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Capability of the Invasive Tree *Prosopis glandulosa* Torr. To Remediate Soil Treated with Sewage Sludge

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Received: 8 March 2019; Accepted: 7 May 2019; Published: 13 May 2019

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Abstract: Sewage sludge improves agricultural soil and plant growth, but there are hazards associated with its use, including high metal(loid) contents. An experimental study was conducted under greenhouse conditions to examine the effects of sewage sludge on growth of the invasive tree *Prosopis glandulosa*, as well as to determine its phytoremediation capacity. Plants were established and grown for seven months along a gradient of sewage sludge content. Plant traits, soil properties, and plant and soil concentrations of N, P, K, Cd, Pb, Cu, Ni, Zn, Cr, Co, As, and Fe were recorded. The addition of sewage sludge led to a significant decrease in soil pH, and Ni, Co, and As concentrations, as well as an increase in soil organic matter and the concentrations of N, P, Cu, Zn, and Cr. Increasing sewage sludge content in the growth medium raised the total uptake of most metals by *P. glandulosa* plants due to higher biomass accumulation (taller plants with more leaves) and higher metal concentrations in the plant tissues. *P. glandulosa* concentrated more Cd, Pb, Cu, Zn, and Fe in its below-ground biomass (BGB) than in its above-ground biomass (AGB). *P. glandulosa* concentrated Ni, Co, and As in both BGB and AGB. *P. glandulosa* has potential as a biotool for the phytoremediation of sewage sludges and sewage-amended soils in arid and semi-arid environments, with a potential accumulation capability for As in plant leaves.

Keywords: alien plant; phytoremediation; metal; plant traits; sludge

1. Introduction

Sewage sludge, a biological by-product of sewage treatments, increases organic matter and macronutrients in agricultural and degraded soils, and is widely used for plant fertilization since it offers the opportunity to recycle organic matter to soil [1,2]. However, concern has increased about the application of municipal sewage sludge for plant fertilization because of the high metal(loid) concentrations in some sewage-amended soils, with potentially deleterious impacts on the ecosystem and human health [3–5].

Because of the potentially dangerous consequences of the presence of metals, data concerning the cleaning techniques of sewage-amended soils are necessary. In this context, plants can be used as biotools to reduce metal loads in soils enriched with sewage sludge or even directly in municipal sewage sludges before they are added to the soil [6]. Phytoremediation is an eco-friendly and inexpensive mechanism for cleaning polluted soils compared to the high cost of chemical and physical remediation techniques, which can be harmful to ecosystems [7]. Phytoremediation simultaneously maintains the biological activity and physical structure of soils, allowing the stabilization and extraction of metals [8]. Basically, phytoremediation refers to the use of plants and associated microbes in soil to reduce pollutant concentrations and/or their toxic effects on the environment [9]. Those plants taking up higher concentrations of metals by their roots and their accumulation in the above-ground biomass (AGB) are suitable biotools for phytoextraction, while those species reducing the mobility and bioavailability of metals in the environment represent biotools for phytostabilization [10]. Recently, much interest has focused on the potential accumulation of phytotoxic metals in plant tissues [11]. Despite this screening, a limited number of such potential plants have been identified [12]. Such plants strongly express metal sequestration mechanisms, can sometimes show high internal requirements for specific metals [13], and are capable of mobilizing metals from less-soluble components in the soil [14]. Metals accumulate and distribute in plant tissues depending on the plant species, the concentration of metals in the soil, and the element species and its bioavailability, which depends on soil pH, organic matter content, and other factors [15]. Over the last decade, the potential use of trees as a suitable solution to remediate metal-contaminated soils has received increasing attention [16–18], especially when it is costly to use other treatments or there is no time pressure to reuse the land [19].

Some of the plants used as biotools in phytoremediation are exotic invasive species [20,21] that are able to grow where the indigenous vegetation is constrained by environmental factors, such as high levels of disturbance, salinity, drought, and pollution [22,23]. This could be the case for *Prosopis glandulosa* Torr. (syn. *Prosopis juliflora* (SW.) DC), an evergreen phreatophytic tree native to southwestern United States and northern Mexico [24]. *P. glandulosa* has invaded and continues to invade millions of hectares of rangeland all around the world, being rated as one of the top 100 least-wanted species worldwide [25]. *P. glandulosa* colonizes arid and semiarid lands, where it exhibits rapid growth, deep and extensive rooting, efficient nutrient uptake, and high biomass production [26,27]. Abiotic stress, such as drought, induces the production of metallothioneins, increasing the tolerance of *P. glandulosa* to metals [28]. These plant traits make *P. glandulosa* a suitable tree for use in the phytoremediation of saline-sodic soils and soils polluted with fluoride and metals [29–32]. However, no previous study has analysed the capability of *P. glandulosa* plants to tolerate, stabilize, and extract metals from sewage sludge and sewage-amended soils. The seedlings of many other species are more sensitive to metal pollution than adult plants [33].

