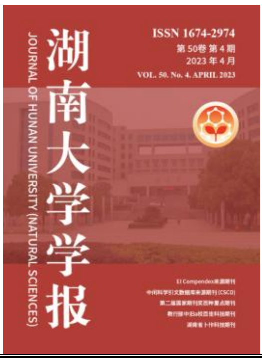

湖南大学学报（自然科学版） 第 50 卷 第 4 期
Journal of Hunan University (Natural Sciences) 2023 年 4 月

Available online at http://jonuns.com/index.php/journal/index Vol. 50 No. 4
April 2023

Open Access Article https://doi.org/10.55463/issn.1674-2974.50.4.6

Phytoremediation Potential of Arbuscular Mycorrhizal Fungi and Berseem in Soil Irrigated with Industrial Wastewater

Sara Nawaz Khan[1]*, Muhammad Nafees[1], Muhammad Imtiaz[2]

[1] Department of Environmental Sciences, University of Peshawar, Peshawar, Pakistan

[2] Soil and Environmental Sciences Division, Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, Pakistan

* Corresponding author: sarahnawaz9@gmail.com

Received: February 16, 2023 ▪ Revised: March 7, 2023 ▪ Accepted: March 14, 2023 ▪ Published: April 28, 2023

Abstract: This article describes a new idea about industrial pollution abatement through berseem (*Trifolium alexandrinum* L.) using arbuscular mycorrhizal fungi (AMF) Glomus Spp. as an accelerating agent. For this purpose, berseem plant was grown on soil irrigated with industrial wastewater in pots. Three cuttings of plants were obtained at different intervals. At each cut, plant biomass, plant height, and rate of mycorrhizal infection were recorded and plant tissues (roots, stems and leaves) were analyzed to determine the concentrations of Cd, Cr, Pb, and Zn as indicators. The results showed that the biomass of the roots, stems, and leaves of the inoculated plants was higher than that of the un-inoculated plants. The height of inoculated plants was higher compared with that of uninoculated plants. The mycorrhizal infection rate was recorded highest at the third cut. Heavy metals in plants were within permissible limits for crops irrigated with wastewater) at each cutting. With inoculation, Zn and Cr concentrations in roots and leaves increased and Pb and Cd enhanced in roots but reduced in leaves as compared to un-inoculated plants, indicating that metals accumulated more in roots due to inoculation. The effectiveness can be confirmed by calculating the difference between the inoculated and non-inoculated plants. The new results showed that AMF inoculation promoted plant biomass and height, reduced metal transfer in leaves and stems, and to roots. This research confirmed that the procedure can be used for protecting plants from toxicity.

Keywords: arbuscular mycorrhizal fungi, heavy metals, biomass, mycorrhizal infection rate, berseem.

丛枝菌根真菌和贝尔森在工业废水灌溉土壤中的植物修复潜力

摘要:

本文介绍了使用丛枝菌根真菌(AMF)球藻的贝西姆(三叶草.)减少工业污染的新思路。作为促进剂。为此，将贝西姆植物种植在用盆中的工业废水灌溉的土壤上。在不同的时间间隔获得三个植物插条。在每次切割时，记录植物生物量、植物高度和菌根感染率，并分析植物组织（根、茎和叶）以确定镉、铬、铅和锌的浓度作为指标。结果表明，接种植株根、茎、叶的生物量均高于未接种植株。与未接种植物相比，接种植物的高度更高。菌根感染率在第三次切割时记录最高。每次收割时，植物中的重金属均在废水灌溉作物允许的限度内）。接种后，与未接种的植物相比，根和叶中的锌和铬浓度增加，根中的铅和光盘浓度增加，而叶片中

的浓度降低，表明由于接种，金属在根中积累更多。有效性可以通过计算接种和未接种植物之间的差异来确认。新结果表明，AMF接种促进了植物生物量和高度，减少了叶子和茎中以及根部的金属转移。该研究证实该程序可用于保护植物免受毒性侵害。

关键词：丛枝菌根真菌，重金属，生物量，菌根感染率，贝西姆。

Introduction

In water-deficient regions, wastewater reuse for irrigation is a growing and common practice as water resources are declining. Due to the discharge of untreated wastewater from industries, areas where urban wastewater is used for irrigation are contaminated with toxic and harmful elements, specifically Pb, Cd, Cr and Zn. Being a potential accumulator in soil and plants, these harmful elements may eventually make their way to the food chain and affect human health [1].

To clean up heavy metal-polluted soils, phytoremediation has proved to be a cost effective and environmentally friendly method compared to different physical and chemical methods [2,3]. To remediate polluted soil, plants store metals in roots or transfer them to shoots (phytostabilization and phytoextraction) when grown on contaminated soil [4]. Symbiotic association of plants with microorganisms also plays a very important role in enhancing a plant's ability to adapt to stressed conditions. Different studies have shown the use of microorganisms with plants for remediation, such as AMF involved in interactions with plants in soil through mycelia [5]. Due to its interaction with plant roots, AMF helps in enhanced nutrient uptake by enlarging the volume of soil [6]. Studies have shown the effect of AMF on the growth of plants, nutritional status, and protection from different stressed conditions such as water stress, salinity, pathogens, and heavy metals, thus protecting plants [7, 8].

The reaction of plants to AMF inoculation depends upon plant species, mycorrhizal species, and soil characteristics [9-12]. The diverse nature and abundance of AMF can be used in agricultural management [13]. In a previous study conducted on berseem, high bioconcentration factor (BCF) from soil to roots was reported for Zn as compared to Pb, Cu, and Cd, and high translocation factor (TF) was recorded for Zn in leaves in a pot experiment [14]. [15] reported higher *T. alexandrinum* productivity in the field and pots with AMF inoculation, and plants showed significantly higher nutrient content compared to control plants.

Symbiotic relationship of AMF with plant roots and berseem being a multi-cut plant, having regrowth ability after cutting can give several cuttings in a season that can be used to determine its heavy metal uptake ability in metalliferous soil and to remediate polluted soil. The aim of this study was to investigate the combined effect of AMF inoculum on berseem in soil irrigated with wastewater. The objectives were to; (i) assess heavy metal concentration in tissues of plants at three different cuttings and (ii) determine plant height, biomass, and mycorrhizal infection rate at each cutting.

1. Material and Methods

1.1. Materials

Wastewater samples for irrigation purpose were selected from 5 main drains of Hayatabad Industrial estate, Peshawar, as these 5 main drains received wastewater from a cluster of different industries. The samples were analyzed for pH using a PHS- 3C pH meter, electrical conductivity (EC) according to standard methods [16] and Cd, Cr, Pb and Zn were determined using an atomic absorption spectrometer (Perkin-Elmer AAS-700). Standard reference materials were used for quality control and assurance for selected heavy metal analysis.

Soil for the experiment was collected from the field at the Nuclear Institute for Food and Agriculture (NIFA) and physicochemical properties of soil were determined. In order to sow seeds, the soil was mixed thoroughly, passed through a sieve and autoclaved at 110 ˚C at 240 kPa pressure for 1 h in order to make the soil free of fungi. Each pot was filled with 3 kg of sterilized soil. For inoculum, AMF-infected roots, hyphae, and soil containing spores (*Glomus* spp) from the rhizosphere of Maize plants were used.

