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Phytoremediation Potential of Selected Ornamental Woody Species to Heavy Metal Accumulation in Response to Long-Term Irrigation with Treated Wastewater

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Abstract: Arid and semiarid environments of Mediterranean countries suffer from scarcity of water resources, which limits their agriculture productivity. Using treated wastewater (TWW) is considered an alternative strategy for irrigation purposes in such areas. However, TWW contains substantial levels of heavy metals (HMs) and contaminants that pollute the environment and soil. The aim of this study is to evaluate the phytoremediation potential of six selected woody tree species under long-term irrigation with TWW. The concentration, bioaccumulation factor (BFC), translocation factor (TF), and comprehensive bioconcentration index (CBCI) of HMs were measured in the various parts (roots, bark, and leaves) of the studied tree species. The results show a general pattern of mineral accumulation in the roots and low translocation to the areal parts of various species. *Cupressus sempervirens*, which is a native species in Mediterranean environments, had higher TF values for Fe, Mn, Cu, Cr, Cd, and Pb metals in its areal parts compared to other tree species. The study shows that *Ficus nitida* has the potential to be a hyperaccumulator for Cd in its bark, with a TF value that exceeds 12. Deciduous trees species (*Populus nigra* and *Robinia pseudoacacia*) were found to have high TF values for Ni and Cd toward their areal parts, whereas a higher TF for Cr (1.21) was only found in *P. nigra* bark. *Cupressus sempervirens* had, significantly, the highest bark and leaf CBCI values (0.83 and 0.82, respectively), whereas *Ficus nitida* had the second-highest values in the bark and leaves (0.56 and 0.51, respectively). Therefore, *Cupressus sempervirens* and *Ficus nitida* are considered good hyperaccumulators for various HMs, and can be used for phytoremediation activities in polluted areas.

Keywords: bioaccumulation factor; translocation factor; comprehensive bioconcentration index; hyperaccumulator; phytoremediation potential

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1. Introduction

Most Mediterranean basin countries are dominated by arid and semiarid environments that suffer from limited water resources [1,2]. In eastern parts of the Mediterranean, Jordan is one of the countries most vulnerable to climate change, and it is considered one of the poorest countries, in terms of water resources, in the world [3]. Furthermore, the annual increase in population densities increases water requirements, especially in urban areas, which has led to the use of water increasing, by very large quantities, in various non-agricultural sectors, resulting in the production of huge volumes of wastewater [4]. Municipal wastewater consists mainly of effluents from homes, institutions, industrial activities, and running water from streets after rainfall or other forms of precipitation [5]. Under such conditions, it has become more imperative to consider the utilization of these non-conventional water resources, such as treated wastewater (TWW), as an essential component of water budget management, especially for agriculture.

Jordan has a total area of 8.9 million ha, with only less than 0.7 million ha of arable land [6]. Forestlands comprise less than 1% of the total area, which is primarily due to limited and variable precipitation, as well as threats due to fire, land degradation, and misuse due to energy shortages in rural areas [7]. Although forest resources are limited in Jordan, they provide important ecosystem services to its inhabitants, such as grazing, wood, and non-wood products, as well as their contribution to the stability of this fragile ecosystem [8]. Restoration of forests plays a vital role in improving the livelihoods of rural communities, in addition to maintaining environmental stability in the face of adverse climatic changes.

The utilization of TWW in forest plantations is considered a valuable strategy for increasing forest areas in Jordan [7]. The non-edible nature of the forest and ornamental woody trees and shrubs reduces the risk of human exposure to heavy metals found in TWW resources [9]. Positive effects were reported for the use of TWW resources on the growth and survival of various woody species [10,11]. For example, it contains large quantities of minerals that are important for plant growth, and, therefore, reduces the need for fertilizer application [12,13]. On the other hand, the negative consequences of TWW reuse in agriculture are associated with the buildup of heavy metals (HMs) in the soil [14]. Such HMs are dissolved in soil water, bound tightly to soil particles, or are exchangeable with the components of the soil matrix, and are thus potentially mobile [15]. Many HMs are highly toxic, and their presence in the soil and water can cause serious health problems [14]; indeed, the accumulation of HMs in the food chain is very harmful and dangerous to human health [16].

The use of TWW in agriculture and forestry might cause the deterioration and contamination of the soil [17,18]. Plant features, soil, and environmental factors are known to have an impact on the bioavailability of these metals [19,20]. Soil characteristics, including soil texture, pH, organic matter content, phosphate content, redox potential, and cation exchange capacity, play a major role in regulating the fractions of soluble and bioavailable metals that determine metal uptake by plants [21].

Woody trees are generally considered beneficial to the environment, as they can transport and fix mineral elements within their tissues and, therefore, are used to remove harmful heavy metals concentrated in water and soil [22]. Woody trees are preferred for the long-term utilization of wastewater resources, as they are considered to be high-biomass-producing plants [11,23]. Woody tree species vary considerably in their metal translocation and their resistance to high concentrations of heavy metals [10,24]. In this context, the hyperaccumulator plants are capable of accumulating potentially phytotoxic elements to concentrations more than 100 times those found in non-accumulator species [25,26]. Therefore, hyperaccumulator tree species can be used for phytoextraction purposes, which is the most prevalent and useful technique for removing HMs from the soil, by accumulating the HMs in their vegetative parts [25].

The bioconcentration factor (BCF) and translocation factor (TF) are used regularly to identify HM accumulation patterns of various plants species, and their phytoremediation capacity [27]. Furthermore, in soils contaminated with multiple HMs, it is more crucial to measure the ability of plants to translocate multiple metals to their areal parts by determining their comprehensive bioconcentration index (CBCI) [28]. In Jordan, the role of different woody species in accumulating heavy metals in response to short-term exposure to wastewater has been studied in different locations [29,30]. However, limited research has been conducted on the assessment of the long-term use of TWW resources on woody trees and shrubs as a measurement of phytoremediation efficiency. The purpose of this study is to assess the ability of different woody trees species to uptake and accumulate heavy metals in their tissues, and, therefore, their usefulness for phytoremediation purposes of contaminated soils in response to long-term irrigation with TWW in arid areas of Jordan.

2. Materials and Methods

2.1. Study Area

This investigation was conducted at the Wadi Musa Wastewater Treatment Plant (WMTP), 31 km to the Northwest of Ma'an city (30°22′27.6″ N 35°26′58.7″ E, with an elevation of 1069 m above sea level), near the historic city of Petra. Wastewater flowing from adjacent communities close to Petra Archaeological Park is collected and handled in this treatment plant. The study site consists of an area of seven hectares cultivated with fodder crops, fruit trees, woody ornamental plants, and forest trees such as willows, poplar, cypress, and eucalypts.

