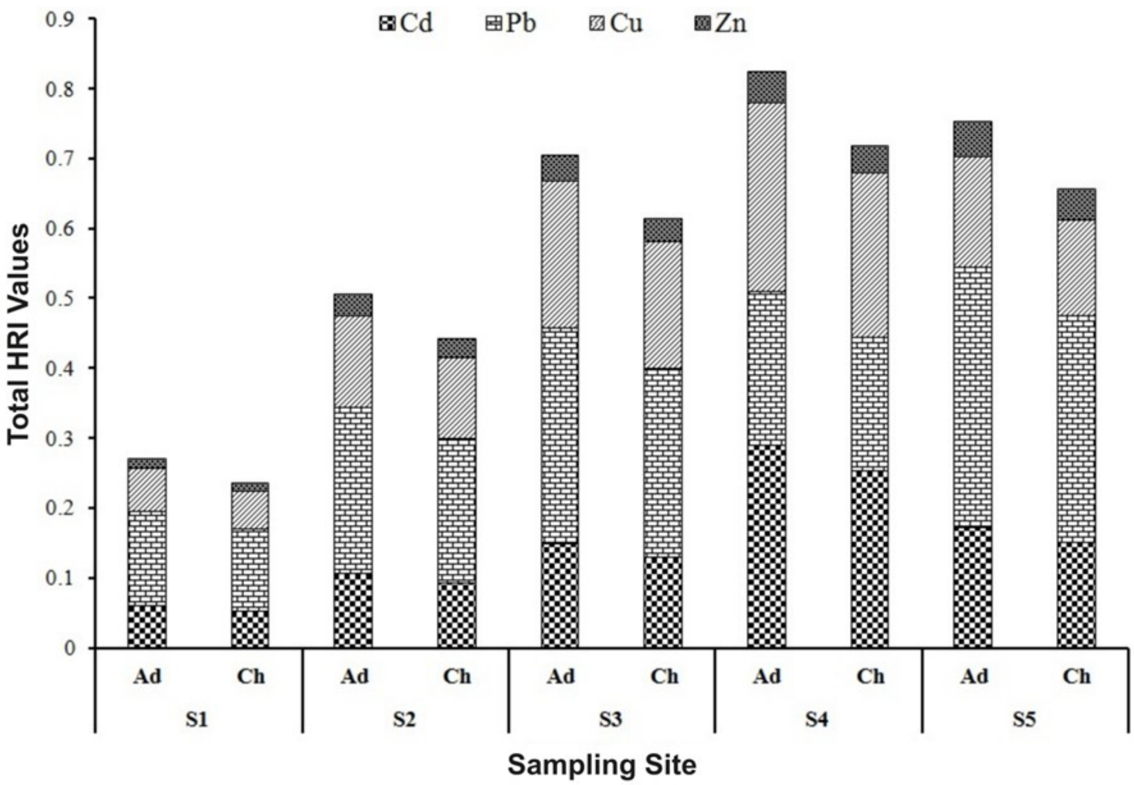

Fig. 4. The total health risk index value for adults (Ad) and children (Ch) across the sampling sites. The HRI for each site (Table S6) was combined to assess non-carcinogenic health risks associated with exposure to heavy metals through food consumption. Data are shown as Mean ± S.D.

DISCUSSION

Heavy Metals In Water and Soil and their Implications

The current findings unequivocally demonstrate significantly elevated HM concentrations in wastewater used for irrigation compared to the control groundwater ($p < 0.05$). This observation aligns with numerous studies highlighting industrial and domestic wastewater as major sources of HM contamination in irrigation water (Sultan *et al.* 2008; Mahmood and Malik 2014; Nafees and Amin 2014). The reducing conditions prevalent in flooded rice paddies can further enhance the solubility and mobility of these metals, increasing their bioavailability for plant uptake (Adamczyk-Szabela and Wolf 2022). Over time, continuous irrigation with HM-laden wastewater leads to the accumulation of these contaminants in agricultural soils.

