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Phytoremediating a Wastewater-Irrigated Soil Contaminated with Toxic Metals: Comparing the Efficacies of Different Crops

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Abstract: A formidable challenge in suburban agriculture is the sustainability of soil health following the use of wastewater for irrigation. The wastewater irrigation likely toxifies the crop plants making them unconsumable. We used a multivariate, completely randomized design in a greenhouse, comparing the phytoextraction capacities of *Brassica juncea*, *Eruca sativa*, *Brassica rapa*, and *Brassica napus*—all grown on silt loam soil irrigated with industrial wastewater, canal water, and a 1:1 mixture, during 2018. The studied *Brassica* plants were generally closely efficient in remediating toxic metals found in wastewater irrigated soil. Substantial differences between *Brassica* and *Eruca* plants/parts were recorded. For example, *B. napus* had significantly higher metal extraction or accumulation compared to *E. sativa* for Zn (71%), Cu (69%), Fe (78%), Mn (79%), Cd (101%), Cr (57%), Ni (92%). and Pb (49%). While the water and plant were the main predictors of metal extraction or accumulation, an interaction between the main effects substantially contributed to Cu, Mn, and Fe extractions from soil and accumulations in plants. Significant correlations between biological accumulation coefficient and biological transfer coefficient for many metals further supported the metal extraction or accumulation efficiencies as: *B. napus* > *B. juncea* > *B. rapa* > *E. sativa*. Root-stem mobility index correlation with stem-leaf mobility index indicated the metal translocation along the root-stem-leaf continuum. Therefore, we suggest that these crops may not be used for human or animal consumption when grown with industrial wastewater of toxic metal concentrations ≥ permissible limits. Rather these plants may serve as effective remediators of toxic metal-polluted soil.

Keywords: *Brassica*; canola; cauliflower; heavy metal; phytoremediation; pollution; soil health; toxic; wastewater

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1. Introduction

Worldwide, surface or groundwater supply for food crop production is limited. The available surface water is irrigating 17% of the arable land with crop production of 34% of the world's food demand. In Pakistan (5th most climate-change-impacted country on Earth), 85% of the food is produced by the lands irrigated with river water, canal water, and/or groundwater [1,2]. Climate warming combined with an increasing population (11.0 billion in 2100 [3]) is frequently predicted to result in diminishing surface and groundwater supplies [4,5]; therefore, irrigation water supplies may become increasingly constrained for food crop production. Hence, there is a tremendous potential to explore alternate but safe irrigation waters as well as their compatible crops to cope with the shortage of irrigation water and uncertainty in food security. While experiments have been conducted to explore alternate irrigation waters and compatible crops for successful phytoextraction or food production, controlled experimentation to compare the efficiencies of these waters and crops remains limited.

Canal water is the surface water which is obtained from an engineered distributary of a river—the distributaries can be thought of as small, constructed rivers. Irrigation water quality of canal water is regarded as the best (Table 1) compared to groundwater or wastewater (sewerage, industrial wastewater). Wastewater is one of the alternate waters drained from industries, for example, leather, tannery, packaging, textile, and pesticide. In addition to nutrients, the wastewater may contain one or more of the essential or non-essential heavy metals, i.e., zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) in excessive (toxic) amounts and may be toxic for soils, plants and/or humans [6–8]. Toxicity thresholds for many micronutrients or metals are reached at considerably lower doses [9]. The wastewater may also contain a large assortment of pollutants including dissolved salts, organic matter, hosts and/or pathogens [10], and unknown human or animal health product residues [11]. The constituents when encountering soil particles, occupy exchange sites, accumulate in the soil over time, and deteriorate soil health, resulting in cropland of low productivity. Crops grown on these lands, uptake the toxic ions and accumulate in roots, stems, leaves or fruits in different proportions.

Soil texture is one of the dominant controls over heavy metal adsorption on soil particle exchange sites and inter-lattice spaces [12]. Light textured soil (sandy, sandy loam, and silt loam) particles exhibit lower surface area, cation exchange capacity (CEC), and surface charge density than those of heavy-textured soils (clay, clay loam, silty clay) [13]. Other soil properties such as pH, redox potential, hydraulic conductivity, and tendency to uptake, accumulate and transport heavy metals to various plant parts also differ with texture [14].

Heavy metals are bonded to particulate organic and inorganic matter in the wastewater [14] and become in equilibrium with the soil solution following the soil is irrigated with wastewater. Heavy metals are mobilized to or accumulate in plant tissues via plant-root-driven mechanisms: (1) plant root secretion of metal-chelating compounds (called phytosiderophores) into the rhizosphere, (2) plant root biochemical reduction of ions bonded to soil particles, and/or (3) plant root rhizosphere acidification by humic substances [15] followed by the exchange of metals with protons [16]. Different plants show significantly different capacities for heavy metal toxicity tolerance using exclusion, inclusion, or selective absorption of metals in amounts equivalent to nutrient requirements [17,18]. These metals are bioaccumulated in plant edible portions—roots, stem, leaves, and/or fruits [19]. Different metals show different threshold levels to become toxic to soil properties or plant tissues. Zn toxicity in plants is commonly indicated by leaf chlorosis [20,21] while Cu toxicity additionally causes suppressed root growth.

Plant species have been used to remediate heavy metal polluted soils—a technique called phytoremediation which involves the use of plants for the extraction of contaminants from soils and accumulating them in their above- and below-ground parts [22]. While phytoextraction rate is dependent on the bioavailability of heavy metals, different phytoextractants (*Brassica juncea*, *Eruca sativa*, *Brassica rapa*, *Brassica napus*) have different efficiencies owing to their distinct growth rates, depth of root systems, and accumulation and translocation capabilities, e.g., [23]. These phytoremediators are amongst the efficient growing and high biomass yielding crops [24], though their efficiencies may differ. For example, *B. juncea*, *B. rapa* and *B. napus* are better at removing Zn than they can remove Cu. *Brassica* taxa are the best at removing a range of heavy metals (Zn, Cu, Fe, Mn, Cd, Cr, Ni, and Pb), though *B. juncea* removed the highest amounts of these metals and accumulated in the stem [23,24]. *E. sativa* is also grown on heavy metals-laden soils and has been found to have good tolerance and adaptability to wastewater irrigated soils [25].

To compare the phytoremediation efficiencies of the test crops (*B. juncea*, *E. sativa*, *B. rapa*, *B. napus*) irrigated with wastewater, we compared ratios of metal concentrations between soil and plant parts as biological accumulation coefficient (BAC); biological transfer coefficient (BTC); mobility index at soil-root (MISR); mobility index at root-stem (MIRS);

mobility index at stem-leaf (MISL) [19]. Ratios of ≥1 indicate effective movement and/or accumulation from soil to root or root to stem or stem to leaf. The higher the coefficient value or ratio, the greater would be the remediation or accumulation efficiency of a crop [19,26–28], and the greater would be the risk to the health of humans consuming these crops.