An arid climate is prevalent in the region, which is a characteristic of the Eastern Mediterranean climate. Data for the monthly averages of maximum and minimum temperatures and average precipitation for the last 10 years at the Wadi Musa Treatment Plant were obtained from Jordan Meteorological Department for the weather station of Wadi Musa, and are summarized in Supplementary Table S1. This area is characterized by low rainfall, occurring mostly in winter (149.5 mm annually), and a hot dry summer that extends from June to September, with mean maximum temperatures surpassing 30 °C during this season. Mean monthly potential evapotranspiration (PET) for this location ranges between 1.8 mm day^{-1} in December to 7.0 mm day^{-1} in Jun and July, with a total annual PET of 1657 mm yr^{-1}.

2.2. Soil and Water Characteristics

The soil texture within the experimental site was sandy loam with EC and pH values of 1.90 dS m^{-1} and 7.9, respectively, and organic matter (OM) and $CaCO_3$ contents of approximately 12.1 g Kg^{-1} and 15.79 g Kg^{-1}, respectively, are reported (Table 1).

Table 1. Main physical and chemical properties of soil at the Wadi Musa Wastewater Treatment Plant experimental site. The data are expressed as the mean ± SD (n = 4).

Soil Analysis	Unit	Measurement
Sand (20–200 µm)	% weight	77.45 ± 1.45
Silt (2–20 µm)	% weight	12.98 ± 0.54
Clay (<20 µm)	% weight	9.58 ± 1.16
EC	dS m^{-1}	1.90 ± 0.16
pH	pH unit	7.93 ± 0.51
OM	g Kg^{-1}	12.10 ± 1.28
$CaCO_3$	g Kg^{-1}	15.79 ± 1.09
CEC	(mEq/100 g)	6.26 ± 0.70

Wastewater analysis was performed on a monthly basis to monitor its chemical and biological properties. Table 2 summarizes the averages of five wastewater analyses performed during 2021 prior to soil and plant tissues sampling. These results comply with the standards of safe use of TWW established by the Jordan Standards and Metrology Organization (JISM, 2013) [31], as well as with those of the World Health Organization (WHO, 2006) [32]. Moreover, the bacterial load in treated wastewater was below the levels that restrict their safe use for irrigation purposes [31,32].

2.3. Plant Material

Six genotypes of ornamental woody trees and shrubs grown in the targeted area were selected for this study. The plant material included four genotypes of 10-year-old evergreen trees and shrubs: Ficus (*Ficus nitida* L.), Mediterranean Cyprus (*Cupressus sempervirens* L.), Weeping Bottlebrush (*Melaleuca viminalis* L.), and Common Oleander (*Nerium oleander* L.), and two genotypes of 10-year-old deciduous trees species; Robenia, (*Robinia pseudoacacia* L.) and Black Poplar (*Populus nigra* L.) (Supplementary Table S2). Trees were irrigated continuously using a drip irrigation system following their cultivation with TWW effluent on a twice-a-week irrigation regime during December, January, February, and March, and every other day for the rest of the year. Average seasonal irrigation water supply was around 1840 mm ha^{-1} (Supplementary Table S1).

Table 2. Treated wastewater analysis of Wadi Musa Wastewater Treatment Plant during 2021.

Parameter	TWW	JISM [1]	WHO [2]
pH	7.61 ± 0.25	6.0–9.0	6.5–8.0
EC (dS m^{-1})	1.85 ± 0.10	1.0–3.0	0.7–3.0
BOD (mg L^{-1})	10.48 ± 1.10	60	300
COD (mg L^{-1})	28.93 ± 1.08	120	500
TDS (mg L^{-1})	829.48 ± 33.42	<2000	450–2000
SAR (ratio)	8.38 ± 0.86	9	<13
Total Coliforms (MPN/100 mL)	1.85 ± 0.06	<10	<9

[1] JISM, Jordan Institution for Standard and Metrology; [2] WHO, World Health Organization. MPN, most probable number.

2.4. Sampling and Analysis of Plant Tissues, Soil, and Water

2.4.1. Plant Sampling

Four trees per grown genotype were selected randomly and sampled for their roots, stem bark, and leaves in June 2021. Fine roots of each tree were sampled using a soil corer of 10 cm diameter. Three samples were taken around each tree 50–60 cm away from the stems to a depth of about 30 cm. After that, the three samples of each tree were mixed together in plastic bags. Roots around 2–3 mm in diameter were sieved, washed, and rinsed with distilled water before drying at 75 °C for approximately 48 h. Three stem bark samples were also collected for each tree around the trunk from the outer 3–5 mm at 1.3–1.5 m above ground level using a 10 mm increment borer. Mature leaves were collected from three selected locations around the canopy of each tree from the upper 5 cm of current-year twigs. All bark and leaf samples of each tree species were also washed thoroughly and rinsed with distilled water before drying at 75 °C for 48 h. Dried roots, stem bark, and leaf samples were ground and prepared for the analysis of various heavy metals, including iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), chromium (Cr), and nickel (Ni), as described in Al-Habahbah et al. [33].

2.4.2. Irrigation Water and Soil Sampling

Irrigation water samples were collected three times during April, May, and June of the 2021 season. Four irrigation water samples were collected per sampling time directly from irrigation lines near selected trees. Water samples were collected in a 1L screw cap glass bottle and stored in an icebox during transportation to the laboratory. Collected samples were prepared and transported to the laboratory for preparation and analysis of heavy metals.

Soil samples were also randomly collected from around each tree at the same time as the tree tissue sampling. Samples were collected to a depth of 40 cm using a soil corer (10 cm in diameter). Collected soil samples were 50 cm distance from a tree trunk. Soil samples were directly sieved through a 2 mm sieve, and the remaining soil of the three samples per tree was mixed together into one composite study sample. Soil samples were dried at 60 °C for 48 h before analysis of heavy metal content, in accordance with Duncan et al. [34].

2.5. Chemical Analysis of Irrigation Water, Soil, and Plant Tissues

For water samples, 100 mL was acidified with a few drops of 70% HNO_3 before filtration through a 0.45 µm membrane filter. Heavy metals were analyzed according to the methods of the water analysis procedure described by Estefan et al. [35]. Irrigation water pH was measured with pH meter 3110, while EC and TDS were also measured with an inoLab Cond 720 (WTW, Weilheim, Germany). Chemical oxidation demand (COD), biochemical oxidation demand (BOD), and sodium adsorption ratio (SAR) were determined by WMTP Service Laboratory, and coliforms were determined as described by Pedrero and Alarcón [36].

