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Heavy Metal Levels in Vegetables Cultivated in Pakistan Soil Irrigated with Untreated Wastewater: Preliminary Results

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Abstract: Unfortunately, vegetables are commonly cultivated with untreated wastewater and consumed by human beings who often ignore their harmful impacts on health. The industrialization and urbanization in developing countries have led to the release of increasing amounts of heavy metals (HM) into the environment. Regular monitoring of metal concentration levels in contaminated soils and edible plants is essential to prevent their excessive build-up in the diet and food chains. This study aimed to determine the concentration and accumulation of selected HM in the soil-plant system from a field located in D.I. Khan, Pakistan. Thereby, determinations of copper (Cu), lead (Pb), cadmium (Cd), chromium (Cr), iron (Fe), nickel (Ni), and cobalt (Co) were assessed in the soil of the field as well as in the roots, stems, leaves, and grains of ridge gourds (RG) and sponge gourds (SG). The gourds were irrigated with untreated wastewater and removed from the soil when completely matured. Their parts were then separated and digested for HM analyses, which were performed by atomic absorption spectrophotometry. Mean HM concentrations of each gourd were compared to each other within the same vegetable or between the vegetables. Intra-analyses depicted a similar quantitative distribution of HM in the RG or SG parts. Independently of the gourds' variety, Fe and Pb were the most concentrated HM, and Pb was particularly concentrated in grains. Mean concentrations of Pb and Co in these vegetables were found to be toxic, since they exceeded the safe limits recommended by the Food and Agriculture Organization/World Health Organization (FAO/WHO). Inter-analyses of HM concentrations performed between RG and SG parts revealed that the mean concentration of Pb was significantly higher in roots of SG compared to that of RG. Nevertheless, mean Cr concentrations were significantly higher in all parts of RG compared to that of SG. The concentrations of Co were insignificantly different between the parts of these two vegetables. Also, the assessment of hyperaccumulation factors demonstrated that these gourds are hyperaccumulators, improper for dietary intake and commercialization, but might be useful for phytoremediation. Taken together, our data shed light on the urgent need for developing sustainable agriculture in Pakistan.

Keywords: wastewater; ridge gourd; sponge gourd; heavy metals; toxicity; phytoremediation; sustainability

1. Introduction

The world is facing environmental pollution as one of the current challenging issues. Although some serious attempts have been made to address this concern, it is still growing with each passing day. For instance, in many areas of the globe, farmers are applying untreated wastewater to grow crops and vegetables. In some cases, it is worth noting that farmers apply high cast of fertilizers and insecticides to grow more crops and satisfy the increasing demand of food needs [1]. The problem is further multiplied when people purchase these vegetables and consume them without knowing their level of contamination [1–6].

Due to a lack of awareness about water pollution and the subsequent use and/or direct consumption of impure water in many countries like Pakistan, diseases are on the rise. The main reasons for using untreated wastewater for irrigation are linked to its economical solution, growing demand for food, and easy ways to eliminate it. Unfortunately, vegetables irrigated with untreated wastewater not only contain disease-causing microorganisms, like viruses, fungi, bacteria, and protozoa [3], but are also contaminated by heavy metals (HM), which may cause genuine toxicity issues for plants, humans (e.g., farmers), and animals as they are chronically accumulated in the topsoil [2,4–9].

HM is a term applied for inorganic elements like Cu, Cr, Cd, Hg, Ni, Pb, and Zn, whose density is greater than 5 g/cm^3 and/or are highly toxic to both plants and animals [10]. These inorganic water pollutants are not only hazardous for plants, humans, and animals, but also affect the environment adversely [11]. Indeed, HM are not easily degraded in the environment and could then produce severe damages both for living organisms and the environment [12]. One should keep in mind that some HM (e.g., Fe) are essential to carry out vital cells' activities/functions, but can become toxic if their concentrations (even low) exceed the safe limits [13].

Nowadays, the costly, user-unfriendly, and inefficient conventional methods (e.g., chemical filtration, precipitation, ion exchange separation) used for the removal of metal ions in wastewater are progressively substituted by cost-effective, eco- and user-friendly advanced wastewater treatment techniques (e.g., biosorption, bioaccumulation through phytoremediation and phytomining) [14–16]. Indeed, the new methods have been proven to remove these nonbiodegradable toxic wastes more completely (i.e., including those found at low concentrations in high-grade contaminated water) without the need for careful disposal or physical and/or chemical purification processes [14–16]. However, it should be emphasized that these methods remain poorly applied in developing countries, such Pakistan, for major reasons that include their availability, the lack of knowledge, training and/or expertise of farmers in these techniques.

Undeniably, non-contaminated soil plays a vital role in sustaining life on Earth, as it provides equally both quality environment and quality food supply. In recent years, due to anthropogenic activities, like urbanization and industrialization, soil has become the prime center of attention. However, the quality of soil and food production are diminished when untreated wastewater is used for crop irrigation. Although food quality remains an important subject worldwide, attention is being diverted towards its safety because of the contamination factor [17]. Food safety, a priority target of investigation in sustainable food systems, involves a reliable path to detect, identify, quantify, characterize, and monitor issues occurring in food. Nowadays, it is widely accepted that toxic HM, widely distributed in the environment and particularly present in soils irrigated by untreated wastewater, are absorbed by different parts of vegetables, which subsequently cause accumulation and translocation [18,19]. Therefore, whereas quality vegetables are considered a balanced diet because they contain carbohydrates, proteins, vitamins, and are supposed to contain other necessary trace elements in proper proportions, vegetables highly contaminated with HM can hurt their consumers, as HM are not easily eliminated from their bodies [20,21].

Unfortunately, Pakistani farmers are amongst the first to be exposed to the hazardous effects of HM because they work in the fields where crop irrigation is made with untreated wastewater [13]. De facto, vegetables contaminated by HM through crop irrigation with wastewater remain a major issue worldwide, subsequently affecting their dietary intake and the food chains which are considered as a major source of pollutant transfer to human beings [22]. Mechanistically, after ingestion of HM, the stomach (acidic medium) converts HM into their stable states, and subsequent reactions occur with proteins and enzymes [23]. Oxidative stress is subsequently produced due to the accumulation of these metals in the human body, and clinical symptoms may be evidenced. Thereby, sore throat, headache, chest pain, cough, dizziness, and lung problems were observed due to acute exposure to Cd, while long-term exposure to Cd can cause cancer and inhibition of protein synthesis [24]. Besides this, chronic Pb poisoning (a.k.a. saturnism) can damage kidneys and reproductive systems, while reducing the intellectual performance in children [25]. Also, Cu toxicity may result in hemolysis, hepatotoxic, and nephrotoxic effects [25]. Moreover, Cr can cause sneezing, itching, runny nose, ulcers when inhaled in excessive concentration [26]. Further, high systemic Co concentrations in humans are characterized by a complex clinical syndrome, including neurological (e.g., hearing and visual impairment), cardiovascular, and endocrine deficits [27]. A recent study reported that Ni contact induces a variety of side effects in humans, such as allergy, cardiovascular and kidney diseases, lung fibrosis, lung and nasal cancer, through molecular mechanisms that would involve mitochondrial dysfunctions and oxidative stress [28]. Fe is considered a paradigmatic HM because it is both essential and toxic for biological systems [29]. Indeed, although its systemic overload (hemochromatosis), by ingestion or transfusion [30], can induce all forms of cell death (i.e., ferroptosis) and clinical complications (e.g., liver damage, liver cirrhosis, pancreatic islet cell damage, diabetes, hypothyroidism, and hypogonadism) [31], it is essential to avoid ferriprivate anemia and a large variety of different processes (e.g., vesicles trapping) serve to prevent its toxic effects [29].

