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Trace Metals in Rice Grains and Their Associated Health Risks from Conventional and Non-Conventional Rice Growing Areas in Punjab-Pakistan

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Abstract: Rice (*Oryza sativa* L.) is cultivated and consumed worldwide, but the contamination of rice grains with trace metals (TMs) could cause adverse impacts on human health. The aims of this study were to determine the concentrations of TMs in different rice varieties available for sale in local markets and to determine whether consumers are likely to be at risk via the consumption of these rice cultivars. For this purpose, samples of rice grains were collected from 12 rice growing districts (administrative units) in Punjab, Pakistan. These districts were further classified based on rice growing methods due to specific soil type. In conventional districts, the puddling method was used, while direct seeding was used for rice cultivation in non-conventional districts. The samples were collected and analyzed for the determination of essential (Cu, Fe, Zn, and Mn) and non-essential (Cd, Ni, and Pb) TMs using an atomic absorption spectrophotometer (AAS). The results showed that the maximum respective concentrations of Cd, Ni, and Pb (0.54, 0.05, 1.10 mg kg^{-1}) were found in rice grains in conventional areas, whereas values of 0.47, 0.20, and 1.20 mg kg^{-1} were found in non-conventional rice growing areas. The maximum concentrations of essential TMs (Cu, Fe, Mn, and Zn) were 4.54, 66.01, 4.82, and 21.51 mg kg^{-1} in conventional areas and 3.76, 74.11, 5.66, 19.63 mg kg^{-1} in non-conventional areas. In the conventional rice growing areas, Fe and Zn concentrations exceeded the permissible limits in the 27 and 7% samples, respectively. In the non-conventional rice areas, the concentrations of Cu, Fe, and Mn exceeded the permissible limits in the 15, 26, and 3% samples, respectively, while its Zn concentration was found within the permissible limits. The estimated weekly intake (EWI) and maximum tolerable dietary intake (MTDI) values for all studied metals were found within the permissible values set by WHO, except for Fe, in both sampled areas. It was concluded that no health risks were associated by utilizing the rice grains. However, the mean values of TMs were found considerably higher in collected rice samples from non-conventional areas than the conventional areas. Therefore, the concentrations of TMs should be monitored properly.

Keywords: fine and coarse rice; food chain; health risks; metals contamination

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1. Introduction

Trace metals (TMs) pollution has become a serious problem worldwide. There is a continuous buildup of TMs in agricultural soils through the application of solid waste and low quality irrigation water [1,2]. The continued use of raw or partially treated wastewater for growing fodder and cereals has resulted in the contamination of food crops, and thus their entry into the food chain [3,4]. The TMs in soils could contaminate the environment and damage human health through various exposure pathways, including direct ingestion, dermal contact, and inhalation [5]. TMs concentration in edible parts of several crops such as spinach, clover, grape vines, shrubs, barley, and wheat have been reported by various reports [6–8]; thus, the risk to humans of excessive TMs in edible parts of food crops should be a matter of concern. The crops absorb these metals from contaminated soils through their roots or from surface-deposited particles [9]. As TMs in crops enter the human body through the food chain and present health risks to humans, food consumption is also an important exposure pathway [10]. Vitamin C, Fe, and other nutrients stored in the body are significantly decreased if humans consume food contaminated by these TMs, leading to a decline in immunity, a deterioration of human function, and disabilities associated with malnutrition [9,11].

As rice is fast growing crop and produces large biomasses, several studies have indicated that rice grown in TM-contaminated soils have higher concentrations of metals than those grown in un-contaminated soils [12,13]. In un-contaminated soils, a major pathway of soil contamination is through the atmospheric deposition of TMs from point sources such as mining, smelting, and industrial activities [14]. Other sources of agricultural soil contamination include agricultural inputs such as fertilizers, pesticides, sewage sludge, organic manures, and composts [15,16]. Therefore, some eco-friendly alternatives could be applied for clean production and sustainable cultivation. The sustainable production of quality food for mankind is a big challenge of the era. Biofertilizers are being used to not only improve the yield but also improve the nutrition status of crop plants. Scientists are using nitrogen-fixing bacteria along with phosphorus-fixing bacteria and getting good results [17]. The use of biofertilizers and compost instead of chemical fertilizers improves soil health and decreases TM contamination in soil, improving plant quality [18]. The balanced use of fertilizer is very effective technique in producing crops and fulfilling their deficiencies [19]. Rice is a major staple food in many countries, particularly in Asian countries such as Bangladesh, India, Thailand, China, Pakistan, and Vietnam, where soil and groundwater pollution with high levels of TMs have been reported [20–23]. Increased levels of TMs in agricultural soils and their uptake in rice, vegetables, and other food crops have become a real health issue in this region [24]. A significant number of studies have focused on studying TM concentrations in Pakistani, Bangladeshi, Chinese, and Indian food items including vegetables [25–28]. The work of Wasim et al. [29] assessed the TM concentrations in rice, but sampling was performed only from one site, i.e., Karachi, Pakistan. Additionally, this study did not involve different rice growing districts and comparisons between conventional and non-conventional rice growing areas from Punjab, Pakistan. The conventional rice growing areas of Punjab are Sheikhupura, Gujranwala, Hafizabad, Sialkot, Narowal, and Gujranwala; rice is grown using the puddling method because of the specific soil type (clay loam), as clay loam soil puddling is easy due to the water retention capacity of soil. However, soil puddling enhances the possibility of TM availability to the plants, as the metals could be solubilized in the water and converted into the more available forms to the plants [30], i.e., zinc sulphide (ZnS) can be converted to cadmium sulphide (CdS) with the exchange of their ions during this practice. The non-conventional rice growing areas are Bahawalnagar, Chiniot, Jhang, Toba Tek Singh, Shorkot, and Mandi Bahauddin; rice is usually grown by using the direct seeded method because the soil is sandy loam. However, soil puddling is difficult in sandy loam because water retention and anaerobic conditions are not possible, moreover, TMs are leached down in these types of soils, thereby reducing the risks of TM contamination. Thus, there is a possibility that conventional rice growing areas could have more TM concentrations than the non-conventional growing areas. This study is based on the same hypothesis, i.e., TMs could behave differently due to the use of conventional and non-conventional rice growing techniques. Previous studies have not reported on this aspect along with the potential health risks to humans. Therefore, the objectives of the present study were to: (a) determine the concentrations of TMs in different rice varieties available for sale in local markets, produced from different conventional and non-conventional rice growing areas of

Punjab, Pakistan; and (b) whether consumers are likely to be at risk of TM exposure via the consumption of these rice cultivars.

2. Materials and Methods

2.1. Sampling

This experiment was conducted to find out the concentrations of different essential (Cu, Fe, Mn, and Zn) and non-essential (Cd, Ni, and Pb) TMs in different types of crop (rice) varieties available in markets from conventional and non-conventional rice growing areas of Punjab, Pakistan. Fifty-five (55) rice samples (in triplicate) from conventional and non-conventional rice growing districts (6 conventional: Sheikhupura, Gujranwala, Hafizabad, Sialkot, Narowal, and Gujranwala; 6 non-conventional: Bahawalnagar, Chiniot, Jhang, Toba Tek Singh, Shorkot, and Mandi Bahauddin) of Punjab, Pakistan were collected during the month of January 2015 (Figure 1, Table 1).