1.2. Experimental Setup

The experiment was conducted in a greenhouse at the Nuclear Institute for Food and Agriculture (NIFA), Peshawar. For this purpose, 12 pots were arranged to grow berseem (*T. alexandrinum*) in soil treated with mycorrhiza. Three pots were for control (untreated, T0), three were for soil treated with mycorrhiza (inoculated, T1), irrigated with fresh water, three pots were for untreated soil, irrigated with wastewater T2, three pots were for soil treated with mycorrhiza (inoculated T3) and irrigated with wastewater. *T. alexandrinum* seeds were sown in pots for a duration of 105 days. Treated pots consist of 23 g mycorrhiza inoculum before sowing. After germination, each pot was thinned to 10 plants per pot. *T. alexandrinum* was cut at three different stages simultaneously after germination. The first cut was done at 45 days (C1), second after 30 days of 1st cutting (C2) and third at 30 days of 2nd cutting (C3). Each cutting plant was removed from each pot for collecting root, stem, and leaves samples and were analyzed for heavy metal concentration and mycorrhiza infection status.

After each cutting, the sample plants were rinsed thoroughly with distill water and oven-dried at 70 °C for 48 hours, ground to fine powder and stored in plastic bags for chemical analysis. For digestion, 0.5 g of plant sample was taken in a flask and 10 ml of acid mixture (nitric acid (HNO_3) and perchloric acid ($HClO_4$) were used.

The percentage of mycorrhizal colonization in root infection was determined by cleaning roots with KOH and staining them with trypan blue to make it transparent for observation [17, 18]. The percentage of mycorrhizal infection was determined using [19] formula and at least 30 root segments of each sample of 1 cm were observed under the microscope.

The percent of mycorrhizal infection = Number of infected root segments × 100 (1)

The plant is widely grown as fodder and is common in the area. The indicator selected for this research was limited to the removal of selected heavy metals. It can be extended to other metals by future researchers.

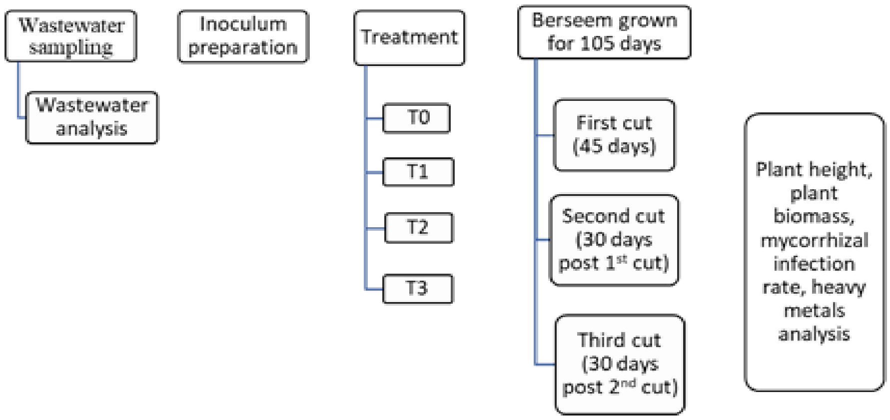

Fig. 1 Flowchart of the experiment

1.3. Statistical Analysis

The experiment was conducted in a factorial design with three replicates. Plant height, biomass, infection rate, and metal concentration data were analyzed using two-way ANOVA and LSD ($P < 0.05$) using Statistix 8.1 and correlation was calculated using 2-tailed Pearson's correlation.

2. Results and Discussion

2.1. Physicochemical Properties of Wastewater Used for Irrigating Berseem

The results of physicochemical parameters of wastewater used for irrigation are given in Table 1. The pH value ranges from 6.44 to 7.31. These values were within limits for irrigation, which ranged from 6 to 9 [20]. The electrical conductivity of wastewater ranged from 1000-1800 µS/cm. By comparison with standards for irrigation, the values were within the permissible limit (3000 µS/cm) [20]. Heavy metals concentration (mg/l) in wastewater ranged between 0.008-0.038 mg/L for Cd, 0.040–0.254 mg/L for Cr, 0.046–0.096 mg/L for Pb and 0.043–1.355 mg/L for Zn. The concentration of Zn was relatively higher as compared to Cr, Pb and Cd. Heavy metals were within permissible limits for Cd of sites 1, 2 and 3.

Table 1 Physicochemical parameters of wastewater used for irrigating berseem (The authors' elaboration)

Sites	pH	EC µS/cm	Pb mg/L	Cr mg/L	Cd mg/L	Zn mg/L
1	6.44 ± 0.101	1627 ± 44.426	0.061 ± 0.048	0.045 ± 0.025	0.016 ± 0.017	0.047 ± 0.028
2	6.64 ± 0.288	1480 ± 41.039	0.096 ± 0.038	0.254 ± 0.148	0.038 ± 0.023	0.436 ± 0.233
3	6.92 ± 0.100	1000 ± 0.00	0.064 ± 0.028	0.048 ± 0.030	0.016 ± 0.009	0.095 ± 0.043
4	6.72 ± 0.108	1800 ± 72.547	0.095 ± 0.051	0.051 ± 0.028	0.008 ± 0.004	1.355 ± 0.335
5	7.31 ± 0.073	1200 ± 0.00	0.046 ± 0.027	0.04 ± 0.018	0.008 ± 0.004	0.043 ± 0.020
[20]	6.5–8.4	3000	5	0.1	0.01	2

2.2. Soil Properties

The physicochemical parameters and heavy metal concentration in soil used in the experiment are shown in Table 2. The soil texture was clay loam and moderately alkaline in nature. In this study, the pH of soil was 7.9, as soil pH range from 6.5 to 7.5 is favorable for plant growth and nutrient availability. However, a greater pH reduces the bioavailability and mobility of metals in soil due to the formation of organic complexes, hydroxides, and carbonate precipitation [21]. The pH of soil also depends upon irrigation water and changes with wastewater irrigation

[22]. The mean concentrations of Cd, Cr and Zn in the experimental soil sample were 0.009 mg/kg, 0.004 mg/kg, 1.71 mg/kg and Pb were below the detection limit, respectively.

Table 2 Soil properties before irrigation (The authors' elaboration)

Parameter	Value
Soil textures	Clay loam
Sand	33 %
Silt	28 %
Clay	39%
pH	7.9
Cd	0.009 mg/kg
Cr	0.004 mg/kg
Pb	BDL
Zn	1.71. mg/kg

2.3. Plant Height

Plant height was significantly higher ($P < 0.05$) for T3 plants (21.4, 25.3 and 32.1 cm) in the first, second, and third cuttings compared with the height of control (T0) plants (16.2, 18.1 and 26.3 cm) (Table 3). In general, plant height was recorded highest at the third cutting, and inoculated plants showed an increase in height compared to uninoculated plants. This increase in height can be attributed to hyphal networks in the soil resulting in high acquisition of nutrients in mycorrhizal inoculated plants. [23] also recorded an increase in the height of maize plants due to inoculation. Similarly, in leguminous plants, high growth was also recorded with AMF inoculation due to increased phosphate uptake in inoculated plants [24].