For soil analysis, pH was measured using saturated soil paste extracts, and electrical conductivity (EC) was measured in aqueous soil extract (5:1). Cation exchange capacity (CEC) was measured using the sodium acetate procedure [37]. Soil carbonate ($CaCO_3$) analysis was conducted via the gas chromatography method described by Amundson et al. [38]. After analyzing organic carbon using a carbon combustion furnace (Leco SC832, Michigan, United States), the organic matter content (OM) was calculated using the conversion equation OM = 1.721 × OC [39]. Furthermore, standard analysis of soil samples for total P and K was performed following the procedure of Estefan et al. [35].

Soil, water, root, bark, and leaf samples were analyzed for their heavy metal content (Fe, Mn, Cu, Zn, Cd, Pb, Cr, and Ni) using the procedure of Estefan et al. [35]. To begin, 0.5 g of dry soil was digested in a mixture of 4 mL of 70% HNO_3 and 1 mL of 62% $HClO_4$ using a high-pressure microwave apparatus (Milestone MLS Ultraclave), and the digested samples were filtered through a 45 μm filter. The final leachates were transferred into a 25 mL volumetric flask, and mixed with a 1% (v/v) HNO_3 solution. Selected heavy metal elements of all samples were measured by atomic absorption spectrophotometer (Analyst 200, PerkinElmer, Waltham, MA, USA). The accuracy of the instrumental analyses and methods of sample extraction were validated using the certified reference material (CRM), Virginia Tobacco Leaves (CTA-VTL-2), for the corresponding elements in triplicate. The results were in good agreement with certified values. The results from the analysis of CRM were all within the 95% confidence limit. Detection limit was defined as the concentration corresponding to three times the standard deviation of ten blanks. Detection limit values of elements as μg L^{-1} were found to be 0.02 for Cd, 0.08 for Cr, 0.07 for Cu, 0.11 for Fe, 0.05 for Mn, 0.14 for Ni, 0.45 for Pb, and 0.02 for Zn.

2.6. Bioconcentration and Translocation Factors, and Comprehensive Bioconcentration Index Analysis

In order to analyze the relationship between various heavy metal content of soils and different trees tissues/organs, the bioconcentration factor (BCF) and translocation factor (TF) were determined to assess the uptake and accumulation of heavy metals in different plant parts, as described by Mellem [40].

$$\mathrm{BCF} = \frac{\text{Concentration of metal in plant tissue}}{\text{Concentration of metal in soil}} \tag{1}$$

$$\mathrm{TF} = \frac{\text{BCF of bark or leaf}}{\text{BCF of root}} \tag{2}$$

A comprehensive bioconcentration index (CBCI) was used to determine the ability of each plant species to accumulate heavy metals, and was calculated as described in Zhao et al. [28].

$$\mathrm{CBCI} = \left(\frac{1}{\mathrm{N}}\right)\sum_{\mathrm{i}=1}^{\mathrm{N}} \mu_{\mathrm{i}} \tag{3}$$

where N is the total number of metals analyzed in this study and μ_i = μ (x) of metal i, which is calculated using the following equation:

$$\mu(\mathrm{X}) = \frac{\mathrm{X} - \mathrm{Xmin}}{\mathrm{Xmax} - \mathrm{Xmin}} \tag{4}$$

where x is the BCF of metal i (Cd, Cu, Cr, Co, Fe, Mn, and Zn) and Xmin and Xmax represent minimum and maximum BCF values for the given metal among the observed plant species.

2.7. Data Analysis

Six genotypes of ornamental woody trees and shrubs were selected in a completely randomized design at the specified location, with four replicates each and a total number of 24 trees associated with the sampling of soil, water, and plant tissues, as described earlier. Data for plant tissue HM concentration, BCF, TF, and CBCI were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS; Version 9.3 for Windows; SAS Institute, Cary, NC, USA). Data means were subjected to Tukey's honestly significant difference Test ($p \leq 0.05$).

3. Results and Discussion

3.1. Chemical Analysis of Irrigation Water and Soil

The concentrations of nutrients and heavy metals in TWW used for irrigation of various tree and shrub species are shown in Table 3. Treated wastewater contained nitrate, phosphate, nitrogen, and potassium, with mean values for each nutrient of 42.3 mg L^{-1}, 16.2 mg L^{-1}, 14.7 mg L^{-1}, and 36.5 mg L^{-1}, respectively (Table 3). The analysis of various nutrient elements found levels to be lower than the standard limits for irrigation water described previously (JISM, 2013; WHO, 2006) [31,32]. Significant quantities of essential elements were reported in treated wastewater when compared to fresh water in the Wadi Musa region, according to previous studies [33,41–43]. Furthermore, higher quantities of Na^{+} and Cl^{-} ions were also found in the TWW with concentrations of 93.0 mg L^{-1} and 134.5 mg L^{-1}, respectively.

Table 3. Chemical analysis of soil and treated wastewater (TWW) used for irrigation of various woody trees and shrubs at the Wadi Musa Treatment Plant compared with Jordanian (JISM) and WHO standard limits for agricultural irrigation water. The data are expressed as the mean ± SD (n = 4).

Element	TWW	JISM [1]	WHO [2]	Element	Soil
N (mg L^{-1})	14.69 ± 2.36	50	5–50	N total (g Kg^{-1})	14.2
NO_3^{-} (mg L^{-1})	42.32 ± 2.19	45	50	K (mg Kg^{-1})	684.2
PO_4^{-3} (mg L^{-1})	16.16 ± 1.93	30	30	P (mg Kg^{-1})	94.8
K (mg L^{-1})	36.50 ± 3.11	80	80	Na (mg Kg^{-1})	374.1
Mg (mg L^{-1})	17.89 ± 1.98	60	60	Cl (mg Kg^{-1})	784.1
Ca (mg L^{-1})	90.04 ± 5.09	400	230	Zn (mg Kg^{-1})	1.26
Na (mg L^{-1})	93.03 ± 2.27	230	69–207	Fe (mg Kg^{-1})	1.55
Cl (mg L^{-1})	134.48 ± 8.16	400	140–350	Mn (mg Kg^{-1})	7.14
Zn (mg L^{-1})	0.81 ± 0.06	2	<2.0	Cu (mg Kg^{-1})	0.30
Fe (mg L^{-1})	4.05 ± 0.34	5	0.1–1.5	Cr (mg Kg^{-1})	0.19
Mn (mg L^{-1})	0.02 ± 0.01	-	0.2	Ni (mg Kg^{-1})	0.08
Cu (mg L^{-1})	0.01 ± 0.01	-	0.2	Cd (mg Kg^{-1})	0.03
Cr (mg L^{-1})	0.72 ± 0.18	-	0.02	Pb (mg Kg^{-1})	2.44
Ni (mg L^{-1})	1.99 ± 0.20	-	0.2		
Cd (mg L^{-1})	1.38 ± 0.10	0.01	<0.01		
Pb (mg L^{-1})	7.00 ± 1.69	5	<5.0		

[1] JISM, Jordan Institution for Standard and Metrology; [2] WHO, World Health Organization.