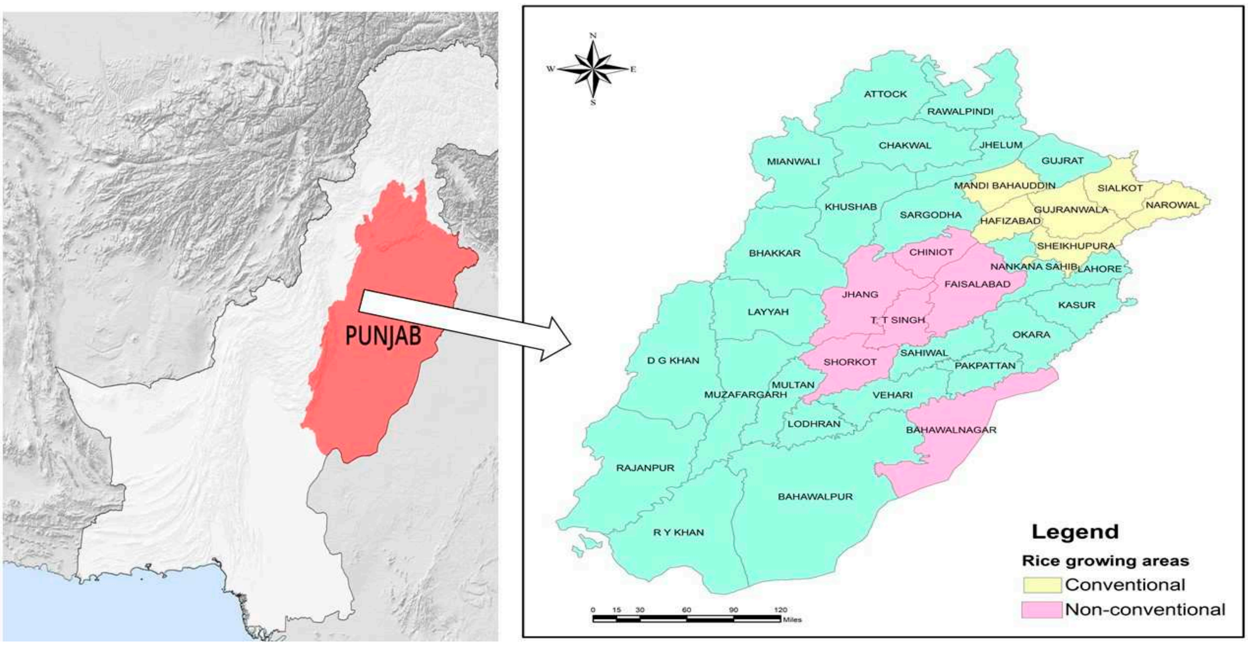

Figure 1. Base map showing sampling districts of Punjab, Pakistan.

Table 1. Coordinates of sampling sites.

Sr. No.	Sampling Location	Longitude	Latitude
1	Chiniot	31.710762	72.992123
2	Bahawalnagar	29.613738	73.137526
3	Jhang	30.843993	71.850886
4	Shorkot	30.844500	72.085357
5	Toba Tek Singh	30.970966	72.479624
6	Faisalabad	31.413924	73.074134
7	Narowal	32.096795	74.862740
8	Gujranwala,	31.982594	74.130777
9	Mandi Bahauddin	32.597835	73.476281
10	Hafizabad	32.052014	73.659907
11	Sialkot	32.386376	74.408410
12	Sheikhupura	31.715192	73.982211

The collected rice varieties were preferred to be cultivated by the farmers of the respective sampling districts. The collected samples details (sampling district, rice variety, and sample ID) are given in Supplementary Table S1. The collected samples were stored at 4 °C prior to further processing. All samples were washed with tap water (three times) followed by deionized (DI) water twice. The samples were then dried in open air for 24 h and in an oven at 65 ± 5 °C for 48 h. Then, the samples were homogenized by grinding with a ceramic mortar and pestle and processed for metals analysis.

2.2. Sample Preparation and Analysis

One gram of ground grain sample was taken in a 25 mL conical flask and kept overnight by adding 10 mL of digestion mixture (2:1 ratio of HNO_3 and $HClO_4$, respectively). The next day, samples were digested by placing on a hot plate at a temperature of 250 ± 5 °C until a clear solution was obtained. Following digestion, tubes were removed, and the samples were diluted to 20 mL with DI water after cooling. Prior to analysis, all samples were filtered through 0.45 µm filter paper (Whatman No. 41). A flame AAS (Solaar S-100, CiSA) was used for the determination of Cd, Cu, Fe, Mn, Ni, Pb, and Zn in rice samples (AOAC, 1990). Elemental concentrations in rice were determined on a dry weight basis.

2.3. Quality Control

The blank reagents and standard reference materials such as Batch 1701-3, BCR no. 150 and Fluka Kamica, Busch Switzerland were used to verify the accuracy and precision of the digestion procedure and subsequent analyses. Each sample batch was analyzed in triplicate under the standard operating conditions within the confidence limit of 95%. The validity of the method was further ascertained by cross method checks, spiked recovery, and replicate analysis.

2.4. Health Risk Assessment

The potential human health risk assessment was conducted by considering the following parameters according to Onsanit et al. [31]. The estimated weekly intake (EWI) and provisional tolerance weekly intake (PTWI) were jointly established by FAO/WHO [32]. The EWI (mg kg^{-1} body weight/week) was calculated using the following equation:

$$\text{EWI} = \text{Crice} \times \frac{\text{WCrice}}{\text{BW}}$$

where C rice = average trace metal concentrations in rice (mg kg^{-1} dry weight), WCrice = weekly rice consumption (g $week^{-1}$) per capita (18 kg/capita/year or ≈50 g per capita per day × 7) (PACRA, 2020), and BW = average body weight (kg) of the Pakistani population (72 kg). The values are expressed in mg $week^{-1}$ $person^{-1}$. The calculated EWI values were compared with levels for typical daily exposure and provisional tolerable weekly intake (PTWI) set by the Codex committee on food additives and contaminants of the joint FAO/WHO food standards program for TM.

2.5. Statistical Analysis

XLSTAT 2018 and Origin 2018 were used for the descriptive statistics and further analysis. Data presented as means ± SD. The Pearson correlation heatmap with dendrograms was developed using Origin v2018 (Origin Lab Corp., Northampton, MA, USA).

3. Results

The mean and range of TM concentrations in rice grains from different conventional and non-conventional growing areas are presented in Supplementary Information (SI) Table S1. All the permissible limits set by WHO/FAO are presented in Table 2 for reference and comparison to our data presented in this paper. The weekly intake rates of essential and non-essential metals are presented in Table 3.

Table 2. Permissible limits of trace metals in rice grains.

Metals	Minimum Value		Maximum Value		Limits (mg kg−1)	References
	CA *	NCA **	CA *	NCA **		
Cd	0	0.10	0.54	0.47	0.2	WHO/FAO [29]
Pb	0.12	0.30	1.10	1.20	0.2	
Ni	0	0.01	0.05	0.20	1.0	
Fe	25.43	31.11	66.01	74.11	15–50	
Cu	1.13	1.01	4.54	3.76	4–15	
Zn	12.61	12.74	21.51	19.63	20	
Mn	1.05	1.13	4.82	5.66	05	

* Conventional Rice Growing Areas; ** Non-Convention Rice Growing Areas.

Table 3. EWI rate of essential and non-essential metals.

Rice Growing Area	Estimated Weekly Intakes (EWI)						
	Essential Metals				Non-Essential Metals		
	Cu	Fe	Mn	Zn	Cd	Ni	Pb
Non-conventional	18.27	360.25	27.51	95.42	2.28	0.97	5.83
Conventional	22	321	23.43	105	2.65	0.24	5.34
EWI by WHO	70 *	315 *	77 *	280 *	7	35	25

EWI: estimated weekly intake for toxic elements, based on WHO guidelines. * There is no PTWI set for Cu, Fe, Mn, and Zn, but it is used as MTDI in mg kg^{-1} body weight in a week).

3.1. Trace Metal Concentration in Rice Grains from Non-Conventional Growing Areas

3.1.1. Essential Metals

There were six non-conventional rice growing areas (Bahawalnagar, Chiniot, Jhang, Shorkot, Toba Tek Singh and Faisalabad), from which we collected rice samples. From non-conventional rice-producing areas, the highest Cu, Fe, Mn, and Zn concentrations in the rice samples were recorded having values of 3.76, 74.11, 5.66, and 19.63 mg kg^{-1}, respectively. The average Cu concentrations were 2.16, 1.57, 1.79, 2.16, 2.93, and 3.25 mg kg^{-1} in Bahawalnagar, Chiniot, Jhang, Shorkot, Toba Tek Singh, and Faisalabad, respectively. Iron (56.38, 40.45, 38.58, 33.90, 36.65, and 50.10 mg kg^{-1}), Mn (2.00, 2.46, 3.32, 2.41, 3.54, and 3.84 mg kg^{-1}), and Zn (16.75, 15.34, 16.72, 16.66, 17.54, and 16.35 mg kg^{-1}) concentrations were recorded, respectively (Figure 2).

3.1.2. Non-Essential Metals

From non-conventional rice-producing areas, the highest Cd, Ni, and Pb concentrations in collected rice samples were recorded having values of 0.47, 0.20, and 1.20 mg kg^{-1}, respectively. The average Cd concentrations were recorded as 0.23, 0.20, 0.31, 0.28, 0.31, and 0.24 mg kg^{-1} in Bahawalnagar, Chiniot, Jhang, Shorkot, Toba Tek Singh, and Faisalabad, respectively. Average Ni (0.08, 0.06, 0.02, 0.04, 0.01, and 0.01 mg kg^{-1}) and Pb (0.59, 0.88, 0.84, 0.98, 0.92, and 0.87 mg kg^{-1}) concentrations were found in the same order of the rice growing areas, respectively (Figure 3).