2.4. Plant Biomass

In this study, the results showed that inoculated plants irrigated with wastewater (T3) showed significantly higher ($P < 0.01$) biomass, 13.01, 29.13, and 41.76 g/pot in the first, second, and third cuttings compared to 8.694, 19.31, and 31.471 g/pot of control (T0) plants; the results showed that the effect of inoculated plants together with wastewater irrigation was more prominent (Table 3). First, second, and third cuts of inoculated plants irrigated with wastewater showed significantly higher ($P < 0.01$) plant biomass as compared to control plants (Table 3). Generally, roots showed the lowest biomass and leaves showed the highest but treatment wise, T3 showed the highest root (4.692 g/ plant), stem (5.235 g/plant), and leaves (9.293 g/plant) biomass as compared to control plants at third cutting. Shoot biomass significantly correlated with plant height in the second ($r= 0.991$, $p < 0.01$) and third cuts ($r = 0.995$, $p < 0.01$), significant positive correlation was observed for stem biomass ($r = 0.987$, $p < 0.05$) and leaves biomass ($r = 0.996$, $p < 0.01$) with root biomass at the third cut, and leaves biomass showed significant correlation ($r = 0.996$, $p < 0.01$) with stem biomass (Table 4).

Higher biomass in wastewater-treated plants can be due to the high nutrient content in wastewater. Similar results were also reported in wheat and forage crops, and the reason was attributed to high N and P in wastewater [25]. An increase in nutrient content and heavy metal concentration in soil irrigated with wastewater was also reported in previous studies [26]. Studies revealed that AMF inoculated plants (wheat) showed an increase in plant biomass as compared to non-mycorrhizal plants and the reason was due to the high content of nutrients in plants due to inoculation [27]. Another research [28] reported an increase in the yield of alfalfa through wastewater irrigation and cuttings and observed a high yield as compared to control plants.

2.5. Mycorrhizal Infection Rate

Mycorrhizae infection appeared only in plants inoculated with AMF; uninoculated and control plants showed no infection (Table 3). In inoculated plants, the highest infection rate was observed for T3 plants, ranging from 27.8-76.6 % compared to 25.3–39.1 % for T1 plants. However, T3 plants showed an infection rate that significantly increased plant height and shoot biomass ($p < 0.05$) (Table 4). This can be attributed to the fact that the availability of nutrients is increased in the soil through wastewater irrigation and, as mycorrhizae are known to improve plant growth by increasing root surface area and increasing nutrient supply to roots from rhizosphere, thus resulting in increased plant biomass and growth of T3 plants as compared to T1 and un-inoculated plants [29-31]. In general, no infection rate was observed except in soil to which the inoculum was added. The results agree with those reported by [32].

Table 3 Plant height, dry biomass (root, stem, and leaves) per plant, shoot biomass per pot, and mycorrhizal infection rate after three different cuttings (The authors' elaboration)

Treatments	Plant height (cm)	Dry biomass per plant (g)			Dry biomass per pot (g) (shoot)	Mycorrhizal infection rate %
		Root	Stem	Leaves		
1st cutting (C1)						
T0	$16.2^{g} \pm 0.624$	$0.522^{h} \pm 0.089$	$0.78^{h} \pm 0.047$	$1.113^{g} \pm 0.038$	$8.694^{l} \pm 0.076$	$0^{g} \pm 0$
T1	$21.1^{e} \pm 0.2$	$0.529^{gh} \pm 0.031$	$0.912^{gh} \pm 0.025$	$1.367^{g} \pm 0.259$	$11.272^{j} \pm 0.141$	$25.3^{f} \pm 0.984$
T2	$18.9^{f} \pm 1.113$	$0.771^{fg} \pm 0.215$	$1.048^{gh} \pm 0.036$	$1.501^{fg} \pm 0.372$	$9.539^{k} \pm 0.016$	$0^{g} \pm 0$
T3	$21.4^{e} \pm 0.264$	$0.803^{f} \pm 0.057$	$1.06^{g} \pm 0.092$	$1.972^{ef} \pm 0.039$	$13.01^{i} \pm 0.087$	$27.8^{e} \pm 0.7$
2nd cutting (C2)						
T0	$18.1^{fg} \pm 0.916$	$2.08^{e} \pm 0.022$	$2.201^{f} \pm 0.103$	$2.35^{e} \pm 0.302$	$19.21^{h} \pm 0.173$	$0^{g} \pm 0$
T1	$22.5^{e} \pm 0.4$	$2.133^{e} \pm 0.092$	$2.79^{e} \pm 0.116$	$2.436^{e} \pm 0.280$	$25.68^{f} \pm 0.382$	$31.7^{d} \pm 0.624$
T2	$21.8^{e} \pm 1.178$	$2.105^{e} \pm 0.072$	$3.416^{d} \pm 0.414$	$3.782^{d} \pm 0.124$	$23.4^{g} \pm 0.792$	$0^{g} \pm 0$
T3	$25.2^{d} \pm 1.014$	$2.271^{e} \pm 0.342$	$3.991^{c} \pm 0.084$	$4.057^{d} \pm 0.104$	$29.13^{e} \pm 1.174$	$45.2^{b} \pm 0.173$
3rd cutting (C3)						
T0	$26.3^{cd} \pm 2.771$	$3.007^{d} \pm 0.005$	$3.01^{e} \pm 0.043$	$3.503^{d} \pm 0.324$	$31.471^{d} \pm 0.029$	$0^{g} \pm 0$

Continuation of Table 3

T1	29.7^{b} ± 2.088	3.351^{c} ± 0.202	3.814^{c} ± 0.226	5.151^{c} ± 0.177	37.99^{b} ± 0.170	39.1^{c} ± 0.264
T2	27.5^{c} ± 0.7	4.177^{b} ± 0.180	4.624^{b} ± 0.194	7.402^{b} ± 0.834	34.52^{c} ± 0.535	0^{g} ± 0
T3	32.1^{a} ± 0.4	4.692^{a} ± 0.080	5.235^{a} ± 0.268	9.293^{a} ± 0.104	41.76^{a} ± 0.308	76.6^{a} ± 1.307
ANOVA						
Treatment	*	*	*	*	*	*
Cutting	*	*	*	*	*	*
Treatment × cutting	ns	*	*	*	*	*

Note: Subscript letters in columns indicate treatments that are significantly different according to LSD (P < 0.05); ns - non-significant (P > 0.05); * P < 0.001; T0 - control; T1 - mycorrhizal-inoculated plants; T2 - wastewater-treated plants; T3 - inoculated plants irrigated with wastewater.

Table 4 Pearson's correlation of plant height, plant biomass (root, stem, leaves and shoot) and infection rate (The authors' elaboration)

	Height	Root	Stem	Leaves	Shoot
First cut					
Root	0.411				
Stem	0.684	0.919			
Leaves	0.752	0.844	0.863		
Shoot	0.91	0.462	0.607	0.862	
Infection	0.889	0.123	0.345	0.625	0.934
The second cut					
Root	0.881				
Stem	0.903	0.817			
Leaves	0.71	0.657	0.944		
Shoot	.991**	0.9	0.845	0.621	
Infection	0.837	0.879	0.574	0.293	0.903
Third cut					
Root	0.684				
Stem	0.741	.987*			
Leaves	0.74	.996**	.996**		
Shoot	.995**	0.711	0.776	0.768	
Infection	.971*	0.491	0.558	0.557	.953*

** p < 0.01; * p < 0.05 level (2-tailed)

2.6. Heavy Metal Concentrations in Different Tissues of Berseem Irrigated with Wastewater

2.6.1. First Cutting

The metal accumulation pattern was Zn > Pb > Cr > Cd at the first cut, as shown in Table 5. Plants analysis showed that the lowest (0.001 mg/kg) Cd concentration was observed in T2 roots and the highest (0.008 mg/kg) in T3 roots, Cr concentration was detected lowest (0.001 mg/kg) in T3 leaves and highest (1.562 mg/kg) in T2 roots, lowest (0.021 mg/kg) Pb concentration was detected in T2 stem and highest (1.903 mg/kg) in T2 roots and lowest (1.010 mg/kg) Zn concentration was observed in T1 stem and highest (13.910 mg/kg) in T1 leaves as compared to T0 plants.

