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Phytoremediation Characterization of Heavy Metals by Some Native Plants at Anthropogenic Polluted Sites in Jeddah, Saudi Arabia

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Abstract: Many anthropogenic activities have lately resulted in soil adulteration by heavy metals (HMs). The assessment of native plant species that grow in the polluted environments is of great importance for using these plants in phytoremediation techniques. This study was conducted in three industrial regions in Jeddah city, Wadi Marik, Bahra, and Khumrah, to assess the HM contamination level in them. This study also evaluated the phytoremediation ability of nine plant species collected from the studied regions. Soil physicochemical properties of the studied sites were investigated. Nine HMs, aluminum (Al), nickel (Ni), zinc (Zn), cobalt (Co), iron (Fe), lead (Pb), manganese (Mn), chromium (Cr), and barium (Ba), have been evaluated in the collected soil, plant shoots, and root samples. Total thiol concentration in the plant shoots and roots was determined. The phytoremediation indexes, such as bioaccumulation factor (BCF) and translocation factor (TF), were estimated. The results show that the soil of all the explored sites was sandy and slightly alkaline. It was found that Ni, Pb, and Cr were above the international permissible limit in all soil samples. The Wadi Marik region recorded the highest HM concentration compared to the other sites. In the Bahra region, Fe, Zn, Co, and Mn in all collected soil samples were below internationally permissible levels. In Khumrah, the highest concentration of Zn was found in the soil sample collected around F. indica plants, while Fe, Co, and Mn in all collected soil samples were below the international permissible limit. Depending on the BCF calculations, most of the investigated species showed phytostabilization ability for most of the studied HMs. Of them, *E. indica*, *T. nubica*, and *P. divisum* recorded the highest BCF values that ranged from 16.1 to 3.4. The BCF values of the studied HMs reduced in the order of Cr > Zn > Mn > Co > Ba > Fe > Al > Pb. Phytoextration of Co and Cr could be achieved by *P. oleracea* and *F. indica*, which showed TF values that reached 6.7 and 6.1, respectively. These plants showed high potential for phytoremediation and can be suggested as protective belts close to the contaminated regions of Jeddah.

Keywords: heavy metals; anthropogenic polluted areas; phytostabilization; phytoextraction bioaccumulation factor; translocation factor; thiols

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1. Introduction

Public health is increasingly concerned about environmental pollution with heavy metals (HMs) because it poses a considerable health risk [1]. Relatively dense metallic elements that are dangerous or toxic, even at low concentrations, are known as heavy metals. These elements refer to metalloids and metals with an atomic concentration more than 4 g/cm^3 or more than five times that of water [2]. Other metals are sometimes classified or treated as "heavy" metals, including aluminum (2.7 g/cm^3) and barium (3.6 g/cm^3) [2,3]. Raychaudhuri et al. [4] reported that Cd, Pb, Hg, Cr, and Al could be regarded as nonessential heavy metals that are not required by plants, even in trace amounts, for any of the metabolic processes. Heavy metal accumulation and distribution in plants are affected strongly by many factors, including ecological conditions; the physical, chemical, and biological properties of soil; plant species; rhizosphere; and root composition in addition to the element type [5].

The main source of HM pollution of soils, rivers, and groundwater is related to many anthropogenic activities, including industry, agricultural, urbanization, transportation, construction, and improper dumping of waste materials [6,7]. In soil, Cd, Ni, Cr, Hg, As, Pb, and Zn are the most commonly found toxic metals [7]. Earlier studies showed that different sites in Jeddah city, viz., Al-Arbaeen and Al-Shabab inlets (central Jeddah coast), were contaminated with high levels of Cu, Zn, Cd, Ni, Cr, and Pb [8]. Abu-Zied et al. [9] reported a possible anthropogenic sourcing for HM contamination in these areas. In addition, sediment samples collected near the Jeddah fish market showed high Zn, Cu, and Pb content. Pan et al. [10] and Bantan et al. [11] also recorded high levels of Mn, Cr, Pb, Zn, and Cu on the Jeddah coast and related this contamination to the influx of domestic and industrial wastewater into this area. These HMs are not degraded biologically; therefore, they endure in the soil through deposition and infiltration of polluted storm water runoff for several years [12]. This long-lasting pollution causes deleterious effects on soil fertility and microbiota [13].

Heavy metals cause many metabolic disorders, including organ damage, central nervous system retardation, and mental disorders, in human beings by disrupting enzyme activity; this effect is based on the period of exposure, dose, and HM type [14,15]. Thus, to avoid human health dangers from consuming contaminated crops, suitable removal methods of HMs from polluted soils are vital to decrease the harmful consequences on edible plants. There are many conventional physical and chemical methods to remove HMs from contaminated environments; however, these remediation procedures come at a high price, are time consuming, and are technically challenging [16]. As a result, different solutions for eliminating HMs from the contaminated soil are required. Phytoremediation is an environmental decontamination technique performed in situ using plant species to minimize the toxic effects of pollutants in water, soil, and air [17]. Thus, in contrast to the conventional engineering methods, phytoremediation offers an eco-friendly and low-cost solution to remediate contaminated soil without causing turbulences to the ecosystem [18,19]. Recently, Nkongolo et al. [20] reported that long-term phytoremediation of metal-contaminated soil increases soil organic matter, microbial abundance, and microbial diversity and function, where newly developed soils can sustain diverse microbial communities and associated vegetation. Phytoremediation is regarded as a promising technique by means of genetic engineering methods to enhance plant tolerance to HM stress and reduce dangerous metal accumulation. Recent investigations used omics technologies to decode the genetic factors underlying the mechanisms of HM tolerance in plants. There are many biotechnological methods used for HM phytoremediation, including the upregulation of several genes that regulate HM uptake, transport, and chelation, which change them into less dangerous and volatile forms [21].

There are several mechanisms involved through the phytoremediation of HMs reviewed by Bhat et al. [5]. Phytostabilization, phytoextraction, rhizofiltration, and phytovolatilization represent the main mechanisms that remediate HMs through phytoremediation. Phytostabilization is a process that uses HM-tolerant plant species for HM immobilization in polluted soils, reducing their bio-accessibility in the soil and hindering their leaching into the groundwater or incorporation into the food cycle [22,23]. On the other hand, phytoextraction is known as the mechanism in which HMs are taken up from the contaminated soil by plant roots and accumulated in the shoots. Therefore, in phytoextraction, HM-tolerant plants could remove HMs from a contaminated environment without changing the soil characteristics, which could happen with physical or chemical remediation methods [21,24].

In HM-tolerant plants, toxic HMs are usually kept in plant tissues, such as cuticles, epidermis, trichomes, or cell vacuoles, to reduce their harmful effects on cellular metabolism. The vacuoles represent the main storage place for HM accumulation, where it occupies up to 95% of the size of the cells [25]. The accumulation of HM ions in the vacuoles of shoots or roots is regarded as a fascinating plant strategy, where this compartment shows restricted activity of metabolism [13]. Toxic HM ions are accumulated in the vacuole after chelation by the binding of HM ions with various chelators and, thus, reducing its possible phytotoxicity [26]. Plants with a promising phytoremediation potential can synthesize various important thiol-rich compounds, such as metallothioneins, glutathiones, and phytochelatins, that could aid in HM uptake and transport [13].