We hypothesized that (1) the type of irrigation water will influence the heavy metal extractions or concentrations in the studied crop plants/parts, and (2) heavy metal BAC, BTC, MISR, MIRS, and MISL of the test crops will be ≥1. Specific research objectives were to (1) compare the phytoextraction capacities of four common crops in response to irrigation with canal water, wastewater, and their 1:1 mix, and (2) compare the different indices for heavy metal uptake (BAC, BTC, MISR, MIRS, and MISL) by these crops.

2. Materials and Methods

2.1. Experimental Design

A pot experiment was conducted in a greenhouse at Cotton Research Station, Multan, Pakistan to quantify and compare the phytoextraction or phytoaccumulation capacities, BAC, BTC, MISR, MIRS, and MISL of four high biomass-producing oil-seed or vegetable species: *B. juncea* (Raya), *E. sativa* (Taramira), *B. rapa* (Toria) and *B. napus* (Canola)—all grown on a homogenous silt loam soil. The authors compared the role of soil texture in phytoextraction or phytoaccumulation by comparing sandy loam and clay loam textures (and their characteristics) in their previous research at this site [29], therefore, in this experiment, the factor already studied at this site is not included to focus on the remaining factors and sustain clarity and brevity of results. Three types of irrigation water were used: canal water (control), industrial wastewater (treatment) and, a 1:1 (*v*/*v*) mix of canal water, and wastewater (treatment).

On 19 September 2018, a total of 36, 20 kg capacity pots were taken—each was filled with 16 kg silt loam soil collected from 0–20 cm deep cultivated/polluted land irrigated with wastewater from Industrial Estate Wastewater Disposal Station (IEWDS), Multan (IEWDS; 30°11′66′′ N, 71°32′67′′ E). The IEWDS collects wastewater from approximately 190 industrial units—mostly of leather, tannery, packaging, textile, and pesticides. Use of the wastewater is preferred by urban/suburban farmers owing to its high concentrations of nutrients and, free and stable year-round availability, which support the livelihood of local farmers [30,31]. The infill soil was uniformly air-dried before filling the pots. For irrigation, canal water was collected from a distributary originating from the Chenab River, and irrigating the Government Agricultural Farms, Multan. Wastewater was obtained from IEWDS, Multan. Treatment of canal + wastewater mixture was prepared by mixing the waters in a 1:1 (*v*/*v*) ratio. The three water types were carried and stored in 25 liter plastic containers. Following Strack et al. 2019 [32], the waters were passed through Whatman 40 and then a series of filters: first through a 1.5 µm glass fiber and then through a 0.4 µm glass fiber filter the same day (continued through the next or following days) to retain any dissolved organic matter and, particulate organic and inorganic matter which may otherwise provide exchange sites for precipitation and cross-precipitation of the organic or inorganic metals and change salinity (ECiw, pHiw). Further, to minimize microbial degradation of heavy metals, the waters were stored in a laboratory at 4 °C before being used for irrigation. Before each irrigation, waters were allowed to warm to normal temperature and tested for any significant changes in salinity. No substantial changes in salinity were recorded ($p \geq 0.05$). Therefore, the waters we used for pot irrigation may have minimal organic matter/metals, changes in salinity, and subsequent precipitation or cross-precipitation—thus, no significant experimental source of uncertainty in results/interpretation was expected [33].

The pot experiment was organized in a completely randomized design [(4 crops × 3 water types) × 3 replications = 36 pots]. All pots were maintained at field capacity for approximately one month from 19 September 2018 to 14 October 2018. On 15 October 2018, a recommended, basal dose of NPK (60:30:30) was mixed with the topsoil, and then, 10 government-certified (disease-free), viable seeds of a study crop were sown in each pot as per the research design. The seeds were obtained from Punjab Seed Corporation, Government of the Punjab, Pakistan. Only the three most viable seedlings were allowed to grow in each pot. The three replicates from each treatment (3 waters $\times$ 4 crops) were subjected to standard agricultural practices during germination and growth. Overall, the plants received 8–9 h of direct or full-spectrum sunlight every day throughout the experiment. The greenhouse had transparent panels which may show an adequate or higher transpiration level [34]. On 30 December 2018, the crop plants, or parts from 36 pots were harvested, placed separately in paper bags (36 bags), and transported to the soil and water testing laboratory, Multan for sample preparation and chemical analyses.

2.2. Sampling and Analysis

Triplicate bulk surface soil samples (0–20 cm) were collected randomly from the Industrial Estate Wastewater Disposal Station-fed area of three acres. The soil was ensured to be enough for filling 16 kg soil in each of the 36 pots. The soil samples were air-dried, crushed, sieved to <2 mm, thoroughly mixed, and stored at room temperature before analyzing for some physicochemical properties and chosen heavy metal concentrations. Soil samples were analyzed for textural class, saturation pastes electrical conductivity (EC_s), and pH (pH_s) following methods described by the US Salinity Laboratory Staff [35]. Based on our laboratory limitations, only Zn, Cu, Fe, Mn, Cd, Cr, Ni, and Pb concentrations in soil and plants could be estimated. To quantify the planned water-soluble heavy metal concentrations in soil, 20 g of dry soil was extracted with 40 mL of deionized water [36] and the extractants were stored for chemical analyses.

Irrigation wastewater/samples were also collected around the soil sampling campaigns and prepared for chemical analyses and irrigation as explained in the 2nd paragraph of this section. Within a week of preparing irrigation water, a 50 mL sample was digested with 10 mL of concentrated HNO_3 at 80 °C until the solution turned clear [37]. The clear solution was then filtered through Whatman No. 42, diluted back to 50 mL using distilled water, and stored for chemical analyses.

Root, stem, and leaf parts of the four test crops were thoroughly cleaned for any dust material, washed sequentially with 1% HCl and double deionized water, air dried in shade for 24 h, and then oven dried at 70 °C until a constant weight was reached. The dry matter was ground to a powder form and sieved to <1 mm. A 1.0 g of the powder was digested with a di-acid mixture of $HClO_4$ and HNO_3 in a 1:2 ratio, respectively. The clear digest was filtered and diluted to 50 mL using distilled water and stored at $\leq$4 °C for chemical analyses.