Therefore, in this study, we aim to determine and analyze the mean concentrations as well as factors (enrichment, translocation, transfer co-efficient, and bioaccumulation) of various selected HM in a soil-gourds system irrigated with untreated wastewater.

2. Materials and Methods

2.1. Research Location Point

Yousaf Raza Gillani Town at Dera Ismail Khan (D.I. Khan) was selected as a research location point. D.I. Khan is situated on the west bank of the Indus River and represents the largest city in the southern part of Khyber Pakhtunkhwa (KP), a province of Pakistan. In the 2017 Census, the total population of this city was 217,457 and it was considered the 37th largest city in Pakistan. Untreated wastewater of D.I. Khan (originating from sewage water, roadside dust, hospital waste, battery workshops in the canal along the roadside) is used to irrigate vegetables, which are commonly consumed by farmers and other beings.

2.2. Soil Sampling and Processing

Collection of soil samples was made at various depths and spots in the field. Soil samples ($N = 7$), weighing 2 kg, were collected at various spots, and thoroughly mixed to produce homogeneous samples. Before analysis, the soil samples were finally dried, ground, and stored in clean polyethylene bags [32].

About 2 mL of each HNO_3 and H_2O_2 was transferred into a beaker of 10 mL and mixed thoroughly with 0.5 g soil. Next day, the samples were filtered using a Whatman # 1 filter paper, and volumes were adjusted with 10 mL distilled water. HM analyses were eventually performed by atomic absorption spectrophotometer [33].

2.3. Vegetables Sampling and Processing

Vegetables selected for the study were ridge gourds *(Luffa acutangula)* and sponge gourds *(Luffa cylindrica)*. These vegetables were grown during the months of May and June at Yosaf Raza Gillani Town at D. I. Khan, Pakistan. They were irrigated with untreated wastewater every week until the study was completed. The content of HM in wastewater was not investigated, as it would have varied anyway in function of the quantity and nature of contaminants (batteries, waste, dust . . .) during the period of vegetables cultivation (60 days). The two varieties of gourds were removed from the soil at maturity and about 20 fresh ripe parts of each gourd (N = 40) were collected to form a homogenous sample. Their roots, stems, leaves, and grains were separated from the main vessel using a clean knife. These parts were then washed with a tap of distilled water to remove dust and dirt, before they were cut into small pieces and exposed to the sun. The sun-dried pieces were subsequently placed in the oven at 105 °C for 48 h. The leaves and grains were converted into powdered mass, whereas the hard-woody roots and stems were further placed in the furnace at 600 to 800 °C to be converted into ash. The powdered samples were eventually placed in labeled polyethylene bags for onward analysis.

One-gram powdered samples of roots, stems, leaves, and grains of each gourd variety were transferred into beakers of 100 mL, separately. A total of 10 mL of 65% concentrated HNO_3 and 4 mL of 20% H_2O_2 were then added into the beakers for digestion. The beakers were covered with watch glasses and boiled at 200 °C on hot plates for 4 h. After boiling, the suspensions were cooled at room temperature (RT). The volume of each suspension was then adjusted to 50 mL with distilled water into a volumetric flask of 50 mL, filtered, kept in cleaned labeled plastic bottles. An overview of the research study design is showed in Figure 1, and placed in the refrigerator until analyses by atomic absorption spectrophotometry were performed.

Similar protocols of digestion of HM were used by other teams [32,33]. Among the main available digestion methods, the nitric acid (HNO_3) procedure was recommended based on recovery analysis, cost- and time-effectiveness; also, the dry ashing method was preconized as a flexible method [7].

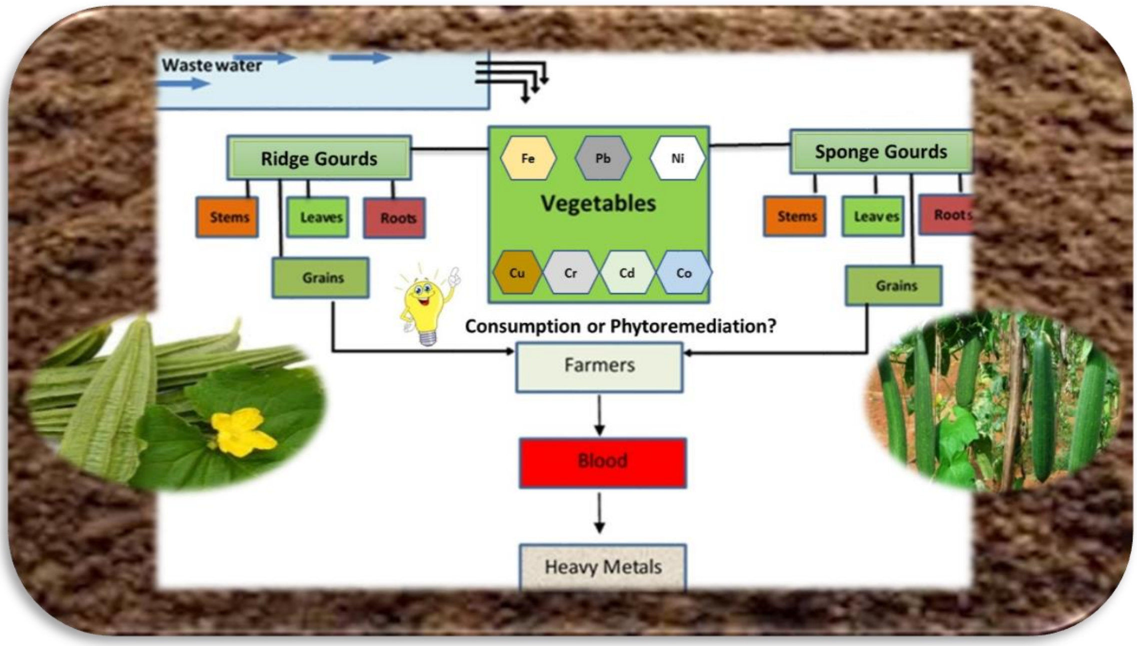

Figure 1. Scheme of the study.

2.4. Enrichment, Translocation, Transfer Co-Efficient, and Bioaccumulation Factors

A hyperaccumulator (i.e., plant that absorbs and tolerates elevated amounts of HM) is defined by EF(Enrichment Factor) or TF (Translocation Factor) > 1 [34]. The determination of such factor metal accumulating species can be used for phytoremediation (i.e., removal of contaminants from soils) or phytomining (i.e., growing plants to harvest the metals) [35].

2.4.1. Enrichment Factor

The EF is calculated as the ratio of metal concentration in the plant to metal concentration in the soil ($[Metal]_{Plant}/[Metal]_{Soil}$) [34]. EF allows the determination of HM accumulation in plants growing on a contaminated site/soil. The formula is given by,

$$EF = Cp/Cs, \quad (1)$$

where EF is the enrichment factor; Cp and Cs represent concentration of metals in the plants and soil, respectively; e.g., $EF_{Cu} = ([Cu_{roots}] + [Cu_{stems}] + [Cu_{leaves}] + [Cu_{grains}])/[Cu_{soil}]$.

2.4.2. Translocation Factor

The translocation factor (TF), also called the mobilization ratio, is the ratio of metal concentration in the shoots to metal concentration in the roots ($[Metal]_{Plant}/[Metal]_{Roots}$) [34]. TF allows the determination of the relative translocation of HM from soil to the roots. The formula is given by:

$$TF = Cs/Cr, \quad (2)$$

where TF is the translocation factor; Cs and Cr represent the concentration of metals in shoots and roots, respectively; e.g., $TF_{Cu} = [Cu_{stems}]/[Cu_{roots}]$.