The aim of this study was to evaluate the tolerance and phytoremediation capacity of the invasive tree *P. glandulosa* to different concentrations of metals when growing on raw sewage sludge and sewage-amended soils. With this aim, we recorded the concentrations of nine metals in the growth medium before and seven months after planting *P. glandulosa* in six different treatments along a gradient of sewage sludge content ranging from 100% agricultural soil to 100% sewage sludge in a greenhouse experiment. We also recorded the concentration of those metals in different plant organs (roots, stems, and leaves) and the growth of *P. glandulosa* plants. We hypothesized that invasive *P. glandulosa* would tolerate high concentrations of metals in the growth medium, being able to phytoremediate them.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Seeds of *Prosopis glandulosa* were collected in September 2016 from multiple mature individuals invading the Wadi Merikwan, Gebel Elba National Park, in the southeast of Egypt (22°14′2″ E–36°36′30.1″ W)(see Ball [34] and Al-Gohary [35] for a site description). A greenhouse experiment was conducted at South Valley University (Qena, Egypt, 26°11′36.5″ N–32°44′38.4″ E) from 1 February to the end of August 2017. Seeds (one seed per pot) of *P. glandulosa* were sown

1 cm deep in the growth media in plastic pots (17 cm deep and 14 cm diameter). A total of 36 pots were used, including six pots (replicate) for each treatment. The growth media were prepared by mixing agricultural soil from the campus of South Valley University with different proportions of sewage sludge from the residual water treatment plant of Qena (26°09´19.8´´ N–32°46´35.8´´ E). The soil consisted of coarse sand (72%), silt (8%), and clay (18%) with a sandy loam texture, pH of 8.25, Ec of 0.87 ds/m, and organic matter content of 4.1%. Treatments were designated as T1 for agricultural soil without sewage sludge (100% soil), T2 (80% soil + 20% sewage sludge), T3 (60% soil + 40% sewage sludge), T4 (40% soil + 60% sewage sludge), T5 (20% soil + 80% sewage sludge), and T6 (100% sewage sludge). Plants were maintained under ambient light in the greenhouse and they were irrigated once a day, avoiding leaching and ensuring a soil moisture value of about 70% of its water holding capacity.

2.2. Growth Media Analyses

The analytical method was assessed using a standard reference material (soil from South Valley University and sewage sludge from Qena governorate), which was included in the triplicate analyses as a part of the quality assurance and quality control protocol (accuracy within 100 ± 10%). Reagent blanks and standards were used to ensure precision and accuracy analysis. Growth medium samples were taken from each pot before planting and after harvesting. pH was determined in a 1:5 (*v*/*v*) suspension of growth medium in water using a pH meter (Crison CM35+, Barcelona, Spain). Organic matter was measured using the LOI method [36]. Samples (1.0 g) were digested with a mixture of HCl and HNO_3 for chemical analysis. The extracts used for chemical analysis followed Tisdall & Oades [37] and each sample was analyzed in triplicate. It is worth mentioning that this method was limited to the pseudo-total, not total, concentrations of metals. Total nitrogen (N) concentration was obtained using the Kjeldahl method. Phosphorous (P) concentration was measured calorimetrically using a UV-VIS spectrophotometer. The pseudo-total concentration of nine metal(loid)s, including Cadmium (Cd), Lead (Pb), Copper (Cu), Nickel (Ni), Zinc (Zn), Chromium (Cr), Cobalt (Co), Arsenic (As), and Iron (Fe), was determined using an atomic absorption spectrometer (Thermo Scientific ICE 3200) (PerkinElmer Atomic Absorption Spectrometers Analyst 400, Waltham, MA, USA).

2.3. Plant Traits

At the end of the experiment (the end of August 2017), the number of leaves per plant of *P. glandulosa* was counted. Plant height was measured from the base of the main stem to the tip of the upper leaf using a ruler. After harvesting, plants were separated into leaves, stems, and roots, and dried at 80 °C for 48 h in a forced-air oven, and their dry weights (DW) were recorded.

For plant chemical analysis after harvesting, 1.0 g DW of plant samples (leaves, stems, and roots) was digested in 20 mL H_2SO_4 (96%) and H_2O_2 (30%), and their concentrations of elements (µg/g DW) were recorded, as previously reported for soil samples. Total element uptake (U) was calculated by multiplying the element concentration measured in the different plant organs (leaves, stems, and roots) by the corresponding biomass (DW) [38]. The bioconcentration factor (*BCF*), an index of metals extracted from soil and accumulated in plant tissues, is the ratio of plant to soil metal concentration and the translocation factor (*TF*), an index of metals translocated from the root to the shoot, is the ratio of plant shoot to root metal concentration [39]. A *TF* > 1 means that the plant has a greater capability to transport metals from the roots to aboveground biomass:

$$BCF = \frac{C_{plant}}{C_{soil}}$$

$$TF = \frac{C_{shoot}}{C_{root}},$$

where C_{plant} is the metal concentration in the plant (µg/g), C_{soil} is the metal concentration in soil, C_{shoot} is the metal concentration in the plant shoots (µg/g), and C_{root} is the metal concentration in roots.

2.4. Statistical Analysis

Statistics were carried out using SPSS release 12.0 (SPSS Inc., Chicago, IL, USA). The data series were tested for homogeneity of variance, using Levene's test, and for normality, using the Kolmogorov-Smirnov test. Differences in plants and growth media between treatments were tested by one-way analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) test as a post-hoc test. The student *t*-test was used to compare growth media properties before planting and after harvesting.

3. Results

3.1. Effects of Sewage Sludge on Growth Media

Increasing the sewage sludge proportion acidified the growth media before planting (ANOVA, F = 30.4, $P < 0.001$) and after harvesting (ANOVA, F = 167.0, $P < 0.001$). The pH was always higher before planting than after harvesting (*t*-test, $P < 0.05$), except for T6 (*t*-test = 4.52, $P = 0.10$) (Table 1). Increasing the sewage sludge content in the growth medium provoked increases in soil organic matter before planting (ANOVA, F = 2417.4, $P < 0.001$) and after harvesting (ANOVA, F = 286.5, $P < 0.001$). Organic matter was slightly lower before planting than after harvesting for T2 (*t*-test = 13.5, $P < 0.05$), showing the opposite response for T4, T5, and T6 (*t*-test, $P < 0.001$) (Table 1).