Plant total and soil and wastewater soluble Zn, Cu, Fe, Mn, Cd, Cr, Ni, and Pb concentrations (mg L^{-1}) were determined from the stored extracts using an atomic absorption spectrophotometer (Model AAS Vario 6, Analytik Jena AG, Jena, Germany). Following Baker (1981); [19]), we calculated the BAC, BTC, MISR, MIRS, and MISL of heavy metals:

$$Biological\ Accumulation\ Coefficient\ (BAC) = \frac{Stem + Leaf\ concentration}{Soil\ concentration} \tag{1}$$

$$Biological\ Transfer\ Coefficient\ (BTC) = \frac{Stem + Leaf\ concentration}{Root\ concentration} \tag{2}$$

$$Mobility\ Index\ at\ Soil - Root\ (MISR) = \frac{Root\ concentration}{Soil\ concentration} \tag{3}$$

$$Mobility\ Index\ at\ Root - Leaf\ (MIRS) = \frac{Stem\ concentration}{Root\ concentration} \tag{4}$$

$$Mobility\ Index\ at\ Shoot - Leaf\ (MISL) = \frac{Leaf\ concentration}{Stem\ concentration} \tag{5}$$

2.3. Statistical Analyses

All data analyses were performed using the SPSS 26.0 package (SPSS, Chicago, IL, USA). We used a two-way (water and crop treatments) multivariate (Zn, Cu, Fe, Mn, Cd, Cr, Ni, Pb) ANOVA for quantifying the individual and interactive effects of the water and crop factors on the response variables of Zn, Cu, Fe, Mn, Cd, Cr, Ni, and Pb concentrations in crops. Separate models were run for plant parts. Regressions and correlations were also performed where meaningful. Data were normalized to $\log_{10}$ values when not normally distributed. Differences were significant when $p < 0.05$.

3. Results

Thoroughly mixed, pot infill soil had uniform texture, salinity (ECs = 3.84 dSm^{-1}, pH = 8.6; Table 1), and toxic levels (mg L^{-1}) of studied heavy metals (Table 1). The original irrigation water types (canal water, wastewater) were different in the EC_{iw} (ANOVA: $F_{1,5} = 121.50$, $p < 0.001$) and pH_{iw} (ANOVA: $F_{1,5} = 121.50$, $p < 0.001$) and heavy metal concentrations. Our study focused on some key drivers of phytoremediation and bioaccumulation, i.e., water type and crops. Other key drivers of phytoremediation or bioaccumulation, such as soil texture have been studied at the experimental site [29].

Table 1. Textural class of soil and, salinity and heavy metal concentrations of the soil and irrigation waters used in the study.

Soil Texture or Water Source	Salinity		Heavy Metal Concentration (mg L^{-1})							
	EC_s/EC_{iw} (dSm^{-1})	pH_s/pH_{iw}	Zn	Cu	Fe	Mn	Cd	Cr	Ni	Pb
Silt loam soil	3.84	8.64	4.52	1.74	15.36	4.62	1.02	0.18	0.20	2.76
Canal water	1.02	7.20	0.01	0.01	0.03	0.04	0.07	0.02	0.02	0.06
Wastewater	4.18	7.41	0.26	0.12	1.28	0.24	0.19	0.11	0.29	1.14
1:1 mix water	2.72	7.34	0.13	0.08	0.67	0.15	0.15	0.07	0.16	0.63
Permissible limits (water) †	1.5	6.5–8.5	2.00	0.20	5.00	0.20	0.01	0.01	0.20	0.50

† Recommended maximum concentration in irrigation water for crops [38,39]. ECs and pHs, and ECiw and pHiw denote electrical conductivity and pH of soil and irrigation water, respectively. For all pots, surface soil (0–20 cm) was collected from Industrial Estate Disposal Station, Multan.

Overall, the main effects of water and crop and their interaction were significant (two-way MANOVA, Wilks' lambda test: $p < 0.001$; Table 2) on heavy metal concentrations in the studied crops irrigated with three water types. However, the interaction was significant only for Cu, Fe, and Mn contents in plants in response to various waters. The Zn, Cd, Cr, Ni, and Pb concentrations did not differ.

More specifically, the irrigation water type was the dominant control over most metal contents in each of the test crops. It reflects that different water types produced different responses in a plant species, with overall highest metal concentrations recorded in response to wastewater followed by the 1:1 mix and canal water in that order. When compared, the four plant species differed in remediation or accumulation capacities, however, similarities were not uncommon, for example, *B. napus* was not different from *E. sativa* for the extraction of Cd, and *B. juncea* for Mn uptake and accumulation. Likewise, *B. juncea* and *B. rapa* had similar responses for their Cr concentrations. Bottom-line, extraction capacities were in the order of *B. napus* > *B. juncea* > *B. rapa* > *E. sativa* in plants and root > stem > leaf in plant parts.

Individually, water type had a significant effect (two-way MANOVA: Table 2; Figure 1A–D) on plant part metal concentrations, except: root Zn ($p = 0.067$), stem Zn ($p = 1.000$), root Cu ($p = 1.000$), stem Cu ($p = 0.678$), leaf Cu ($p = 0.211$), leaf Fe ($p = 1.000$), root Cd ($p = 0.001$), root Cr ($p = 0.424$), and leaf Cr ($p = 1.000$). Wastewater had the highest impact on extraction of all metals by roots > stem > leaves, followed by the impact of 1:1 combined water mix in the same order on plant parts.

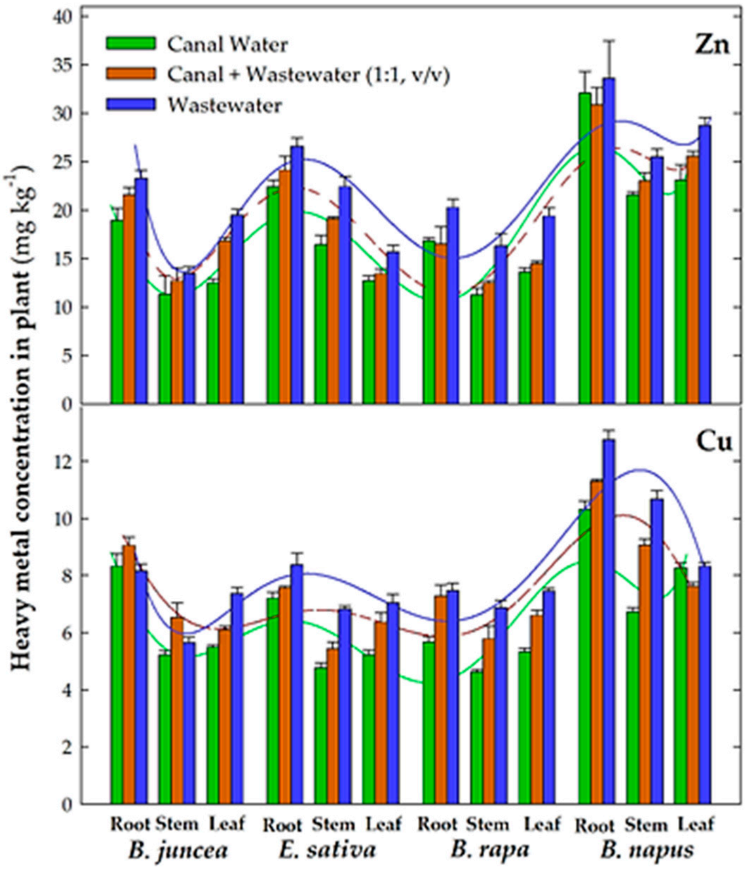
Table 2. Results of a two-way MANOVA for phytoextraction of heavy metals by root, stem, and leaf of study crops (*B. juncea*, *E. sativa*, *B. rapa*, and *B. napus*) irrigated with canal water, wastewater, and canal + wastewater (1:1; *v*/*v*) in Multan.