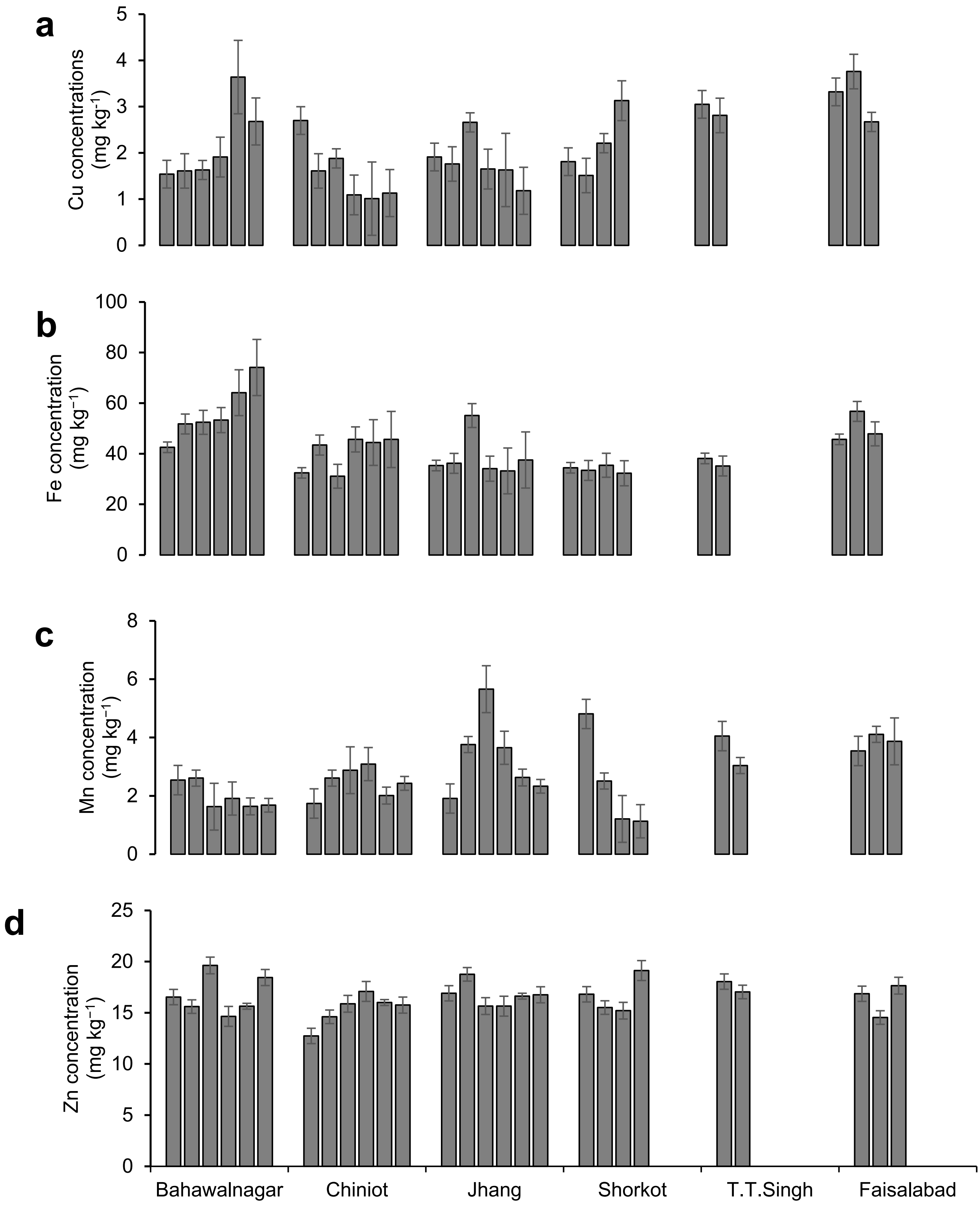

Figure 2. Concentrations of (**a**) Cu, (**b**) Fe, (**c**) Mn, and (**d**) Zn in rice grain samples from non-conventional rice growing districts; Bahawalnagar, Chiniot, Jhang, Shorkot, Toba Tek Singh (T.T. Singh), and Faisalabad, respectively; No. of bars show no. of samples taken from each district, and height shows metals' concentrations; error bars show standard deviation.

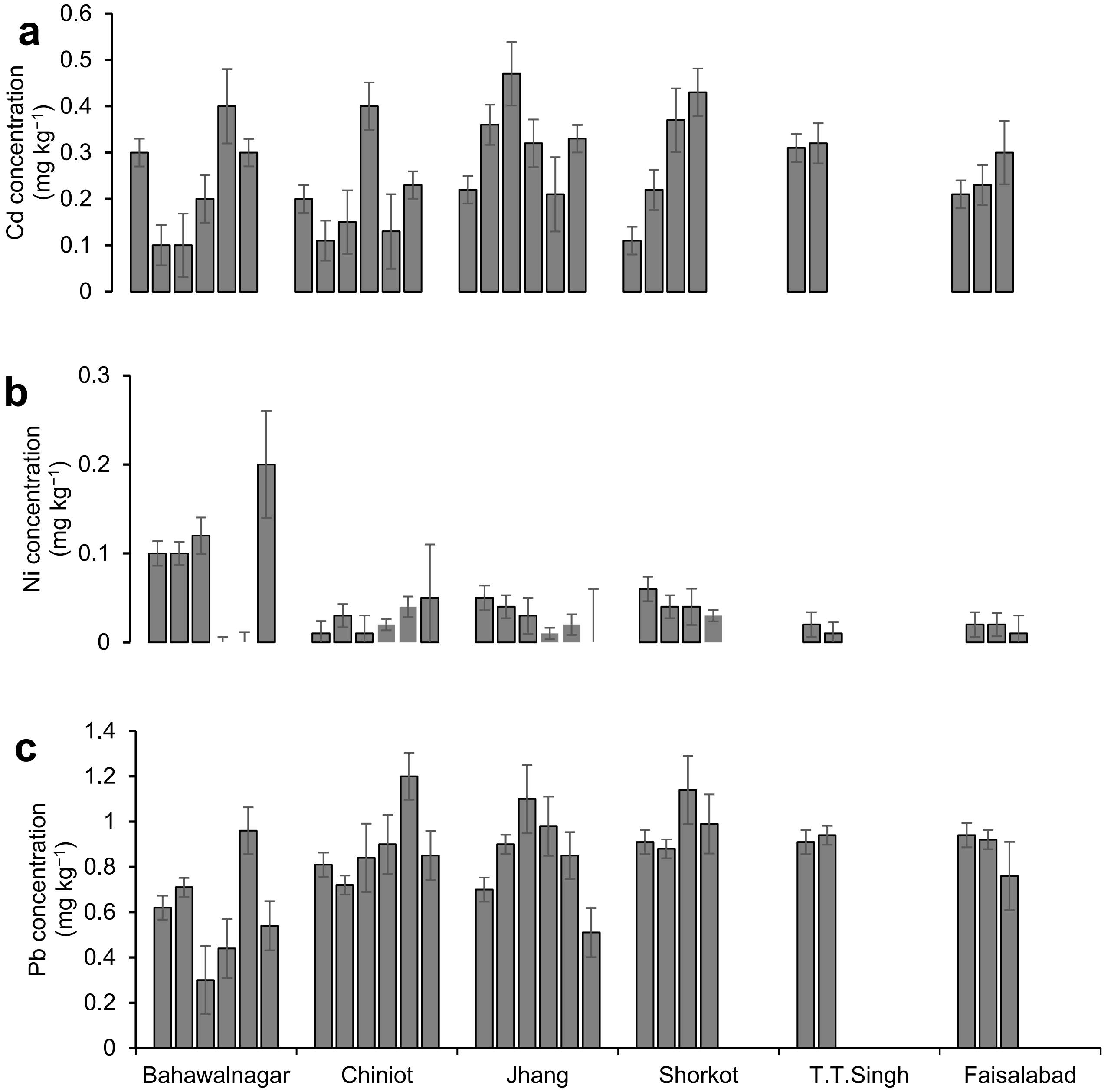

Figure 3. Concentrations of (**a**) Cd, (**b**) Ni, and (**c**) Pb in rice grain samples from non-conventional rice growing districts; Bahawalnagar, Chiniot, Jhang, Shorkot, Toba Tek Singh (T.T. Singh), and Faisalabad, respectively; No. of bars show no. of samples taken from each district, and height shows metals' concentrations; error bars show standard deviation.