Notably, while most HMs remained within permissible limits in the irrigation water, cadmium (Cd) consistently exceeded these guidelines across all wastewater-irrigated sites. This is a significant finding, as even at lower concentrations in water, Cd's high mobility and bioavailability can lead to substantial accumulation in soil and subsequently in crops (Feszterová *et al.* 2021). The levels of other HMs in water were generally within reported ranges from previous studies in similar settings (Sultan *et al.* 2008; Mahmood and Malik 2014; Nafees and Amin 2014), suggesting Cd as a particularly concerning contaminant in the wastewater sources of the study area.

Soil pH plays a critical role in HM speciation, solubility, and thus, bioavailability (Adamczyk-Szabela and Wolf 2022). While the pH of the contaminated soils was slightly altered by wastewater irrigation, the impact on HM mobility appeared uneven. For instance, higher accumulation of Zn and Pb in rice grown in municipal wastewater-irrigated soil (S5), which had a relatively lower pH (6.1 to 7.6), suggests that slightly acidic to neutral conditions might have enhanced their solubility and uptake. However, this relationship is complex and can be influenced by other soil properties and the specific metal (Adamczyk-Szabela and Wolf 2022). The overall higher HM concentrations in wastewater-irrigated soils compared to the control ($p < 0.05$), with Cd exceeding permissible limits (Awasthi 2000; EU 2002; SEPA 2005), underscores the detrimental impact of using these water sources for irrigation.

The elevated Cd levels in the study area could be attributed to various anthropogenic sources beyond wastewater, including the potential use of compost derived from Cd-containing waste, as well as the application of phosphate fertilizers and Cd-containing pesticides, which are practices reported in other studies in the region (Nafees *et al.* 2009; Ullah *et al.* 2016a). The comparison with non-agricultural soil (Background soil HM; Table 2) further supports the notion that agricultural activities, particularly wastewater irrigation and fertilizer use, are significant contributors to HM accumulation. The identified potential sources of HMs in the study area, including marble industries, car wash centers, petrol pumps, restaurants/hotels, municipality discharges, industrial emissions, and agricultural inputs (Ullah *et al.* 2016b), highlight the complex and multi-faceted nature of HM contamination in agricultural ecosystems. The common use of manure and sewage sludge as organic fertilizers in the studied area (as observed) further warrants investigation as potential sources of HM input.

Heavy Metal Uptake and Translocation in Rice

The observed variations in HM concentrations across different parts of the rice plant (roots > shoots > grains) are consistent with typical plant physiology, where roots are the primary point of uptake, followed by translocation to the shoots, with limited transfer to the grains as a defense mechanism (Pandey *et al.* 2012; Satpathy *et al*., 2014). However, the presence of HMs in the grains across all sampling sites, even the control, is concerning for food safety. The higher HM concentrations in plants from contaminated sites directly reflect the elevated levels in the irrigation water and soil. The potential contribution of atmospheric deposition from vehicular emissions and industrial activities (as observed with outdated vehicles in the area) to HM contamination in paddy fields cannot be entirely discounted (Islam *et al.* 2017). These airborne pollutants can settle on plant surfaces and in the soil, potentially contributing to uptake. The significant positive correlations observed between HM pairs in both soil and rice grains suggest a common origin, likely the wastewater used for irrigation and possibly the agrochemicals applied (Okoro *et al.* 2017). This co-occurrence implies that management strategies should address multiple contaminants simultaneously.

Implications of Bioaccumulation and Enrichment Factors

The EF values greater than 1 across all contaminated sites clearly indicate a significant anthropogenic contribution to HM accumulation in the soil (Izah *et al.* 2017). The higher EF values in contaminated sites compared to the control highlight the impact of wastewater irrigation. The magnitude of EF is influenced by HM concentration and bioavailability in the soil, the chemical properties of the metal, and plant uptake characteristics (Almasoud *et al.* 2015; Balkhair and Ashraf 2016). The considerably higher EF values observed in this study compared to some previous reports could be attributed to the specific characteristics of the wastewater sources in Lower Dir, including potentially high HM loads, or the specific soil conditions enhancing metal availability.

The BAF values indicate the efficiency of rice plants in transferring HMs from soil to the edible grains (Zhuang *et al.* 2009). The BAF order (Pb < Zn < Cd < Cu) suggests that copper is most readily translocated to the grains relative to its soil concentration, followed by cadmium. The higher BAFs in contaminated sites underscore the increased risk of HM transfer to the food chain when wastewater is used for irrigation. Even BAF values less than 1 can still lead to significant HM accumulation in grains if soil concentrations are sufficiently high, as observed with Cd and Zn exceeding food safety limits despite relatively moderate BAFs.