Table 1. Soil pH and soil contents of organic matter (OM), Nitrogen (N), Phosphorus (P), Potassium (K), Cadmium (Cd), Lead (Pb), Copper (Cu), Nickel (Ni), Zinc (Zn), Chromium (Cr), Cobalt (Co), arsenic (As), and Iron (Fe) for different mixtures of agricultural soil and sewage sludge (100% soil (T1), 80% soil + 20% sewage sludge (T2), 60:40 (T3), 40:60 (T4), 20:80 (T5), and 100% sewage sludge (T6)) before planting and after harvesting of *Prosopis glandulosa*. Different letters indicate significant differences between treatments before planting or after harvesting (ANOVA, $P < 0.05$; Tukey's honest significant difference (HSD) test, $P < 0.05$) and asterisks indicate significant differences between before planting and after harvesting (*t*-test, $P < 0.05$).

		T1	T2	T3	T4	T5	T6
pH	Before planting	8.25 a *	7.85 b *	7.29 c *	7.43 c *	7.12 cd *	6.86 d
	After harvesting	7.98 a	7.10 b	6.85 c	6.70 cd	6.74 cd	6.60 d
OM (%)	Before planting	4.10 d *	5.00 d *	8.03 c	17.60 c *	18.10 b *	27.57 a *
	After harvesting	5.10 d	5.30 d	8.03 c	10.44 b	10.50 b	16.80 a
N (%)	Before planting	0.19 c *	0.19 c	0.29 c	0.52 b	0.64 b *	0.80 a *
	After harvesting	0.14 e	0.20 de	0.30 d	0.51 c	1.09 b	1.71 a
P (%)	Before planting	0.13 c	0.33 ab	0.25 b	0.10 c *	0.30 b *	0.43 a *
	After harvesting	0.11 e	0.21 de	0.31 d	0.83 c	1.44 b	1.84 a
K (%)	Before planting	0.67 a *	0.68 a *	0.65 ab *	0.53 bc *	0.40 c	0.24 d *
	After harvesting	0.26 b	0.26 b	0.24 b	0.28 b	0.47 a	0.51 a
Cd (µg/g)	Before planting	1.34 a	1.73 a *	1.84 a	1.37 a	1.43 a	1.93 a
	After harvesting	0.60 a	0.80 a	1.23 a	1.23 a	1.47 a	0.93 a
Pb (µg/g)	Before planting	9.94 a	15.50 a	15.08 a	16.47 a	11.83 a	13.53 a
	After harvesting	6.93 a	7.00 a	7.63 a	10.37 a	14.13 a	8.40 a
Cu (µg/g)	Before planting	21.57 e *	25.46 e *	36.63 d *	51.87 c *	64.93 b *	96.00 a *
	After harvesting	4.90 f	8.63 e	13.17 d	24.50 c	46.47 b	65.45 a
Ni (µg/g)	Before planting	115.54 a *	95.23 ab *	83.70 b *	87.93 b *	70.47 bc *	54.63 c *
	After harvesting	20.07 a	17.13 a	11.90 a	2.70 b	1.36 b	1.21 b
Zn (µg/g)	Before planting	75.82 d *	93.63 d *	156.65 d *	254.83 c *	511.90 b *	1530.1 a *
	After harvesting	20.13 e	23.47 e	35.93 d	42.93 c	59.70 b	83.55 a
Cr (µg/g)	Before planting	116.15 d *	127.90 c *	129.27 c *	129.87 c *	141.30 b *	177.3 a *
	After harvesting	79.33 c	100.87 ab	106.67 ab	91.50 bc	113.63 a	109.6 ab
Co (µg/g)	Before planting	55.96 ab *	59.60 a *	67.74 a *	48.70 ab *	44.97 ab *	34.23 b *
	After harvesting	23.23 a	10.13 b	26.20 a	10.27 b	8.40 b	1.80 c
As (µg/g)	Before planting	0.234 a *	0.280 a *	0.334 a *	0.235 a *	0.200 a	0.166 a
	After harvesting	0.124 c	0.039 b	0.140 c	0.025 a	0.188 d	0.095 cd
Fe (µg/g)	Before planting	2.084 a *	2.055 a *	2.042 a *	1.643 a *	1.229 a *	1.408 a *
	After harvesting	0.770 a	0.432 ab	0.384 ab	0.353 b	0.459 ab	0.461 ab

The concentration of N, Cu, Zn, and Cr increased with increasing sewage sludge content before planting (ANOVA, $P < 0.001$) and after harvesting (ANOVA, $P < 0.01$) (Table 1). In contrast, the concentration of K decreased gradually with increasing sewage sludge content before planting (ANOVA, F = 38.91, $P < 0.001$) and increased for T5 and T6 after harvesting (ANOVA, $P < 0.001$; HSD-test, $P < 0.05$) (Table 1). The concentration of Ni and Co decreased with increasing sewage sludge content before planting (ANOVA, $P < 0.01$) and after harvesting (ANOVA, $P < 0.001$) (Table 1). The concentration of As decreased with a higher sewage sludge content before planting, with its maximum reached for T3 (ANOVA, F = 40.7, $P < 0.001$; HSD-test, $P < 0.05$), without showing significant differences between treatments after harvesting (ANOVA, $P > 0.05$) (Table 1). The addition of sewage sludge provoked no significant effects on the concentrations of Cd, Pb, and Fe in the growth media (ANOVA, $P > 0.05$) (Table 1).

Nitrogen and P concentrations in the growth media were higher after harvesting than before planting for T5 and T6, and T4, T5, and T6, respectively (*t*-test, $P < 0.001$) (Table 1). In contrast, the K concentration was lower after harvesting than before planting for T1–4 (*t*-test, $P < 0.01$) and only higher for T6 (*t*-test = 10.9, $P < 0.001$) (Table 1). The concentrations of Cu, Ni, Zn, Cr, Co, As, and Fe were lower after harvesting than before planting for every treatment (*t*-test, $P < 0.05$) (Table 1). The concentrations of Cd and Pb showed no significant differences during the experiment (Table 1).