3.2. Trace Metal Concentration in Rice Grains from Conventional Rice Growing Areas

3.2.1. Essential Metals

From conventional rice-producing areas, the highest Cu, Fe, Mn, and Zn concentrations in collected rice samples were recorded as 4.54, 66.01, 4.82, and 21.51 mg kg^{-1}, respectively. The average concentrations of Cu were found as 2.12, 2.89, 2.74, 1.97, 3.62, and 2.21 mg kg^{-1} in Narowal, Gujranwala, Hafizabad, Mandi Bahauddin, Sialkot, and Sheikhupura, respectively. Iron (42.75, 48.31, 34.00, 44.70, 45.19 and 45.81 mg kg^{-1}), Mn (2.53, 2.89, 1.99, 2.76, 4.37, and 3.89 mg kg^{-1}), and Zn (18.69, 14.64, 14.74, 16.51, 15.87 and 16.59 mg kg^{-1}) concentrations were found in the same order of the conventional rice growing areas, respectively (Figure 4).

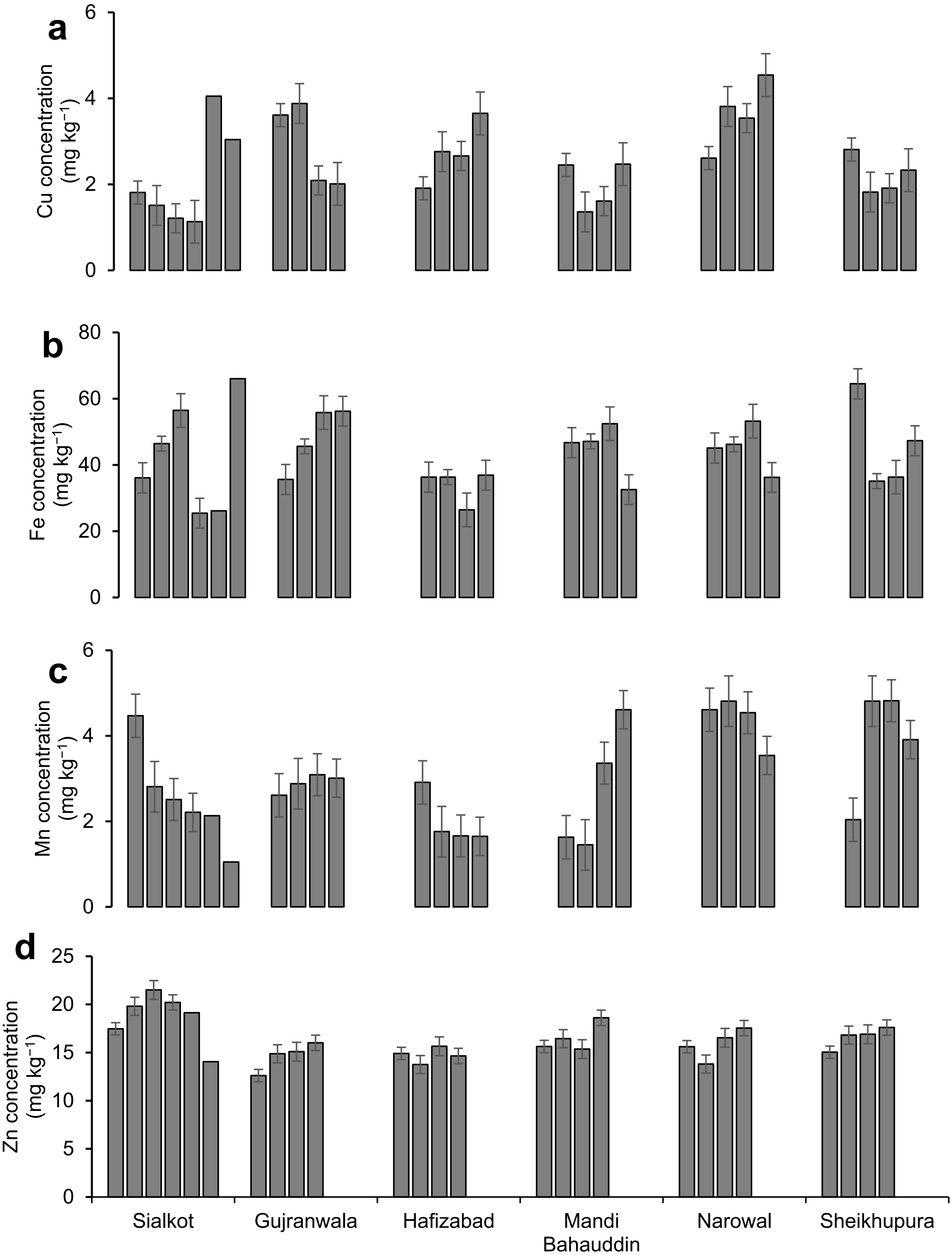

Figure 4. Concentrations of (**a**) Cu, (**b**) Fe, (**c**) Mn, and (**d**) Zn, in rice grain samples from conventional rice growing districts; Sialkot, Gujranwala, Hafizabad, Mandi Bahauddin, Narowal, and Sheikhupura, respectively; No. of bars show no. of samples taken from each district, and height shows metals' concentrations; error bars show standard deviation.

3.2.2. Non-Essential Metals

From conventional rice-producing areas, the highest Cd, Ni and Pb concentrations in the collected rice samples were recorded as 0.54, 0.05, and 1.10 mg kg^{-1}, respectively. The average concentrations of Cd were found as 0.37, 0.31, 0.09, 0.29, 0.30, and 0.36 mg kg^{-1} in Narowal, Gujranwala, Hafizabad, Mandi Bahauddin, Sialkot, and Sheikhupura, respectively. Nickel (0.02, 0.01, 0.01, 0.02, 0.01, and 0.02 mg kg^{-1}) and Pb (0.85, 0.82, 0.80, 0.66, 0.78 and 0.81 mg kg^{-1}) concentrations were found in the same order of the conventional rice growing areas, respectively (Figure 5).

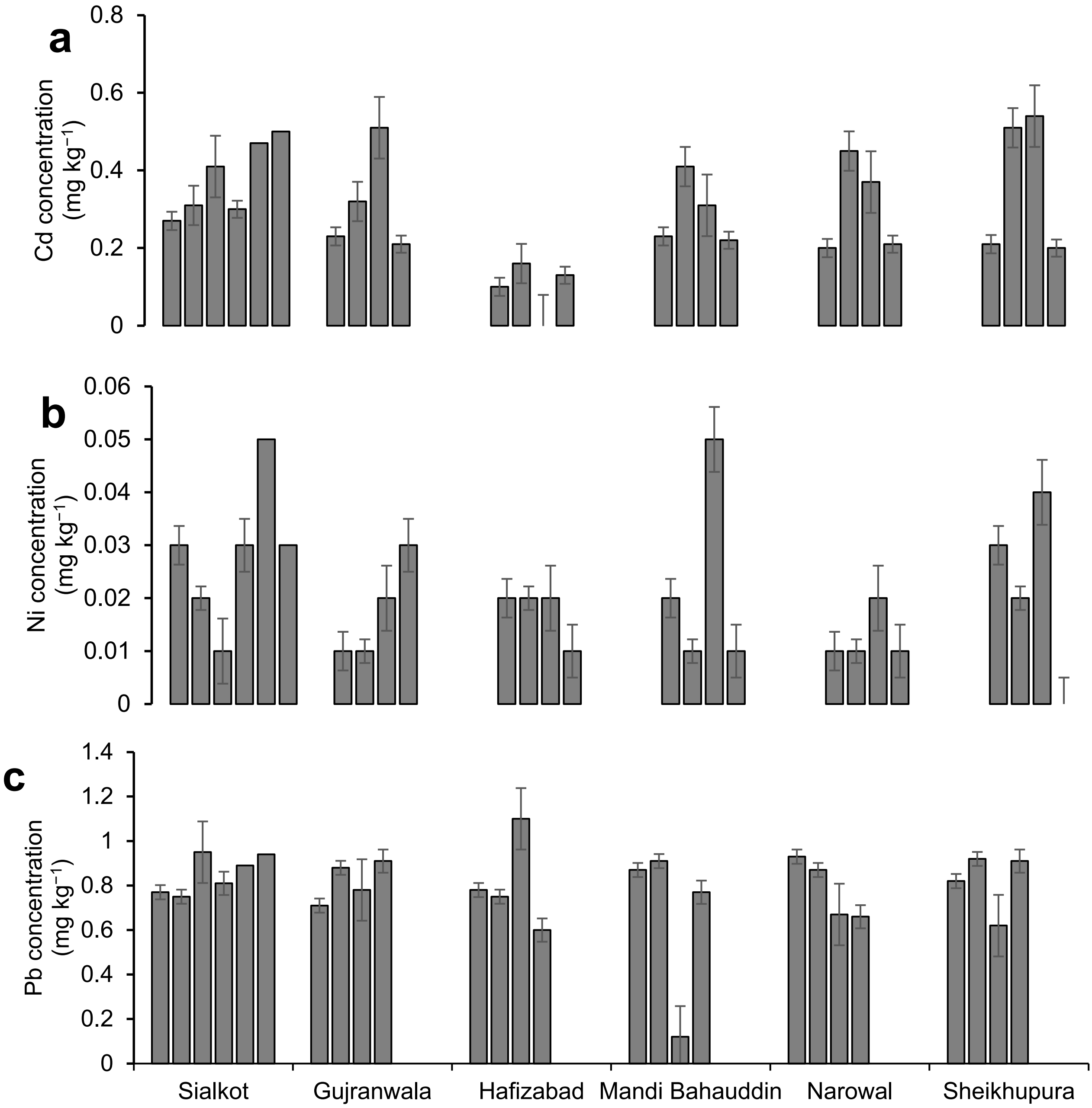

Figure 5. Concentrations of (**a**) Cd, (**b**) Ni, and (**c**) Pb in rice grain samples from conventional rice growing districts; Sialkot, Gujranwala, Hafizabad, Mandi Bahauddin, Narowal, and Sheikhupura, respectively; No. of bars show no. of samples taken from each district, and height shows metals' concentrations; error bars show standard deviation.