2.4.3. Transfer Coefficient Factor

The transfer co-efficient (TC) is the ratio of absorption of metals from edible parts to the roots of vegetables ($[Metal]_{Ep}/[Metal]_{Root}$) [36]. The formula is given by:

$$TC = Cep/Cr, \quad (3)$$

where TF is the transfer co-efficient factor; Cep and Cr represent the concentration of metals in edible parts and roots, respectively; e.g., $TC_{Cu} = [Cu_{graines}]/[Cu_{roots}]$.

2.4.4. Bioaccumulation Factor

The bioaccumulation factor/coefficient (BAF or BAC) is the ratio of metal concentration from shoots to the soil of vegetables ($[Metal]_{shoots}/[Metal]_{soil}$) [36]. BAF represents an index of the ability of the plant to tolerate and accumulate a particular metal with respect to its concentration in the soil substrate. It is then used to evaluate the effectiveness of a plant in metal accumulation and translocation. The formula is given by:

$$BAF = Cs/Csoil, \quad (4)$$

where BAF is the bioaccumulation factor; Cs and Csoil represent the concentration of metals in shoots and soil, respectively; e.g., $BAF_{Cu} = [Cu_{stems}]/[Cu_{soil}]$.

2.5. Statistical Analysis

The Statistical Package for the Social Sciences (SPSS), version 17.0, was used for the calculation of standard deviation, mean, factor, and cluster analysis. The data (e.g., heavy metal concentration in each part of the tested vegetables) were evaluated as Mean ± Standard Deviation (SD) of at least three independent experiments. ANOVA (analysis of variance) test was performed to compare groups based on their metal concentrations. Thereby, intra-analyses (i.e., comparisons of metal concentrations in each vegetable), and inter-analyses (i.e., comparisons of a metal concentrations between the two vegetables). *p*-values of less than 0.05 ($p < 0.05$) were regarded as statistically significant.

3. Results and Discussion

FAO/WHO researchers discussed the impacts of metals accumulation in vegetables and edited a guideline for contaminants and toxins in food [37]. In Pakistan, the human and environmental impacts of toxic metal concentrations, including in vegetables originating from untreated wastewater, remain a challenging matter in terms of research studies, food quality, food safety, and sustainability [13,38,39].

In the present study, untreated wastewater was used by farmers to irrigate edible vegetables in a field from a relatively small town from Pakistan, belonging to D.I. Khan district located in the southern part of KP. It should be mentioned that ridge gourds (RG) and sponge gourds (SG) are the basis of human nutrition in the study area, where no pesticides or any additional chemical compounds are used. As mentioned earlier, HM concentrations were not studied in untreated wastewater that served to crop irrigation because, contrary to soil and plants which are subjected to HM enrichment and accumulation, the quantity of these inorganic pollutants vary in untreated wastewater depending on the presence of batteries, waste, dust during the two months of vegetables cultivation. The concentrations of selected HM were then investigated in soil and in various parts (i.e., roots, stems, leaves, and grains) of each gourd variety. The main aim was to check whether the local plant-soil system was proper (or not) for vegetables consumption. Additional goals consisted of determining whether the gourds could be defined as hyperaccumulators, which might have an important application in phytoremediation. Besides this, the direct impacts of HM-related toxicity on human health were evaluated (data not shown).

The concentration (mg/kg) of HM in soil and vegetables is reported in Tables 1 and 2. The results indicated that the soil accumulated selected metals in mg/kg in descending order of Fe (65.41) > Pb (2.313) >Ni (1.471) > Co (0.742) > Cu (0.598) > Cr (0.242) > Cd (0.022), and a closed HM concentration pattern could be rapidly observed between the soil and the pooled parts of cultivated vegetables, i.e.,:

Fe (22.213) > Pb (0.717) > Cr (0.225) > Ni (0.154) > Cu (0.141)> Co (0.065) > Cd (0.011) in RG (1)

Fe (25.308) > Pb (0.930) > Ni (0.208) > Cu (0.112) > Cr (0.074) > Co (0.053)> Cd (0.005) in SG (2),

when the mean of mean concentration (MOM) of a given metal from a part of a given vegetable (i.e., mean concentration after pooling the concentrations of a given HM obtained either from roots, stems, leaves, or grains) was calculated, clearly indicating that the soil contaminated RG and SG in a similar fashion.

Table 1. Intra-analysis of the mean concentrations (±SD) of heavy metals (mg/kg) in different parts of the cultivated ridge gourds (RG).

	Copper	Lead	Cadmium	Chromium	Iron	Nickel	Cobalt
Root	0.085 ± 0.001 [a]	0.194 ± 0.058 [b]	0.001 ± 0.001 [c]	0.163 ± 0.083 [b]	15.06 ± 0.696 [d]	0.101 ± 0.025 [a]	0.070 ± 0.049 [a]
Stem	*0.344 ± 0.004* [e]	0.631 ± 0.187 [f]	0.021 ± 0.008 [c]	0.290 ± 0.047 [b]	25.54 ± 3.460 [h]	*0.412 ± 0.104* [e]	0.053 ± 0.081 [a]
Leaf	0.059 ± 0.011 [a]	0.505 ± 0.012 [f]	0.008 ± 0.009 [c]	0.234 ± 0.046 [b]	24.46 ± 13.86 [h]	0.031 ± 0.134 [a]	0.047 ± 0.088 [a]
Grain	0.077 ± 0.001 [a]	*1.538 ± 0.113* [i]	0.014 ± 0.005 [c]	0.212 ± 0.049 [b]	23.79 ± 0.463 [h]	0.071 ± 0.049 [a]	0.090 ± 0.093 [a]
MOM (±SD)	0.141 ± 0.136	0.717 ± 0.577	0.011 ± 0.009	0.225 ± 0.053	22.213 ± 4.823	0.154 ± 0.175	0.065 ± 0.019
Soil	0.598 ± 0.007	2.313 ± 0.367	0.022 ± 0.005	0.242 ± 0.024	65.410 ± 3.958	1.471 ± 0.014	0.742 ± 0.466
(M/S)%	23.62	31.00	50.00	**92.87**	33.96	10.45	08.76

The mean of the mean concentrations (MOM ± SD) of each metal present in the selected parts of RG as well as the concentrations of each HM present in the soil that served to cultivate RG are mentioned. (M/S)% corresponds to the ratio between MOM of a given HM found in the vegetable (when the metal concentration levels of the vegetable parts are pooled) and the concentration level of the given HM found in the soil. The highest (M/S)% is indicated in bold. Basically, an M/S = 25% corresponds to an EF = 1. For intra-analysis, statistical significance ($p < 0.05$) of the HM concentration present in a part of RG is represented with different letters. Conversely, when the concentration level of a HM in a part of the vegetable is statistically insignificant ($p > 0.05$), then same letter is used. In italic, the significant highest concentration of a given HM in a specific part of the vegetable is indicated.

Table 2. Intra-analysis of the mean concentrations (±SD) of heavy metals (mg/kg) in different parts of the cultivated sponge gourds (SG).