Significant levels of HMs were also detected in TWW used for irrigation purposes, with higher concentrations of Pb and Cr detected compared to Cd and Ni (Table 3). On the other hand, no significant concentrations of Cu, Zn, or Mn were observed in the irrigation water samples (Table 3). High levels of Pb, Cr, Cd, and Ni were found to exceed the acceptable Jordanian (JISM) and WHO standard limits for irrigation water, which agrees with Al-Hababha et al. [33]. Variability in the elemental composition of TWW was reported in Jordan depending on the source of municipal or industrial water effluents. Maximum values of Fe and Cu were recorded, with values reaching 200 and 85 mg L^{-1}, respectively [44,45]. Manasreh and Alzaydien [46] found similar values of Fe and Cu, in addition to high concentrations of Zn, Pb, Cd, and Cr, with values of 90, 21, 2.1, and 2 mg L^{-1}, respectively.

Soil collected from the vicinity of woody trees and shrubs was found to contain considerably high levels of nitrate, phosphate, total nitrogen, potassium, sodium, and chlorine (Table 3). The buildup of elements in the soil samples could be attributed to the long-term use of TWW for irrigation. Estimated amounts of N, P, and K added by continuous irrigation with TWW in this study might reach 105, 30, and 67 Kg ha^{-1} yr^{-1} for these elements, respectively. Treated wastewater is well-known for its high content of ions, particularly nitrate and phosphate [47]. Furthermore, the higher contents of organic matter that exist in TWW could also be responsible for increasing ion availability, especially N, P, and K [48,49]. Such an increase in the concentrations of essential nutrients usually has positive effects on the growth of various plant species [10,28]. However, although high levels of Na (374 mg Kg^{-1}) and Cl (784 mg Kg^{-1}) were detected in TWW-irrigated soil, no toxicity symptoms were observed on the leaves of the studied trees and shrub species. It is known that the use of TWW for long periods leads to the accumulation of significant quantities of toxic metals in the soil [42]. In this study, the results of the soil heavy metal analysis showed comparatively low heavy metal levels in the soil around the trees and shrubs (Table 3), which might suggest the possibility of higher uptake of these elements by plants. Higher Fe and Cr levels were detected, with values of 2.03 mg L^{-1} and 0.20 mg L^{-1}, respectively (Table 3).

3.2. Effects of Irrigation with TWW on HM Content in Different Tissues of Woody Trees

The average concentrations of HMs in roots, bark, and leaves of six woody trees irrigated with TWW are summarized in Table 4. The concentrations of Zn, Fe, Mn, and Cu in roots differed considerably among tree species, with the highest content of Zn detected in *F. nitida* roots at a mean value of 54.05 mg Kg^{-1}, while the lowest mean value (11.1 mg Kg^{-1}) was found in *P. nigra* roots. For root Fe concentrations, *R. pseudoacacia* was significantly the highest (625.5 mg Kg^{-1}) among all tree species, while *C. sempervirens* contained the lowest Fe concentrations (120.5 mg Kg^{-1}). Mn concentrations in *N. oleander, R. pseudoacacia*, and *F. nitida* roots were also significantly higher than the rest of the tree species, with mean values of 31.9 mg Kg^{-1}, 26.4 mg Kg^{-1}, and 27.3 mg Kg^{-1}, respectively, while the lowest concentration of Mn (8.99 mg Kg^{-1}) was found in the roots of *P. nigra*. From Table 4, it is clear that *F. nitida* and *M. viminalis* contained, significantly, the highest concentrations of Cu in their roots, with mean values of 41.25 mg Kg^{-1} and 34.86 mg Kg^{-1}, respectively, whereas Cu concentrations in the roots of *C. sempervirens* were, significantly, the lowest, with a mean value of 7.13 mg Kg^{-1}. The Cr and Ni root concentrations reached 4.47 and 4.95 mg Kg^{-1}, respectively, with no significant differences between different tree species, except for *P. nigra*, which had the lowest concentrations of 1.82 mg Kg^{-1} and 2.06 mg Kg^{-1} for Cr and Ni, respectively. Cd root concentrations were as high as 0.71 mg Kg^{-1}, except for *F. nitida* and *C. sempervirens*, where they were significantly lower, with values of 0.16 mg Kg^{-1} and 0.18 mg Kg^{-1}, respectively. It was clear that all roots of all tree species accumulated high concentrations of Pb, with a maximum content of 45.64 mg Kg^{-1} in *M. viminalis* (Table 4). The trends in mean concentrations for each metal in the roots of different trees were in the order of Fe > Pb > Zn > Mn > Cu > Ni > Cr > Cd, with average values, in mg Kg^{-1}, of 355.63, 29.00, 23.24, 20.20, 17.72, 3.82, 3.81, 0.40, respectively. High levels of Pb and Fe correlates with their high levels in TWW (Pb > Fe > Ni > Cd > Zn > Cr > Mn > Cu) and in the soil content (Mn > Pb > Fe > Zn > Cu > Cr > Ni > Cd).