3.3. Relation between the Toxic Metals

Table 4 shows the Pearson correlation (R) between essential and non-essential toxic metals found in rice grains from conventional and non-conventional rice growing areas. At both places, the TMs displayed varied responses. In non-conventional areas, Cd had a significant positive effect with Zn (R^2 = 0.22, $p < 0.01$) and Cu (R^2 = 0.34, $p < 0.01$), whereas Ni had positive relation with Fe (R^2 = 0.47, $p < 0.05$), as well as Cd with Pb (R^2 = 0.35, $p < 0.05$). In the conventional areas, although the R^2 showed slight effect, the Zn had a significant positive effect on Mn (R^2 = 0.14, $p < 0.01$), Cd had positive relationship with Fe (R^2 = 0.27, $p < 0.01$), Mn (R^2 = 0.23, $p < 0.01$) and Zn (R^2 = 0.18, $p < 0.01$), while Ni also had positive relation with Cd (R^2 = 0.30, $p < 0.01$).

Table 4. Correlation matrix of metals in rice grains from conventional and non-conventional growing areas.

Metals	Essential Metals				Non-Essential Metals		
	Cu	**Fe**	**Mn**	**Zn**	**Cd**	**Ni**	**Pb**
Metal concentrations in rice grains from non-conventional growing areas							
Cu	1.00						
Fe	0.30	1.00					
Mn	0.14	−0.02	1.00				
Zn	0.00	0.05	0.00	1.00			
Cd	0.34 *	0.08	0.11	0.22 **	1.00		
Ni	−0.15	0.47 *	−0.22	0.40 *	−0.21	1.00	
Pb	0.23	−0.28	0.30	−0.21	0.35 *	−0.42	1.00
Metal concentrations in rice grains from conventional growing areas							
Cu	1.00						
Fe	−0.10	1.00					
Mn	−0.01	−0.11	1.00				
Zn	−0.42	−0.20	0.14 **	1.00			
Cd	−0.11	0.27 *	0.23 *	0.18 *	1.00		
Ni	−0.18	−0.02	−0.10	0.09	0.30 *	1.00	
Pb	0.01	−0.03	−0.19	0.12	−0.03	−0.38	1.00

* Values with single asterisk are statistically significant at $p < 0.00$; ** values with double asterisk are statistically significant at $p < 0.005$.

3.4. Principal Component Analysis and Heatmap

The first three components of the PCA accumulated 61.7% of the total variance (Figure 6). The first principal component explained 24.4% of the variance and reflected the negative coordination with Pb and positive with Ni. The second principal component explained 19.0% of the variance and reflected a covariation of Zn and Cd with loading factors of 0.75 and 0.67, respectively. The third principal component explained 18.3% of the variance and was linked to Fe and Cu (loading factor 0.78 and 0.56). The biplot shows the overlapping of the confidence eclipse for conventional and non-conventional rice growing areas that means the differences in metal concentrations, with respect to areas, were non-significant. The points near the lines originating from the center depicted higher values as compared to distant points. The heat map (Figure 7) gives the clearer grouping of metals on the basis of the Pearson's correlation. It can be seen from Figure 5 that Pb and Cu; Mn, Cd, and Zn; and Ni and Fe were grouped together in three clusters.

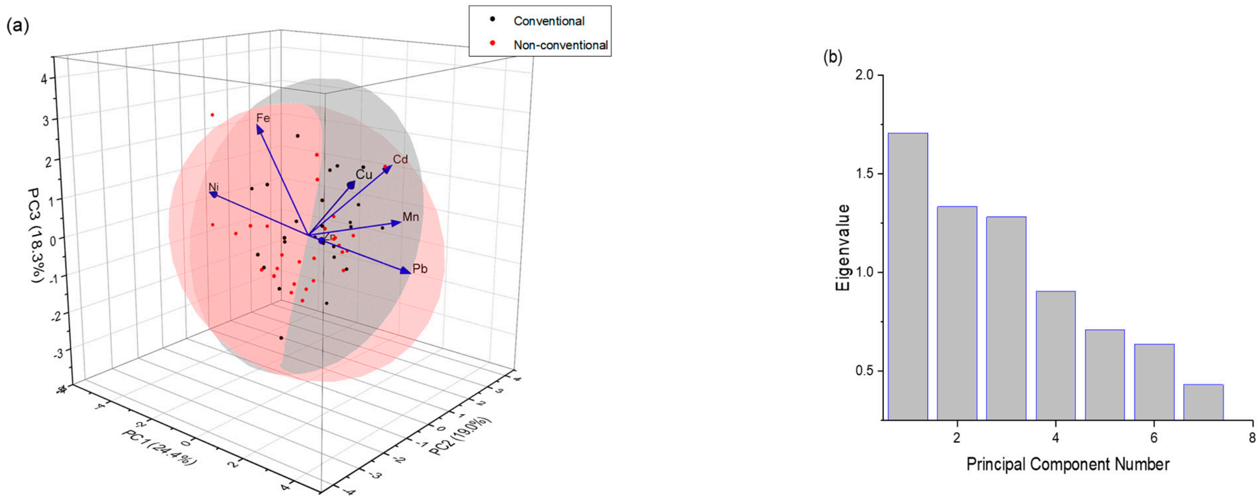

Figure 6. Principal component analysis of studied metals analyzed in rice varieties grown at conventional and non-conventional rice growing areas of Punjab Pakistan. (**a**) Biplot between PC1, PC2, and PC3. The blue lines originating from central point of biplot indicate positive or negative correlations of different metals. The black and red dots represent the tested collected rice samples from conventional and non-conventional areas, respectively. Gray and pink circles represent the 95% confidence eclipse for conventional and non-conventional areas. (**b**) Variance decomposition of principal components.