	Copper	Lead	Cadmium	Chromium	Iron	Nickel	Cobalt
Root	0.092 ± 0.002 [a]	0.634 ± 0.163 [b]	0.001 ± 0.003 [c]	0.090 ± 0.064 [a]	26.74 ± 13.82 [d]	0.284 ± 0.057 [e]	0.036 ± 0.052 [a]
Stem	*0.204 ± 0.003* [f]	0.914 ± 0.206 [b]	0.009 ± 0.002 [c]	0.086 ± 0.069 [a]	30.05 ± 14.05 [d]	0.320 ± 0.018 [e]	0.108 ± 0.037 [a]
Leaf	0.059 ± 0.002 [a]	0.481 ± 0.176 [b]	0.010 ± 0.006 [c]	0.039 ± 0.049 [a]	22.69 ± 10.62 [d]	0.161 ± 0.054 [e]	0.043 ± 0.075 [a]
Grain	0.092 ± 0.003 [a]	*1.691 ± 0.035* [g]	0.001 ± 0.002 [c]	0.080 ± 0.051 [a]	21.75 ± 9.980 [d]	0.067 ± 0.046 [a]	0.023 ± 0.053 [a]
MOM (±SD)	0.112 ± 0.063	0.930 ± 0.538	0.005 ± 0.005	0.074 ± 0.024	25.308 ± 3.832	0.208 ± 0.116	0.053 ± 0.038
Soil	0.598 ± 0.007	2.313 ± 0.367	0.022 ± 0.005	0.242 ± 0.024	65.41 ± 3.958	1.471 ± 0.014	0.742 ± 0.466
(M/S)%	18.69	**40.21**	23.86	30.48	38.69	14.14	7.08

The mean of the mean concentrations (MOM ± SD) of each metal present in the selected parts of SG as well as the concentrations of each HM present in the soil that served to cultivate SG are mentioned. (M/S)% corresponds to the ratio between MOM of a given HM found in the vegetable (when the metal concentration levels of the vegetable parts are pooled) and the concentration level of the given HM found in the soil. The highest (M/S)% is indicated in bold. Basically, an M/S = 25% corresponds to EF = 1. For intra-analysis, statistical significance ($p < 0.05$) of the HM concentration present in a part of SG is represented with different letters. Conversely, when the concentration level of a HM in a part of the vegetable is statistically insignificant ($p > 0.05$), then same letter is used. In italic, the significant highest concentration of a given HM in a specific part of the vegetable is indicated.

Also, it should be mentioned that the MOM concentration of Cr in RG was the only significantly higher ($p < 0.05$) HM compared to that of SG. In agreement with this observation, the ratio MOM/Soil (M/S) for Cr reported a tremendously higher (about three-fold) enrichment of this HM in RG (92.87%) compared to that of SG (30.48%).

Interestingly, the data revealed that the proportion (%) of HM absorbed by the vegetables from the soil occurred in the following descending order:

Cr (92.87) > Cd (50.00) > Fe (33.96) > Pb (31.00) > Cu (23.62) > Ni (10.45) > Co (8.76) in RG

Pb (40.21) > Fe (38.69) > Cr (30.48) > Cd (23.86) > Cu (18.69) > Ni (14.14) > Co (7.08) in SG,

indicating that both vegetables enriched HM in a different order for four HM: Fe, Pb, Cr, and Cd. Thereby, RG would rather hyperaccumulate Cr and Cd compared to that of SG.

From these preliminary observations, we sought to perform detailed intra-analysis and inter-analysis to evaluate the significant differences in HM concentration levels in and between the parts of both vegetables. We also checked whether the highest HM concentrations could be considered as toxic, according to FAO/WHO recommendations [37], and determined factors allowing the characterization of each gourd as a potential hyperaccumulator.

Thereby, from the mean concentration (mg/kg) of each selected HM obtained from roots, stems, leaves, and grains of RG, it was clearly noticed that this gourd concentrated the selected HM in all of its parts (Table 1).

Indeed, when the mean concentrations of HM were compared to each other within or between a given part of this vegetable, it was noticed that the roots of RG concentrated HM in mg/kg in the following descending order:

Fe (15.06) > Pb (0.194) > Cr (0.163) > Ni (0.101) > Cu (0.085) > Co (0.070) > Cd (0.001).

It is interesting to note that this sequence is identical to the sequence (1) obtained once MOM of each metal was calculated from each part of RG, suggesting that the roots may reflect a qualitative and quantitative hierarchization of HM in the whole plant. Although the mean concentration of Fe was significantly higher ($p < 0.05$) compared to that of other studied HM, the mean concentration of Pb was insignificantly higher ($p > 0.05$) compared to that of Cr. Both Pb and Cr mean concentration levels were significantly higher ($p < 0.05$) compared to that of Ni, Cu, and Co, which were insignificantly different ($p > 0.05$) between themselves but significantly higher ($p < 0.05$) than Cd. Thus, the order of mean [HM] in roots of RG has been corrected as:

Fe > Pb = Cr > Ni = Cu = Co > Cd (3).

Besides this, it is worth noting that the concentration level of Fe in roots was significantly the lowest ($p < 0.05$) when compared to that of other parts of the same vegetable.

Also, the stems of RG concentrated HM in mg/kg followed the descending order of:

Fe (25.54) > Pb (0.631) > Ni (0.412) > Cu (0.344) > Cr (0.290) > Co (0.053) > Cd (0.021).

The mean concentration of Fe was significantly higher ($p < 0.05$) compared to that of Pb, which was significantly higher ($p < 0.05$) than the mean concentrations of other HM. The mean concentrations of Ni and Cu were found to be insignificantly different ($p > 0.05$) between themselves. The mean concentrations of Ni and Cu were significantly higher ($p < 0.05$) than the mean concentration of Cr, which was significantly higher ($p < 0.05$) than that of Co and Cd. The mean concentration of Co was significantly higher ($p < 0.05$) than that of Cd. Thus, the order of mean [HM] in stems of RG has been corrected as:

Fe > Pb > Ni = Cu > Cr > Co > Cd (4).

Besides, the mean concentration of Cr in stems was insignificantly different ($p > 0.05$) from the mean concentration of Cr in roots. Also, the mean concentration of Co in stems was insignificantly different ($p > 0.05$) compared to that of Co and Cu in roots. It is worth noting that the mean concentrations of Cu and Ni in stems are much higher ($p < 0.05$) compared to that of other parts of the same vegetable.

Furthermore, the leaves of RG concentrated HM in mg/kg following the descending order of:

Fe (24.46) > Pb (0.505) > Cr (0.234) > Cu (0.059) > Co (0.047) > Ni (0.031) > Cd (0.008).

The mean concentration of Fe was significantly higher ($p < 0.05$) than that of Pb, which was significantly higher ($p < 0.05$) than the mean concentrations of other HM. The mean concentration of Cr was significantly higher ($p < 0.05$) than that of Cu, which was insignificantly higher ($p > 0.05$) than Co and Ni. The mean concentration of Ni was significantly higher ($p < 0.05$) than that of Cd. Thus, the order of mean [HM] in leaves of RG has been corrected as:

Fe > Pb > Cr > Cu = Co = Ni > Cd (5).

Besides this, the mean concentrations of Fe, Pb, Cr, and Co in leaves were insignificantly different ($p > 0.05$) from those measured in stems. Also, the mean concentrations of Cu, Co, Ni, and Cd were insignificantly different ($p > 0.05$) from those measured in roots.

Eventually, the grains of RG concentrated HM in mg/kg following the descending order of:

Fe (23.79) > Pb (1.538) > Cr (0.212) > Cu (0.077) > Ni (0.071) > Co (0.090) > Cd (0.014).

The mean concentration of Fe was significantly higher ($p < 0.05$) than that of Pb, which was significantly higher ($p < 0.05$) than the mean concentrations of other HM. The mean concentration of Cr was significantly higher ($p < 0.05$) than that of Cu, which was insignificantly higher ($p > 0.05$) than that of Ni and Co. The mean concentration of Co was significantly higher ($p < 0.05$) than that of Cd. Thus, the order of mean [HM] in grains of RG has been corrected as:

Fe > Pb > Cr > Cu = Co = Ni > Cd (6).