Health Risk Assessment, Long-Term Food Safety, and Mitigation Strategies

While the individual HRI values for each HM were below the non-carcinogenic risk threshold of 1 (US EPA 1999; Muhammad *et al.* 2011), the elevated HRI for Pb and the overall higher HRIs compared to some previous studies (Korre *et al.* 2002; Liu *et al.* 2011; Khan *et al.* 2013; Jaishankar *et al*., 2014) raise concerns about potential long-term health implications, especially considering the cumulative exposure to multiple HMs. The THRI, which considers the additive effects of all four metals, approached the risk threshold in several contaminated sites, particularly those irrigated with stone processing and municipal wastewater. This suggests that the combined exposure to these metals through rice consumption may pose a greater risk than exposure to individual metals alone (Hallenbeck 1993). The higher THRI values for adults compared to children are likely due to higher rice consumption rates. The finding that Pb is the major contributor to THRI is significant and warrants further investigation into the sources and behavior of Pb in the local environment. The potential for bioaccumulation and biomagnification of these HMs over time further exacerbates the long-term food safety concerns for the local population relying on rice as a staple food (Zhang *et al.* 2017; Zia *et al.* 2017).

Dietary intake of contaminated food is a major route of HM exposure in humans, accounting for a substantial portion (*e.g.*, up to 70% for Cd) (Zhang *et al.* 2017; Zia *et al.* 2017; Jan *et al*., 2010). The DIM for Cd was observed to be higher in adults compared to children, which was likely due to the higher rice consumption rates in adults. However, the DIM for Cd in both groups was below the tolerable daily intake (1.0^{-03} mg/person/day). Similarly, DIM values for Pb, Cu, and Zn were also below their respective tolerable daily intake levels (Pb: 3.5^{-03} mg/person/day; Cu: 4.0^{-02} mg/person/day; Zn: 3.0^{-01} mg/person/day), consistent with findings from previous studies in similar contexts (Khan *et al.* 2013; Mahmood and Malik 2014; Ullah *et al.* 2016a).

The THRI, which represents the cumulative non-carcinogenic risk from the combined intake of all four HMs, provides a more comprehensive assessment of potential health impacts (Hallenbeck 1993; Qing *et al*., 2015). The THRI values of 0.612 for adults and 0.533 for children indicate a potential for adverse health effects in the future, particularly considering the likelihood of increased HM accumulation in the soil and rice over extended periods due to continuous irrigation with contaminated wastewater. The processes of bioaccumulation in the food chain and biomagnification of HMs can exacerbate these risks over time, leading to higher concentrations in human tissues (Hallenbeck 1993). Therefore, while immediate risks may appear low based on individual HRIs, the trend of increasing HM levels and the cumulative risk indicated by THRI necessitate proactive measures to mitigate HM contamination in the agricultural ecosystem of Lower Dir to safeguard long-term public health.

The study's findings underscore the urgent need for improved soil and water management practices in the studied region. Untreated or poorly treated wastewater from industrial (marble and stone processing) and domestic sources should not be directly used for irrigation without proper treatment to remove or reduce HM loads. Implementing wastewater treatment technologies, such as chemical precipitation, adsorption, or biological methods (Balkhair and Ashraf, 2016), is crucial to minimize HM contamination of irrigation water and subsequently the soil and crops. Government should offer economic incentives (*e.g*., tax breaks, subsidies) to factories that implement closed-loop water recycling systems, minimizing both discharge volume and overall water abstraction from the Swat River.

Promoting sustainable agricultural practices, including the judicious use of fertilizers and pesticides, and exploring alternative, less contaminated organic fertilizers, can also help mitigate HM input into the soil (Khan *et al.* 2008; Bourliva *et al.*, 2017). Regular monitoring of HM concentrations in irrigation water, soil, and rice crops is essential to assess the effectiveness of any implemented mitigation strategies and to ensure long-term food safety. Furthermore, research into rice cultivars with lower HM uptake and accumulation potential (phytoremediation or biofortification approaches) could offer a long-term solution for reducing dietary exposure (Feszterová *et al.* 2021). Public awareness campaigns regarding the risks associated with wastewater irrigation and the importance of safe agricultural practices are also crucial for protecting public health in the region.