Source	F/*p*	Zn	Cu	Fe	Mn	Cd	Cr	Ni	Pb
Water ($df_{2,24}$)	$p\leq$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
root	F	13.81	64.06	44.35	134.21	4.71	8.17	3.68	12.47
	$p\leq$	0.000	0.000	0.000	0.000	0.020	0.000	0.040	0.000
stem	F	57.28	207.92	186.86	268.56	8.91	23.26	16.04	61.93
	$p\leq$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
leaf	F	170.84	179.71	236.73	398.76	7.59	31.51	4.91	33.17
	$p\leq$	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.000
Crop ($df_{2,24}$)	$p\leq$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
root	F	121.30	429.21	861.94	80.14	86.18	17.21	11.29	65.25
	$p\leq$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
stem	F	243.51	305.18	640.30	333.54	31.82	33.66	9.05	114.00
	$p\leq$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
leaf	F	497.12	189.28	537.08	273.62	15.78	23.48	8.59	263.15
	$p\leq$	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Water × Crop ($df_{6,24}$)	$p\leq$	ns	0.000	0.000	0.00	ns	ns	ns	ns
root	F	0.99	14.85	3.29	9.71	0.80	0.95	0.06	0.32
	p	ns	0.00	0.02	0.00	ns	ns	ns	ns
stem	F	2.48	27.80	13.61	16.51	0.27	1.70	0.24	0.81
	p	0.05	0.00	0.00	0.00	ns	ns	ns	ns
leaf	F	6.83	24.04	1.18	26.50	0.43	1.45	0.08	0.35
	p	0.00	0.00	ns	0.00	ns	ns	ns	ns

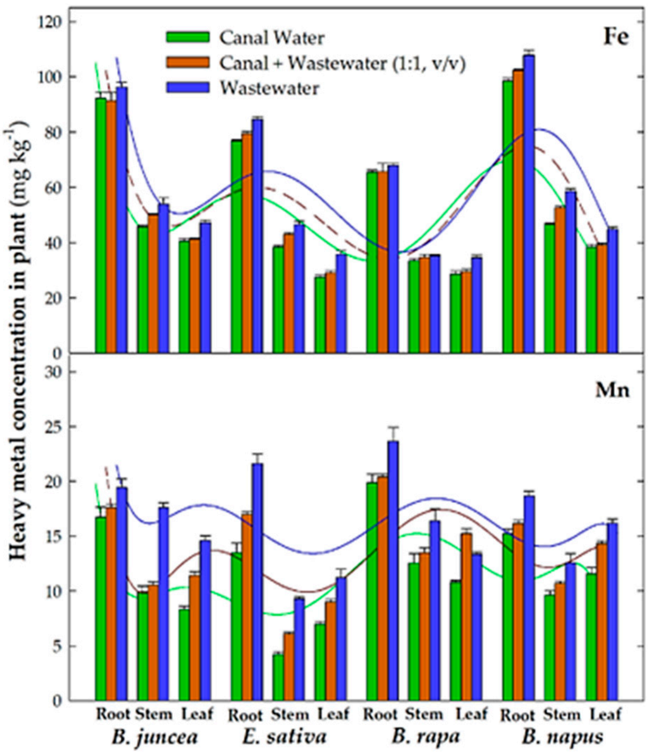
F_{stat} = variation between sample means/variation within samples; Higher the F value greater is the variation between sample means relative to the variation within the samples. df represents the degree of freedom to choose from the number of observations available for a variable. $p \leq 0.05$ means we are 95% confident to reject the null hypothesis which states no difference among the group means (for example, Zn extraction in response to three water types). n.s represents non-significant. Overall, the effects of water and crop and their interaction were significant ($p < 0.001$; Wilks' lambda test) on heavy metal concentrations in the root, stem, and leaf of the study crops irrigated with three different water types. Exceptions are debated in the discussion section.

The bioaccumulations by plant parts were also significantly different between crops (two-way MANOVA: Table 2; Figure 1A–D), except: *B. juncea* did not differ from *B. napus* for leaf Zn (p = 1.000), stem Cu (p = 0.773), and leaf Fe (p = 0.609), from *E. sativa*, *B. rapa* and *B. napus* for root Cd (p = 1.000, 1.000, 0.711, respectively) and from *E. sativa*, *B. rapa* for leaf Cr concentrations (p = 0.338, 0.817, respectively); *E. sativa* did not differ from *B. rapa* for *B. rapa* for stem and leaf Zn (p = 0.130), root and stem Cu (p = 0.424, 1.000, respectively), stem Cd (p = 0.314) and root Cr concentrations (p = 1.000).

Overall, the effects of water and crop treatments (individually and interactively) on BAC, BTC, MISR, MIRS and MISL were significant (two-way MANOVA, Wilks' lambda test: $F_{26, 22}$ = 283.97, $p < 0.001$; $F_{39, 33}$= 898.25, $p < 0.001$; $F_{78, 66}$ = 14.61, $p < 0.001$, respectively). Comparisons between the water treatments showed that all the wastewater irrigated crops had the highest values of BAC and BTC followed by the lower coefficient values of the 1:1 mixed water irrigated crops while the canal irrigated crops had the lowest BAC and BTC values (Table 3). Although water treatment had a significant effect on overall MI ($p < 0.001$), however, crop to crop comparisons for MISR, MIRS, and MISL had mixed results; however, many of the indexes had values higher than 1.0 (Figure 2).

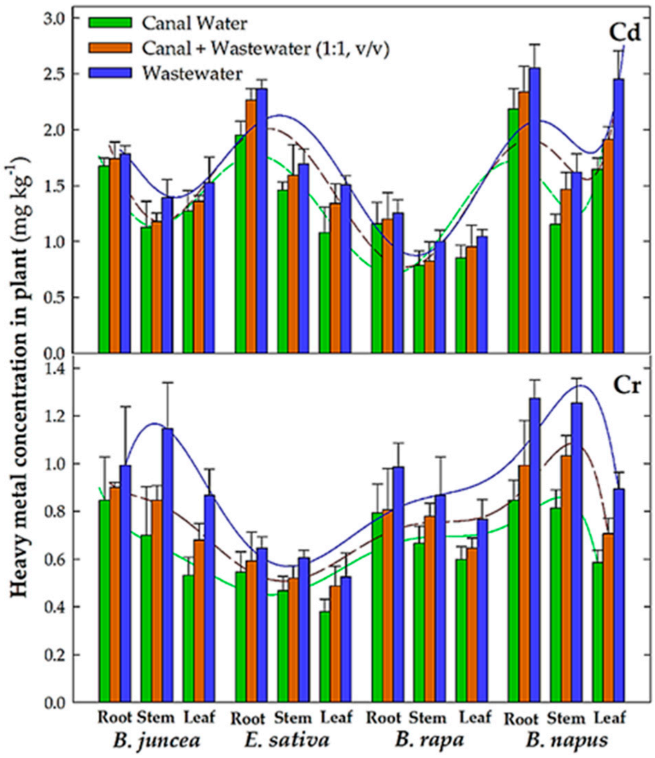


(**A**)