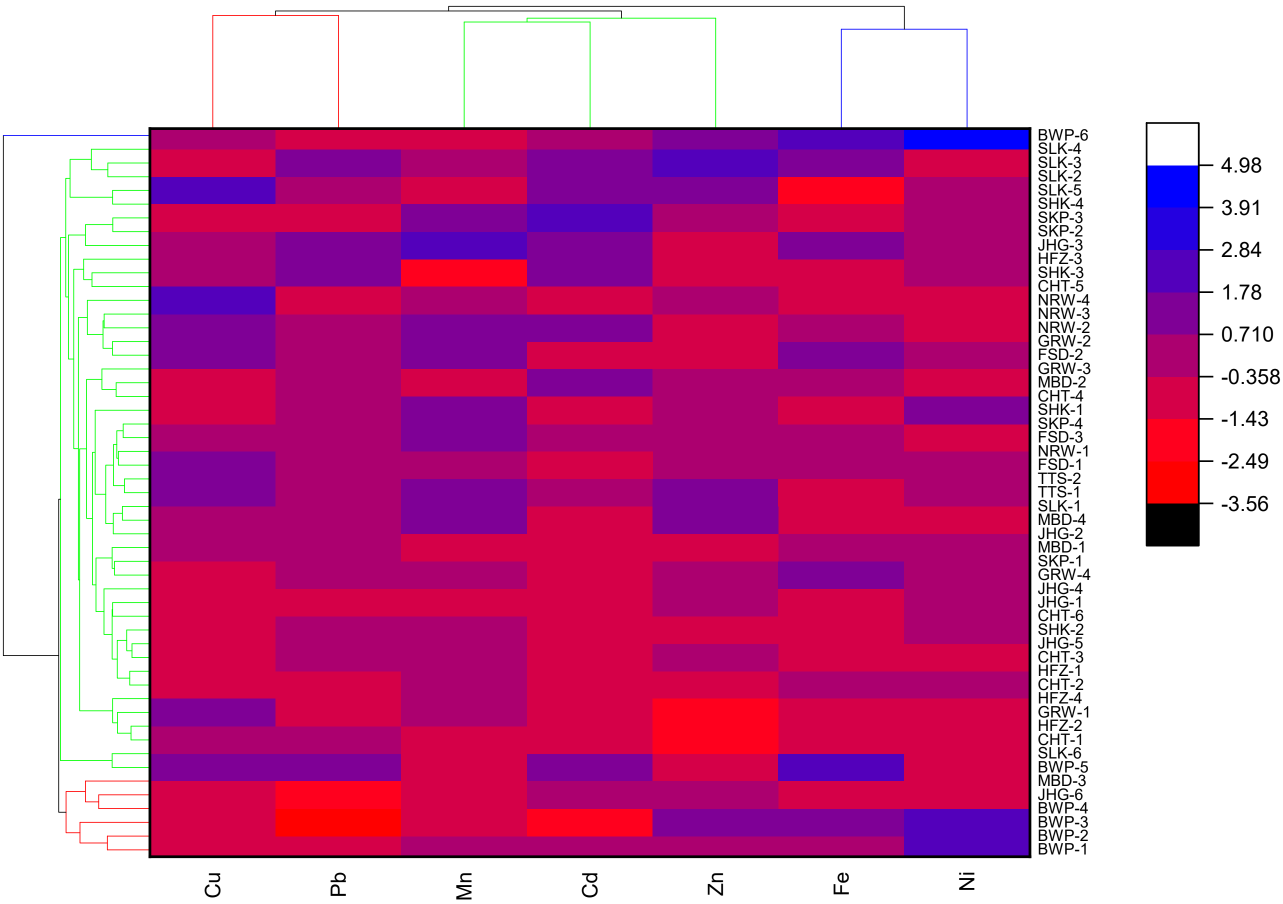

Figure 7. The Heatmap based on Pearson's correlation.

4. Discussion

4.1. Sources of Metals in Rice Growing Areas of Pakistan

Agricultural soil contaminations with TMs have gradually increased in the last decade due to urban sprawl, population increase, and industrialization [33,34]. It is considered a major global environmental issue because of their persistence and associated risks to both the biotic and abiotic factors of the environment. Trace metals such as Cd, Cu, Fe, Mn, Ni, Pb, and Zn are naturally present in soil through rocks weathering and volcanic eruptions, but their concentrations may be locally elevated as a result of nearby anthropogenic activities such as mining, resource extraction, industrial and vehicular emissions, etc. [35,36]. Additionally, soil preparation, crop growing, and management practices also greatly influence the TM concentration in rice growing areas. In conventional rice growing areas, puddling is done before the rice nursery transplanting. Puddling could increase the bioavailability of TMs to rice crop by enhancing their solubilization [30]. Higher concentrations of TMs in agricultural soils are responsible for their transfer from soil to food crops and grains, leading into long-term risks to the ecosystem. Vegetables, grains, and other essential foods may have a range of toxic and essential trace metals [37,38].

Additionally, TM pollution in rice growing areas is attributed to adjacent industrial sites and their effluent's use to irrigate agricultural soils in Pakistan, as indicated by Nawaz et al. [39], Azam et al. [40], and Qi et al. [41]. In addition to the use of industrial wastewater for irrigation, some farmers are over-using fertilizers and pesticides recommended for crop growth [42,43]. Phosphatic fertilizers are a rich source of Cd, and their repetitive application results in the magnification of Cd in soil and the edible parts of plants [44,45]. Moreover, submerged conditions during rice growth and development stages result in more mobility of TMs in soil solutions and their translocation to plants' vegetative and edible parts [46,47].

4.2. Non-Essential Metals in Rice

Cadmium is a metallic element that occurs naturally at low levels in the environment. Food, rather than air or water, represents the major source of Cd exposure [48,49]. The highest Cd concentration in the rice grains was found as 0.54 mg kg^{-1} (Supplementary Table S1). Cd concentrations in all the rice samples in the present study ranged from 0 to 0.47 mg kg^{-1}. The Cd concentration ranged from 0 to 0.54 in conventional areas, while it was 0.10–0.47 mg kg^{-1} in non-conventional sites. Thus, the mean Cd concentrations were considerably higher in non-conventional areas as compared to conventional areas. The Cd concentrations in rice samples from non-conventional areas were found higher than the WHO limits in Bahawalnagar and Chiniot (33%), Jhang (100%), 75% of the samples from Shorkot, and 100% samples from Toba Tek Singh and Faisalabad, respectively. These results are supported by the previous work of Dehghani and Mosaferi [50], who found higher Cd and Pb concentrations in imported Pakistani rice grains available in Irani markets and Cd, Cr, Ni, Fe, and Pb in Shakargarh Tehsil, District Sialkot, Pakistan [51]. This is probably due to fact that non-conventional areas receive relatively more contaminated irrigational water as compared to conventional areas. The conventional areas fall in upper Punjab, Pakistan, which has more slopes compared to the non-conventional areas (higher than sea level, due to which the water flow is high, minimizing the chances of water contamination) [52]. Secondly, conventional areas are not densely populated and are mainly agricultural lands [53], thus the risks of water contamination from sewage effluents are rare. In contradiction, the rice in non-conventional areas is mostly grown in the peri-urban areas and regularly receives sewage water for use in irrigation. The maximum concentrations of Cd in rice from conventional areas were found to be higher in 75% samples from Narowal, 100% from Gujranwala, 25% from Hafizabad, and 100% in Mandi Bahauddin, Sialkot, and Sheikhupura. Khan et al. [33] reported similar results of TM (Cd, Pb and Ni) contaminations in the Khyber Pakhtunkhwa Province of Pakistan. Nickel concentrations in rice grains from conventional areas of Punjab, Pakistan were 0–0.05 and 0.01–0.05 mg kg^{-1} in non-conventional areas. All the samples collected from conventional and non-conventional areas remained within the permissible limits (1.0 mg kg^{-1}) of the WHO. Lead ranged from 0.12 to 1.10 and 0.30 to 1.20 mg kg^{-1} in conventional and non-conventional areas, respectively. All the samples from non-conventional areas exceeded Pb concentration limits in rice prescribed by WHO (except one sample from Mandi Bahauddin), indicating a significantly higher Pb pollution level in the Punjab. The higher Pb levels could be due to the aerial deposition of Pb from vehicular emissions and their subsequent entry to rice grains through plant tissues and mixing in soil solution and uptake [54].

4.3. Essential Metals in Rice

Copper concentration in rice grains was found highest (4.54 mg kg^{-1}) in conventional areas (Figure 4). A previous study by Xu et al. [55] reported a mean Cu concentration in Chinese rice of up to 18 mg kg^{-1}, which is considerably higher than that found in the present study. Mean Mn concentrations in the conventional and non-conventional areas were 43.57 and 44.03 mg kg^{-1}, respectively. It was notable that Mn concentrations in the rice samples exceeded permissible limits. Roychowdhury [56] reported a mean Mn concentration of 9.9 mg kg^{-1} in rice in West Bengal, India. The mean Zn concentrations in rice in the present study did not differ significantly for different areas. The highest mean Zn concentration (16.42 mg kg^{-1}) in rice grains was found in the conventional area, which was comparable to that in the non-conventional areas' rice grains 16.41 mg kg^{-1}.

4.4. Health Risk Assessment

As peer reviewed data on all the parameters used for the health risk assessment of rice in this study are not available, we could not estimate the health risks associated with the consumption of essential metals (Cu, Fe, Mn, and Zn), but we calculated the health risks associated with consumption of non-essential metals (Cd, Pb, and Ni) through comparing PTWI values devised by the FAO/WHO. The same procedures were used by Naseri et al. [57] and Ghoreishy et al. [58] in Iran. Based on the results, it was recorded that the EWI of the samples from both areas fell within the permissible limits, except for Fe, whose EWI limits exceeded the MTDI in the conventional as well as non-conventional areas (Table 3).

5. Conclusions

In this study, most of the mean metal concentrations (Cd, Pb, Fe, Zn, and Mn) were found to be substantially higher in conventional rice growing areas, followed by non-conventional areas. Specifically, the mean Cd concentrations were considerably higher in rice grains collected from non-conventional areas compared to conventional rice growing areas. The Cd, Pb, and Ni concentrations in rice samples from non-conventional areas were found higher than the WHO limits in the 70, 100, and 0% collected samples, respectively. In conventional areas, Cd, Pb, and Ni concentrations were found higher than WHO limits in the 77, 99, and 0% samples, respectively. A health risk assessment indicated that all the EWI and/or MTDI values were found within the permissible values set by the WHO due to less rice consumption by Pakistani people, except for Fe in both areas.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su15097259/s1, Table S1: title: Details of the sampling districts, IDs along with concentrations of essential and non-essential metals.