Besides this, the mean concentrations of Fe, Cr, Cu, Ni, Co, and Cd in grains were insignificantly different ($p > 0.05$) from those measured in leaves. It is also worth noting that the mean concentration level of Pb in grains was significantly highest ($p < 0.05$) compared to that of other parts of the same vegetable.

Taken together, from the data obtained from RG, it was found that:

Fe > Pb = Cr > Ni = Cu = Co > Cd in roots (3)

Fe > Pb > Ni = Cu > Cr > Co > Cd in stems (4)

Fe > Pb > Cr > Cu = Co = Ni > Cd in leaves (5)

Fe > Pb > Cr > Cu = Co = Ni > Cd in grains (6)

This intra-analysis in RG clearly showed that the quantitative distribution of HM in the different parts of RG followed a similar pattern, especially for Cr, Co, and Cd ($p > 0.05$). The mean concentrations (mg/kg) of Fe and Pb were significantly the highest compared to those of other studied HM ($p < 0.05$), independently of the vegetable parts. The mean concentration level of Fe was significantly much higher compared to that of Pb ($p < 0.05$), independently of the parts of the same vegetable. Fe was tremendously concentrated in stems, leaves, and grains compared to that of roots ($p < 0.05$), while the significantly highest concentration in Pb was incredibly noticed in grains compared to that of other parts of RG ($p < 0.05$). The significantly highest mean concentrations of Cu and Ni were observed in stems ($p < 0.05$).

In the meantime, we analyzed the mean concentration (mg/kg) of each selected HM in the four parts of SG. Like RG, it was clearly observed that SG concentrated all selected HM in its roots, stems, leaves, and grains (Table 2). Thereby, when the concentrations of HM were compared to each other within or between a given part of this vegetable, it was observed that the roots of SG concentrated HM in mg/kg in the following descending order of:

Fe (26.74) > Pb (0.634) > Ni (0.284) > Cu (0.092) > Cr (0.090) > Co (0.036) > Cd (0.001).

It is interesting to note that this sequence is identical to the sequence (2) obtained once MOM was calculated for each metal in all parts of the vegetable, SUGGESTING that roots can be the part of the vegetable that may reflect a qualitative and quantitative hierarchization of HM in the whole plant. The mean concentration of Fe was significantly higher ($p < 0.05$) than that of Pb, which was significantly higher ($p < 0.05$) than the mean concentrations of other HM. The mean concentration of Ni was significantly higher ($p < 0.05$) than that of Cu, which was insignificantly different ($p > 0.05$) from the mean concentrations of Cr and Co. Cr and Co concentrations were significantly higher ($p < 0.05$) than that of Cd. Thus, the order of mean [HM] in roots of SG has been corrected as:

Fe > Pb > Ni > Cu = Cr = Co > Cd (7)

Also, the stems of SG concentrated HM in mg/kg following the descending order of:

Fe (30.05) > Pb (0.914) > Ni (0.320) > Cu (0.204) > Co (0.108) > Cr (0.086) > Cd (0.009).

The concentration of Fe was significantly higher ($p < 0.05$) than to that of Pb, which was significantly higher ($p < 0.05$) than the mean concentrations of other HM. The mean concentration of Ni was significantly higher ($p < 0.05$) than that of Cu, which was significantly ($p < 0.05$) higher than that of Co, Cr, and Cd. The mean concentrations of Co and Cr were insignificantly different ($p > 0.05$) when compared to each other but were significantly higher ($p < 0.05$) than the mean concentration of Cd. Thus, the order of mean [HM] in stems of RG has been corrected as:

Fe > Pb > Ni > Cu > Co = Cr > Cd (8).

Besides this, the mean concentrations of Fe, Pb, Ni, Co, Cr, and Cd in stems were insignificantly different ($p > 0.05$) compared to that of those found in roots. Also, the mean concentration of Cu in stems was significantly the highest ($p < 0.05$) when compared to that of other parts of SG.

Further, the leaves of SG concentrated HM in mg/kg following the descending order of:

Fe (22.69) > Pb (0.481) > Ni (0.161) > Cu (0.059) > Co (0.043) > Cr (0.039) > Cd (0.010).

The mean concentration of Fe was significantly higher ($p < 0.05$) compared to that of Pb, which was significantly higher ($p < 0.05$) than the mean concentrations of other HM. The mean concentration of Ni was significantly higher ($p < 0.05$) than the mean concentrations of Cu, Co, and Cr, which all were insignificantly different ($p < 0.05$) between themselves but significantly higher ($p < 0.05$) than that of Cd. Thus, the order of mean [HM] in leaves of SG has been corrected as:

Fe > Pb > Ni > Cu = Co = Cr > Cd (9).

Besides this, the concentrations of Fe, Pb, Ni, Co, Cr, and Cd in leaves of SG were insignificantly different ($p > 0.05$) compared to that of those measured in stems and roots. Although the concentration of Cu in leaves was insignificantly different ($p > 0.05$) from the concentration of Cu in roots, it was significantly lower ($p < 0.05$) compared to that of Cu in leaves.

Eventually, the grains of SG concentrated HM in mg/kg following the descending order of:

Fe (21.75) > Pb (1.691) > Cu (0.092) > Cr (0.080) > Ni (0.067) > Co (0.023) > Cd (0.001).

The mean concentration of Fe was significantly higher ($p < 0.05$) than that of Pb, which was significantly much higher ($p < 0.05$) than the mean concentrations of other HM. The mean concentration of Cu was insignificantly higher ($p > 0.05$) than those of Cr, Ni, and Co, which were insignificantly different ($p > 0.05$) between themselves but significantly higher ($p < 0.05$) than that of Cd. Thus, the order of mean [HM] in grains of RG has been corrected as:

Fe > Pb > Cu = Cr = Ni = Co > Cd (10).

Besides this, it was found that the mean concentrations of Fe, Cr, Co, and Cd in grains were insignificantly different ($p > 0.05$) compared to that of those measured in leaves, stems, and roots. It is worth noting that the concentration level of Pb in grains was significantly the highest ($p < 0.05$) when compared to that of other parts of SG. Conversely, the mean concentration of Ni in grains was significantly the lowest ($p < 0.05$) compared to that of other parts of SG. The mean concentration of Cu in grains was insignificantly different ($p > 0.05$) compared to that of those measured in leaves and roots but was significantly lower ($p < 0.05$) when compared to that of Cu in stems.

Taken together, from the data obtained from SG, it was found that:

Roots: Fe > Pb > Ni > Cu = Cr = Co > Cd (7)

Stems: Fe > Pb > Ni > Cu > Co = Cr > Cd (8)

Leaves: Fe > Pb > Ni > Cu = Co = Cr > Cd (9)

Grains: Fe > Pb > Cu = Cr = Ni = Co > Cd (10)

This intra-analysis in SG clearly showed that the quantitative distribution of HM in the different parts of SG followed a similar pattern, particularly for Fe, Cr, Co, and Cd ($p > 0.05$). The mean concentrations (mg/kg) of Fe, Pb, and Ni were significantly the highest compared to that of other studied HM ($p < 0.05$), independently of the vegetable parts. The mean concentration level of Fe in SG was significantly much higher than that of Pb ($p < 0.05$), independently of the parts of the same vegetable. Fe was tremendously concentrated in all the studied parts of SG, while the significantly highest concentration in Pb was again incredibly noticed in grains when compared to other parts of SG ($p < 0.05$). Conversely, the mean concentration of Ni was significantly the lowest in grains when compared to other parts of SG ($p < 0.05$).