Table 5 Metal concentration in berseem after first cut (45 days after sowing) (The authors' elaboration)

Metals	Tissue	T0	T1	T2	T3
Cd mg/kg	Leaves	ND^{d}	ND^{d}	ND^{d}	ND^{d}
	Stem	ND^{c}	ND^{c}	ND^{c}	ND^{c}
	Roots	ND^{e}	ND^{e}	0.001^{e} ± 0.001	0.008^{e} ± 0.001
Cr mg/kg	Leaves	ND^{e}	ND^{e}	ND^{e}	0.001^{de} ± 0.001
	Stem	ND^{e}	ND^{e}	0.007^{d} ± 0.002	0.02^{c} ± 0.003
	Roots	ND^{e}	ND^{e}	1.562^{c} ± 0.012	1.097^{d} ± 0.088
Pb mg/kg	Leaves	ND^{c}	ND^{c}	ND^{c}	ND^{c}
	Stem	ND^{d}	ND^{d}	0.021^{c} ± 0.002	ND^{d}
	Roots	ND^{f}	ND^{f}	1.903^{a} ± 0.095	0.509^{d} ± 0.024
Zn mg/kg	Leaves	11.072^{d} ± 0.267	13.910^{c} ± 0.163	10.512^{ef} ± 0.300	10.201^{f} ± 0.248
	Stem	2.51^{b} ± 0.214	1.01^{f} ± 0.023	1.22^{ef} ± 0.031	1.541^{de} ± 0.233
	Roots	8.012^{h} ± 0.084	11.289^{g} ± 0.171	10.711^{g} ± 0.363	10.833^{g} ± 0.198

Notes: Subscript letters show the significant difference of P < 0.05 (LSD); T0 - control; T1 - mycorrhizal-inoculated plants: T2 - wastewater-treated plants; T3 - inoculated plants irrigated with wastewater; ND - not detected.

2.6.2. The Second Cutting

The metal accumulation pattern was Zn > Cr > Pb > Cd; metals were found within permissible limits as shown in Table 6. Plants analysis showed that the lowest (0.004 mg/kg) Cd concentration was observed in T2 leaves and the highest (0.075 mg/kg) in T2 roots, Cr concentration was detected lowest (0.018 mg/kg) in T3 stem and highest (2.141 mg/kg) in T2 roots, lowest (0.003 mg/kg) Pb concentration was detected in T3 stem and highest (1.010 mg/kg) in T3 roots and lowest Zn concentration was observed lowest in T2 (0.191 mg/kg) stem and highest (19.216 mg/kg) in T3 leaves as compared to T0 plants.

Table 6 Metal concentration in berseem after the second cut (30 days after the first cut) (The authors' elaboration)

Metals	Tissue	T0	T1	T2	T3
Cd mg/kg	Leaves	ND^{d}	ND^{d}	0.004^{c} ± 0.001	ND^{d}
	Stem	ND^{c}	ND^{c}	ND^{c}	ND^{c}
	Roots	ND^{e}	0.001^{e} ± 0.001	0.075^{c} ± 0.005	0.02^{d} ± 0.006
Cr mg/kg	Leaves	ND^{e}	ND^{e}	0.075^{d} ± 0.014	1.016^{c} ± 0.011
	Stem	ND^{e}	ND^{e}	0.101^{a} ± 0.002	0.018^{c} ± 0.003
	Roots	ND^{e}	ND^{e}	2.141^{b} ± 0.022	1.105^{d} ± 0.071

Continuation of Table 6

Pb mg/kg	Leaves	ND^{c}	ND^{c}	ND^{c}	ND^{c}
	Stem	ND^{d}	ND^{d}	0.499^{b} ± 0.024	0.003^{d} ± 0.001
	Roots	ND^{f}	ND^{f}	0.15^{e} ± 0.038	1.01^{c} ± 0.007
Zn mg/kg	Leaves	13.8^{c} ± 0.268	16.03^{a} ± 0.158	10.884^{de} ± 0.164	13.611^{c} ± 0.316
	Stem	1.08^{ef} ± 0.069	1.16^{ef} ± 0.236	0.191^{g} ± 0.159	3.89^{a} ± 0.809
	Roots	10.551^{g} ± 0.666	14.062^{de} ± 0.044	12.079^{fg} ± 1.930	19.216^{b} ± 0.095

Notes: Subscript letters show the significant difference (P < 0.05) (LSD); T0 - control; T1 - mycorrhizal-inoculated plants; T2 - wastewater-treated plants; T3 - inoculated plants irrigated with wastewater; ND - not detected.

2.6.3. The Third Cutting

The metal accumulation pattern was Zn > Cr > Pb > Cd; metals were found within permissible limits as shown in Table 7. Plants analysis showed that the lowest (0.002 mg/kg) Cd concentration was observed in T2 stem and the highest (0.135 mg/kg) in T3 roots, Cr concentration was detected lowest (0.001 mg/kg) in T1 stem and highest (3.053 mg/kg) in T3 leaves, lowest (0.001 mg/kg) Pb concentration was detected in T3 leaves and highest (1.074 mg/kg) in T3 roots and lowest (0.736 mg/kg) Zn concentration was observed in T2 stem and highest (21.872 mg/kg) in T3 roots as compared to T0 plants.

Metal concentrations in this study were found within permissible limits (FAO, 1992) (0.2 mg/kg Cd, 0.005 mg/kg Cr, 0.005 mg/kg Pb and 60 mg/kg Zn). In Table 8, analysis of variance (ANOVA) showed a highly significant effect ($p < 0.01$) of cuttings and treatments and their interactive effect on metals (Cd, Cr and Pb) in roots, stems. The effect of cutting was significant on Zn in leaves but showed non-significant results for treatment ($P > 0.05$) and on the interactive effect of treatment and cutting on leaves ($P > 0.05$).

The results showed that Zn was high in plants compared with Cd, Cr, and Pb in both mycorrhizal and nonmycorrhizal plants. As metals come directly in contact with roots of plants, the uptake and translocation of metals in plants is affected by the interaction of metals and competition for adsorption due to their similar properties [33, 34]. Zn showed a significantly high correlation between plant biomass (leaves, stem and roots) compared to Cd, Cr and Pb. The reason for the high concentration of Zn in roots and leaves is that Zn is an essential nutrient for plant physiological processes. Thus, it was found highest in plants compared to Cd, Cr and Pb which are non-essential for plants [35]. Generally, in this study, inoculation enhanced Pb and Cd in roots and Zn and Cr in roots, stem, and leaves. Cd and Pb transfer to leaves and stems were reduced. The reason might be heavy metal storage in fungi structures, thus immobilizing Cd and Pb in roots as vesicles, arbuscles and spores act as a storage for heavy metals in hyphae [36, 37]. Previous studies showed that AMF enhances the retention of heavy metals in roots and reduces the transfer from root to stem, which is similar to this study [38]. The retention of metals in roots might be due to the change in the bioavailability of heavy metals due to the changes induced by AMF [39].