3.2. Effect of Sewage Sludge on Plant Traits

Table 2 presents the ANOVA of plant traits under different mixtures of agricultural soil and sewage sludge. The plants of *Prosopis glandulosa* were taller for T4, T5, and T6 than for T1, T2, and T3 (ANOVA, F = 16.1, $P < 0.001$; HSD-test, $P < 0.05$) (Figure 1). The number of leaves per plant reached its maximum for T2 and T6 and minimum for T1 (ANOVA, F = 11.3, $P < 0.001$; HSD-test, $P < 0.05$) (Figure 1). The biomass of leaves, shoots, and roots tended to be higher for T5 and T6 than for the other treatments, without showing significant differences (ANOVA, $P > 0.05$) (Figure 1).

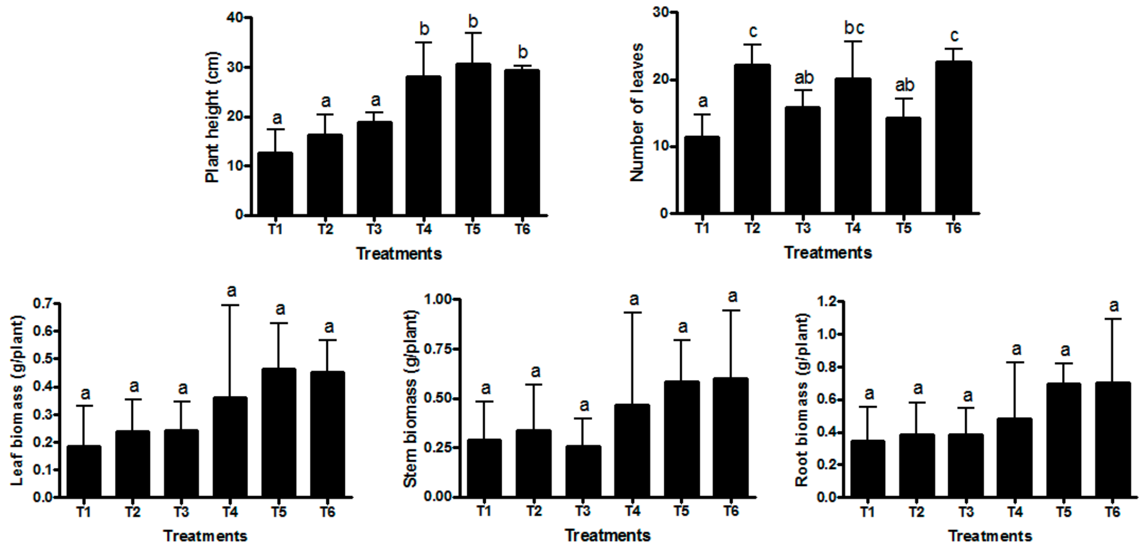

Figure 1. Plant height, number of leaves, and leaf, stem, and root biomass of *Prosopis glandulosa* grown on mixtures of agricultural soil and sewage sludge (100% soil (T1), 80% soil + 20% sewage sludge (T2), 60:40 (T3), 40:60 (T4), 20:80 (T5), and 100% sewage sludge (T6)). Different letters indicate significant differences between treatments (ANOVA, $P < 0.05$; HSD-test, $P < 0.05$).

Table 2. One-way analysis of variance (ANOVA) for plant height (cm), number of leaves, and leaves and roots biomass of *Prosopis glandulosa* under different soil and sewage sludge mixtures.

	DF	SS	MS	F. Value
Plant height	5	1771.6	354.3	16.1 **
Number of leaves	5	623.6	124.7	11.3 **
Leaf biomass	5	0.423	0.085	2.59 *
Root biomass	5	0.782	0.156	2.40 n.s.

DF: degree of freedom; SS: sum of squares; MS: mean of squares; * $P < 0.05$; ** $P < 0.001$, n.s. not significant.

The concentration of N in leaves was the highest for T2, T3, and T6 (ANOVA, F = 8.63, $P < 0.01$; HSD-test, $P < 0.05$), whereas the concentration of P was significantly higher for T6 compared to the other treatments (ANOVA, F = 20.64, $P < 0.001$; HSD-test, $P < 0.05$). The foliar concentration of Cu, Ni, Zn, Cr, Co, As, and Fe increased with increasing sewage sludge content, reaching their maximum values for T4, except for Fe, which reached its maximum for T5 (ANOVA, $P < 0.001$) (Figure 2). No significant differences were shown among treatments for the foliar concentration of K, Cd, and Pb (ANOVA, $P > 0.05$) (Figure 2).