Importantly, the intra-analyses in RG and SG, revealed that, except for the mean concentration of Pb in roots of RG, the mean concentrations of Pb and Co were significantly higher ($p < 0.05$) than the safe limits provided by FAO/WHO, indicating that these HM are highly toxic. Indeed, the safe and permissible limit of FAO/WHO for Pb and Co is 0.3 mg/kg and 0.01 mg/kg, respectively [37]. Other HM were found in their low concentrations, and thus, were not considered as toxic. Thereby, even mean Fe concentration levels, which were the highest ($p < 0.05$) in any of the studied gourds, could not be considered as a toxic metal. This fact could be explained by the large variety of different processes (e.g., vesicles trapping) that are implicated in preventing Fe-related toxicity effects, at least in humans [29]. It should be pointed out that metal ion contamination of wastewater, often used to irrigate fields, is a serious ongoing problem in developing countries such as India [16], Bangladesh [17], and Pakistan [13,38,39], especially when concentrations of metals such as Pb and Cd are exceeded. Pb is absorbed by plants and this absorption depends on its concentration in the soil [39]. The huge concentration level of Pb in grains of both gourd varieties can be explained by the fact that they were grown near the roadside, where the presence of workshops of lead storage batteries was noticed on the way to the study area/field. Indeed, runoff from the road contains Pb, which is absorbed by plants when they are irrigated with wastewater [40]. Sewage and industrial effluents along with waste from lead storage batteries also contain Pb in high concentration [38,39,41]. Moreover, the mean concentration levels of Co in all parts of both gourd varieties were beyond the safe limits, and this could be explained by the sewage water, urban and agricultural runoff, which are also known to contain considerable amounts of Co [42,43].

In a further step, we decided to perform inter-analyses by analyzing the mean concentrations of HM present in a specific part of a gourd (e.g., RG) compared to that of the corresponding part in the other gourd (e.g., SG) (Table 3, Figures 2 and 3).

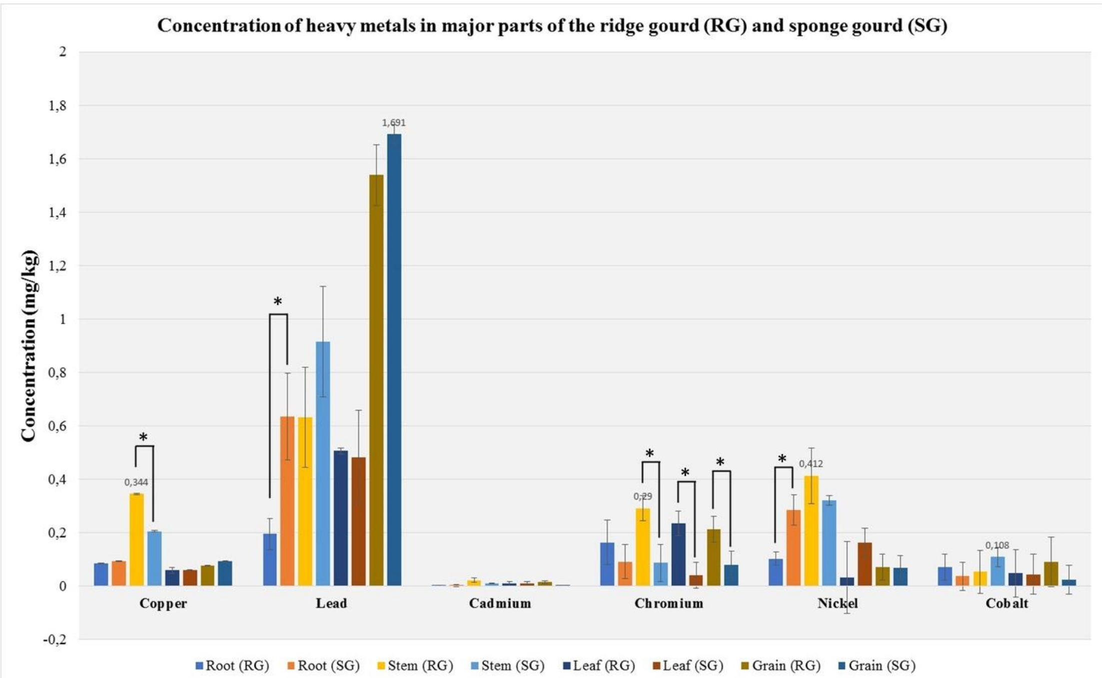

Figure 2. Comparative concentration of heavy metals (mg/kg) in selected parts of the ridge and sponge gourds. Mean concentration of HM, present in each part of RG, was statistically compared to that of SG. For instance, the concentration of Pb in roots of RG was compared to the concentration of Pb in roots of SG. All p-values less than 0.05 ($p < 0.05$) are summarized with one asterisk (*).

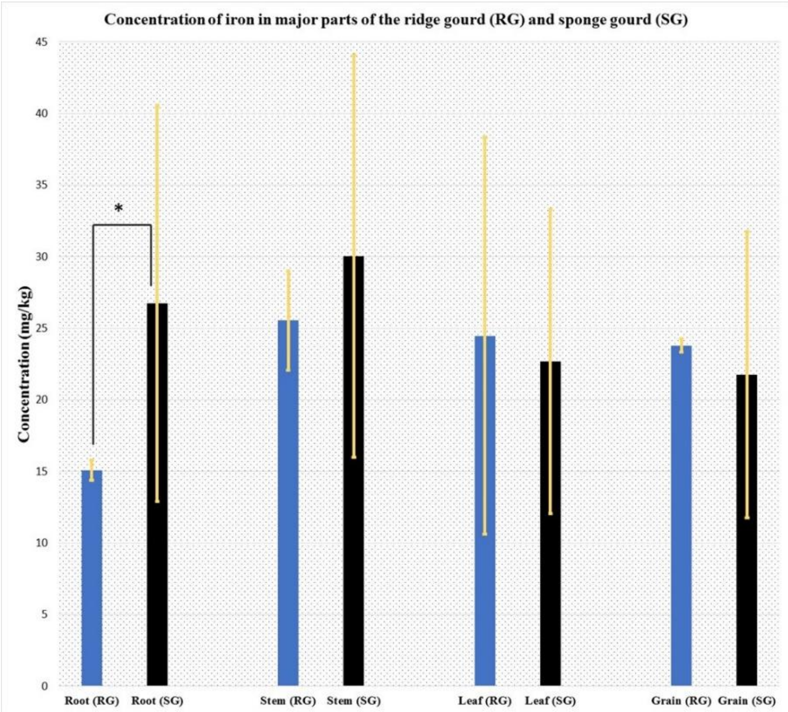

Figure 3. Comparative concentration of iron (mg/kg) in selected parts of the ridge and sponge gourds. Mean concentration of Fe, present in each part of RG, was statistically compared to that of SG. All *p*-values less than 0.05 ($p < 0.05$) are summarized with one asterisk (*).

Thereby, it should be underlined that the mean concentrations of Fe and Pb were significantly higher ($p < 0.05$) only in roots of SG compared to that of RG. The mean concentrations of Fe, which were the highest among the studied HM, were quite similar ($p > 0.05$) in other parts of RG compared to those of SG. This observation was expected, as Fe is commonly abundant in most cultivated soils with concentrations ranging from 20 to 40 $g \cdot kg^{-1}$ [44]. Again, contrary to Pb, Fe cannot be defined as a toxic metal as it did not exceed the safety limits allowed by the WHO/FAO, charged with protecting consumer health and ensuring fair practices in the food trade by establishing standards for safety levels through their joint initiative of Codex Alimentarius [37]. Further, the mean concentration of Ni was significantly higher ($p < 0.05$) in roots and leaves of SG compared to that of RG. Conversely, the mean concentration of Cr was significantly higher ($p < 0.05$) in all parts of RG compared to that of SG. Also, the mean concentration of Cu was significantly higher ($p < 0.05$) in stems of RG compared to that of SG. Besides this, it may be noted that the mean concentrations of Co and Cd were insignificantly adsorbed ($p > 0.05$) by any parts of both gourds. These data are in accordance with our previous observations, and it should be recalled here that about 93% of Cr could be transferred from the soil to RG while only a third was done with SG.