The concentrations of various measured heavy metals in the bark tissues significantly varied among the tree species in response to TWW irrigation (Table 4). *Robinia pseudoacacia* bark was found to have the highest Zn (45.31 mg Kg^{-1}) and Mn (68.89 mg Kg^{-1}) concentrations among the studied tree species. On the contrary, *P. nigra* concentrations of Zn and Mn were the lowest, with mean values of 8.64 mg Kg^{-1} and 7.03 mg Kg^{-1}, respectively. The highest concentrations of Fe, Cu, Cr, Ni, and Cd were found in *C. sempervirens* bark, with mean concentrations of 227.95, 20.68, 4.08, 4.14, 1.84 mg Kg^{-1}, respectively. The lowest Fe and Ni contents were found in the bark of *P. nigra* trees, with mean values of 91.03 mg Kg^{-1} and 2.10 mg Kg^{-1}, respectively, whereas the bark of *M. viminalis* trees contained the lowest

Cu and Cr contents, with mean values of 2.50 mg Kg^{-1} and 0.83 mg Kg^{-1}, respectively. On the other hand, Cd content was the lowest in the bark of *N. oleander* trees (0.35 mg Kg^{-1}), while Pb content was the highest in *M. viminalis* (26.43 mg Kg^{-1}), and the lowest was in *P. nigra* (3.19 mg Kg^{-1}). The trends in the mean concentrations of each metal in the bark of different genotypes were in the order of Fe > Mn > Zn > Pb > Cu > Ni> Cr > Cd, with average values, in mg Kg^{-1}, of 159.17, 24.56, 18.22, 14.72, 8.74, 3.15, 2.20, 1.20, respectively. Mn and Cd concentrations in bark ranged from 122% to 301% of those in roots, respectively.

Table 4. Concentration (mg Kg^{-1}) of Zn, Fe, Mn, Cu, Cr, Ni, Cd, Pb in the roots, stem bark, and leaves of six woody trees species irrigated continuously with TWW from the Wadi Musa Wastewater Treatment Plant.

Part/Species	Zn	Fe	Mn	Cu	Cr	Ni	Cd	Pb
Roots								
F. nitida	54.05 a *	419.38 b	27.25 ab	41.25 a	4.47 a	4.95 a	0.16 b	24.94 cd
C. sempervirens	25.58 b	120.53 d	15.59 ab	7.13 d	4.08 a	4.74 ab	0.18 b	18.05 de
M. viminalis	15.87 cd	375.38 b	11.11 ab	34.86 b	4.08 a	4.37 ab	0.71 a	45.64 a
N. oleander	13.46 cd	360.98 b	31.90 a	11.54 d	3.82 a	3.38 bc	0.55 a	33.80 bc
R. pseudoacacia	19.39 c	625.53 a	26.38 ab	17.92 c	4.56 a	3.41 bc	0.42 a	36.96 ab
P. nigra	11.09 d	232.00 c	8.99 b	10.60 d	1.82 b	2.06 c	0.36 a	14.60 e
Bark								
F. nitida	20.65 b	215.31 a	14.16 c	13.55 b	2.37 b	3.94 a	1.81 ab	10.70 d
C. sempervirens	13.64 c	227.95 a	19.45 bc	20.68 a	4.08 a	4.14 a	1.84 a	18.79 b
M. viminalis	9.95 d	110.11 b	22.50 b	2.50 d	0.83 d	4.14 a	1.71 ab	26.43 a
N. oleander	11.14 cd	191.45 a	15.30 c	6.11 c	2.24 bc	2.24 b	0.35 c	13.45 c
R. pseudoacacia	45.31 a	119.19 b	68.89 a	5.49 c	1.46 c	2.35 b	1.05 b	15.75 bc
P. nigra	8.64 d	91.03 c	7.03 d	4.08 cd	2.23 bc	2.10 b	0.46 bc	3.19 e
Leaves								
F. nitida	13.69 a	277.93 ab	57.63 b	17.68 ab	2.04 a	4.14 ab	0.77 a	11.91 b
C. sempervirens	16.15 a	326.70 a	101.86 a	19.55 ab	1.73 ab	4.22 ab	0.69 a	22.13 a
M. viminalis	10.83 b	188.50 c	38.22 bc	7.54 c	1.28 b	3.73 b	0.73 a	19.68 a
N. oleander	17.18 a	228.06 bc	48.50 b	11.38 bc	2.26 ab	5.56 a	0.26 b	17.93 a
R. pseudoacacia	17.50 a	252.88 b	142.19 a	22.01 a	1.31 b	4.30 ab	0.79 a	21.66 a
P. nigra	9.55 b	180.28 c	29.83 c	5.56 c	0.61 c	4.06 ab	0.40 b	6.06 c

* Different letters for the same metal ion and the same plant part (root, bark, leaves) represent significantly different means of the different tree genotypes, using one-way ANOVA and Tukey's honestly significant difference test at the 5% significance level.

The leaves of the various tree species also exhibited variable HM contents, as shown in Table 4. High concentrations of Zn, Mn, Cu, and Cd were detected in *R. pseudoacacia* leaves, with mean values of 17.50, 142.19, 22.01, and 0.79 mg Kg^{-1}, respectively, whereas *N. oleander* leaves were found to have the highest concentrations of Cr and Ni, with mean values of 2.26 mg Kg^{-1} and 5.56 mg Kg^{-1}, respectively. Fe and Pb were the highest in *C. sempervirens* leaves, with mean values of 326.70 mg Kg^{-1} and 22.13 mg Kg^{-1}, when compared to other tree species leaves. *Populus nigra* leaf contents of Zn, Fe, Mn, Cu, Cr, and Pb were the lowest among all tree species (Table 4). The lowest mean value of leaf Ni content (3.73 mg Kg^{-1}) was found in *M. viminalis*, whereas the lowest Cd content (0.26 mg Kg^{-1}) was found in *N. oleander* leaves. The trends in mean concentrations of each metal in the leaves of different trees were in the order of Fe > Mn > Pb > Zn > Cu > Ni > Cr > Cd, with average values of 242.39, 69.71, 16.56, 14.15, 13.95, 4.34, 1.54, 0.61 mg Kg^{-1}, respectively. Mn, Ni, and Cd concentrations of leaves were higher than those for roots, with values of 345%, 113%, and 152% compared to roots, respectively. The results also show that Fe accumulation was the highest in roots, as compared to leaves and bark, with the exception of *C. sempervirens* (Table 4).

Absorption of heavy metals by plants depends primarily on their concentration and bioavailability in soil, and the rate at which elements transfer from solid to liquid phases and to plant roots [50]. Plants can potentially accumulate metal ions in their roots at magnitudes greater than the surrounding medium [51]. This can be explained by the fact that one of the normal functions of the roots is to absorb ions from the aqueous solution of the rhizosphere as compared to the aerial parts [52]. Soil pH and organic matter content are major factors that affect the solubility and availability of metal ions in contaminated areas, and therefore, their absorption [53]. Phytoaccumulator plants have developed efficient mechanisms to absorb metal ions from their environment, such as the ability of their roots to induce soil pH changes and redox reactions that increase the solubility of these metals, and therefore, increase their absorption rates [54]. Furthermore, soil microorganisms (bacteria and fungi) that live in the vicinity of the rhizosphere may contribute to metal solubility and bioavailability in the soil [55]. This occurs primarily due to the altering of soil pH and the production of chelators, such as siderophores and organic acids [56].