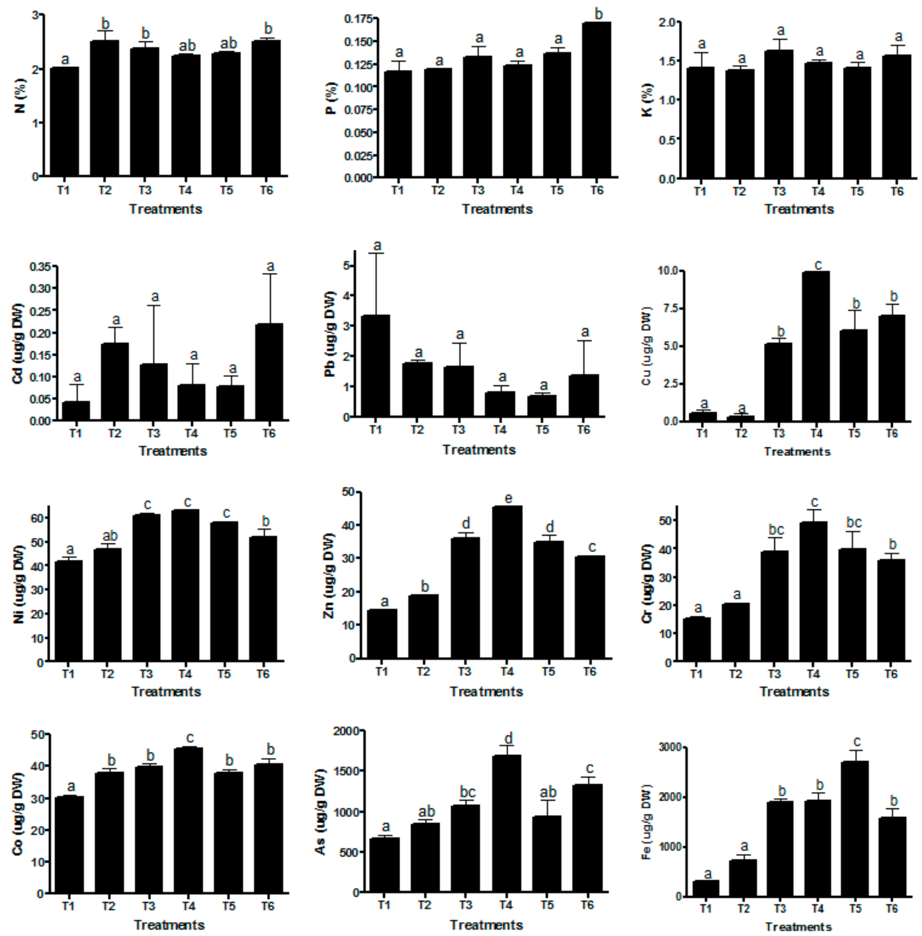

Figure 2. Nitrogen (N), Phosphorus (P), Potassium (K), Cadmium (Cd), Lead (Pb), Copper (Cu), Nickel (Ni), Zinc (Zn), Chromium(Cr), Cobalt (Co), Arsenic (As), and Iron (Fe) contents in leaves of *Prosopis glandulosa* grown on mixtures of agricultural soil and sewage sludge (100% soil (T1), 80% soil + 20% sewage sludge (T2), 60:40 (T3), 40:60 (T4), 20:80 (T5), and 100% sewage sludge (T6)). Different letters indicate significant differences between treatments for the same plant part (ANOVA, $P < 0.05$; HSD-test, $P < 0.05$).

Regarding the roots, there were no significant differences between treatments for N, K, and Pb concentrations (ANOVA, $P > 0.05$). The concentration of P in the roots tended to increase with increasing sewage sludge content; however, it showed its maximum for T2 (ANOVA, F = 75.67, $P < 0.001$). The concentrations of Ni, Cr, Co, and As showed their maximum values for T3 and T6 (Figure 3). The concentration of Cd in the roots increased under T6 compared to all other treatments (ANOVA, F = 72.79, $P < 0.001$; HSD-test, $P < 0.05$), whereas the concentrations of Cu, Zn, and Fe in the roots rose with increasing sewage sludge content (ANOVA, $P < 0.001$) (Figure 3).