It is important to use native plants for phytoremediation because the plant that mediates the clean-up should be adapted to the soil properties, toxicity level, and climate of the contaminated site [27]. Herbaceous species usually adapt faster to these conditions than do other plant species because their shorter life cycles allow for them to produce various genotypes in a shorter time [28]. Previous studies have investigated the phytoremediation potential of various plants in greenhouse experiments. However, only a few studies have been carried out under field conditions. Alzahrani et al. [29] found *Aerva javanica* to be an accumulator of Cu, Cr, and Ni in HM-contaminated industrial sites. Hyperaccumulation of Cd, Cr, and As was found in *Portulaca oleracea* naturally growing in an industrial effluent irrigated area [30]. El-Sherbiny et al. [31] showed that *T. coccineum* had a BCF of more than 1 for Zn; therefore, it can be considered a Zn accumulator. Eben et al. [32] reported that several native herbaceous plants on an HM-contaminated site had the potential for Cd, Cr, Cu, Ni, Pb, and Zn accumulation. Therefore, it is possible to identify metal-tolerant pioneer herbaceous plants among the natural vegetation of field sites contaminated with heavy metals.

Saudi Arabia is progressively influenced by numerous anthropogenic activities, which make it susceptible to pollution. Phyto-management techniques using native plant species permit the remediation of polluted soils using an inexpensive manner and avoid the environmental hazards accompanying the use of non-native species [33]. Therefore, this study aimed to assess the HM contamination level in three industrial regions in Jeddah, including Wadi Marik, Bahra, and Khumrah. Furthermore, this study evaluated the phytoremediation potential of nine native herbaceous plant species collected from the studied regions. These species are *A. iavanica*, *P. oleracea*, *E. indica*, *C. ciliaris*, *T. nubica*, *D. glaucoma*, *P. divisum*, *T.coccinea*, and *F. indica*.

2. Materials and Methods

2.1. Plant Collection and Identification

Nine different plant taxa belonging to 6 families were collected from 9 different sampling sites from 3 study areas (Wadi Marik, 21.53744° N, 39.29701° E; Bahra, 21°24′06″ N, 39°27′03″ E; and Khumrah, 21.33543° N, 39.24858° E), which are located in south and east of Jeddah, Saudi Arabia (Figure 1). The samples were collected in March 2020, the temperature ranged from 19 to 35 °C, the humidity was approximately 58%, and the speed of the wind was 27 km/h. These sites are located at the industrial region in Jeddah city. The collected plant species are *Avera javanica* (Family: Amaranthaceae); *Portulaca oleracea* (Family: Portulacaceae); *Tephrosia nubica* (Family: Fabaceae); *Dipterygium glaucoma* (Family: Capparaceae); *Tetraena coccinea* and *Fagonia indica* (Family: Zygophyllaceae); and *Eleusine indica*, *Cenchrus ciliaris*, and *Pennisetum divisum* (Family: Poaceae). For identification, whole plant samples were dried and preserved properly. For HM analysis, the shoot and root samples were washed gently using deionized water and dried at 70 °C for two days. They were separately powdered using an electronic grinder to a fine powder. Other samples were freshly frozen for total thiols determination at −80 °C.

Figure 1. Map showing study sites. Source: Google Map (Software used by Map maker 3.9.0_723). The blue symbol refers to the location of Jeddah city.

2.2. Soil Collection

The soil samples were collected using soil sampling tubes at a depth of 25 cm from each sampling point at each plant zone in the three study sites. Then, the samples were brought into the laboratory and oven-dried to a constant mass for 3 days at 60 °C. Then, the dried samples were grinded for a composite sample, sieved through a 2 mm screen to remove the stone pieces and large root particles, and used for soil analysis.

2.3. Soil Physicochemical Properties Analysis

Soil texture was determined following the method of Al-Yamani and Al-Desoki [34]. The soil pH was measured by a pH meter (METTLER-S220). Soil organic matter (OM) was investigated as described previously by Walz et al. [35]. Soil electrical conductivity and water content were determined according to Yousef [36] and Conklin [37].

2.4. Heavy Metal Analysis

The concentrations of Al, Ni, Zn, Co, Fe, Pb, Mn, Cr, and Ba were analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS). A total of 0.5 g of soil, shoot, and root samples was digested in 5 mL of HNO_3 and H_2O_2 (5:2, v/v) mixture as described by Tuzen et al. [38]. Calibration solutions were prepared by appropriate dilution of 10 mg/L multi-elemental stock solution in 5% HNO_3 (Multi-Element Calibration Standard 3, PerkinElmer, Cambridge, MA, USA). The calibration curves were constructed in the following concentration ranges: 0.1–100 g/L for Al and Fe and 0.01–20 g/L for Ni, Zn, Co, Pb, Mn, Cr, and Ba. The limits of detection values were as follows: Al 0.12 g/kg, Fe 0.32, Ni 0.09 g/Kg, Zn and Co 0.01 g/kg, Pb 0.05 g/kg, Mn and Cr 0.006, and Ba 0.007 g/kg.

2.5. Phytoremediation Indices Calculation

To evaluate the plant's efficiency for HM accumulation from soil, the bioaccumulation factor (BCF) was calculated. To investigate the plant's ability to translocate HMs from roots to shoots, the translocation factor (TF) was calculated. These phytoremediation indices were calculated based on the following formulas [39]:

$$\text{BCF} = \text{HM concentration in root}/\text{HM concentration in soil}$$

$$\text{TF} = \text{HM concentration in shoot}/\text{HM concentration in root}$$

BCF and TF are usually used to assess the effectiveness of a plant species to accumulate HMs and evaluate the potential of plants for phytostabilization and phytoextraction [40,41]. Plant species with BCF values more than 1, referring to the accumulation of a certain HM in the root, have the potential of phytoremediation. TF values more than 1 indicate that the plants translocate metals efficiently from roots to shoots. Therefore, plant species that have a BCF and TF more than 1 are appropriate for phytoextraction purposes, while if only the BCF is above 1, they exhibit phytostabilization potential [42].

2.6. Total Thiols Determination

Sulfhydryl (-SH) groups in plant samples were determined using Ellman's spectrophotometric technique. In liquid N2, 0.1 g of fresh shoots and roots was crushed into a fine powder. The samples were then extracted in 1.5 mL of phosphate buffer pH (7.4) having 0.02% MEDTA. Then, the extracts were centrifuged for 15 min at 4 °C at 14,000 g after being mixed with a vortex. A total of 0.5 mL of the supernatant was combined with 1.5 mL of Tris buffer 0.2 M, pH (8.2) and 1 mL of (5,5 Dithiobis 2-Nitrobenzoic Acid (0.01 M) generated in the 0.05 M Na-acetate. Before measuring the absorbance, the reaction was allowed to progress for 20 min at room temperature. At 405 nm, the absorbance was measured; the thiol content was calculated based on the extinction coefficient of 13.100 M^{-1} cm^{-1} [43].