Metal contents of most plant species are quite variable. Essential metalloid micronutrient concentrations have been averaged to be in the range of 2–20 $\mu g\ g^{-1}$ for Cu, 70–700 $\mu g\ g^{-1}$ for Fe, 20–70 $\mu g\ g^{-1}$ for Mn, and 20–40 $\mu g\ g^{-1}$ for Zn for many plant species [57,58]. These elements have a significant role in plant growth and development [59], including ionic balance, membrane stability, coenzymes, protein synthesis, and participation in major cellular redox reactions [58,59]. In comparison with previous studies, the accumulation of nutrient elements in tree species irrigated with WWT was in the range of normal levels, except for zinc concentrations in the roots of *C. sempervirens* and *F. nitida*, where the values exceeded the normal range [58,60]. Furthermore, Cu concentrations exceeded the normal values for *F. nitida* and *M. viminalis* in their roots. Upper critical concentrations of Zn and Cu were reported for plant leaves with values up to 300 $\mu g\ g^{-1}$ and 100 $\mu g\ g^{-1}$ of dry weight, respectively [60,61]. These levels are much higher than those found in all studied species in our analysis. Moreover, no toxicity symptoms were evident in any of the studied tree species (data not shown).

On the other hand, non-essential heavy metals levels in crop plants occur in the range of 0.2–2.4 $\mu g\ g^{-1}$ for Cd, 1–5 $\mu g\ g^{-1}$ for Ni, 1–13 $\mu g\ g^{-1}$ for Pb, and 1–10 $\mu g\ g^{-1}$ for Cr [62,63]. Ni, Cr, and Cd concentrations in all parts of the six tree species were found to be within the normal range, whereas the concentrations of Pb were in excess of the normal limits, but still below phytotoxic levels (50 $\mu g\ g^{-1}$) [64].

3.3. Effects of Irrigation with TWW on HM Bioconcentration and Translocation Factors in Different Parts of Woody Trees

The uptake of heavy metals and their translocation and partitioning into various plant parts is summarized by the measurements of their BCF and TF values. As shown in Table 5, BCF mean values of Zn, Fe, Mn, Cu, Cr, Ni, Cd, and Pb were 23, 233.7, 3.4, 54.3, 22, 55, 18.5, 12.6 for roots, whereas for bark they were 15.8, 108.9, 3.6, 30.6, 13.5, 45.8, 55.7, 6.1, and 13.5, 162.2, 11.8, 45.7, 9.1, 64.2, 30.2, 7.2 for leaves, respectively. Roots showed higher accumulation ability for Fe, Cu, Cr, and Pb, whereas bark accumulation ability was the highest for Cd and Zn; in contrast, for leaves, higher accumulation ratios were observed for Ni and Mn. According to the calculated values of BCF, the capability of tree species to accumulate metals was in the order of Fe > Ni > Cu > Cd > Zn > Cr > Pb > Mn (Table 5). *Ficus nitida*, *R. pseudoacacia*, and *P. nigra* were found to have the highest Zn BCF in the roots (54.87), bark (25.69), and leaves (23.21), respectively. *Robinia pseudoacacia* also exhibited the highest Fe BCF (347) in its roots, whereas for *C. sempervirens*, maximum Fe BCF occurred in the bark (163) and leaves (233). *Nerium oleander*, *R. pseudoacacia*, and *C. sempervirens* were also found to have the highest BCF values of Mn in the roots (5.68), bark (7.49), and leaves (23.74), respectively. Maximum Cu BCF was found in the roots of *F. nitida* (97.66), while the highest values in bark (90.09) and leaves (85.19) were found in *C. sempervirens*. On the other hand, *F. nitida* showed the maximum BCF of Cr for both roots (38.02) and leaves (17.34), as compared to *C. sempervirens* bark (29.21). The highest Ni BCF values were also found in the roots (94.7) and bark (82.75) of *C. sempervirens*, as compared to the high BFC values in the leaves of *P. nigra* and *M. viminalis*. Maximum Cd BCF values in roots and bark were found in *R. pseudoacacia* and *M. viminalis*, while the maximum BCF in leaves was found in *R. pseudoacacia* (Table 5). High Pb BCF was also detected in the roots of *P. nigra* (17.85), and in the bark and leaves of *C. sempervirens* (10.27 and 12.1, respectively). In summary, and according to the obtained averages of the BCF for overall plant parts, *C. sempervirens* and *F. nitida* showed an active accumulation of different heavy metals from the surrounding soil.

Table 5. Bioconcentration factor (BCF) of Zn, Fe, Mn, Cu, Cr, Ni, Cd, and Pb in the roots, stem bark, and leaves of six woody trees species irrigated continuously with TWW from the Wadi Musa Wastewater Treatment Plant.

Part/Species	Zn	Fe	Mn	Cu	Cr	Ni	Cd	Pb
Roots								
F. nitida	54.87 a *	283.84 ab	3.40 bc	97.66 a	38.02 a	84.57 a	5.64 bc	12.72 ab
C. sempervirens	28.04 b	85.94 c	3.63 bc	31.05 cd	29.23 a	94.70 a	2.14 c	9.87 b
M. viminalis	9.52 c	291.67 a	0.83 d	84.80 ab	17.78 b	29.28 b	35.63 a	13.73 ab
N. oleander	7.48 c	279.88 ab	5.68 a	31.06 cd	17.47 b	52.94 ab	10.05 bc	10.87 b
R. pseudoacacia	10.99 c	346.55 a	2.87 c	53.68 bcd	20.06 ab	23.28 b	33.40 a	10.33 b
P. nigra	26.94 b	114.37 bc	3.95 bc	27.41 d	9.21 c	44.92 ab	24.17 ab	17.85 a
Bark								
F. nitida	20.96 a	145.73 ab	1.77 cd	42.34 b	20.19 a	67.35 a	65.82 ab	5.46 b
C. sempervirens	14.95 a	162.53 a	4.53 ab	90.09 a	29.21 a	82.75 a	61.25 ab	10.27 a
M. viminalis	5.97 b	85.56 bc	1.68 d	7.61 d	3.60 d	27.76 ab	85.63 a	7.95 a
N. oleander	6.19 b	148.44 ab	2.73 b	16.44 bcd	10.24 b	35.10 ab	6.41 c	4.32 b
R. pseudoacacia	25.69 a	66.03 bc	7.49 a	16.44 bcd	6.44 c	16.04 b	84.00 a	4.40 b
P. nigra	20.99 a	44.88 c	3.09 bc	10.54 cd	11.28 b	45.90 ab	30.83 b	3.90 b
Leaves								
F. nitida	13.90 ab	188.10 a	7.20 c	55.23 ab	17.34 a	70.73 a	27.91 b	6.08 b
C. sempervirens	17.70 ab	232.94 a	23.74 a	85.19 a	12.42 a	84.35 a	22.92 b	12.10 a
M. viminalis	6.50 c	146.46 ab	2.85 d	22.96 b	5.56 b	24.96 b	36.25 b	5.92 b
N. oleander	9.56 bc	176.83 ab	8.64 c	30.62 b	10.33 ab	87.25 a	4.68 c	5.76 b
R. pseudoacacia	9.92 bc	140.10 ab	15.46 b	65.96 ab	5.78 b	29.35 b	63.00 a	6.05 b
P. nigra	23.21 a	88.87 b	13.10 b	14.36 c	3.08 c	88.74 a	26.50 b	7.41 b