Table 3. Inter-analysis of the mean concentrations (±SD) of heavy metals (mg/kg) in different parts of the cultivated ridge gourds (RG) and sponge gourds (SG).

	Copper	**Lead**	**Cadmium**	**Chromium**	**Iron**	**Nickel**	**Cobalt**
Root RG	0.085 ± 0.001 [a]	0.194 ± 0.058 [b]	0.001 ± 0.001 [c]	0.163 ± 0.083 [b]	15.06 ± 0.696 [d]	0.101 ± 0.025 [a]	0.070 ± 0.049 [a]
Root SG	0.092 ± 0.002 [a]	0.634 ± 0.163 [e]	0.001 ± 0.003 [c]	0.090 ± 0.064 [a]	26.74 ± 13.82 [f]	0.284 ± 0.057 [b]	0.036 ± 0.052 [a]
Stem RG	*0.344 ± 0.004* [g]	0.631 ± 0.187 [e]	0.021 ± 0.008 [c]	*0.290 ± 0.047* [b]	25.54 ± 3.460 [f]	*0.412 ± 0.104* [b]	0.053 ± 0.081 [a]
Stem SG	0.204 ± 0.003 [b]	0.914 ± 0.206 [e]	0.009 ± 0.002 [c]	0.086 ± 0.069 [a]	30.05 ± 14.05 [f]	0.320 ± 0.018 [b]	0.108 ± 0.037 [a]
Leaf RG	0.059 ± 0.011 [a]	0.505 ± 0.012 [e]	0.008 ± 0.009 [c]	0.234 ± 0.046 [b]	24.46 ± 13.86 [f]	0.031 ± 0.134 [a]	0.047 ± 0.088 [a]
Leaf SG	0.059 ± 0.002 [a]	0.481 ± 0.176 [e]	0.010 ± 0.006 [c]	0.039 ± 0.049 [a]	22.69 ± 10.62 [f]	0.161 ± 0.054 [b]	0.043 ± 0.075 [a]
Grain RG	0.077 ± 0.001 [a]	1.538 ± 0.113 [h]	0.014 ± 0.005 [c]	0.212 ± 0.049 [b]	23.79 ± 0.463 [f]	0.071 ± 0.049 [a]	0.090 ± 0.093 [a]
Grain SG	0.092 ± 0.003 [a]	*1.691 ± 0.035* [h]	0.001 ± 0.002 [c]	0.080 ± 0.051 [a]	21.75 ± 9.980 [f]	0.067 ± 0.046 [a]	0.023 ± 0.053 [a]

HM concentration present in a part of RG is statistically compared to that of SG. Significant differences ($p < 0.05$) are represented with different letters; Conversely, the same letter indicated insignificant differences ($p > 0.05$). In italic, the significatively highest concentration of a given HM, either in RG or SG, is indicated.

Interestingly, and in line with our findings on Fe and Cu, Arora et al. (2008) reported a substantial, albeit not toxic, build-up of HM (i.e., Fe, Mg, Cu, Zn) in vegetables (e.g., spinach, mint, carrots) cultivated in a field from India [20]. This presence was attributed to irrigation with water from different sources, and the concentration range of Fe and Cu in wastewater-irrigated plants was 116–378 mg/kg and 5.2–16.8 mg/kg, respectively [20]. It was preconized that the regular monitoring of metal concentration levels from effluent and sewage, in vegetables and in other food materials, is essential to prevent excessive build-up of these metals in the food chains [22]. Also, Demirezen and Aksoy (2006) determined HM (i.e., Pb, Cu, Zn, Ni, Cd) in various vegetables produced in urban and rural areas of Turkey and found that the range concentrations of Cu and Ni were within safe limits [32]. However, those for Pb and Cd did not meet the recommended international standards, and their enhanced concentrations in vegetables were more likely related to their soil concentration [32]. Indeed, one possible explanation related to excess Pb in vegetables is that the Pb uptake can be promoted by the pH of soil and the levels of organic matter [32]. It is worth mentioning that like in India [16], the industrialization and urbanization in Pakistan have led to the release of increasing amounts of HM into the environment [38,39]. Untreated wastewater is commonly used by the farmers in the study field and is assumed to be the unique cause of soil and subsequent vegetable contaminations [13]. Indeed, it should be recalled here that the local farmers in this field do not apply chemical (e.g., phosphate/phosphoric) fertilizers, herbicides, or pesticides.

Eventually, the choice of living biomass (e.g., plants) as valuable bioadsorbents in reducing toxic metals from soils [16] was illuminated. The concept of bioadsorption, which allows a biomass to adsorb toxic HM from wastewater or soils contaminated by irrigation with untreated wastewater, was well-defined and reviewed by Mathew et al. (2016) [16]. It should be stressed that this phytoremediation method [45] would be a great fit in Pakistan for both sustainable agriculture and health safety. The main reason is that the existing technologies for wastewater treatment remain too costly for developing countries, whereas the use of bioadsorbents (living or dead biomasses) is a cheap, eco-friendly, and effective method [16]. Following this idea, the role of the gourds as potential bioadsorbents was investigated by determining EF, TF, TC, and BAF, which represent key parameters to compare accumulation of HM in vegetables [34–36,43,45]. Indeed, the definition of metal hyperaccumulation must take into consideration not only the metal concentration in the aboveground biomass but also the metal concentration in the soil. A hyperaccumulator plant is defined as when these parameters are greater than 1 [34,36]. At least, both enrichment factor (EF) and translocation factor (TF) must be considered while evaluating whether a plant is a metal hyperaccumulator [34]. These factors could then provide important clues for choosing the best soil decontamination strategies (e.g., phytoremediation, phytomining), while ensuring a quality and safe production [16,34,35,45]. Thus, we did apply them for RG and SG. The data are summarized in Table 4.

Thereby, EF of HM in RG followed a descending order from Cr to Co, as:

Cr (3.715) > Cd (2.00) > Fe (1.358) > Pb (1.240) > Cu (0.945) > Ni (0.418) > Co (0.350),

while EF in SG followed a descending order from Pb to Co, as:

Pb (1.608) > Fe (1.548) > Cr (1.219) > Cd (0.954) > Cu (0.747) > Ni (0.566) > Co (0.283).

These results (Table 4a) indicate that RG was enriched (from a contaminated soil) with Cr, Cd, Fe, and Pb, while SG was enriched with Cr, Fe, and Pb. Thus, both RG and SG are hyperaccumulators, which might be used as bioadsorbents for the highly toxic Pb. Indeed, it has been reported that EF is an important criterion for the selection of suitable crop species, which can be selected for cultivation in a field with a higher level of metal contamination or receiving industrial effluent [36]. Here, we found the highest EF value for Cr in RG compared to that of Pb in SG. This is somewhat in accordance with another study led by Singh et al. (2011), who reported a maximal enrichment of Cr in soil and root, among eight evaluated metals [36].

Also, TF in RG was observed in descending order from Cd to Co, as:

Cd (21.00) > Ni (4.079) > Cu (4.047) > Pb (3.252) > Cr (1.780) > Fe (1.696) > Co (0.757),

while TF in SG followed a descending order from Cd to Cr, as:

Cd (9.000) > Co (3.000) > Cu (2.217) > Pb (1.442) > Ni (1.127) > Fe (1.124) > Cr (0.955).