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References

1. Mehmood, A.; Mirza, M.A.; Choudhary, M.A.; Kim, K.-H.; Raza, W.; Raza, N.; Lee, S.S.; Zhang, M.; Lee, J.-H.; Sarfraz, M. Spatial distribution of heavy metals in crops in a wastewater irrigated zone and health risk assessment. *Environ. Res.* **2019**, *168*, 382–388. [CrossRef]
2. Nzediegwu, C.; Prasher, S.; Elsayed, E.; Dhiman, J.; Mawof, A.; Patel, R. Effect of biochar on heavy metal accumulation in potatoes from wastewater irrigation. *J. Environ. Manag.* **2019**, *232*, 153–164. [CrossRef] [PubMed]
3. Onakpa, M.M.; Njan, A.A.; Kalu, O.C. A review of heavy metal contamination of food crops in Nigeria. *Ann. Glob. Health* **2018**, *84*, 488. [CrossRef] [PubMed]
4. Sharma, S.; Nagpal, A.K.; Kaur, I. Heavy metal contamination in soil, food crops and associated health risks for residents of Ropar wetland, Punjab, India and its environs. *Food Chem.* **2018**, *255*, 15–22. [CrossRef] [PubMed]
5. Sarim, M.; Jan, T.; Khattak, S.A.; Mihoub, A.; Jamal, A.; Saeed, M.F.; Soltani-Gerdefaramarzi, S.; Tariq, S.R.; Fernández, M.P.; Mancinelli, R. Assessment of the Ecological and Health Risks of Potentially Toxic Metals in Agricultural Soils from the Drosh-Shishi Valley, Pakistan. *Land* **2022**, *11*, 1663. [CrossRef]
6. Ikram, M.; Ali, N.; Jan, G.; Jan, F.G.; Rahman, I.U.; Iqbal, A.; Hamayun, M. IAA producing fungal endophyte *Penicillium roqueforti* Thom., enhances stress tolerance and nutrients uptake in wheat plants grown on heavy metal contaminated soils. *PLoS ONE* **2018**, *13*, e0208150. [CrossRef]
7. Zhu, H.; Teng, Y.; Wang, X.; Zhao, L.; Ren, W.; Luo, Y.; Christie, P. Changes in clover rhizosphere microbial community and diazotrophs in mercury-contaminated soils. *Sci. Total Environ.* **2021**, *767*, 145473. [CrossRef] [PubMed]
8. Shilev, S.; Babrikova, I.; Babrikov, T. Consortium of plant growth—Promoting bacteria improves spinach (*Spinacea oleracea* L.) growth under heavy metal stress conditions. *J. Chem. Technol. Biotechnol.* **2020**, *95*, 932–939. [CrossRef]
9. Xiao, R.; Wang, S.; Li, R.; Wang, J.J.; Zhang, Z. Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi, China. *Ecotoxicol. Environ. Saf.* **2017**, *141*, 17–24. [CrossRef]
10. Adimalla, N.; Chen, J.; Qian, H. Spatial characteristics of heavy metal contamination and potential human health risk assessment of urban soils: A case study from an urban region of South India. *Ecotoxicol. Environ. Saf.* **2020**, *194*, 110406. [CrossRef]
11. Mishra, S.; Bharagava, R.N.; More, N.; Yadav, A.; Zainith, S.; Mani, S.; Chowdhary, P. Heavy metal contamination: An alarming threat to environment and human health. In *Environmental Biotechnology: For Sustainable Future*; Sobti, R., Arora, N., Kothari, R., Eds.; Springer: Singapore, 2019; pp. 103–125.
12. Du, F.; Yang, Z.; Liu, P.; Wang, L. Accumulation, translocation, and assessment of heavy metals in the soil-rice systems near a mine-impacted region. *Environ. Sci. Pollut. Res.* **2018**, *25*, 32221–32230. [CrossRef] [PubMed]
13. Wang, Z.; Qin, H.; Liu, X. Health risk assessment of heavy metals in the soil-water-rice system around the Xiazhuang uranium mine, China. *Environ. Sci. Pollut. Res.* **2019**, *26*, 5904–5912. [CrossRef]
14. Fan, Y.; Zhu, T.; Li, M.; He, J.; Huang, R. Heavy metal contamination in soil and brown rice and human health risk assessment near three mining areas in central China. *J. Health Eng.* **2017**, *2017*, 4124302. [CrossRef] [PubMed]
15. Barau, B.; Abdulhameed, A.; Ezra, A.; Muhammad, M.; Kyari, E.; Bawa, U. Heavy metal contamination of some vegetables from pesticides and the potential health risk in Bauchi, northern Nigeria. *Int. J. Sci. Technol.* **2018**, *7*, 1–11. [CrossRef]
16. Tian, D.; Li, Z.; O'Connor, D.; Shen, Z. The need to prioritize sustainable phosphate—Based fertilizers. *Soil Use Manag.* **2020**, *36*, 351–354. [CrossRef]
17. Kahil, A.; Hassan, F.; Ali, E. Influence of bio-fertilizers on growth, yield and anthocyanin content of *Hibiscus sabdariffa* L. plant under Taif region conditions. *Annu. Res. Rev. Biol.* **2017**, *17*, 1–15. [CrossRef]
18. Hassan, F.; Ali, E.; Mahfouz, S. Comparison between different fertilization sources, irrigation frequency and their combinations on the growth and yield of coriander plant. *Aust. J. Basic. Appl. Sci.* **2012**, *6*, 600–615.
19. Saboor, A.; Ali, M.A.; Ahmed, N.; Skalicky, M.; Danish, S.; Fahad, S.; Hassan, F.; Hassan, M.M.; Brestic, M.; El Sabagh, A. Biofertilizer-based zinc application enhances maize growth, gas exchange attributes, and yield in zinc-deficient soil. *Agriculture* **2021**, *11*, 310. [CrossRef]
20. Wagh, V.M.; Panaskar, D.B.; Mukate, S.V.; Gaikwad, S.K.; Muley, A.A.; Varade, A.M. Health risk assessment of heavy metal contamination in groundwater of Kadava River Basin, Nashik, India. *Model Earth Syst. Environ.* **2018**, *4*, 969–980. [CrossRef]
21. Chiamsathit, C.; Auttamana, S.; Thammarakcharoen, S. Heavy metal pollution index for assessment of seasonal groundwater supply quality in hillside area, Kalasin, Thailand. *Appl. Water Sci.* **2020**, *10*, 1–8. [CrossRef]