The cut-wise metal accumulation pattern was; Zn > Pb > Cr > Cd at the first cutting and Zn > Cr > Pb > Cd at the second and third cuttings. This trend might be due to the nutrients required by plants and availability of metals in soil and water used for irrigation. Similar trend of metals concentration was also reported in previous studies on food crops [40] and high Zn and lowest Cd concentrations were observed in vegetables [41]. Third, cutting metals accumulated the highest in inoculated plants. The results corroborate with the results of [36], which reported an increase in metal concentration in Alfalfa with wastewater irrigation as compared to tube well water, and that metal concentration was recorded highest at third cutting.

The results showed that heavy metal accumulation in plants depends upon availability in soil, as wastewater was used for irrigation, so metals accumulated in wastewater irrigated plants (either inoculated or un-inoculated), but AMF inoculation greatly affected the transfer of metals to different tissues (Table 5, 6 and 7).

Table 7 Metal concentration in berseem after the third cut (30 days after the second cut) (The authors' elaboration)

Metals	**Tissue**	**T0**	**T1**	**T2**	**T3**
Cd mg/kg	Leaves	ND^{d}	0.004^{c} ± 0.001	0.018^{a} ± 0.001	0.011^{b} ± 0.000
	Stem	ND^{c}	ND^{c}	0.002^{b} ± 0	0.004^{a} ± 0.001
	Roots	0.003^{e} ± 0.002	0.007^{e} ± 0.003	0.099^{b} ± 0.008	0.135^{a} ± 0.013
Cr mg/kg	Leaves	ND^{e}	ND^{e} ±	1.102^{b} ± 0.148	3.053^{a} ± 0.024
	Stem	ND^{e}	0.001^{e} ± 0.001	0.003^{e} ± 0.002	0.024^{b} ± 0.003
	Roots	ND^{e}	0.004^{e} ± 0.003	2.199^{b} ± 0.015	2.381^{a} ± 0.247
Pb mg/kg	Leaves	ND^{c}	ND^{c}	0.004^{a} ± 0.002	0.001^{b} ± 0.001
	Stem	ND^{d}	ND^{d}	0.736^{a} ± 0.016	0.008^{cd} ± 0.002
	Roots	ND^{f}	ND^{f}	0.121^{e} ± 0.021	1.074^{b} ± 0.039
Zn mg/kg	Leaves	15.010^{b} ± 0.070	16.505^{a} ± 0.235	10.901^{de} ± 0.600	13.953^{c} ± 0.327
	Stem	2.110^{bc} ± 0.011	2^{cd} ± 0.364	0.736^{f} ± 0.210	1.050^{ef} ± 0.019
	Roots	12.980^{ef} ± 1.461	17.002^{c} ± 1.716	15.203^{d} ± 0.618	21.872^{a} ± 1.584

Notes: Subscript letters show the significant difference (P < 0.05) (LSD); T0 - control; T1 - mycorrhizal-inoculated plants; T2 - wastewater-treated plants; T3 - inoculated plants irrigated with wastewater; ND - not detected.

Table 8 Significance the effects of different factors and their interactions on variables (ANOVA) (The authors' elaboration)

Variables	Leaves				Stem				Roots			
	Cd	Cr	Pb	Zn	Cd	Cr	Pb	Zn	Cd	Cr	Pb	Zn
Cuttings	*	*	*	*	*	*	*	*	*	*	*	*
Treatments	*	*	*	ns	*	*	*	*	*	*	*	*
Cuttings × Treatment	*	*	*	ns	*	*	*	*	*	*	*	*

Notes: Ns - non-significant (P > 0.05); * P < 0.01

3. Conclusion

In this study, the AMF inoculum had a positive effect on biomass, growth, and storage of heavy metals in roots. The below permissible limits of metals in plants at each cutting showed that the regrowth ability of berseem crop can be used to remediate soil without exceeding heavy metal limits. The plants exhibited a reduced concentration of toxic heavy metals in the above - ground biomass of inoculated plants plants.

The positive performance of inoculum for plant biomass and plant growth against contaminated soil showed that it can be used to enhance plant biomass, but for remediation of heavy metals in contaminated soil, further screening for AM inoculum with the potential to enhance metal uptake might help to remediate polluted soil. The study is focused on a limited number of heavy metals.

Acknowledgment

The authors gratefully acknowledge NIFA (Nuclear institute for food and agriculture) for providing laboratory facilities. We are also grateful to the staff members who provided technical assistance, support, and guidance during the study.

References

[1] HAJJAMI K., ENNAJI M. M., FOUAD S., OUBRIM N., and COHEN N. Wastewater reuse for irrigation in Morocco: Helminth eggs contamination's level of irrigated crops and sanitary risk (a case study of Settat and Soualem regions). *Journal of Bacteriology and Parasitology*, 2013, 4(1): 1-5. http://dx.doi.org/10.4172/2155-9597.1000163

[2] LOMBI E., ZHAO F. J., DUNHAM S. J., and MCGRATH S. P. Phytoremediation of heavy metal–contaminated soils: Natural hyperaccumulation versus chemically enhanced phytoextraction. *Journal of Environmental Quality*, 2001, 30(6): 1919-1926. https://doi.org/10.2134/jeq2001.1919

[3] SRIVASTAVA S., & THAKUR I. S. Evaluation of bioremediation and detoxification potentiality of Aspergillus niger for removal of hexavalent chromium in soil microcosm. *Soil Biology and Biochemistry*, 2006, 38(7): 1904-1911. https://doi.org/10.1016/j.soilbio.2005.12.016

[4] BHATTI S. S., KUMAR V., SAMBYAL V., SINGH J., and NAGPAL A. K. Comparative analysis of tissue compartmentalized heavy metal uptake by common forage crop: a field experiment. *Catena*, 2018, 160: 185-193. https://doi.org/10.1016/j.catena.2017.09.015

[5] KHAN A. G., KUEK C., CHAUDHRY T. M., KHOO C. S., and HAYES W. J. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. *Chemosphere*, 2000, 41(1-2): 197-207. https://doi.org/10.1016/S0045-6535(99)00412-9

[6] SMITH S. E., & READ D. J. *Mycorrhizal symbiosis*. Academic Press, New York, 2008. https://doi.org/10.2136/sssaj2008.0015br

[7] AUGÉ R. M. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 2001, 11(1): 3-42. https://doi.org/10.1007/s005720100097

[8] GRAHAM J. H. What do root pathogens see in mycorrhizas? *New Phytologist*, 2001: 357-359. https://doi.org/10.1046/j.1469-8137.2001.00077.x

[9] GEORGE E. Nutrient uptake. In KAPULNIK Y., & DOUDS D. D. (eds.) *Arbuscular mycorrhizas: physiology and function*. Springer, Dordrecht, 2000: 307-343. https://doi.org/10.1007/978-94-017-0776-3_14