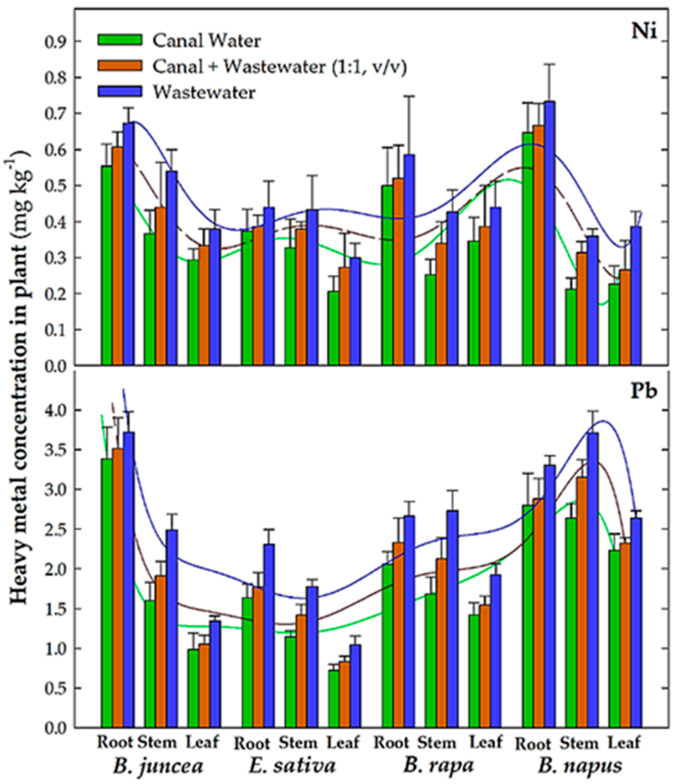


(**B**)

Figure 1. *Cont.*



(**C**)



(**D**)

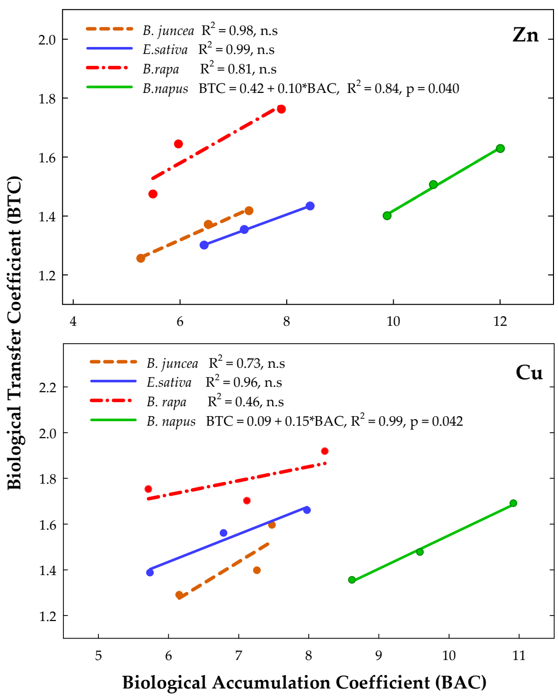
Figure 1. Impact of irrigation water type on Zn and Cu (**A**), Fe and Mn (**B**), Cd and Cr (**C**), and Ni and Pb (**D**) concentrations (mg kg^{-1} dry weight) in the root, stem, and leaf of *B. juncea*, *E. sativa*, *B. rapa* and *B. napus* in a pot experiment. Bars and line of same color represent one water type. The statistical significances or *p* values (instead of significance letters on bars) are provided in Table 2.

Table 3. Results of a two-way MANOVA for biological accumulation coefficient (BAC) and biological transfer coefficient (BTC) of studied crops, and mobility index (MI; soil-root, root-stem, and stem-leaf) of these crops irrigated with canal water, wastewater, and canal + wastewater in Multan.

Source	F/*p*	Zn	Cu	Fe	Mn	Cd	Cr	Ni	Pb
Water ($df_{2,24}$)									
BAC	F	164.65	322.04	427.26	690.84	14.89	34.19	20.59	98.36
	p	0.000	0.000	0.007	0.001	0.007	0.000	0.002	0.001
BTC	F	11.41	41.91	65.83	83.42	1.33	1.13	3.68	4.562
	p	0.000	0.006	0.007	0.007	ns	ns	0.042	0.021
MI (soil-root)	F	22.20	60.70	20.10	118.08	1.73	5.69	2.79	16.72
	p	0.000	0.005	0.005	0.009	ns	0.010	0.081	0.000
MI (root-stem)	F	3.69	29.86	54.87	94.57	1.84	1.16	4.71	10.53
	p	0.040	0.004	0.001	0.007	ns	ns	0.022	0.000
MI (stem-leaf)	F	0.57	22.43	22.89	40.80	0.24	0.00	0.34	2.19
	p	ns	0.000	0.001	0.001	ns	ns	ns	ns
Crop ($df_{3,24}$)									
BAC	*p*	0.002	0.000	0.000	0.000	0.004	0.000	0.000	0.000
BTC	*p*	0.001	0.000	0.001	0.000	0.029	ns	0.000	0.000
MI (soil-root)	*p*	0.000	0.000	0.000	0.001	0.002	0.001	0.000	0.000
MI (root-stem)	*p*	0.000	0.001	0.009	0.000	0.068	ns	0.001	0.005
MI (stem-leaf)	*p*	0.001	0.008	0.005	0.000	0.008	0.007	0.011	0.088
Water × Crop ($df_{6,24}$)									
BAC	*p*	ns	0.000	0.000	0.004	ns	ns	ns	ns
BTC	*p*	ns	0.000	ns	0.002	ns	ns	ns	ns
MI (soil-root)	*p*	ns	0.005	ns	0.000	ns	ns	ns	ns
MI (root-stem)	*p*	ns	0.010	0.010	0.000	ns	ns	ns	ns
MI (stem-leaf)	*p*	0.0100	0.001	0.000	0.007	ns	ns	ns	ns