22. Jalees, M.I.; Farooq, M.U.; Anis, M.; Hussain, G.; Iqbal, A.; Saleem, S. Hydrochemistry modelling: Evaluation of groundwater quality deterioration due to anthropogenic activities in Lahore, Pakistan. *Environ. Dev. Sustain.* **2021**, *23*, 3062–3076. [CrossRef]
23. Qiao, J.; Zhu, Y.; Jia, X.; Niu, X.; Liu, J. Distributions of arsenic and other heavy metals, and health risk assessments for groundwater in the Guanzhong Plain region of China. *Environ. Res.* **2020**, *181*, 108957. [CrossRef] [PubMed]
24. Pateriya, A.; Verma, R.K.; Sankhla, M.S.; Kumar, R. Heavy metal toxicity in rice and its effects on human health. *Lett. Appl. NanoBio. Sci.* **2020**, *10*, 1833–1845.
25. Sultana, M.S.; Rana, S.; Yamazaki, S.; Aono, T.; Yoshida, S. Health risk assessment for carcinogenic and non-carcinogenic heavy metal exposures from vegetables and fruits of Bangladesh. *Cogent Environ. Sci.* **2017**, *3*, 1291107. [CrossRef]
26. Bi, C.; Zhou, Y.; Chen, Z.; Jia, J.; Bao, X. Heavy metals and lead isotopes in soils, road dust and leafy vegetables and health risks via vegetable consumption in the industrial areas of Shanghai, China. *Sci. Total Environ.* **2018**, *619*, 1349–1357. [CrossRef]
27. Rehman, Z.U.; Sardar, K.; Shah, M.T.; Brusseau, M.L.; Khan, S.A.; Mainhagu, J. Transfer of heavy metals from soils to vegetables and associated human health risks at selected sites in Pakistan. *Pedosphere* **2018**, *28*, 666–679. [CrossRef]
28. Gupta, N.; Yadav, K.K.; Kumar, V.; Krishnan, S.; Kumar, S.; Nejad, Z.D.; Khan, M.M.; Alam, J. Evaluating heavy metals contamination in soil and vegetables in the region of North India: Levels, transfer and potential human health risk analysis. *Environ. Toxicol. Pharm.* **2021**, *82*, 103563. [CrossRef]
29. Wasim, A.A.; Naz, S.; Khan, M.N.; Fazalurrehman, S. Assessment of heavy metals in rice using atomic absorption spectrophotometry–A study of different rice varieties in Pakistan. *Pak. J. Anal. Environ.* **2019**, *20*, 67–74. [CrossRef]
30. Huang, B.; Li, Z.; Li, D.; Yuan, Z.; Chen, Z.; Huang, J. Distribution characteristics of heavy metal (loid) s in aggregates of different size fractions along contaminated paddy soil profile. *Environ. Sci. Pollut. Res.* **2017**, *24*, 23939–23952. [CrossRef]
31. Onsanit, S.; Ke, C.; Wang, X.; Wang, K.-J.; Wang, W.-X. Trace elements in two marine fish cultured in fish cages in Fujian province, China. *Environ. Pollut.* **2010**, *158*, 1334–1342. [CrossRef]
32. Food and Agriculture Organization of the United Nations (FAO); World Health Organization (WHO). *Principles and Methods for the Risk Assessment of Chemicals in Food; Chapter 6 Dietary Exposure Assessment of Chemicals in Food*; FAO: Rome, Italy; WHO: Geneva, Switzerland, 2009; Volume 68.
33. Khan, A.Z.; Khan, S.; Khan, M.A.; Alam, M.; Ayaz, T. Biochar reduced the uptake of toxic heavy metals and their associated health risk via rice (*Oryza sativa* L.) grown in Cr-Mn mine contaminated soils. *Environ. Technol. Innov.* **2020**, *17*, 100590. [CrossRef]
34. Tran, L.D.; Phung, L.D.; Pham, D.V.; Pham, D.D.; Nishiyama, M.; Sasaki, A.; Watanabe, T. High yield and nutritional quality of rice for animal feed achieved by continuous irrigation with treated municipal wastewater. *Paddy Water Environ.* **2019**, *17*, 507–513. [CrossRef]
35. Hussain, A.; Alamzeb, S.; Begum, S. Accumulation of heavy metals in edible parts of vegetables irrigated with waste water and their daily intake to adults and children, District Mardan, Pakistan. *Food Chem.* **2013**, *136*, 1515–1523.
36. Karim, Z.; Qureshi, B.A. Health risk assessment of heavy metals in urban soil of Karachi, Pakistan. *Hum. Ecol. Risk Assess* **2014**, *20*, 658–667. [CrossRef]
37. Mahmood, A.; Malik, R.N. Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan. *Arab. J. Chem.* **2014**, *7*, 91–99. [CrossRef]
38. Ullah, H.; Noreen, S.; Rehman, A.; Waseem, A.; Zubair, S.; Adnan, M.; Ahmad, I. Comparative study of heavy metals content in cosmetic products of different countries marketed in Khyber Pakhtunkhwa, Pakistan. *Arab. J. Chem.* **2017**, *10*, 10–18. [CrossRef]
39. Nawaz, A.; Khurshid, K.; Arif, M.S.; Ranjha, A. Accumulation of heavy metals in soil and rice plant (*Oryza sativa* L.) irrigated with industrial effluents. *Int. J. Agric. Biol.* **2006**, *8*, 391–393.
40. Azam, I.; Afsheen, S.; Zia, A.; Javed, M.; Saeed, R.; Sarwar, M.K.; Munir, B. Evaluating insects as bioindicators of heavy metal contamination and accumulation near industrial area of Gujrat, Pakistan. *Biomed. Res. Int.* **2015**, *2015*, 942751. [CrossRef]
41. Qi, D.; Yan, J.; Zhu, J. Effect of a reduced fertilizer rate on the water quality of paddy fields and rice yields under fishpond effluent irrigation. *Agric. Water Manag.* **2020**, *231*, 105999. [CrossRef]
42. Khan, K.; Lu, Y.; Khan, H.; Ishtiaq, M.; Khan, S.; Waqas, M.; Wei, L.; Wang, T. Heavy metals in agricultural soils and crops and their health risks in Swat District, northern Pakistan. *Food Chem. Toxicol.* **2013**, *58*, 449–458. [CrossRef] [PubMed]
43. Tariq, S.R.; Shafiq, M.; Chotana, G.A. Distribution of heavy metals in the soils associated with the commonly used pesticides in cotton fields. *Scientifica* **2016**, *2016*, 7575239. [CrossRef] [PubMed]
44. Park, H.J.; Kim, S.U.; Jung, K.Y.; Lee, S.; Choi, Y.D.; Owens, V.N.; Kumar, S.; Yun, S.W.; Hong, C.O. Cadmium phytoavailability from 1976 through 2016: Changes in soil amended with phosphate fertilizer and compost. *Sci. Total Environ.* **2021**, *762*, 143132. [CrossRef] [PubMed]
45. Murtaza, G.; Javed, W.; Hussain, A.; Wahid, A.; Murtaza, B.; Owens, G. Metal uptake via phosphate fertilizer and city sewage in cereal and legume crops in Pakistan. *Environ. Sci. Pollut. Res.* **2015**, *22*, 9136–9147. [CrossRef]
46. Jho, E.H.; Youn, Y.; Yun, S.H. Effect of CO2 exposure on the mobility of heavy metals in submerged soils. *Appl. Biol. Chem.* **2018**, *61*, 617–623. [CrossRef]
47. Wan, Y.; Huang, Q.; Camara, A.Y.; Wang, Q.; Li, H. Water management impacts on the solubility of Cd, Pb, As, and Cr and their uptake by rice in two contaminated paddy soils. *Chemosphere* **2019**, *228*, 360–369. [CrossRef] [PubMed]
48. Dennis, K.K.; Judd, S.E.; Alvarez, J.A.; Kahe, K.; Jones, D.P.; Hartman, T.J. Plant food intake is associated with lower cadmium body burden in middle-aged adults. *Eur. J. Nutr.* **2021**, *60*, 3365–3374. [CrossRef]

49. Li, N.; Feng, A.; Liu, N.; Jiang, Z.; Wei, S. Silicon application improved the yield and nutritional quality while reduced cadmium concentration in rice. *Environ. Sci. Pollut. Res.* **2020**, *27*, 20370–20379. [CrossRef]
50. Dehghani, M.; Mosaferi, F. Determination of heavy metals (cadmium, arsenic and lead) in Iranian, Pakistani and Indian rice consumed in Hormozgan Province, Iran. *J. Maz. Univ. Med.* **2016**, *25*, 363–367.
51. Tariq, S.R.; Rashid, N. Multivariate analysis of metal levels in paddy soil, rice plants, and rice grains: A case study from Shakargarh, Pakistan. *J. Chem.* **2013**, *2013*, 539251. [CrossRef]
52. Flood Map Pro. Pakistan Flood Map: Elevation Map, Sea Level Rise Map. 2020. Available online: https://www.floodmap.net/?ct=PK (accessed on 18 August 2021).
53. World Bank. Population, Total-Pakistan. 2021. Data. Available online: https://data.worldbank.org/indicator/SP.POP.TOTL?end=2020&locations=PK&start=1960&view=chart (accessed on 5 August 2022).
54. Zeng, F.; Wei, W.; Li, M.; Huang, R.; Yang, F.; Duan, Y. Heavy metal contamination in rice-producing soils of Hunan province, China and potential health risks. *Int. J. Environ. Res.* **2015**, *12*, 15584–15593. [CrossRef]
55. Xu, J.; Yang, L.; Wang, Z.; Dong, G.; Huang, J.; Wang, Y. Toxicity of copper on rice growth and accumulation of copper in rice grain in copper contaminated soil. *Chemosphere* **2006**, *62*, 602–607. [CrossRef] [PubMed]
56. Roychowdhury, T. Impact of sedimentary arsenic through irrigated groundwater on soil, plant, crops and human continuum from Bengal delta: Special reference to raw and cooked rice. *Food Chem. Toxicol.* **2008**, *46*, 2856–2864. [CrossRef] [PubMed]
57. Naseri, M.; Vazirzadeh, A.; Kazemi, R.; Zaheri, F. Concentration of some heavy metals in rice types available in Shiraz market and human health risk assessment. *Food Chem.* **2015**, *175*, 243–248. [CrossRef] [PubMed]
58. Ghoreishy, F.; Salehi, M.; Fallahzade, J. Cadmium and lead in rice grains and wheat breads in Isfahan (Iran) and human health risk assessment. *Hum. Ecol. Risk Assess* **2019**, *25*, 924–934. [CrossRef]