[10] STREITWOLF-ENGEL R., VAN DER HEIJDEN M. G. A., WIEMKEN A., and SANDERS I. R. The ecological significance of arbuscular mycorrhizal fungal effects on clonal reproduction in plants. *Ecology*, 2001, 82(10): 2846-2859. https://doi.org/10.1890/0012-9658(2001)082[2846:TESOAM]2.0.CO;2

[11] VAN DER HEIJDEN M. G., & SANDERS I. R. Mycorrhizal ecology: synthesis and perspectives. In HEIJDEN M. G. A., & SANDERS I. R. (eds.) *Mycorrhizal ecology*. Springer, Berlin, 2002: 441-456. https://doi.org/10.1007/978-3-540-38364-2_17

[12] KLIRONOMOS J. N. Variation in plant response to native and exotic arbuscular mycorrhizal fungi. *Ecology*, 2003, 84(9): 2292-2301. https://doi.org/10.1890/02-0413

[13] KOIDE R. T., & MOSSE B. A history of research on arbuscular mycorrhiza. *Mycorrhiza*, 2004, 14(3): 145-163. https://doi.org/10.1007/s00572-004-0307-4

[14] ALI H., NASEER M., and SAJAD M. A. Phytoremediation of heavy metals by Trifolium alexandrinum. *International Journal of Environmental Sciences*, 2012, 2(3): 1459. http://dx.doi.org/10.6088/ijes.00202030031

[15] PELLEGRINO E., BEDINI S., AVIO L., BONARI E., and GIOVANNETTI M. Field inoculation effectiveness of native and exotic arbuscular mycorrhizal fungi in a Mediterranean agricultural soil. *Soil Biology and Biochemistry*, 2011, 43(2): 367-376. https://doi.org/10.1016/j.soilbio.2010.11.002

[16] BAIRD R. B., EATON A. D., and CLESCERI L. S. (2012). *Standard methods for the examination of water and wastewater (Part 10).* American public health association, Washington DC, 1999. https://beta-static.fishersci.com/content/dam/fishersci/en_US/documents/programs/scientific/technical-documents/white-papers/apha-water-testing-standard-methods-introduction-white-paper.pdf

[17] KOSKE R. E., & GEMMA J. N. A modified procedure

for staining roots to detect VA mycorrhizas. Mycological Research, 1989, 92(4): 486-488. https://doi.org/10.1016/S0953-7562(89)80195-9

[18] PHILLIPS J. M., & HAYMAN D. S. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British mycological Society*, 1970, 55(1): 158-161. https://doi.org/10.1016/S0007-1536(70)80110-3

[19] BIERMANN B., & LINDERMAN R. G. Quantifying vesicular-arbuscular mycorrhizae: a proposed method towards standardization. *New Phytologist*, 1981, 87(1): 63-67. https://doi.org/10.1111/j.1469-8137.1981.tb01690.x

[20] PESCOD M. B. *Wastewater treatment, and use in agriculture – FAO irrigation and drainage paper 47*. Food and agriculture organization of the united nations, Rome, 1992: 25-35. https://www.fao.org/3/T0551E/T0551E00.htm

[21] SU W., GU C., YANG G., CHEN S., and ZHEN F. Measuring the impact of urban sprawl on natural landscape pattern of the Western Taihu Lake watershed, China. *Landscape and Urban Planning*, 2010, 95(1-2): 61-67. https://doi.org/10.1016/j.landurbplan.2009.12.003

[22] NIGAM R., SRIVASTAVA S., PRAKASH S., and SRIVASTAVA M. M. Cadmium mobilisation and plant availability – the impact of organic acids commonly exuded from roots. *Plant and Soil*, 2001, 230(1): 107-113. https://doi.org/10.1023/A:1004865811529

[23] DING C., ZHAO Y., ZHANG Q., LIN Y., XUE R., CHEN C., ZENG R., CHEN D., and SONG Y. Cadmium transfer between maize and soybean plants via common mycorrhizal networks. *Ecotoxicology and Environmental Safety*, 2022, 232: 113273. https://doi.org/10.1016/j.ecoenv.2022.113273

[24] TABASSUM Y., TANVIR B., and FARRUKH H. Effect of arbuscular mycorrhizal inoculation on nutrient uptake, growth and productivity of chickpea (Cicer arietinum) varieties. *International Journal of Agronomy and Plant Production*, 2012, 3(9): 334-345. https://doi.org/10.5897/ajb10.1494

[25] RUSAN M. J. M., HINNAWI S., and ROUSAN L. Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination*, 2007, 215(1-3): 143-152. https://doi.org/10.1016/j.desal.2006.10.032

[26] RATTAN R. K., DATTA S. P., CHHONKAR P. K., SURIBABU K., and SINGH A. K. Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater—a case study. *Agriculture, ecosystems & environment*, 2005, 109(3-4): 310-322. https://doi.org/10.1016/j.agee.2005.02.025

[27] KANWAL S., BANO A., & MALIK R. N. Role of arbuscular mycorrhizal fungi in phytoremediation of heavy metals and effects on growth and biochemical activities of wheat (Triticum aestivum L.) plants in Zn contaminated soils. *African Journal of Biotechnology*, 2016, 15(20): 872-883. https://doi.org/10.5897/ajb2016.15292

[28] KHAN M. J., KHAN N. U., KHAN F., AFZAL M., and JAN M. T. Effect of amendments on bioavailability of heavy metals to alfalfa and biomass yield irrigated with wastewater. *Journal of Environmental Engineering*, 2016, 142(8): 04016038. https://doi.org/10.1061/(asce)ee.1943-7870.0001113

[29] OGUNKUNLE C. O., EL-IMAM A. A., BASSEY E., VISHWAKARMA V., and FATOBA P. O. Co-application of indigenous arbuscular mycorrhizal fungi and nano-TiO2 reduced Cd uptake and oxidative stress in pre-flowering cowpea plants. *Environmental Technology & Innovation*, 2020, 20: 101163. https://doi.org/10.1016/j.eti.2020.101163

[30] MEDDAD-HAMZA A., BEDDIAR A., GOLLOTTE A., LEMOINE M. C., KUSZALA C., and GIANINAZZI S. Arbuscular mycorrhizal fungi improve the growth of olive trees and their resistance to transplantation stress. *African Journal of Biotechnology*, 2010, 9(8): 1159-1167. https://doi.org/10.5897/ajb09.1282

[31] KABIR Z., & KOIDE R. T. The effect of dandelion or a cover crop on mycorrhiza inoculum potential, soil aggregation and yield of maize. *Agriculture, Ecosystems & Environment*, 2000, 78(2): 167-174. https://doi.org/10.1016/S0167-8809(99)00121-8

[32] EL KINANY S., ACHBANI E., FAGGROUD M., OUAHMANE L., EL HILALI R., HAGGOUD A., and BOUAMRI R. (2019). Effect of organic fertilizer and commercial arbuscular mycorrhizal fungi on the growth of micropropagated date palm cv. Feggouss. *Journal of the Saudi Society of Agricultural Sciences*, 18(4): 411-417. https://doi.org/10.1016/j.jssas.2018.01.004