2.7. Statistical Analysis

SPSS Statistics V22.0 was utilized to statistically analyze the results. To evaluate the significant differences between plants, roots, and soils, the data were assessed using some means and one-way ANOVA and were examined at $p \leq 0.05$ significance levels using a post hoc test.

3. Results and Discussion

3.1. Physical and Chemical Characteristics of Soil

Zhou et al. [44] and Rosenfeld et al. [45] reported that HM bioavailability to plants is affected by soil properties, including soil acidity or alkalinity, soil texture, cation exchange capacity, water and organic matter content, etc. The soil characteristics of the study area in this study are shown in Table 1. No signifcant variations were recorded in soil pH value and water and organic matter (%) content between the studied sites. On the other hand, significant variations were registered in the soil's electrical conductivity (µS/cm) value between the studied sites. It was found that the soil samples collected around *C. ciliaris* roots have the highest EC value (1.71) followed by soil samples collected around *E. indica* roots (1.18). The least EC value (0.28) was recorded in the soil samples collected sround *P. oleracea* root. The soil texture of all collected soil samples were found to have the same profile: sand > coarse sand > silt > clay. The soils showed a slight alkaline condition. The soil alkalinity in this study is in accordance with the findings presented by other studies in different locations south and west of Jeddah [46,47]. Soil pH is an important variable controlling the availability of a given element in the soil for plant uptake; it impacts mainly the solubility of metals. Low soil pH increases HM content in the soil solution [48]. By increasing soil acidity, the hydrogen ion concentration will increase, which rises the cation exchange capacity for HMs, and the desorption process accelerates the penetration of HMs from the bulk into the soil surface [48]. Nevertheless, HM activation is not always generated by soil pH reduction for all toxic elements [49]. Heavy metal solubility also rises by increasing soil organic matter, which encourages metal chelation, improves nutrient amounts, and boosts cation exchange efficiency, which, finally, increases metal accessibility to plants [50–52].

Table 1. Physicochemical characteristics of soil samples collected from different sampling points at the study sites.

Site	*** Sampling Point**	**PH**	**EC (µS/cm)**	**WC (%)**	**OM (%)**	**Soil Texture**			
						Coarse Sand (%)	**Sand (%)**	**Silt (%)**	**Clay (%)**
Wadi Marik	A	8.00	0.34	49.21	4.86	28.97	60.46	6.21	0.68
	B	7.79	0.28	49.33	4.82	12.21	78.21	8.95	0.63
	C	7.74	1.18	49.34	4.83	14.72	78.51	5.63	0.57
Bahra	D	7.84	1.71	49.13	4.79	18.16	70.54	9.57	0.01
	E	7.93	0.34	48.95	4.74	16.33	78.17	4.44	0.61
	F	7.70	0.74	49.09	4.75	12.38	78.64	6.17	1.55
Khumrah	G	7.67	0.46	49.68	4.86	22.44	63.03	10.57	3.47
	H	7.62	1.10	49.42	4.88	25.99	66.9	4.41	1.19
	I	7.69	0.40	49.80	4.90	23.1	68.93	5.53	1.07

* Sampling points A, B, C, D, E, F, G, H, and I represent the soil samples collected around the roots of *A. javanica*, *P. oleracea*, *E.indica*, *C. ciliaris*, *T. nubica*, *D. glaucoma. divisum*, *T. coccinea*, and *F. indica*, respectively.

3.2. Heavy Metals in Soil

The level of soil contamination with HMs is usually indicated by their concentration [53]. The soil HM concentrations in the sites under this investigation are presented in Table 2. In the three studied areas, Al, Ni, Zn, Co, Fe, Pb, Mn, Cr, and Ba concentrations ranged from 68.23 to 10.05, 0.41 to 0.20, 0.67 to 0.05, 0 to 0.04, 61.56 to 8.24, 0.68 to 0.30, 1.58 to 0.15, 0.11 to 0.3, and 0.42 to 0.04 g/kg, respectively. It was remarkable that all studied HMs showed a similar distribution, whose concentrations recorded the highest HM values in d in the soil samples collected around the plant species collected from Wadi Marik, while the least concentrations were recorded around the Bahra plants. Ni and Cr far exceeded the standard tolerable level for soil as indicated by Lee et al. [54], demonstrating that the soil samples of all sites were severely contaminated with these HMs. The soil samples collected around *A. javanica* roots showed the highest concentrations of Al, Co, Fe, Mn, Cr, and Ba. On the other hand, the soil samples collected around *T. nubica* showed the least contents of Al, Zn, Fe, Mn, and Ba.

All the studied sites in this investigation are located at the industrial region in the south and west of Jeddah city. The possible sources of HMs in the studied areas could be anthropogenic activities released from the improper disposal of industrial and municipal wastes. Halawani et al. [55] recently reported that the south of the Jeddah coast is construed to be contaminated by the wastes of the industrial activity in the industrial city. Industry, transportation, waste burning, and power generation are regarded as the main sources of HM accumulation into various environments [56]. In the same context, Halawani et al. [55] described in their investigation that the sediments at different sites in the middle and south locations in Jeddah were polluted with high levels of various HMs, including Pb (150.59), Mn (37.72), Zn (23.94), Cr (9.56), Cu (9.18), and Ni (4.54) in mg/kg. The southern corniche of Jeddah has also been recorded to be contaminated with many HMs, including Fe, Mn, Cu, Pb, Cr, Zn, Ni, and Co, due to the disposal of domestic and industrial wastes [10]. Alhogbi and Alsolame [57] also recorded toxic levels of As, Co, Cr, Hg, Ni, V, Pb, and Zn in many areas in Jeddah city due to the indiscriminate influx of dangerous waste materials.

3.3. Heavy Metals in Plants

Heavy metal accumulation in the shoots of the plants gathered from the different sites is shown in Table 3. The shoots of the plant species collected from Wadi Marik had the highest values of HMs in all investigated plant species. On the other hand, the shoots of the plant species collected from Khumra had the least values of HMs in all investigated plant species. *A. javanica* shoots have the highest values of Fe, Pb, and Mn. *P. oleracea* shoots have the highest values of Al, Ni, Cr, and Ba, while *E. indica* showed the highest values of Zn and Co. However, *T. coccinea* shoots showed the least concentrations of Al, Zn, Fe, Pb, Mn, and Ba. In the same context, *F. indica* shoots recorded the least concentrations of Ni, while the least concentrations of Co and Cr were recorded in *D. glaucoma* and *T. nubica* shoots, respectively. Fe was not detected in all soil samples collected from Bahra. The concentration of all investigated HMs in all samples collected from Wadi Marik was much higher than the allowable concentrations in plants [58–60]. The Ni and Cr concentrations in the shoot samples of all plant species collected from Bahra and Khumrah were also higher than the allowable concentrations in plants (Table 3).