CONCLUSIONS

1. The heavy metals (HMs) were present in the soil and water samples in the order of Cd < Pb < Cu < Zn. The concentration of Cd in the wastewater and soil samples was observed to be higher than the suggested permissible limits.
2. The HMs concentration was significantly higher in sampling sites that had been irrigated with wastewater as compared to the control site. Furthermore, a strong significant correlation was observed in HMs concentrations between soil and grains. While Zn concentration was consistently higher, and Cu was generally elevated, Pb concentration in the rice grains from wastewater-irrigated fields significantly exceeded food safety standards. For instance, the mean Pb concentration in grains from sites S3, S4, and S5 ranged from 1.3 to 2.2 μg/g. When compared to the international limit (*e.g*., FAO/WHO standard of 0.3 μg/g), the measured levels were 4 to 7 times higher.

3. Consequently, the risk assessment showed that there were no health-risks in the studied region for most HMs except Pb, which showed a high level of HRI (2.5×10^{-1} and 2.2×10^{-1} for adults and children, respectively). This high value of HRI might pose a potential health risk to the consumers.
4. It is hence recommended that the rice grains from the contaminated sites should not be consumed without appropriate treatment. Regular analysis of HMs should be carried out in all food crops to appraise any health-risks, guarantee food-safety, and protect local masses from potential health risks.
5. The Government and environmental protection agencies/organizations should properly plan future environmental management strategies.
6. Strict environmental laws should be implemented to avoid and control HMs contaminations. Moreover, further deterioration of the soil should also be assured in order to ensure health risks free agricultural foods/crops consumption.
7. It is important to acknowledge the limitations of this study, including the data being collected only over a single cropping season, the use of composite sampling, and the absence of metal speciation analysis, which would provide greater insight into the bioavailability and mobility of the heavy metals.

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Competing Interests

The authors declare that there are no conflicts of interest.

Availability of Data and Material

All the data generated in this research work has been included in this manuscript.

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APPENDIX
Supplementary Information

Fig. S1. Sampling location in the studied area

Table S1. pH and EC (μS/cm) of the Water Samples, Collected From the Specified Sites

	Sampling Sites									
	S1		S2		S3		S4		S5	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
pH	6.9-7.6	7.4±0.21	6.7-7.8	7.6±0.32	7.7–8.4	8.2±0.29	7.6-8.2	7.9±0.25	6.5-7.2	6.9±0.19
EC	70.3-131.7	54.2±10.4^{c}	89.2-172.8	72.3±15.7^{c}	70.4-169.4	69.4±12.3^{c}	104.4-234.5	104±18.3^{b}	154-313.6	225.8±22.3^{a}

Data is shown as Mean ± SD

Table S2. pH and EC of the Soil Samples, Collected From the Specified Sampling Sites

	Units	Sampling Sites									
		S1		S2		S3		S4		S5	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
pH	------	6.7-7.8	7.5±0.6	6.3-7.4	7.2±0.8	7.7-8.1	7.8±0.3	7.6-8.2	7.9±0.5	6.2-7.6	6.6±1.1
EC	μS/cm	263-403	360±70.3[d]	487-732	561±201[c]	382-872	740±54[b]	345-757	651±105[c]	579-921	822±92.2[a]

Data is shown as Mean ± SD

Table S3. Correlation Coefficient Matrix for the HMs in Soil Samples

	Cd	*Pb*	*Cu*	*Zn*
Cd	1			
Pb	0.293	1		
Cu	**0.924**	0.388	1	
Zn	**0.753**	**0.835**	**0.744**	1

Bold figures show a positive significant correlation at P < 0.05 (2-tailed)

Table S4a. Comparison of the Studied HMs for the Soil Samples Against the Permissible Limits

