

Vegetables Irrigated with Untreated Wastewater

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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.

Wastewater

Heavy metals

Toxicity

Vegetables cultivation

Sustainability

Sustainable food

Food safety

Food quality

Bioremediation

Hyperaccumulator

Agriculture

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][2][3][4][5][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][5][6][7][8][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/cm³ 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][15][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][15][16]. However, it should be emphasized that these methods remain poorly applied in developing countries, such as 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 ferriprive anemia and a large variety of different processes (e.g., vesicles trapping) serve to prevent its toxic effects [29].

2. Discussion

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 [32] 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 [33][34][35][36][32]. 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 [33][35]. At least, both enrichment factor (EF) and translocation factor (TF) must be considered while evaluating whether a plant is a metal hyperaccumulator [33]. 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][33][34][32]. Thus, we did apply them for RG and SG. The data are summarized in [Table 1](#).

Table 1. 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

Translocation Factor (TF)							
(b)	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
Transfer Coefficient (TC)							
(c)	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
Bioaccumulation Factor (BAF)							
(d)	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).

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 1a](#)) 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 [\[35\]](#). 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 [\[35\]](#).

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 1b](#)) 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 [\[35\]](#). 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 [\[35\]](#). 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 [\[35\]](#). The metal translocation process in plant species is also a crucial factor in determining the metal distribution in different plant tissues [\[35\]](#). Several factors, including anatomical, biochemical, and physiological factors, contribute to HM accumulation and distribution in the upper vegetative parts [\[35\]](#).

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 1c](#)) 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 1d](#)) 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 [\[35\]](#). Principally, the food chain (soil-plant-human) pathway is recognized as one of the major pathways for human exposure to soil contamination [\[35\]](#).

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) [\[37\]](#)[\[38\]](#). This study also provided evidence that sustainable solutions for safe agriculture, at least in Pakistan, are urgently required to protect present and future generations.

3. 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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