Heavy metal accumulation in the roots of the plants collected from the different sites is shown in Table 4. It was observed that the Ni concentration was less than the detection limit in the roots of all examined plant species. The roots of *P. divisum* collected from Khumrah had the highest values of Al, Mn, Cr, and Ba in all investigated plant species, while the roots of *E. indica* had the highest concentrations of Zn, Co, and Fe. The highest concentration of Pb was recorded in *P. oleracea* roots. On the other hand, *F. indica* roots indicated the lowest concentrations of Co, Mn, and Cr. The lowest concentrations of Zn, Fe, Pb, and Ba were recorded in the roots of *A. javanica*, *P. oleracea*, *T. coccinea*, and *E. indica*, respectively (Table 3). The concentrations of Zn, Fe, Pb, and Cr in the roots of all investigated plant species were much higher than the allowable concentrations in plants [58–60]. Furthermore, the Mn concentration in *P. oleracea*, *E. indica*, *C. ciliaris*, *T. nubica*, and *P. divisum* roots was higher than the allowable concentrations in plants. Co concentration in *E. indica* and *C. ciliaris* roots was higher than the allowable concentrations in plants (Table 4). Zhu et al. [24] also found that Ni and Cr contents were higher than the normal levels, which were 0.1–10 mg/kg for Ni and 0.2–0.8 mg/kg for Cr.

The variation in the total HM concentrations that is shown in Tables 3 and 4 and their bio-accessibility could result in differential metal accumulation in plants. Root pressure and leaf transpiration are the main factors that control metal translocation in plants from roots to shoots. Except for Ni, the HM concentrations in the plant roots were much higher than those in the shoots for the investigated species. Therefore, based on the results of this study and as confirmed by other findings, herbaceous plants accumulate HMs mainly in the roots rather than in the aerial parts [33,61,62]. Plant roots can immobilize HMs through adsorption of precipitation in rhizosphere soil [63]. The studied plants in this investigation had variable tolerance and accumulation features for Al, Ni, Zn, Co, Fe, Pb, Mn, Cr, and Ba based on the plant species. After a long period of natural selection, native plant species could have established a defense strategy against the elevated HM levels to establish HM tolerance mechanisms and support their growth and development [64,65]. The native herbaceous plants that were investigated in this study are tolerant to Al, Ni, Zn, Co, Fe, Pb, Mn, Cr, and Ba and could be utilized as pioneer species or potential phytoremetiators for polluted soils. These plants are capable of withstanding the harsh mine environment around them with their small and light seeds that are easily and widely distributed by the wind. Moreover, they generally grow densely, can form a single species, and can also easily build a community with other plants [66]. Thus, they were often applied for HM phytoremediation in polluted soils [67].

Table 2. Heavy metal concentration (g/kg) in the soil samples collected from different sampling points at the study sites.

Site	* Sampling Point	Al	Ni	Zn	Co	Fe	Pb	Mn	Cr	Ba
Wadi Marik	A	68.23 ± 3.40 ^{a}	0.24 ± 0.01 ^{d}	0.24 ± 0.01 ^{c}	0.04 ± 0 ^{bc}	61.56 ± 5.90 ^{a}	0.47 ± 0.02 ^{b}	1.58 ± 0.03 ^{a}	0.11 ± 0 ^{ab}	0.42 ± 0 ^{a}
	B	20.04 ± 0.94 ^{fg}	0.41 ± 0.01 ^{a}	0.12 ± 0 ^{d}	0.04 ± 0 ^{abc}	18.97 ± 1.70 ^{g}	0.68 ± 0 ^{a}	0.36 ± 0.02 ^{ef}	0.03 ± 0 ^{gh}	0.08 ± 0 ^{g}
	C	28.32 ± 0.73 ^{d}	0.32 ± 0.02 ^{bc}	0.15 ± 0.01 ^{d}	0.03 ± 0 ^{bcd}	25.87 ± 2.60 ^{de}	0.37 ± 0.02 ^{c}	0.55 ± 0 ^{bc}	0.09 ± 0 ^{bc}	0.12 ± 0 ^{bcd}
Bahra	D	34.53 ± 1.53 ^{c}	0.34 ± 0.01 ^{abc}	0.23 ± 0.01 ^{c}	0.06 ± 0 ^{a}	29.21 ± 1.33 ^{cd}	0.32 ± 0.01 ^{cde}	0.56 ± 0.02 ^{bc}	0.07 ± 0 ^{cde}	0.13 ± 0 ^{bc}
	E	10.05 ± 1.87 ^{h}	0.27 ± 0.04 ^{cd}	0.05 ± 10 ^{e}	0.04 ± 0 ^{abcd}	8.24 ± 0.55 ^{h}	0.45 ± 0.02 ^{b}	0.15 ± 0.03 ^{h}	0.04 ± 0.01 ^{fgh}	0.04 ± 0 ^{h}
	F	14.77 ± 0.40 ^{gh}	0.20 ± 0.1 ^{d}	0.11 ± 0 ^{d}	0.04 ± 0 ^{bcd}	11.43 ± 1.50 ^{h}	0.30 ± 0 ^{de}	0.22 ± 0.07 ^{gh}	0.06 ± 0.01 ^{de}	0.04 ± 0 ^{h}
Khumrah	G	24.8 ± 0.89 ^{ef}	0.32 ± 0.01 ^{bc}	0.03 ± 0 ^{e}	0.02 ± 0 ^{de}	21.78 ± 3.90 ^{fg}	0.36 ± 0.01 ^{cd}	0.38 ± 0.01 ^{ef}	0.03 ± 0 ^{h}	0.08 ± 0 ^{fg}
	H	33.8 ± 0.46 ^{cd}	0.38 ± 0.05 ^{ab}	0.25 ± 0 ^{c}	0.02 ± 0 ^{cde}	26.97 ± 4.20 ^{de}	0.37 ± 0.04 ^{c}	0.45 ± 0 ^{de}	0.09 ± 0.01 ^{bc}	0.07 ± 0 ^{g}
	I	49.80 ± 2.19 ^{b}	0.34 ± 0.02 ^{abc}	0.67 ± 0.03 ^{a}	LD **	35.42 ± 1.55 ^{b}	0.34 ± 0 ^{cde}	0.61 ± 0.02 ^{b}	0.10 ± 0 ^{a}	0.12 ± 0 ^{bcd}
Tolerable Level (g/kg) for soil [54]		-	0.04	0.30	0.10	50	0.10	2	0.004	-

* Sampling points A, B, C, D, E, F, G, H, and I represent the soil samples collected around the roots of *A. javanica*, *P. oleracea*, *E.indica*, *C. ciliaris*, *T. nubica*, *D. glaucoma. divisum*, *T. coccinea*, and *F. indica*, respectively. ** LD = Less than detection limit. Different lowercase letters mean the values are significantly different at $p \leq 0.05$, based on the post hoc test.

Table 3. Heavy metal concentration (g/kg DW) in the shoots of investigated plants at the study sites.