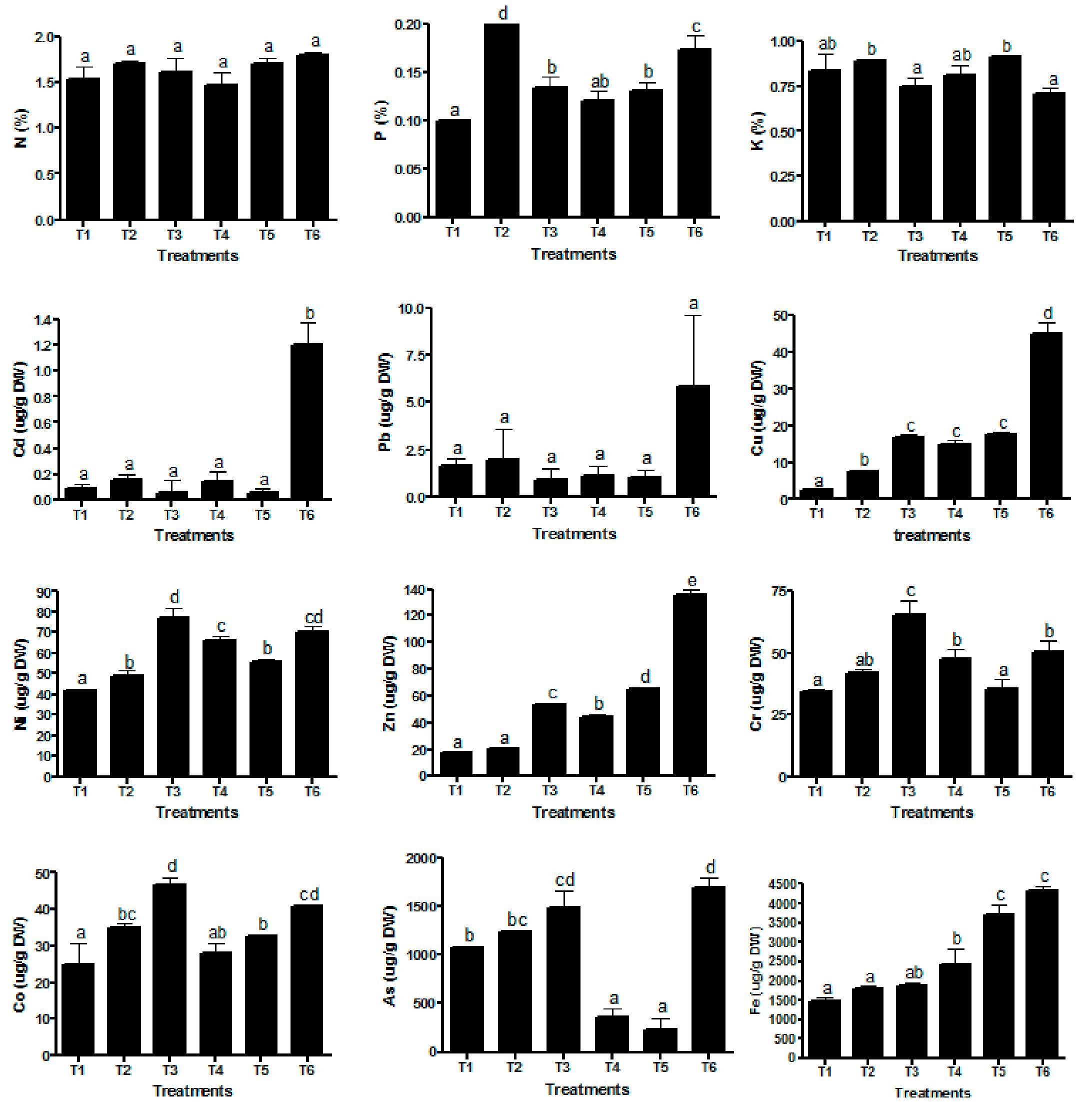

Figure 3. Nitrogen (N), Phosphorus (P), Potassium (K), Cadmium (Cd), Lead (Pb), Copper (Cu), Nickel (Ni), Zinc (Zn), Chromium(Cr), Cobalt (Co), Arsenic (As), and Iron (Fe) contents in roots of *Prosopis glandulosa* grown on mixtures of agricultural soil and sewage sludge (100% soil (T1), 80% soil + 20% sewage sludge (T2), 60:40 (T3), 40:60 (T4), 20:80 (T5), and 100% sewage sludge (T6)). Different letters indicate significant differences between treatments for the same plant part (ANOVA, $P < 0.05$; HSD-test, $P < 0.05$).

The total uptake (U) of every element by *P. glandulosa* increased greatly when increasing the sewage sludge content in growth medium. The highest U values were recorded for T6, where the highest accumulated metal was Fe, followed by As > Zn > Ni > Co > Cr > Cu > Pb > Ni. Under T1 (100% soil), the highest accumulated metal was As followed by Fe > Ni > Co > Cr > Zn > Pb > Cu > Cd. The metal accumulation under T6 was more than 3000-times that for Cu, 2000-times that for Cd, 1000-times that for Zn, and 600-times that for Fe when compared with T1. The other elements increased between 265- and 413-times under T6 when compared to T1 (Figure 4).