These results (Table 4b) showed that RG translocated (from roots to stems) Cr, Cd, Fe, Pb, Ni, and Cu, while SG did so with Cd, Fe, Pb, Ni, Cu, and Co. TF is one of the key components of human exposure to metals through the food chain [36]. Besides this, the hyperaccumulation of Pb and Cr by both gourds was confirmed. Even though the mean concentrations of Cd in gourds were not found to be above the safety limits, it appeared that the highest TF value in both gourd varieties was obtained for Cd. One of the reasons for these results is that Cd occurs with Zn in nature and Cd(II) is retained less strongly by the soil than the other toxic cations [36]. Establishing a pattern of translocation of metals from root to other parts of a plant species may be useful in biological monitoring of HM contamination as well as in the selection of metal accumulator or tolerant species [36]. The metal translocation process in plant species is also a crucial factor in determining the metal distribution in different plant tissues [36]. Several factors, including anatomical, biochemical, and physiological factors, contribute to HM accumulation and distribution in the upper vegetative parts [36].

Further, TC in RG was observed in descending order from Cd to Ni, as:

Cd (14.00) > Pb (7.930) > Fe (1.580) > Cr (1.300) > Co (1.286) > Cu (0.906) > Ni (0.703),

while TC in SG followed a descending order from Pb to Ni, as:

Pb (2.667) > Cd (1.000) = Cu (1.000) > Cr (0.888) > Fe (0.813) > Co (0.639) > Ni (0.236).

These data (Table 4c) reveal that RG transferred (from roots to grains) Cr, Cd, Fe, Pb, and Co, while SG did so with Cd, Pb, and Cu. Therefore, Cd and Pb were found again to be hyperaccumulated in both gourds.

Eventually, BAF in RG was observed in descending order from Cr to Co, as:

Cr (1.198) > Cd (0.955) > Cu (0.575) > Fe (0.390) > Ni (0.280) > Pb (0.273) > Co (0.071),

while BAF in SG followed a descending order from Fe to Co, as:

Fe (0.459) > Cd (0.409) > Pb (0.395) > Cr (0.355) > Cu (0.341) > Ni (0.217) > Co (0.146).

These results (Table 4d) indicate that RG accumulated (from soil to stems) Cr only, while SG did not produce any accumulation, and strongly suggest that the metal bioavailability was relatively low at the experimental site. Bioaccumulation (soil-to-plant transfer) is a process through which concentration of elements is increased through food chains [36]. Principally, the food chain (soil-plant-human) pathway is recognized as one of the major pathways for human exposure to soil contamination [36].

Table 4. Heavy metals hyperaccumulation factors in ridge gourds (RG) and sponge gourds (SG).

(a)	**Enrichment Factor (EF)**						
	Copper	**Lead**	**Cadmium**	**Chromium**	**Iron**	**Nickel**	**Cobalt**
RG	0.945	**1.240**	**2.000**	**3.715**	**1.358**	0.418	0.350
SG	0.747	**1.608**	0.954	1.219	**1.548**	0.566	0.283
(b)	**Translocation Factor (TF)**						
	Copper	**Lead**	**Cadmium**	**Chromium**	**Iron**	**Nickel**	**Cobalt**
RG	**4.047**	**3.252**	**21.00**	**1.780**	**1.696**	**4.079**	0.757
SG	**2.217**	**1.442**	**9.000**	0.955	**1.124**	**1.127**	**3.000**
(c)	**Transfer Coefficient (TC)**						
	Copper	**Lead**	**Cadmium**	**Chromium**	**Iron**	**Nickel**	**Cobalt**
RG	0.906	**7.930**	**14.00**	**1.300**	**1.580**	0.703	**1.286**
SG	**1.000**	**2.667**	**1.000**	0.888	0.813	0.236	0.639
(d)	**Bioaccumulation Factor (BAF)**						
	Copper	**Lead**	**Cadmium**	**Chromium**	**Iron**	**Nickel**	**Cobalt**
RG	0.575	0.273	0.955	**1.198**	0.390	0.280	0.071
SG	0.341	0.395	0.409	0.355	0.459	0.217	0.146

EF, TF, TC, and BAF were calculated. The gourds were considered as hyperaccumulators for a given metal when the score was higher than 1 (bold numbers).

Importantly, corroborating with our present findings, preliminary data obtained from an ongoing cohort study from our lab revealed a toxic concentration level of Pb in the blood of farmers working in the field and who consumed the gourds (contaminated with HM from the soil irrigated with untreated wastewater).

Taken together, we demonstrated that harvesting gourds for consumption from the said experimental area is unwelcome unless environmental chemistry and pollution are prevented (e.g., reducing emissions and quantity of runoff while maintaining acceptable runoff water quality) [46,47]. This study also provided evidence that sustainable solutions for safe agriculture, at least in Pakistan, are urgently required to protect present and future generations.

4. Conclusions

The industrialization and urbanization in Pakistan have led to the release of increasing amounts of HM into the environment. Regular monitoring of metal concentration levels from effluents and sewage, in vegetables and in other foods, is essential to prevent excessive build-up of these metals in the food chains, which can subsequently impact the health of the farmers and other populations.

In this study, detailed investigations were carried out to determine concentrations of HM in different parts (i.e., roots, stems, leaves, and grains) of RG and SG cultivated in a field located in D.I. Khan, Pakistan. To the best of our knowledge, this study field/area was not exposed to phosphoric fertilizers, pesticides, or other chemicals from the farmers, who humbly used untreated wastewater to irrigate their field.

Thereby, intra-analyses and inter-analyses of the two vegetables depicted a similar quantitative distribution of HM (especially for Co and Cd) in the different parts of these vegetables. Indeed, in both gourd varieties, Fe and Pb were the most concentrated HM and Pb was particularly concentrated in grains. Mean concentrations of Pb and Co in gourds were found to be toxic because they exceeded the safe limits recommended by the FAO/WHO. It was also interesting to note that the mean concentrations of Cr were significantly higher in all parts of RG compared to that of SG. Also, the assessment of hyperaccumulation factors demonstrated that both gourds can be considered as useful hyperaccumulators, at least for the highly toxic Pb.

Since the consumption of vegetables cultivated in such an environment should be cautioned against, their use as possible bioadsorbents should be further explored. Indeed, it should be emphasized that phytoremediation (i.e., use of green plants to decontaminate soils in situ) is an emerging cost-effective environmental restoration technology that might use hyperaccumulators to reduce the concentrations of toxic HM. Meantime, the use of untreated wastewater for irrigation must be obviously avoided.

It is widely accepted that a contaminated edible plant-soil system is not fit for quality, health and safety, and sustainable agricultural development, and this study aimed to shed light the importance of food safety in addition to food quality.

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Conflicts of Interest: Authors have not any financial conflict of interest.

Research Involving Animals: All procedures performed in this study involving animals (humans) were in accordance with the ethical standards of the institution where experiments were conducted. All the experimental procedures were approved by the local institutional Ethical Review Board (approval no. 196/ERB/QEC).

Abbreviations

[ ]	Concentration(s)
BAF	Bioaccumulation factor
Cd	Cadmium
Cr	Chromium
Co	Cobalt
Cu	Copper
EF	Enrichment factor
FAO	Food and Agriculture Organization (of the United Nations)
Fe	Iron
HM	Heavy metals
Ni	Nickel
Pb	Lead
RG	Ridge gourds
RT	Room temperature
SG	Sponge gourds
TC	Transfer coefficient
TF	Translocation factor
WHO	World Health Organization

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