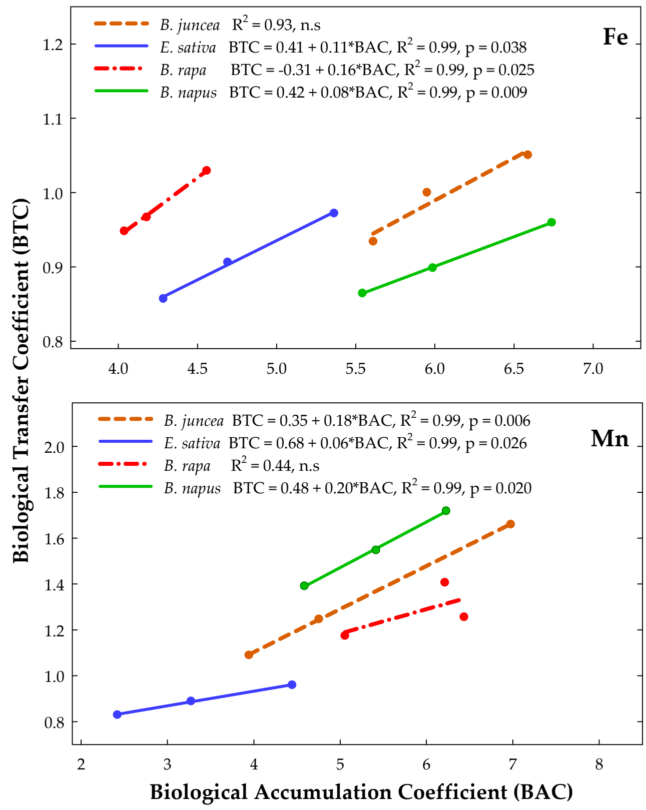
Overall, the effects of water and crop and their interaction were significant ($p < 0.001$; Wilks's lambda test) on BAC, BTC, and MI of the four crops irrigated with three different water types. n.s represents non-significant.

The BAC-BTC Pearson correlations were found significant for Zn, Cu, Fe, Mn, and Cd contents of *B. napus*, Zn, Fe, and Mn contents of *E. sativa*, Mn, and Cr contents of *B. juncea*, and only Fe contents of *B. rapa*. (Figure 2). While most MIRS–MISL correlations (R^2) for the studied metals and crops were significant and positive, we found some relationships were negative but meaningful, for example, Mn MIRS–MISL relationship in *E. sativa* and Ni MIRS–MISL relationship in *B. juncea* (Figure 3).

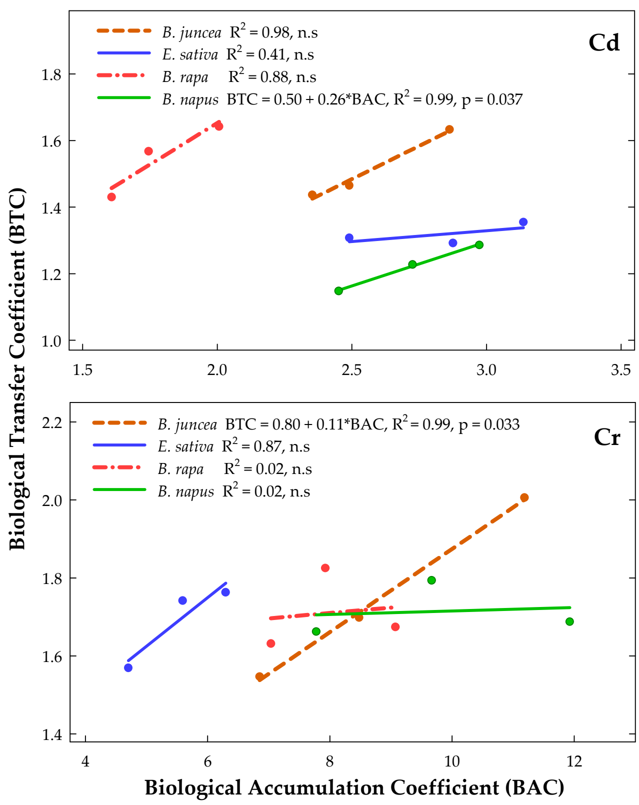


(A)

Figure 2. *Cont.*

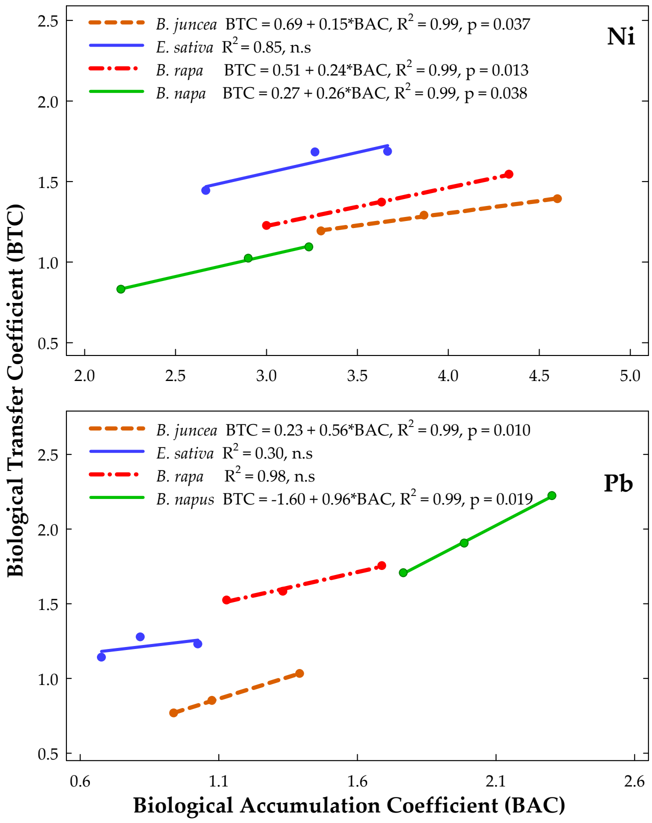


(**B**)



(**C**)

Figure 2. *Cont.*



(**D**)

Figure 2. Relationships between biological accumulation coefficient (BAC) and biological transfer coefficient (BTC) of test crops for Zn and Cu (**A**), Fe and Mn (**B**), Cd and Cr (**C**), and Ni and Pb (**D**) concentrations (mg kg^{-1} dry weight). Relationship is significant at $p < 0.05$. n.s represents non-significant ($p > 0.05$).