Site	Plant Species	Al	Ni	Zn	Co	Fe	Pb	Mn	Cr	Ba
Wadi Marik	*A. javanica*	0.95 ± 0.10 ^{cd}	0.37 ± 0.01 ^{ab}	0.08 ± 0 ^{c}	0.05 ± 0 ^{ab}	2.72 ± 0.26 ^{a}	0.50 ± 0.03 ^{b}	0.11 ± 10 ^{a}	0.30 ± 0 ^{c}	0.06 ± 0 ^{b}
	P. oleracea	1.13 ± 0.07 ^{bc}	0.42 ± 0.03 ^{a}	0.15 ± 0 ^{b}	0.04 ± 0 ^{d}	2.27 ± 0.02 ^{ab}	0.20 ± 0.01 ^{f}	0.06 ± 0 ^{b}	0.42 ± 0.01 ^{a}	0.11 ± 0 ^{a}
	E. indica	0.86 ± 0.08 ^{d}	0.41 ± 0.01 ^{a}	0.26 ± 0.02 ^{a}	0.05 ± 0 ^{ab}	1.63 ± 0.03 ^{bc}	LD	0.05 ± 10 ^{bc}	0.19 ± 0.01 ^{e}	0.03 ± 0 ^{d}
Bahra	*C. ciliaris*	0.47 ± 0.01 ^{e}	0.30 ± 0.01 ^{cd}	0.06 ± 0 ^{f}	0.04 ± 0 ^{bc}	LD	0.27 ± 0 ^{e}	LD	0.13 ± 0.02 ^{f}	0.01 ± 0 ^{ef}
	T. nubica	0.71 ± 0.1 ^{de}	0.30 ± 0.01 ^{cd}	0.03 ± 0 ^{ef}	0.03 ± 0 ^{d}	LD	0.20 ± 0 ^{f}	0.01 ± 0 ^{cd}	0.19 ± 0.01 ^{e}	0.01 ± 0 ^{ef}
	D. glaucoma	0.39 ± 0 ^{ef}	0.25 ± 0.01 ^{de}	0.03 ± 0 ^{ef}	LD	LD	0.17 ± 0 ^{g}	0.02 ± 0 ^{bcd}	0.12 ± 0 ^{f}	0.01 ± 0 ^{ef}
Khumrah	*P. divisum*	0.19 ± 0.07 ^{f}	0.34 ± 0.01 ^{bc}	0.04 ± 0 ^{de}	0.02 ± 0 ^{e}	0.48 ± 0 ^{d}	0.07 ± 0 ^{h}	LD	0.34 ± 0 ^{b}	0.01 ± 0 ^{e}
	T. coccinea	LD *	0.35 ± 0 ^{bc}	0.02 ± 0 ^{f}	0.03 ± 0 ^{e}	LD	LD	LD	0.29 ± 0.02 ^{c}	LD
	F. indica	1.41 ± 0 ^{a}	0.22 ± 0.01 ^{e}	0.05 ± 0 ^{de}	0.02 ± 0 ^{e}	0.54 ± 0.11 ^{d}	0.30 ± 0 ^{d}	0.01 ± 0 ^{cd}	0.24 ± 0 ^{d}	0.01 ± 0 ^{f}
Tolerable Level (g/kg) for plants [58–60]		-	0.01	0.05	0.05	0.07	0.0001–0.0003	0.5	0.0001–0.0003	-

* LD = Less than detection limit. Different lowercase letters mean the values are significantly different at $p \leq 0.05$, based on the post hoc test.

Table 4. Heavy metal concentration (g/kg DW) in the roots of investigated plants at the study sites.

Site	Plant Species	Al	Ni	Zn	Co	Fe	Pb	Mn	Cr	Ba
Wadi Marik	*A. javanica*	6.47 ± 0.13 ^{g}	LD *	0.08 ± 0.01 ^{h}	0.03 ± 0 ^{ef}	11.59 ± 0.10 ^{g}	0.38 ± 0 ^{b}	0.35 ± 0 ^{g}	0.52 ± 30 ^{a}	0.21 ± 0 ^{c}
	P. oleracea	3.34 ± 0.02 ^{hi}	LD	0.99 ± 0.01 ^{c}	LD	7.05 ± 0.01 ^{h}	0.38 ± 0.01 ^{b}	1.11 ± 0.01 ^{d}	0.45 ± 0.02 ^{b}	0.30 ± 0 ^{b}
	E. indica	8.61 ± 0.14 ^{f}	LD	1.92 ± 0.01 ^{a}	0.11 ± 0 ^{a}	68.65 ± 0.61 ^{a}	0.20 ± 0 ^{ef}	1.39 ± 0.01 ^{c}	0.19 ± 0.01 ^{d}	0.13 ± 30 ^{e}
Bahra	*C. ciliaris*	44.03 ± 1.96 ^{b}	LD	1.75 ± 0.11 ^{b}	0.07 ± 0 ^{b}	53.12 ± 0.18 ^{b}	0.35 ± 0.01 ^{b}	2.09 ± 0.07 ^{b}	0.30 ± 0.02 ^{c}	0.35 ± 0 ^{a}
	T. nubica	31.47 ± 0.30 ^{c}	LD	0.31 ± 0.02 ^{d}	0.02 ± 0 ^{f}	29.91 ± 0.38 ^{d}	0.23 ± 0 ^{de}	0.77 ± 0 ^{f}	0.14 ± 0.01 ^{de}	0.19 ± 0 ^{c}
	D. glaucoma	11.98 ± 0.13 ^{e}	LD	0.17 ± 0.01 ^{efg}	0.02 ± 0 ^{f}	14.70 ± 0.19 ^{f}	0.36 ± 0.01 ^{b}	0.31 ± 0.01 ^{g}	0.03 ± 0.02 ^{gh}	0.06 ± 0 ^{fg}
Khumrah	*P. divisum*	85.02 ± 0.34 ^{a}	LD	0.28 ± 0 ^{d}	0.05 ± 0 ^{c}	47.71 ± 0.19 ^{c}	0.29 ± 0 ^{c}	2.62 ± 0.02 ^{a}	0.52 ± 0.02 ^{a}	0.35 ± 0 ^{a}
	T. coccinea	2.87 ± 0.11 ^{ij}	LD	0.12 ± 0 ^{gh}	0.03 ± 0 ^{ef}	4.91 ± 0.11 ^{i}	0.19 ± 0 ^{f}	0.11 ± 0 ^{i}	LD	0.04 ± 0 ^{gh}
	F. indica	4.87 ± 0.05 ^{h}	LD	0.25 ± 0 ^{def}	0.01 ± 0 ^{gh}	6.48 ± 0.07 ^{h}	0.25 ± 0.01 ^{d}	0.16 ± 0 ^{hi}	0.10 ± 0.04 ^{ef}	0.05 ± 0 ^{gh}

* LD = Less than detection limit. Different lowercase letters mean the values are significantly different at $p \leq 0.05$, based on the post hoc test.

3.4. Heavy Metal Accumulation and Translocation Potential Assessment

Where the soil HM content in the study sites was much higher than the standard tolerable level for soil, as indicated by Lee et al. [54], it is essential to investigate the potential of HM accumulation and transport by the studied plant species. The BCF and TF of HMs in nine native plants collected from the study area are demonstrated in Figures 2 and 3. The Ni concentration in the roots of all investigated species was less than the detection limit; therefore, the BCF and TF were not calculated. Within the studied plant species, the main values of the BCFs of the HMs gradually reduced in the order of Cr > Zn > Mn > Co > Ba > Fe > Al > Pb. It is interesting to remark that, for all investigated HMs, there is one or more plant species with BCF > 1. *P. divisum*, *E. indica*, and *T. nubica* showed the strongest enrichment ability to most of the studied HMs. They showed high potential in eliminating HMs from the soil, where the BCFs of *P. divisum* ranged from 16.1 (for Cr) to 3.4 (for Al), the BCFs of *E. indica* ranged from 12.3 (for Zn) to 5.6 (for Co), and the BCFs of *T. nubica* ranged from 4.3 (for Ba) to 3.9 (for Fe). (Figure 2).