* Different letters for the same metal ion and the same plant part (root, bark, leaves) represent significantly different means of the different tree genotypes using one-way ANOVA and Tukey's honestly significant difference test at the 5% significance level.

In addition to BCF, the TFs of various HMs in the bark and leaves of the woody trees were also measured (Table 6). According to Anderson [65], plants are considered to be accumulators and hyperaccumulators once their TF values exceed one (TF > 1). *Robinia pseudoacacia* appeared to be an accumulator for Zn in their bark, as compared to other species that had TF values > 1. Similarly, *R. pseudoacacia, C. sempervirens, and M. viminalis* were also considered to be accumulators for Mn. Furthermore, *C. sempervirens* was found as an accumulator for Fe, Cu, Cd, Pb, and Cr, whereas *P. nigra* had the highest TF values for Cr in their bark. *Cupressus sempervirens* was considered a hyperaccumulator plant for metalloids in bark tissue, except for Zn and Ni; on the contrary, *N. oleander* was clearly an excluder plant. *Cupressus sempervirens* had the highest TF values for leaves, and was evidently a hyperaccumulator for Fe, Mn, Cu, Cd, and Pb, with TF mean values of 2.76, 6.73, 2.72, 10.40, and 1.24, respectively (Table 6). On the contrary, all TF values of Cr for all tree species leaves were < 1, with no significant differences among them. *Populus nigra* and *N. oleander* leaves possessed the highest TF values for Ni, at 1.96 and 1.65, respectively. Consequently, it is evident that *C. sempervirens* can be considered a hyperaccumulator plant for the different heavy metals in its bark and leaves, and it can be recommended as a good choice for soil remediation in the long term.

Table 6. Translocation factor (TF) of Zn, Fe, Mn, Cu, Cr, Ni, Cd, Pb in stem bark and leaves of six woody tree species irrigated continuously with TWW from the Wadi Musa Wastewater Treatment Plant.

Part/Species	Zn	Fe	Mn	Cu	Cr	Ni	Cd	Pb
Bark								
F. nitida	0.38 d *	0.52 b	0.52 b	0.34 b	0.53 b	0.87 a	12.49 b	0.46 b
C. sempervirens	0.54 cd	1.92 a	1.31 ab	3.00 a	1.00 a	0.90 a	27.63 a	1.06 a
M. viminalis	0.63 bc	0.30 bc	2.12 ab	0.07 b	0.21 b	1.00 a	2.80 c	0.62 b
N. oleander	0.83 b	0.54 b	0.48 b	0.53 b	0.60 b	0.66 a	0.64 c	0.41 bc
R. pseudoacacia	2.38 a	0.19 c	2.75 a	0.32 b	0.34 b	0.69 a	2.76 c	0.43 c
P. nigra	0.80 b	0.40 bc	0.83 ab	0.38 b	1.21 a	1.03 a	1.30 c	0.22 bc
Leaves								
F. nitida	0.26 d	0.69 bc	2.12 b	0.44 d	0.46 a	0.91 c	5.43 ab	0.52 b
C. sempervirens	0.64 c	2.76 a	6.73 a	2.72 a	0.43 a	0.91 c	10.40 a	1.24 a
M. viminalis	0.70 c	0.51 bc	3.37 b	0.22 d	0.32 a	0.88 c	1.28 b	0.46 b
N. oleander	1.28 a	0.64 bc	1.53 b	1.00 bc	0.63 a	1.65 ab	0.48 b	0.53 b
R. pseudoacacia	0.95 b	0.41 c	5.56 a	1.24 b	0.31 a	1.27 bc	1.95 b	0.59 b
P. nigra	0.85 bc	0.78 b	3.27 b	0.51 cd	0.32 a	1.96 a	1.37 b	0.40 b

* Different letters for the same metal ion and the same plant part (bark, leaves) represent significantly different means of the different tree genotypes using one-way ANOVA and Tukey's honestly significant difference test at the 5% significance level.

The translocation of heavy metals into plants occurs either through energy-dependent active transport using special membrane-bound transporters, or passively through the transpiration stream [66]. Increased transpiration losses in arid environments may be responsible for the increased uptake of metal ions and their accumulation in various plant parts [66]. Furthermore, decreased temperature and metabolic rates also result in the decreased accumulation of metals, such as Cd in the leaves of tobacco plants. Heavy metal accumulation in hyperaccumulator genotypes have been associated with high translocation rates to areal parts [67].

Previously, BCF and TF were utilized by researchers to select plant species based on their phytoremediation capabilities [28,68]. The selection of species with high translocation rates of harmful metalloids to aerial parts, primarily to stem tissues that can be harvested later and utilized for various purposes, is crucial for phytoremediation studies. Peuke and Rennenberg [69] suggested that an effective economical phytoremediation process requires the use of plants that have metal BCF values of 10–20. The majority of forest species in this study have shown a prevalent pattern of metal accumulation and compartmentalization in the roots, and low translocation to areal parts. As illustrated in Table 6, *C. sempervirens* had higher TF values for several heavy metals in their areal parts than the other tree species. In addition to exhibiting a TF > 1 for Fe, Mn, Cu, Cr, Cd, and Pb in their leaves and bark, *C. sempervirens* was also found to have the ability to transfer the high molecular weight Pb to its bark and leaves. This is in agreement with the results of several reports indicating that *C. sempervirens* are phytoaccumulator trees and showed high translocation potentials for Pb, Zn, Mn, Cu, and Cd [70–72].