[33] SHARMA R. K., AGRAWAL M., and MARSHALL F. Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotoxicology and Environmental Safety*, 2007, 66(2): 258-266. https://doi.org/10.1016/j.ecoenv.2005.11.007

[34] SINHA P., DUBE B. K., SRIVASTAVA P., and CHATTERJEE C. Alteration in uptake and translocation of essential nutrients in cabbage by excess lead. *Chemosphere*, 2006, 65(4): 651-656. https://doi.org/10.1016/j.chemosphere.2006.01.068

[35] KABATA-PENDIAS A. Soil–plant transfer of trace elements—an environmental issue. *Geoderma*, 2004, 122(2-4): 143-149. https://doi.org/10.1016/j.geoderma.2004.01.004

[36] GAMALERO E., LINGUA G., BERTA G., and GLICK B. R. Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. *Canadian Journal of Microbiology*, 2009, 55(5): 501-514. https://doi.org/10.1139/W09-010

[37] FERROL N., GONZÁLEZ-GUERRERO M., VALDERAS A., BENABDELLAH K., and AZCÓN-AGUILAR C. Survival strategies of arbuscular mycorrhizal fungi in Cu-polluted environments. *Phytochemistry Reviews*, 2009, 8(3): 551-559. https://doi.org/10.1007/s11101-009-9133-9

[38] HUANG S., JIA X., ZHAO Y., BAI B., and CHANG Y. Elevated CO2 benefits the soil microenvironment in the rhizosphere of Robinia pseudoacacia L. seedlings in Cd-and Pb-contaminated soils. *Chemosphere*, 2017, 168: 606-616. https://doi.org/10.1016/j.chemosphere.2016.11.017

[39] ZHAN F., LI B., JIANG M., YUE X., HE Y., XIA Y., and WANG Y. Arbuscular mycorrhizal fungi enhance antioxidant defense in the leaves and the retention of heavy metals in the roots of maize. *Environmental Science and Pollution Research*, 2018, 25(24): 24338-24347. https://doi.org/10.1007/s11356-018-2487-z

[40] CHAOUA S., BOUSSAA S., El GHARMALI A., and BOUMEZZOUGH A. Impact of irrigation with wastewater on accumulation of heavy metals in soil and crops in the region of Marrakech in Morocco. *Journal of the Saudi Society of Agricultural Sciences*, 2019, 18(4): 429-436. https://doi.org/10.1016/j.jssas.2018.02.003

[41] RADWAN M. A., & SALAMA A. K. Market basket survey for some heavy metals in Egyptian fruits and vegetables. *Food and Chemical Toxicology*, 2006, 44(8): 1273-1278. https://doi.org/10.1016/j.fct.2006.02.004

参考文:

[1] HAJJAMI K., ENNAJI M. M., FOUAD S., OUBRIM N., 和 COHEN N. 摩洛哥灌溉废水再利用：灌溉作物的蠕虫卵污染水平和卫生风险（塞塔特和苏阿莱姆地区的案例研究）。细菌学和寄生虫学杂志, 2013, 4(1): 1-5. http://dx.doi.org/10.4172/2155-9597.1000163

[2] LOMBI E., ZHAO F. J., DUNHAM S. J., 和 MCGRATH S. P. 重金属污染土壤的植物修复：自然超积累与化学强化植物提取。环境质量杂志, 2001, 30(6): 1919-1926. https://doi.org/10.2134/jeq2001.1919

[3] SRIVASTAVA S., 和 THAKUR I. S. 黑曲霉去除土壤微观世界中六价铬的生物修复和解毒潜力评价。土壤生物学和生物化学, 2006, 38(7): 1904-1911. https://doi.org/10.1016/j.soilbio.2005.12.016

[4] BHATTI S. S., KUMAR V., SAMBYAL V., SINGH J., 和 NAGPAL A. K. 普通牧草作物对组织区室化重金属吸收的比较分析：田间试验。系列, 2018, 160: 185-193. https://doi.org/10.1016/j.catena.2017.09.015

[5] KHAN A. G., KUEK C., CHAUDHRY T. M., KHOO C. S., 和 HAYES W. J. 植物、菌根和植物螯合剂在重金属污染土地修复中的作用。化学圈, 2000, 41(1-2): 197-207. https://doi.org/10.1016/S0045-6535(99)00412-9

[6] SMITH S. E., 和 READ D. J. 菌根共生。学术出版社, 纽约, 2008. https://doi.org/10.2136/sssaj2008.0015br

[7] AUGÉ R. M. 水关系、干旱和水泡-丛枝菌根共生。菌根, 2001, 11(1): 3-42. https://doi.org/10.1007/s005720100097

[8] GRAHAM J. H. 根部病原体在菌根中看到什么？新植物学家，2001: 357-359. https://doi.org/10.1046/j.1469-8137.2001.00077.x

[9] GEORGE E. 营养吸收。在 KAPULNIK Y., 和 DOUDS D. D. (编辑。) 丛枝菌根：生理学和功能。斯普林格，多德雷赫特, 2000: 307-343. https://doi.org/10.1007/978-94-017-0776-3_14

[10] STREITWOLF-ENGEL R., VAN DER HEIJDEN M. G. A., WIEMKEN A., 和 SANDERS I. R. 丛枝菌根真菌对植物克隆繁殖影响的生态学意义。生态, 2001, 82(10): 2846-2859. https://doi.org/10.1890/0012-9658(2001)082[2846:TESOAM]2.0.CO;2

[11] VAN DER HEIJDEN M. G., 和 SANDERS I. R. 菌根生态学：综合与展望。在 HEIJDEN M. G. A., 和 SANDERS I. R.（编辑）菌根生态学。施普林格，柏林, 2002: 441-456. https://doi.org/10.1007/978-3-540-38364-2_17

[12] KLIRONOMOS J. N. 植物对本地和外来丛枝菌根真菌反应的变化。生态, 2003, 84(9): 2292-2301. https://doi.org/10.1890/02-0413

[13] KOIDE R. T., 和 MOSSE B. 丛枝菌根研究的历史。菌根, 2004, 14(3): 145-163. https://doi.org/10.1007/s00572-004-0307-4

[14] ALI H., NASEER M., 和 SAJAD M. A. 三叶草对重金属的植物修复。国际环境科学杂志, 2012, 2(3): 1459. http://dx.doi.org/10.6088/ijes.00202030031

[15] PELLEGRINO E., BEDINI S., AVIO L., BONARI E., 和 GIOVANNETTI M. 地中海农业土壤中本地和外来丛枝菌根真菌的田间接种效果。土壤生物学和生物化学, 2011, 43(2): 367-376. https://doi.org/10.1016/j.soilbio.2010.11.002

[16] BAIRD R. B., EATON A. D., 和 CLESCERI L. S. (2012). 水和废水检验的标准方法（第10部分）。美国公共卫生协会，华盛顿特区, 1999. https://beta-static.fishersci.com/content/dam/fishersci/en_US/documents/programs/scientific/technical-documents/white-papers/apha-water-testing-standard-methods-introduction-white-paper.pdf

[17] KOSKE R. E., 和 GEMMA J. N. 用于检测水泡-丛枝菌根的染色根的改进程序。真菌学研究, 1989, 92(4): 486-488. https://doi.org/10.1016/S0953-7562(89)80195-9