In general, the chief concern of studying these herbaceous species could be using them in phytostabilization practices. The phytostabilization capability of the herbaceous plant species has been recorded and deliberated in many other investigations that could confirm the outcomes of this study. For example, Eben et al. [32] recorded that many herbaceous species exhibited a very high BCF for Cd, Cr, Cu, Ni, Pb, and Zn: *Thlaspi caerulescens* (22.3), *Avena sativa* (2.29), *Trifolium pratense* (2.06), *Pisum sativum* (1.86), and *Thlaspi caerulescens* (1.88). Therefore, these plants were projected for phytostabilization to remediate HM-contaminated soil. Based on the BCF, some herbaceous plants, including *Boehmeria nivea*, *Chrysanhemum indicum*, *Miscanthus floridulus*, *Conyza canadensis*, *Rubus setchuenensis*, *Senecio scandens*, and *Arthraxon hispidus*, displayed amazing phytostabilization capacities of Cr, Cd, Ni, and Cu, which could be utilized as prospective phytoremediation species [62].

Phytoextraction is regarded as the main mechanism behind HM transport from the roots to the shoots of the plants, which is especially important for phytoremediation [68]. The highest value of TF among the studied plants was recorded for Co (6.7), while the lowest value was recorded for Fe and Mn (0.32). The main values of the TFs of the studied HM declined in the order of Co > Cr > Pb > Zn > Ba > Al > Fe > Mn. As shown in Figure 3, *A. javanica* (for Co, Pb, Zn), *F. indica* (for Co, Cr, Pb), *T. nubica* (for Co, Cr), *P. oleracea* (for Co), and *D. glaucoma* and *T. coccinea* (for Cr) showed strong transfer ability (TF > 1). *P. oleracea* and *F. indica* had the highest TF values, reaching 6.7 and 6.1 for Co and Cr, respectively, showing an enhanced accumulation of HMs in the shoot related to the roots; thereby, they could be qualified as hyperaccumulators. Alzaheani et al. [29] also mentioned that *A. javanica* was observed to accumulate Cr, Ni, and Pb in the leaves. Moreover, accumulation of Cr t in plant shoots is in accordance with the results of Pourkhabbaz et al. [69] and Iqbal et al. [70], who reported that Cr content was higher in the shoots of *Platanus orientals* and *Aloe vera* species growing in a contaminated zone. In the same context, some herbaceous plants recorded a TF of more than one, e.g., *Phalaris arundinacea* with 1.36 for Zn and *Brassica juncea* with 1.61 for Cd [32]. It is known that plants can synthesize metabolites that promote the translocation of these HMs to the aerial parts; these include chelators, metallothioneins, glutathione, and phytochelatins [71].

On the other hand, the TF is below 1 in all studied plants in this investigation for Al, Fe, Mn, and Ba, indicating that these HMs were minimally translocate to the shoots and accumulated mostly in the roots (Figure 3). The major accumulation of HMs in the roots more than in shoots shows limited movement of HM ions through the plants. These findings are supported by the results of previous studies [72,73]. This is regarded as an important strategy for tolerating increased concentrations of HMs used by metal excluders, which accumulate high amounts of HMs in the roots and limit their transfer to aerial parts [74]. In phytoremediation, HM contamination in the soil could be steadily decreased via phytoextraction with high sequestration and transport capability. Phytoremediation could be achieved also by phytostabilization with a high BCF and low TF [75].

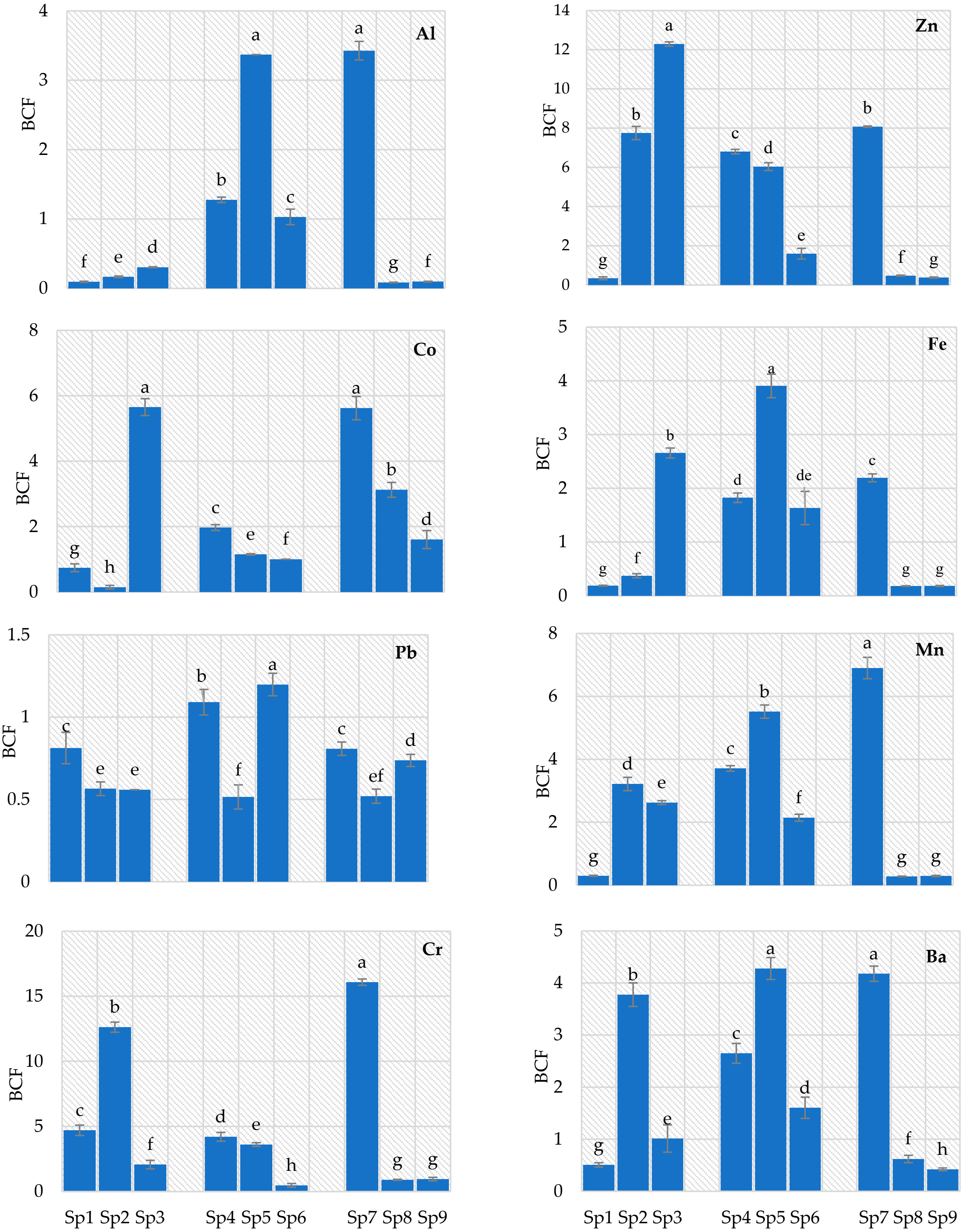

Figure 2. Bioaccumulation factor (BCF) of investigated plants in the study areas. Vertical bar represents SD ($n = 3$), while different lowercase letters mean the values are significantly different at $p \leq 0.05$, based on the post hoc test. SP1, Sp2, Sp3, Sp4, Sp5, Sp6, Sp7, Sp8, and Sp9 refer to *A. javanica*, *P. oleracea*, *E. indica*; *C. ciliaris*, *T. nubica*, *D. glaucoma*, *P. divisum*, *T. coccinea*, and *F. indica*, respectively.