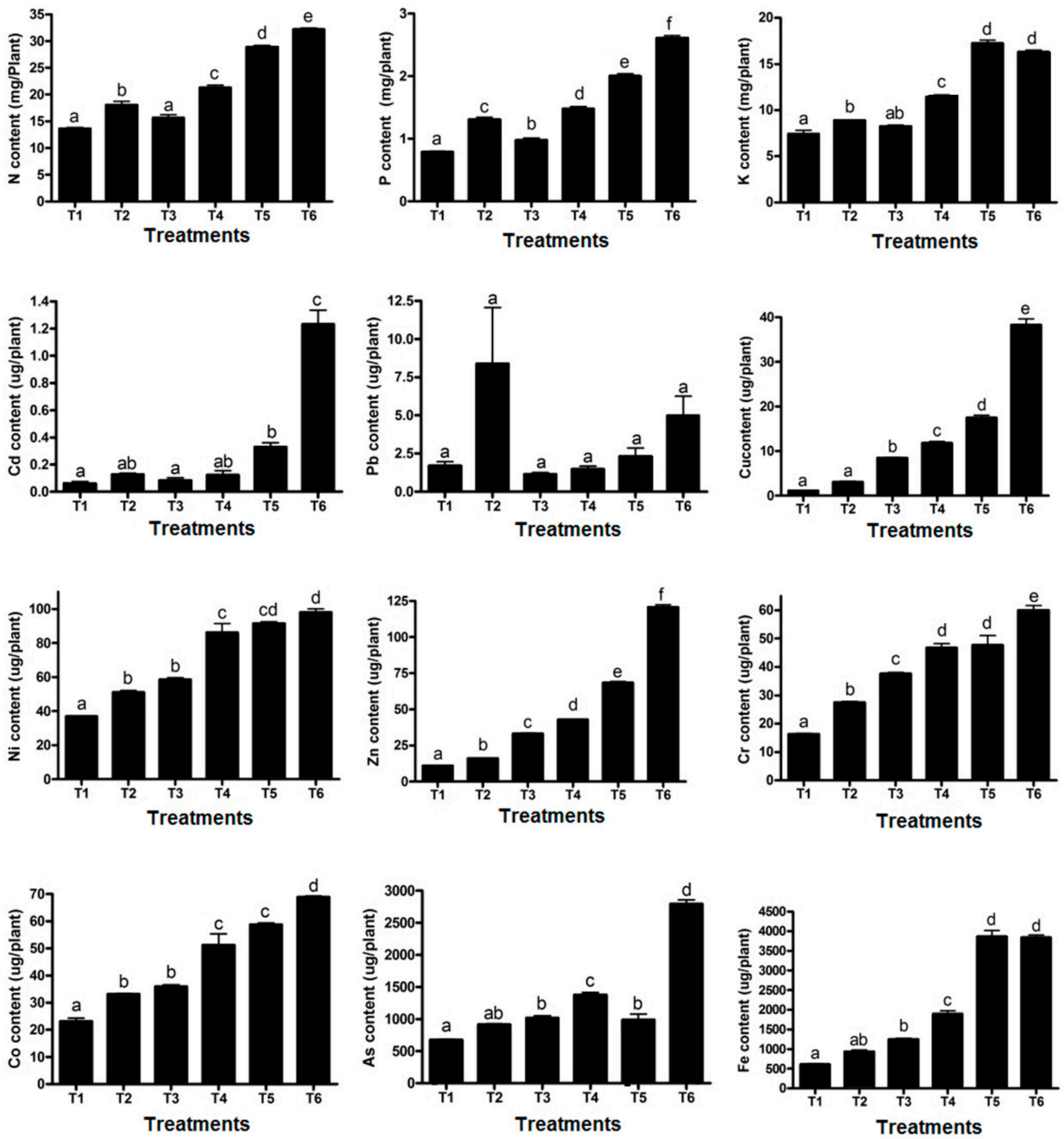

Figure 4. Total metal content for plants of *Prosopis glandulosa* under different soil and sewage sludge mixtures: (T1) (control, 100% soil), (T2) (80% soil+20% sewage sludge), (T3) (60% soil+40% sewage sludge), (T4) (40% soil+60% sewage sludge), (T5) (20% soil+80% sewage sludge), and (T6) (100% sewage sludge). Different letters indicate significant differences between treatments (ANOVA, $P < 0.05$; HSD-test, $P < 0.05$).

The TF was lower than 1 for Cu, Zn, Cr, and Fe metals in all treatments, but other metals showed TF values of more than 1 under some treatments (Table 3). The highest TF values were shown at T5 for Cd (4.829), T2 for Pb and Ni (4.192 and 1.157, respectively), and T4 for Co and As (1.613 and 4.098, respectively). On the other hand, the BCF increased with sewage sludge addition. The highest BCF values were shown at T6 for Cd, Pb, Ni, and Co (0.749, 0.337, 46.077, and 21.753, respectively); at T3 for Cu, Zn, and Cr (0.724, 1.046 and 0.400, respectively); and at T4 for As (4.186). The BCF of metals showed gradual increases with increasing sewage sludge content (Table 3). It is worth mentioning that the concentrations of Co and Ni in plants were more than 21- and 42-times higher, respectively, when compared to soil with T6 treatment, and were more than four-times higher for As with T3.

Table 3. Bioconcentration factor (BCF) and translocation factor (TF) of metals under different soil and sewage sludge mixtures: T1 (control, 100% soil), T2 (80% soil+20% sewage sludge), T3 (60% soil+40% sewage sludge), T4 (40% soil+60% sewage sludge), T5 (20% soil+80% sewage sludge), and T6 (100% sewage sludge).

	Cd	Pb	Cu	Ni	Zn	Cr	Co	As	Fe
Bioconcentration Factor (BCF)									
T1	0.118	0.295	0.280	2.235	0.660	0.250	1.208	0.661	0.099
T2	0.163	0.166	0.374	3.131	0.724	0.286	3.426	2.464	0.228
T3	0.073	0.170	0.724	5.581	1.046	0.400	1.553	1.017	0.369
T4	0.078	0.108	0.370	25.733	0.764	0.392	3.816	4.186	0.411
T5	0.129	0.095	0.216	38.576	0.663	0.241	4.019	0.302	0.484
T6	0.749	0.337	0.333	46.077	0.822	0.312	21.753	1.674	0.474
Translocation Factor (TF)									
T1	0.586	1.393	0.251	1.133	0.580	0.271	1.219	0.588	0.141
T2	0.729	4.192	0.051	1.157	0.701	0.484	0.993	0.622	0.254
T3	1.816	1.761	0.216	0.747	0.469	0.378	0.776	0.593	0.574
T4	0.485	0.945	0.371	0.998	0.596	0.608	1.613	4.098	0.371
T5	4.829	1.373	0.295	0.914	0.344	0.625	1.055	3.584	0.330
T6	0.301	0.141	0.137	0.651	0.179	0.461	0.936	0.889	0.176

4. Discussion

The environmental factors, especially soil properties, are the key factors limiting metal bioavailability. Chiroma et al. [40] determined the permissible level of heavy metals in soil as follows: Cd (3 µg/g); As (20 µg/g); Co and Ni (50 µg/g); Cr, Cu, and Pb (100 µg/g); Zn (300 µg/g); and Fe (50000 µg/g). Thus, the solubility of many metals increases in acidic soils [41], whereas their availability is reduced by high levels of organic matter due to metal-organic complications [42]. In our study, organic matter increased and the growth medium tended to be more acidic when increasing its sewage sludge content, which may have resulted in reducing the negative impacts of metal availability, raising the cation exchange capacity in the soil and the nutrient accessibility for plant production due to the decomposition of the organic matter [43]. In this sense, pH decreased about 10%, organic matter content decreased about 40%, and N and P concentrations in the growth medium increased significantly after harvesting in comparison with before planting under high sewage sludge contents. Thus, the application of sewage sludge improved edaphic properties; however, it also increased the concentrations of some metals in the soil, as reported in previous studies [2,44]. Nevertheless, the presence of the least concentrated metals in the sewage sludge did not change (Cd and Pb) or were lowered (Ni, Co, and As) by its addition. Before planting *Prosopis glandulosa*, the recorded concentrations of Cd, Pb, Cu, and Fe in the growth medium were below their permissible limits for every treatment according to the levels set by the World Health Organization (WHO) [40]. The concentration of Co in the growth medium decreased under its permissible limit after the addition of 80%–100% of sewage sludge. The concentrations of Ni and As were higher than its permissible levels for every treatment, even when levels decreased with an increase of the sewage sludge content. The concentrations of Cr and Zn increased with the sewage sludge content in the growth medium before planting, and were always higher than its permissible level for Cr and at higher sewage sludge contents (80%–100%), for Zn (Table 1).