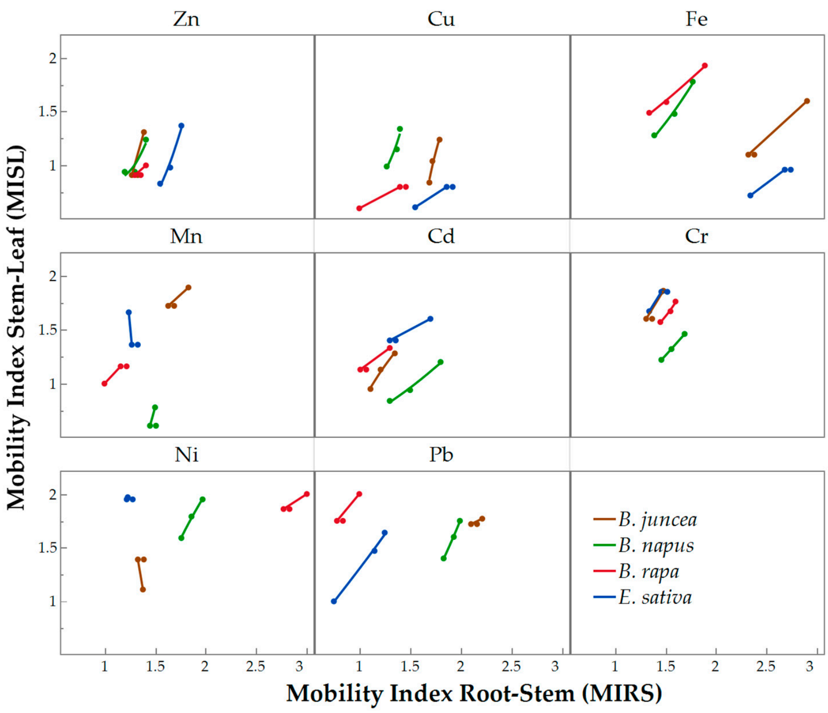


Figure 3. Relationships between mobility index at root-stem (MIRS) and mobility index at stem-leaf (MISL) of crops for heavy metals concentrations (mg kg^{-1} dry weight).

4. Discussion

Unlike most previous work, we conducted multivariate research—it involved wastewater-fed soil, polluted with toxic mineral heavy metals. Four different crops variably remediated the metals that accumulated in the root, stem, and leaf parts. While the three *Brassica* and one *Eruca* plant species and their parts were generally effective for phytoextraction and phytoaccumulation (respectively), significant differences between species and among parts for metal extraction or accumulation were not uncommon—though, *B. juncea*, *B. rapa*, and *B. napus* were closer for metal removal efficiencies compared to *Eruca* plants. While the disparity in metal extraction or accumulation function is generally attributed to the type of irrigation water, nature of the toxic metals, plant species/physiology, and/or soil/plant redox potentials [40], why the different water types produced different phytoremediation responses in the studied plants is discussed in detail in the following paragraphs. Our findings have important implications for (1) urban and suburban farmers who use industrial wastewater for cropping and/or planning to remediate their polluted soils, and (2) industrial ecologists who seek to remediate toxic heavy metal contaminated soils or treat contaminated waters using treatment wetlands in industrial settings.

4.1. Types of Irrigation Water and Phytoremediators

Based on the heavy metal toxicity criteria for irrigation water (Table 1), the wastewater concentrations of Cd, Cr, Ni, and Pb were well above the permissible limits and significantly higher than those in canal water. The 1:1 combined water still had higher than normal concentrations, except Ni. We did not manipulate the excessive metal concentrations in the 1:1 mix, but rather adopted a holistic approach and used the 1:1 mix as such to make this work practically useful for growers. Using the 1:1 mix without further dilution provided us with an opportunity to assess the extraction or accumulation responses of test plants to waters of varying toxic metal concentrations. Additionally, the metal accumulations by roots, stems, and leaves in response to these abnormal waters were also measured. Four important crop plants belonging to the *Brassicaceae* family (mustards) namely *Juncea* (Raya), *rapa* (Toria), *napus* (Canola), and *sativa* (Taramira) were used as phytoremediators.

4.2. Effect of Wastewater on Phytoremediation or Phytoaccumulation

Overall, different water types produced different responses in plants. The plant metal concentrations responded to wastewater > 1:1 mix > canal water in all plants with the corresponding concentrations of Cd (1.6, 1.4, 1.3 mg kg^{-1}), Cr (0.9, 0.7, 0.6 mg kg^{-1}), Ni (0.5, 0.4, 0.3 mg kg^{-1}), and Pb (2.5, 2.1, 1.8 mg kg^{-1}), all respectively. *B. napus* extracted or accumulated the highest quantity of Cd, Cr, Ni, and Pb in response to wastewater (1.9, 1.2, 0.5, 3.2 mg kg^{-1}), 1:1 combined (1.6, 0.9, 0.4, 2.7 mg kg^{-1}), and normal water (1.5, 0.7, 0.4, 2.6 mg kg^{-1}) and *E. sativa* has the lowest contents. The plant responses reflect that the irrigation water type may be one of the strongest controls on heavy metal concentrations in each of the four, test plants. Another reason that water type is one of the strongest controls is that plants/roots absorb water from soil following the rise in negative water potential in plants. The higher the concentration of a metal in water, the higher could be the corresponding accumulation in plants. The reason is the mineral metals are uptaken by roots and mobilized to and accumulated in the stem and leaves, e.g., [41] with sap-flow and via the mass flow of water [42,43]. Though, the metal extraction (e.g., Ni, Pb), root accumulation, and subsequent transport to and accumulation in stem and leaves may be suppressed by the competitive concentrations of contemporary metals and/or nutrients in soil/water, for example, [44,45]. Differential uptake and accumulations may also be the results of exclusion or inclusion mechanisms, for example, [46]. However, few plants, for example, *B. napus* have evolved as a hyperaccumulator of Cd and Pb [47], which supports our finding of plant accumulations of the same metals in addition to Zn, Cu, Fe, and Cr we found accumulated in root > stem > leaf. The difference in plant metabolisms may also be one of the reasons different water types produced different metal content responses in plants [34]. But studying the plant metabolism, physiology or phenology was beyond the scope of this experiment. Other drivers of plant metal concentration, such as soil texture, are already published by authors [29]. Moreover, in this experiment, the soil texture may not be a contributor to the difference in plant response to different water types because we used a single homogenous soil/texture under controlled conditions (pot experiment in a greenhouse). As some of the experimental materials (soil texture, ECiw, pHiw) were the same for all treatments, therefore we agree with (Indoriaetal.(2013) [48]) who reported that plant genes involved in tolerance to heavy metals in *Brassica* plants are different from *Eruca* plants while the genes for tolerance to other biotic or abiotic stresses may not be different. However, the genes for plant tolerance to salinity and excessive concentrations of Pb and Ni may modulate each other's functions in some plants [49,50].

The increases in metal concentrations of root > stem > leaf were higher in response to wastewater compared to canal water. The influences of 1:1 mixed water on the metal contents of plant parts were diverse. Mechanisms involved in the movement of heavy metals along the soil–root–stem–leaf continuum comprise the uptake by roots and transportation into the root cells via transmembrane carriers for nutritional ions—the metals are further diffused into the xylem vessels and unloaded into the xylem sap, thereby reaching the aboveground parts of plants [40,51,52]. That is why most of the BAC and MIRS ratios were also found to be ≥ 1, except for Cd, which was similar to the previously reported data [45]. The exceptional response of plants to Cd uptake, transport, and accumulation might be the result of dominance by the contemporary nutrient or other competitive toxic metal concentrations [44,45]. In the absence of competition, the Cd is reported to have high water solubility and mobility through plant parts [44]. Cd concentrations in plants/parts (in response to different waters) we present are aligned with earlier research findings, e.g., [18,23,45]. Non-significance of BTC, MISR, MIRS, and MISL for Cd in this study compares with previous research findings (although involving different crop plants) reported by Baker [19].