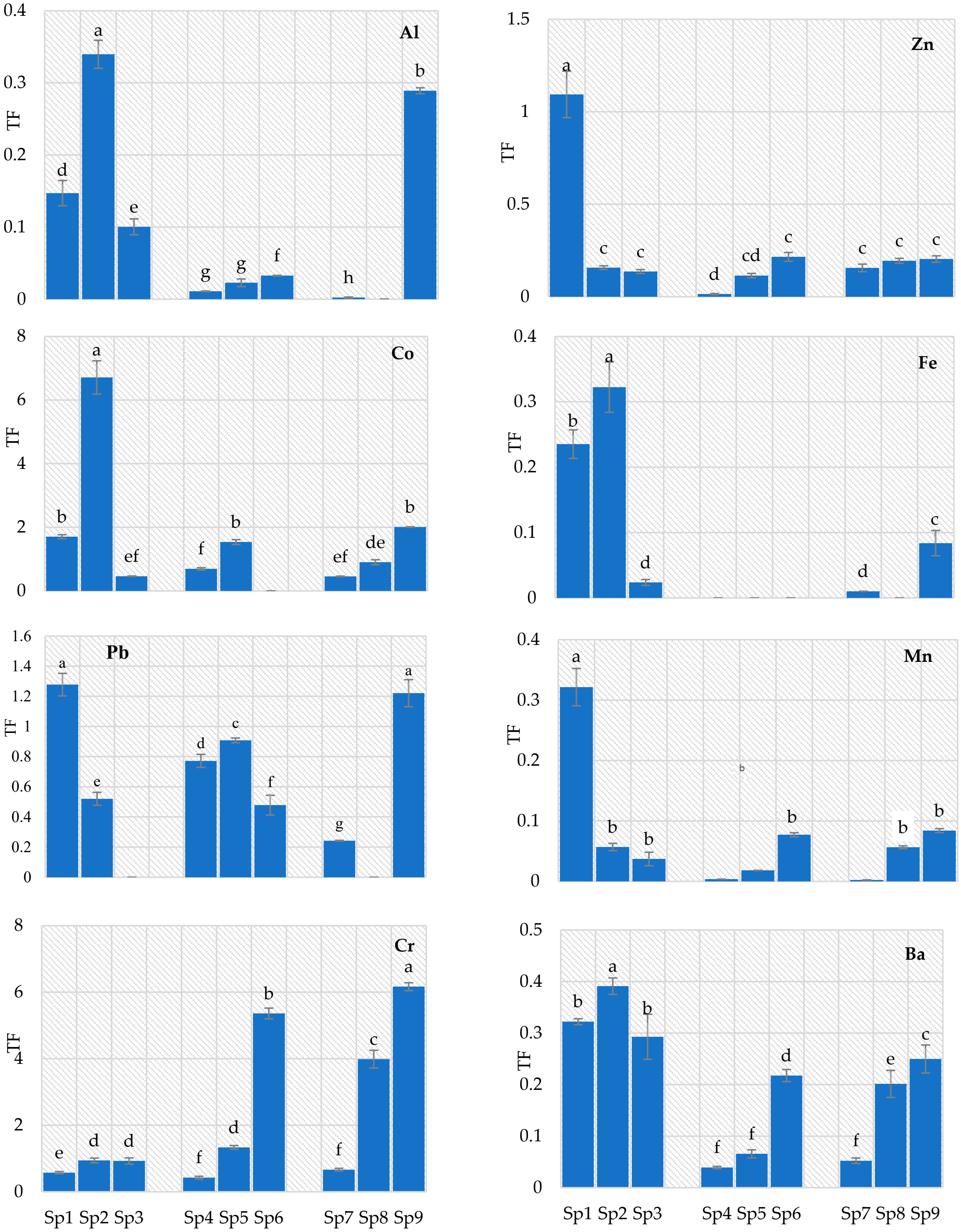

Figure 3. Transfer factor (TF) of investigated plants in the study areas. Vertical bar represents SD ($n = 3$), while different lowercase letters mean the values are significantly different at $p \leq 0.05$, based on the post hoc test. SP1, Sp2, Sp3, Sp4, Sp5, Sp6, Sp7, Sp8, and Sp9 refer to *A. javanica*, *P. oleracea*, *E. indica*; *C. ciliaris*, *T. nubica*, *D. glaucoma*, *P. divisum*, *T. coccinea*, and *F. indica*, respectively.

Plant reaction to HMs in soil is influenced by plant type, metal bioavailability, and metal content of the soil [76]. The results recorded in Table 5 conclude a solid capacity of the native plant *P. divisum* for accumulation of Zn, Co, Fe, Mn, and Ba. Some other native plants were also recorded with a comparable metal enrichment capability with a low TF and high BCF value (*P. oleracea* for Cd, Zn, Mn, Cr, and Ba; *E. indica* for Zn, Co, Fe, Mn, and Cr; *C. ciliaris for* Zn, Co, Fe, Mn, Cr, and Ba; *T. nubica* for Zn, Fe, Mn, Cr, and Ba; and *D. glaucoma* for Zn, Fe, Pb, Mn, and Ba) that can absorb and stabilize HMs in the rhizosphere. Generally, the soil in the study area is contaminated to varying degrees by Al, Ni, Zn, Co, Fe, Pb, Mn, Cr, and Ba. Four species showed good phytoextraction ability, including *F. indica*, *D. glaucoma*, *T. coccinea*, *T. nubica* for Cr, and *P. oleracea* for Co, while *A. javanica*, *P. oleracea*, *E. indica*, *C. ciliaris*, *T. nubica*, *D. glaucoma*, *P. divisum*, and *T. coccinea* showed notable phytostabilization ability. Due to the herbaceous nature of the investigated plants, they could produce a large quantity of biomass, settle, grow, and reproduce in the harsh environment rapidly. Therefore, they are suggested as phytoremediation candidates to clean HM-contaminated soils in this area. The species that showed BCF and TF values more than 1 are promising to be applied in HM phytoextraction [77,78]. Therefore, they will be most applicable to soil contaminations that are not so deep from the soil surface, are relatively non-leachable, and cover a large area. Moreover, integrating phytoextraction with complementary techniques, such as phytostabilization and bioremediation, may offer sustainable land rehabilitation efforts with synergistic benefits to conserve soil as a resource, bring soil back into beneficial use, prevent the spread of pollution to air and water, and reduce the pressure for development on green or agricultural field sites.