HMs	Sampling Sites					Standards		
	S1	S2	S3	S4	S5	SEPA[a]	Indian Std.[c]	WHO[b]
Zn	7.03	15.9	18.9	22.1	25.1	100	50	9.4
Cu	4.2	8.9	14.2	18.2	10.8	20	30	73.3
Pb	0.8	1.4	1.8	1.3	2.2	9	2.5	0.3
Cd	0.1	0.18	0.25	0.49	0.29	0.1 to 0.2	1.5	NA[d]

[a] SEPA (2005); [b] FAO/WHO (2001); [c] Indian standards (Awashti 2000); [d] NA = not available

Table S4b. Correlation Coefficient Matrix for the HMs in Grain Samples

	Cd	*Pb*	*Cu*	*Zn*
Cd	1			
Pb	**0.764**	1		
Cu	**0.981**	**0.682**	1	
Zn	**0.623**	**0.966**	**0.519**	1

Bold figures show a positive significant correlation at P < 0.05 (2tailed)

Table S5. DIM Values for the HMs for Adults and Children Across the Sampling Sites

HMs	Individuals	Sampling Sites					ORD
		S1	S2	S3	S4	S5	
Cd	Adults	5.9×10^{-5}	1.1×10^{-4}	1.5×10^{-4}	2.9×10^{-4}	1.7×10^{-4}	(1.0×10^{-3})[a]
	Children	5.2×10^{-5}	9.3×10^{-5}	1.3×10^{-4}	2.5×10^{-4}	1.5×10^{-4}	
Pb	Adults	4.7×10^{-4}	8.3×10^{-4}	1.1×10^{-3}	7.7×10^{-4}	1.3×10^{-3}	(3.5×10^{-3})[b]
	Children	4.1×10^{-4}	7.2×10^{-4}	9.4×10^{-4}	6.7×10^{-4}	1.1×10^{-3}	
Cu	Adults	2.5×10^{-3}	5.3×10^{-3}	8.4×10^{-3}	1.1×10^{-2}	6.4×10^{-3}	(4.0×10^{-2})[a]
	Children	2.2×10^{-3}	4.6×10^{-3}	7.3×10^{-3}	9.4×10^{-3}	5.5×10^{-3}	
Zn	Adults	4.2×10^{-3}	9.4×10^{-3}	1.1×10^{-2}	1.3×10^{-2}	1.5×10^{-2}	(3.0×10^{-1})[a]
	Children	3.6×10^{-3}	8.2×10^{-3}	9.7×10^{-3}	1.1×10^{-2}	1.3×10^{-2}	

Daily Intake (DI, mg/kg/day), Oral reference dose (ORD, mg/kg/day); [a][80] [b][81]

Table S6. HRI From HMs Consumption *via* Rice for Adults and Children Across All Sampling Sites

HMs	Individual	Sampling Sites					Mean
		S1	S2	S3	S4	S5	
Cd	Adults	6.0×10^{-2}	1.1×10^{-1}	1.5×10^{-1}	2.9×10^{-1}	1.7×10^{-1}	1.6×10^{-1}
	Children	5.2×10^{-2}	9.3×10^{-2}	1.3×10^{-1}	2.5×10^{-1}	1.5×10^{-1}	1.3×10^{-1}
Pb	Adults	1.3×10^{-1}	2.4×10^{-1}	3.1×10^{-1}	2.2×10^{-1}	3.7×10^{-1}	2.5×10^{-1}
	Children	1.2×10^{-1}	2.1×10^{-1}	2.7×10^{-1}	1.9×10^{-1}	3.2×10^{-1}	2.2×10^{-1}
Cu	Adults	6.2×10^{-2}	1.3×10^{-1}	2.1×10^{-1}	2.7×10^{-1}	1.6×10^{-1}	1.7×10^{-1}
	Children	5.4×10^{-2}	1.2×10^{-1}	1.8×10^{-1}	2.4×10^{-1}	1.4×10^{-1}	1.4×10^{-1}
Zn	Adults	1.2×10^{-2}	3.1×10^{-2}	3.7×10^{-2}	4.3×10^{-2}	5.0×10^{-2}	3.5×10^{-2}
	Children	1.2×10^{-2}	2.7×10^{-2}	3.2×10^{-2}	3.8×10^{-2}	4.3×10^{-2}	3.1×10^{-2}
∑ HI	Adults	0.6115					
	Children	0.5329					