The role of different Ficus species (*Ficus stranglers*, *Ficus infectoria* Roxb, *Ficus palmata* Forsk, *Ficus religiosa* L.) in phytoremediation studies has indicated marginal potential to phytoextract soil contaminants [73,74]. Calculated TF values < 1 for Cd, Cr, Co, Cu, Fe, Mn, Pb, and Zn were reported [73,74]. On the contrary, this study indicates that *F. nitida* has the potential to be hyperaccumulators for Cd in their bark, since the TF value exceeds 12.

Although TF values were < 1 for Zn, Mn, Cu, and Ni in oleander tree bark, higher values (>1) were found in their leaves, suggesting the potential of oleander shrubs for removing such contaminants from the soil, and for use in the phytoextraction process. These results are in agreement with previous studies indicating that oleander trees had BCF and TF < 1 for metals such as Pb, and TF > 1 for Cd and Zn, in their areal parts [75–77]. The deciduous trees, Poplar and Robinia, were not considered hyperaccumulators, nevertheless, they were recommended as an effective species for soil remediation due to their fast-growing behavior and deep root systems [69,78]. The results of this study indicate that Poplar and Robinia have a high TF for Ni and Cd toward their areal parts, whereas a high TF for Cr was observed in Poplar bark only (1.21). These results agree with those of Bhargava et al. [79], who also concluded that poplar trees preferentially accumulated high Ni and Cd in their leaves and bark, respectively.

3.4. Comprehensive Metal Accumulation Index for Tree Species

Applying the CBCI concept to HM acquisition for each of the investigated species is a useful indicator for their ability to accumulate multiple metals in their areal parts. The CBCI data for each of the investigated species shows that *C. sempervirens* has the highest bark and leaf CBCI values (0.83 and 0.82, respectively) (Figure 1). The bark and leaf CBCI values were also high for *F. nitida*, but they were significantly lower than *C. sempervirens* CBCI values. The CBCI mean values of the other four species were considerably lower than *C. sempervirens* and *F. nitida* (Figure 1). Calculated CBCI values showed that native species possessed a higher ability than other tree species to accumulate different minerals simultaneously [28]. Surprisingly, we found that *C. sempervirens*, which is a native species in the Mediterranean region, had, significantly, the highest bark and leaf CBCI values (0.83 and 0.82, respectively). Moreover, *F. nitida* had the second-highest values in their bark and leaves (0.56 and 0.51, respectively). In addition to the large size of *F. nitida*, these trees grow well in the Mediterranean region climate, as this species can withstand temperatures down to zero degrees [80]. Thus, when considering the multi-metal accumulation ability of *C. sempervirens* and *F. nitida*, these trees are considered one of the best options for the long-term phytoremediation of contaminated soils irrigated by wastewater. On the other hand, the deciduous trees *R. Pseudoacacia* and *P. nigra* showed lower potential for accumulating multiple metals in their aerial parts. Several studies have shown that phytoextraction by fast-growing, high-biomass-producing tree species should be recognized as a recommended method for phytoremediation processes [81–83].

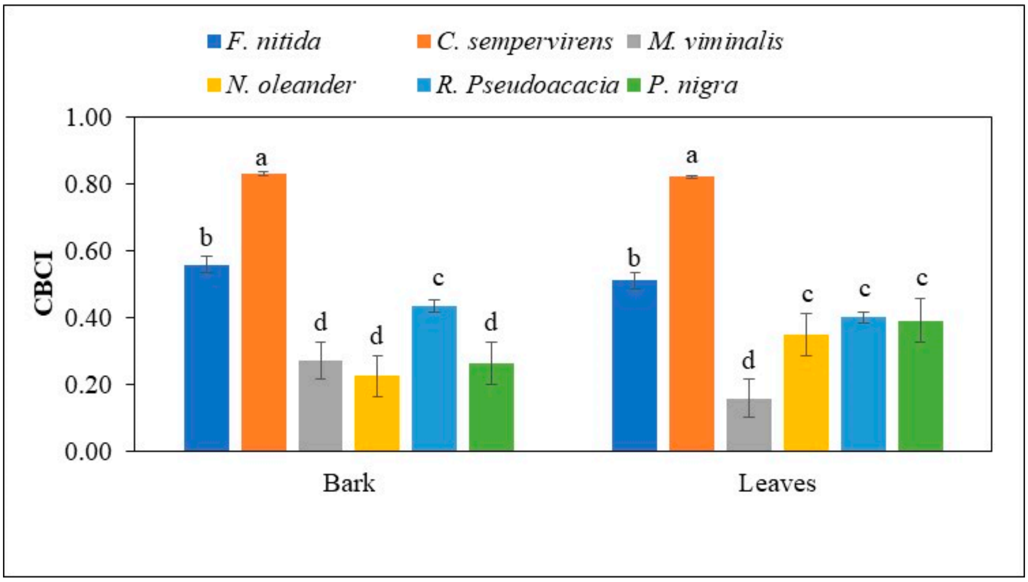

Figure 1. Comprehensive metal accumulation index (CBCI) of Zn, Fe, Mn, Cu, Cr, Ni, Cd, Pb in stem bark and leaves of six woody tree species irrigated continuously with TWW from the Wadi Musa Wastewater Treatment Plant. Bars represent mean ± SE. Different letters for the same plant part (bark, leaves) represent significantly different means among the different tree genotypes using one-way ANOVA and Tukey's honestly significant difference test at the 5% significance level.

4. Conclusions

The use of tree species in phytoremediation has promising potential for the clean-up of contaminated lands by HMs. The high accumulation capacity of different tree species indicates their application potential for the biomonitoring of HM contaminants in lands irrigated with wastewater.

Our data suggest that *C. sempervirens* and *F. nitida* have the highest ability to accumulate HMs from the soil, and consequently, are considered to be hyperaccumulators, as evident from their high TF and CBCI values. Moreover, the use of fast-growing species such as *P. nigra* and *R. pseudoacacia* might be a successful choice for phytoremediation regardless of their lower TF and CBCI values. Their fast-growing ability could cause a rapid uptake of harmful metalloids, especially Ni, Cr, and Cd, from contaminated soil.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14132086/s1. Table S1: Monthly means of min. and max. temperatures, rainfall, and wastewater irrigation amounts; Table S2: Selected woody tree species used to study the long-term effects of irrigation with treated wastewater effluent in WMTP. References [84–88] are cited in the supplementary materials.

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