Table 5. The heavy metal removal potential of the investigated plants at the study sites.

Site	Plant Species	Heavy Metal	Function
Wadi Marik	*A. javanica*	Cr Co	Phytostabilization, Phytoextraction
	P. oleracea	Zn, Mn, Cr, Ba Co	Phytostabilization, Phytoextraction
	E. indica	Zn, Co, Fe, Mn, Cr	Phytostabilization
Bahra	*C. ciliaris*	Zn, Co, Fe, Mn, Cr, Ba	Phytostabilization
	T. nubica	Zn, Fe, Mn, Cr, Ba Cr	Phytostabilization, Phytoextraction
	D. glaucoma	Zn, Fe, Pb, Mn, Ba Co, Cr	Phytostabilization, Phytoextraction
Khumrah	*P. divisum*	Zn, Co, Fe, Mn, Ba	Phytostabilization
	T. coccinea	Co Cr	Phytostabilization, Phytoextraction
	F. indica	Co, Cr	Phytoextraction

3.5. Total Thiols

The total concentration of thiols in the studied plant species is shown in Figure 4. It is worth mentioning that the thiol concentration in the plant roots was three times higher than that in the shoots in all species. The highest thiol concentration was recorded in *T. nubica* root (1.63 mg/g fresh weight), followed by *A. javanica* root (1.51 mg/g FW). No considerable differences were recorded in thiol concentration between the roots of the other species. On the other hand, marked differences were observed in thiol concentration in shoot samples between the examined species. *A. javanica* shoot recorded the highest value of approximately 0.52 mg/g FW, followed by *D. glaucoma* (0.45 mg/g FW) and *T. nubica* (0.41 mg/g FW). The least concentration of total thiols was recorded in *C. ciliaris* and *P. divisum* shoots, which reached a value of approximately 0.33 mg/g FW (Figure 4).

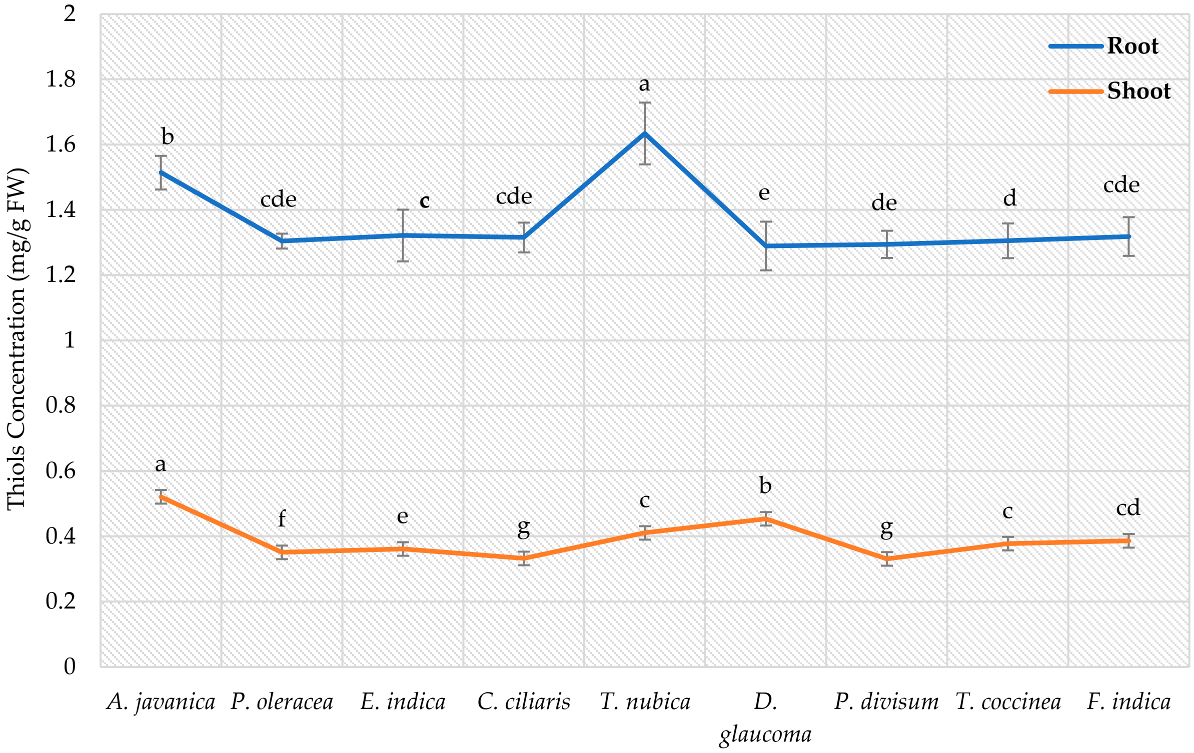

Figure 4. Total thiol concentration in roots and shoots of the investigated plant species. Vertical bar represents SD ($n = 3$), while different lowercase letters mean the values are significantly different at $p \leq 0.05$, based on the post hoc test.

Heavy metal detoxification or sequestration is enhanced in tolerant plants by the production of various thiols, such as MTs, PCs, and GSH, which are used as metal chelators that bind to the HMs through their sh-groups [79]. For example, the upregulation of the pea MT gene PsMTA enhanced Cu accumulation in plants [80]. As can be concluded from the findings of Čabala et al. [81], the tolerant *Vicia faba* cultivar exhibited a stronger capacity to accumulate Cd in the roots, which related to enhanced synthesis of PCs compared to the sensitive cultivar. The stimulation of PC synthesis in response to HM exposure was also recorded in recent studies for Cd and Pb in *Brassica parachinensis* and *B. juncea*, respectively [82,83]. After chelation, HMs might be compartmentalized into root vacuoles or transported to plant shoot systems through the xylem [84].

4. Conclusions

Nine herbaceous plants belonging to six families collected from three study areas were investigated in this study. All soil samples in this study are sandy, slightly alkaline, and contaminated mainly by Al, Ni, Zn, Co, Fe, Pb, Mn, Cr, and Ba. Wadi Marik recorded the highest values for all studied HMs. The concentration of all investigated HMs in all shoot samples collected from Wadi Marik was much higher than the allowable concentrations in plants. The Ni and Cr concentrations in the shoot samples of the plant species collected from Bahra and Khumrah were also higher than the allowable concentrations in plants. Zn, Fe, Pb, and Cr concentrations in the roots of all investigated plant species far exceeded the allowable concentrations in plants. All plant species accumulated thiols in the root three times higher than those in the shoot, which could illustrate the higher phytostabilization capacity of the studied species. Based on HM content, BCF, and TF in the plants, it could be concluded that *P. divisum*, *T. nubica*, and *E. indica* showed remarkable phytostabilization potential for most of the studied HMs. Furthermore, *P. oleracea* and *F. indica* species showed high phytoextraction ability to Co and Cr. Therefore, the investigated plants could be utilized to create environmental isolation coverage in this area by sequestration or avoid the distribution of HMs in order to remediate the HM-polluted soil in the meanwhile.

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