4.3. Metal Extraction or Accumulation by Plants

Extraction of heavy metals was significantly different among plants: the highest by *B. napus* followed by *B. juncea* whereas, *E. sativa* extracted the lowest amounts of metals (not different from *B. rapa*), though root accumulated the highest amounts of metals followed by stem and the lowest by leaf in all crop plants (Figure 1). More specifically, *B. napus* had significantly higher metal extraction or accumulation compared to *E. sativa* for Zn (71%), Cu (69%), Fe (78%), Mn (79%), Cd (101%), Cr (57%), Ni (92%), and Pb (49%). The hyperaccumulation function of *B. napus* has been reported by Rattan, Datta [45], and is comparable with the findings of this study. Some exceptions were also recorded, for example, *B. napus* was not different from *E. sativa* for the accumulation of Ni, and *B. Juncea* for Mn. Likewise, *B. juncea* and *B. rapa* were similar in Cr concentrations. Overall, phytoextraction capacities were in the order of: *B. napus* > *B. juncea* > *B. rapa* > *E. sativa* in plants and root > stem > leaf in plant parts. The BAC, BTC, MISR, MIRS, and MISL values of all metals were also highest for *B. napus* except for Mn, which was the highest in *B. juncea*; however, the lowest Mn values were found in *B. rapa* which findings agree with those reported by Broadley et al. (2001; [53]) but disagree with Neilson and Rajakaruna (2012; [54]) who concluded that the rhizosphere processes, soil management, and the role of chelators may be more important than the physiological mechanisms (given in above paragraph) for the extraction and accumulation of metals. Contrary to their report, many accept that most crops accumulate heavy metals because of mass flow, exclusion, and/or inclusion mechanisms along the soil–root–stem–leaf continuum [47].

4.4. Metal Transfer or Accumulation along the Root-Stem-Leaf Continuum

Different water types produced different transfer or accumulation responses (BAC and BTC) in plants. Wastewater produced the highest values followed by the 1:1 combined water and canal water in that order (Table 3). Further, the metal transports or accumulatios (MISR, MIRS, MISL) increased as the irrigation water heavy metal concentrations increased—potentially, owing to the consistently higher negative water potential in the leaves compared to the potentials in stem and roots [55]. Additionally, dynamic evapotranspiration from leaves may result in in situ metal accumulations followed by lower accumulations in the stem and roots [55] in that order.

The four test plants had variably ≥1 BAC, BTC, MISR, MIRS, and MISL values which reflect that some crops were more efficient than others for the extractions, transports, or accumulations of some studied metals in response to the different water types. 1:1 BAC-BTC relationships showed that *B. napus* had the significant extraction, transport, or accumulation capacity for the largest number of test metals, including Zn, Cu, Fe, Mn, and Cd contents (Figure 2). The next greatest number of metal transports or accumulations (Mn, Cr) were shown by *B. juncea* and only Fe accumulation was found in *B. rapa*. Besides, these plant- and metal-specific, BAC-BTC significant regression models, the Pearson correlation coefficients shown by different crops were mostly very strong with a range of 0.76–0.99. Contrastingly, *B. rapa* did not show transfer–accumulation relationships for Cr and Cu, *B. napus* had no such relationship for Cr, and similarly, *E. sativa* did not reflect the relationship for Cd and Pb—reasons could be diverse based on previous reports, for example, the direct leaching nature of Ni, Cd, and Pb from coarse soils [40,56]. More specifically, the transitional nature and 10 valence electrons of Ni prefer long-term chelation with root exudates and/or organic acids secreted or present in the rhizosphere. Therefore, Ni or other metals leach or percolate with the mass flow of water or adhere to the root surface (due to the proton pump) until they are mineralized with free electrons required to move through the root membrane of the plant [56,57].

While most MIRS–MISL correlations (R^2) for the studied metals and crops were positive and significant, we found some relationships were negative but meaningful, for example, Mn MIRS–MISL relationship in *E. sativa* and Ni MIRS–MISL relationship in *B. juncea*. Based on most ratios ≥1 which reflect effective movement and/or accumulation from soil to root or root to stem or stem to leaf. The higher the coefficient value or ratio, the greater would be the phytoremediation or bioaccumulation efficiency of a crop [19,26–28], and the greater would be the risk to the health of humans consuming these vegetables. Further, there were significant correlations between most MIRS–MISL which reveal that movements or accumulations of most metals were maintained along the root-stem-leaf continuum, except the movements or accumulations of Mn in *E. sativa* and Ni in *B. juncea* were in the reverse direction—from leaves to roots. Mehes-Smith and Nkongolo [58] justified the exception of more Ni or other metal accumulations in roots than aboveground parts. Exposure to toxic metal irrigation water disturbs the plant cytological stability and causes significant mitotic disruption which leads to a heavy accumulation of toxic metals in roots. Dierssen, Herault [59] added that long-term exposure of roots to excessive metal concentrations may cause a variety of severe phenotypic syndromes. While the metal accumulation we debate, the plant/parts senescence or maturity could be one of the reasons for lower accumulation in aboveground plant parts [60,61]. While this research spanned only one growing season, repeated measures (seasons) research with the addition of soil texture and salinity variables may show consistency between the regression significance and Pearson correlation for most metal transports or accumulations along the root-stem-leaves route to generalize the results of this work. Further research is strongly recommended to investigate the edibility or valorization of the food crops irrigated with wastewater containing toxic metal concentrations.

5. Conclusions

The multivariate, toxic metal phytoextraction or accumulation we studied with the predictors of water and crop has important implications for (1) urban and suburban farmers who use wastewater for crops and/or planning to remediate their polluted soils, and (2) industrial ecologists who seek to remediate toxic heavy metal contaminated lands or treat contaminated waters using treatment wetlands in industrial settings. Irrigation with industrial wastewater contributes to an overall increase (potentially toxic levels) in heavy metal concentrations in plant roots > stem > leaf of all study crops such that *B. napus* > *B. juncea* > *B. rapa* > *E. sativa*. Overall, the BAC, BTC, MISR, MIRS, and MISL of the four plants or their parts were also affected by the wastewater irrigation in the same order. Therefore, these crops may not be used for human or animal consumption when grown with industrial wastewater of heavy metal concentrations ≥ permissible limits. Rather, these plants may be used for effective remediation of wastewater irrigated, heavy metal-contaminated soil. While this research spanned a single growing season, more repeated measures (seasons) research works with the addition of soil texture and salinity variables may show consistency between the BAC-BTC regression significance and Pearson correlation for most metal transports or accumulations along the root–stem–leaves route to support the results of this work. Further research is recommended to investigate the edibleness and/or valorization of the food crops irrigated with wastewater.

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