The plants of *P. glandulosa* showed a high tolerance to metals, which was reflected by taller plants with more leaves that tended to accumulate more biomass, even when grown on raw sewage sludge. *P. glandulosa* harbors some metal-resistant bacteria and arbuscular mycorrhizal in the rhizosphere and endosphere that improve its tolerance and efficiency of phytoremediation of metal-degraded soils [45,46]. In general, the macronutrients concentration in *P. glandulosa* tissues did not show clear relationships with the addition of sewage sludge. Thus, the concentration of N increased in leaves by 20%–40% at 100% sewage sludge, without showing significant differences between treatments in roots. The concentration of P increased in leaves when growing on raw sewage sludge and in the roots for 20% sewage sludge, and the concentration of K was similar for every treatment in leaves and roots. On the other hand, the abundance of Pb and Cd, the less concentrated metals in the growth medium independent of the sewage sludge content, were similar among treatments in the leaves and roots, except for Cd in roots for the highest sewage sludge contents. This may be related to the presence of a transport peptide in this species that seems to have an important role in Cd uptake [47]. In contrast, the concentration of Cu, Zn, Cr, Ni, Co, and Fe in leaves and roots increased with the addition of sewage sludge. Mokgalaka-Matlala [48] recorded the reduced growth of *Prosopis* sp. due to high As concentrations. *P. glandulosa* has a deep and extensive root system [49] that allows it to explore a high volume of soil, where it may interact with metals.

The total uptake of most metals by *P. glandulosa* plants increased with the addition of sewage sludge, which may be induced by an increment of their availability, probably related to the acidification and their increasing concentrations in the growth medium [4,50]. As a result of this increase in metal uptake and the tendency to accumulate more biomass at higher sewage sludge contents, the *P. glandulosa* plants accumulated many more metals in their biomass due to the addition of sewage sludge. Because of this, *P. glandulosa* was able to extract enough metals from the growth media to significantly reduce their concentration after seven months of planting. Consequently, *P. glandulosa* reduced the concentration of Cu, Ni, Zn, and Co to below their permissible level following the WTO standards [40].

The TF was less than 1 under all treatments for Cu, Zn, Cr, and Fe, representing a low capability to transport metals from roots to AGB. This was associated with BCF values of less than 1 for those metals, with the exception of Zn under T3 treatment. *P. glandulosa* concentrated more Cd, Pb, Ni, Co, and As in its AGB than in its BGB, when grown under some treatments of sewage sludge. The TF was less than 1 for all metals under T6 (100% sewage sludge). Root activity can act as a barrier for metal translocation to the photosynthetic organs [51]. The higher concentrations of metals (Cu, Zn, Cr, and Fe) in the roots, rather than ABB, pointed to the low capacity of *P. glandulosa* to phytoextract such metals when growing under high concentrations. Ni, Co, and As accumulation increased in both BGB and AGB with increasing sewage sludge concentration, however, the concentrations of both Ni and Co were rather low. BCF and TF also increased gradually with increases of sewage sludge concentration, these increments are suggested to be artificial since increasing the sludge concentration made it easier for the plant to uptake such metals. These results support the capability of *P. glandulosa* to phytostabilize such metals when growing under high concentrations. Hammond et al. [52] recorded the sequestration of As in the epidermis and the vacuoles of the roots of *P. glandulosa*. Although the concentration of As in soil was extremely law, its concentration in leaves increased to much more than the permissible level (1000 µg/g) in some treatments, as well as the increments of BCF and TF that suggested the promise of *P. glandulosa* for As phytoextraction [39,53,54]. The significant decreases of most metals' concentration in soil after seven months of planting *P. glandulosa* were associated with an increase of those metals in plants and increasing BCF, representing the capability of plants to accumulate those metals in their tissues. Our results are consistent with previous findings, which showed that *P. glandulosa* is an effective remediator of polluted soils and tannery sludges polluted with different metals [29–31]. Despite the capability of *P. glandulosa* to remediate metals and metalloids from soil and accumulate them in plant tissue, potential accumulation was only shown for As when its content was more than 1000 µg/g under high sewage sludge contents.

5. Conclusions

In view of our results, *P. glandulosa* has potential for the phytoremediation of sewage sludges and sewage-amended soils, limited to sandy loam soil, when they are polluted with metals such as Cd, Zn, Ni, Co, and As, with a potential accumulation capability of As. Due to the ability of *P. glandulosa* to colonize semi-arid and arid lands, and because its tolerance to metals is improved by abiotic stress [28], this invasive tree could be used to remediate metal-polluted sewage sludge, agricultural soils, and mine tailings in arid and semi-arid environments. In this context, *P. glandulosa* shows different ecotypes with a contrasted tolerance to metals [53]. Because of the high invasive capacity of *P. glandulosa* [25], we recommend that, if this species is grown outside of its native range, only young plants should be used before they have reached their reproductive stage. At the same time, for phytoremediation, *P. glandulosa* may be used as a multipurpose crop during its cultivation, for example, for carbon storage, erosion control, and fertility maintenance, and, once removed from the remediated site, it may be valuable for energetic use in an integrated phytomanagement strategy [55].

Author Contributions: Conceptualization, A.M.A., J.M.C. and W.S.S.; methodology, S.M.E.-A.; software, S.K.A.-E.; validation, J.M.C., A.M.A. and W.S.S.; formal analysis, N.E.-T.; investigation, A.M.A., J.S.C. and W.S.S.; resources, A.M.A.; data curation, S.K.A.-E.; writing—original draft preparation, W.S.S.; writing—review and editing, J.M.C.; visualization, J.M.C.

Funding: This research received no external funding.

Acknowledgments: The authors thank Mansour Hussein for his work in the greenhouse facilities of South Valley University. The authors would like to express their gratitude to King Khalid University, Saudi Arabia, for providing administrative and technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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