[18] PHILLIPS J. M., 和 HAYMAN D. S. 改进的清除根和染色寄生和水泡-丛枝菌根真菌的程序，以快速评估感染。英国真菌学会汇刊, 1970, 55(1): 158-161. https://doi.org/10.1016/S0007-1536(70)80110-3

[19] BIERMANN B., 和 LINDERMAN R. G. 量化水泡-丛枝菌根：一种拟议的标准化方法。新植物学家, 1981, 87(1): 63-67. https://doi.org/10.1111/j.1469-8137.1981.tb01690.x

[20] PESCOD M. B. 废水处理和在农业中的使用—粮农组织灌溉和排水论文47。联合国粮食及农业组织，罗马, 1992: 25-35. https://www.fao.org/3/T0551E/T0551E00.htm

[21] SU W., GU C., YANG G., CHEN S., 和 ZHEN F. 测量城市扩张对中国西太湖流域自然景观格局的影响。景观与城市规划, 2010, 95(1-2): 61-67. https://doi.org/10.1016/j.landurbplan.2009.12.003

[22] NIGAM R., SRIVASTAVA S., PRAKASH S., 和 SRIVASTAVA M. M. 镉动员和植物可用性—通常从根部渗出的有机酸的影响。植物和土壤, 2001, 230(1): 107-113. https://doi.org/10.1023/A:1004865811529

[23] DING C., ZHAO Y., ZHANG Q., LIN Y., XUE R., CHEN C., ZENG R., CHEN D., 和 SONG Y. 镉通过常见的菌根网络在玉米和大豆植物之间转移。生态毒理学和环境安全, 2022, 232: 113273. https://doi.org/10.1016/j.ecoenv.2022.113273

[24] TABASSUM Y., TANVIR B., 和 FARRUKH H. 丛枝菌根接种对鹰嘴豆(西芹)品种养分吸收、生长和生产力的影响。国际农学与植物生产杂志, 2012, 3(9): 334-345. https://doi.org/10.5897/ajb10.1494

[25] RUSAN M. J. M., HINNAWI S., 和 ROUSAN L. 饲料作物废水灌溉对土壤和植物质量参数的长期影响。海水淡化, 2007, 215(1-3): 143-152. https://doi.org/10.1016/j.desal.2006.10.032

[26] RATTAN R. K., DATTA S. P., CHHONKAR P. K., SURIBABU K., 和 SINGH A. K. 污水灌溉对土壤、农作物和地下水中重金属含量的长期

影响—案例研究。农业、生态系统和环境, 2005, 109(3-4): 310-322. https://doi.org/10.1016/j.agee.2005.02.025
[27] KANWAL S., BANO A., 和 MALIK R. N. 丛枝菌根真菌在重金属植物修复中的作用以及对锌污染土壤中小麦(夏小麦)植物生长和生化活性的影响。非洲生物技术杂志, 2016, 15(20): 872-883. https://doi.org/10.5897/ajb2016.15292
[28] KHAN M. J., KHAN N. U., KHAN F., AFZAL M., 和 JAN M. T. 改良剂对紫花苜蓿重金属生物有效性和污水灌溉生物量产量的影响。环境工程学报, 2016, 142(8): 04016038. https://doi.org/10.1061/(asce)ee.1943-7870.0001113
[29] OGUNKUNLE C. O., EL-IMAM A. A., BASSEY E., VISHWAKARMA V., 和 FATOBA P. O. 土着丛枝菌根真菌和纳米二氧化钛的共同应用减少了开花前豇豆植物的镉吸收和氧化应激。环境技术与创新, 2020, 20: 101163. https://doi.org/10.1016/j.eti.2020.101163
[30] MEDDAD-HAMZA A., BEDDIAR A., GOLLOTTE A., LEMOINE M. C., KUSZALA C., 和 GIANINAZZI S. 丛枝菌根真菌可改善橄榄树的生长及其对移植胁迫的抵抗力。非洲生物技术杂志, 2010, 9(8): 1159-1167. https://doi.org/10.5897/ajb09.1282
[31] KABIR Z., 和 KOIDE R. T. 蒲公英或覆盖作物对菌根接种潜力、土壤聚集和玉米产量的影响。农业、生态系统与环境, 2000, 78(2): 167-174. https://doi.org/10.1016/S0167-8809(99)00121-8
[32] EL KINANY S., ACHBANI E., FAGGROUD M., OUAHMANE L., EL HILALI R., HAGGOUD A., 和 BOUAMRI R. (2019). 有机肥和商业丛枝菌根真菌对微繁殖枣椰树生长的影响。多汗的。沙特农业科学学会杂志, 18(4): 411-417. https://doi.org/10.1016/j.jssas.2018.01.004
[33] SHARMA R. K., AGRAWAL M., 和 MARSHALL F. 印度瓦拉纳西郊区土壤和蔬菜的重金属污染。生态毒理学和环境安全, 2007, 66(2): 258-266. https://doi.org/10.1016/j.ecoenv.2005.11.007
[34] SINHA P., DUBE B. K., SRIVASTAVA P., 和 CHATTERJEE C. 过量铅改变卷心菜中必需营养素的吸收和转运。化学圈, 2006, 65(4): 651-656. https://doi.org/10.1016/j.chemosphere.2006.01.068
[35] KABATA-PENDIAS A. 微量元素的土壤-植物转移—一个环境问题。地皮, 2004, 122(2-4): 143-149. https://doi.org/10.1016/j.geoderma.2004.01.004
[36] GAMALERO E., LINGUA G., BERTA G., 和 GLICK B. R. 植物生长促进细菌和丛枝菌根真菌对植物对重金属胁迫反应的有益作用。加拿大微生物学杂志, 2009, 55(5): 501-514. https://doi.org/10.1139/W09-010
[37] FERROL N., GONZÁLEZ-GUERRERO M., VALDERAS A., BENABDELLAH K., 和 AZCÓN-AGUILAR C. 丛枝菌根真菌在铜污染环境中的生存策略。植物化学评论, 2009, 8(3): 551-559. https://doi.org/10.1007/s11101-009-9133-9
[38] HUANG S., JIA X., ZHAO Y., BAI B., 和 CHANG Y. 升高的二氧化碳有利于镉和铅污染土壤中刺槐幼苗根际的土壤微环境。化学圈, 2017, 168: 606-616. https://doi.org/10.1016/j.chemosphere.2016.11.017
[39] ZHAN F., LI B., JIANG M., YUE X., HE Y., XIA Y., 和 WANG Y. 丛枝菌根真菌增强叶片的抗氧化防御能力和玉米根部对重金属的保留。环境科学与污染研究, 2018, 25(24): 24338-24347. https://doi.org/10.1007/s11356-018-2487-z
[40] CHAOUA S., BOUSSAA S., El GHARMALI A., 和 BOUMEZZOUGH A. 废水灌溉对摩洛哥马拉喀什地区土壤和作物中重金属积累的影响。沙特农业科学学会杂志, 2019, 18(4): 429-436. https://doi.org/10.1016/j.jssas.2018.02.003
[41] RADWAN M. A., 和 SALAMA A. K. 埃及水果和蔬菜中某些重金属的市场篮子调查。食品和化学毒理学, 2006, 44(8): 1273-1278. https://doi.org/10.1016/j.fct.2006.02.004