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Phytoremediation of Soil Contaminated with Heavy Metals via Arbuscular Mycorrhiza (*Funneliformis mosseae*) Inoculation Ameliorates the Growth Responses and Essential Oil Content in Lavender (*Lavandula angustifolia* L.)

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Abstract: Phytoremediation of heavy metals (HMs) is an efficient methodology to remove toxic metals from the soil. On the other hand, arbuscular mycorrhizal fungi (AMF) are utilized as biological fertilizers as they improve root expansion, nutrient uptake, shoot growth, and plant biological performance. In this study, the effect of AMF inoculation on the morphological traits, macro- and micronutrient contents, essential oil content (EOC), and essential oil yield (EOY) of lavender (*Lavandula angustifolia* L.) was investigated, under HM (Pb and Ni) stress in greenhouse conditions. The performed treatments were as follows: AMF (*Funneliformis mosseae*) inoculation (5 g kg^{-1} soil), and HM stress, including Pb (150 and 225 mg kg^{-1} soil from $Pb(NO_3)_2$) and Ni (220 and 330 mg kg^{-1} soil from $Ni(NO_3)_2$). The controls were the absence of AMF and HM treatments. The results showed that the contamination with Pb and Ni decreased plant height, branch number, fresh and dry weights of shoots and roots, and P, K, Mg, Fe, Zn, and Mn contents. At the same time, AMF inoculation modulated the adverse effects of Pb and Ni treatments. AMF inoculation and lower concentrations of Pb and Ni increased the EOC and EOY of lavender plants, whereas the higher levels of HMs reduced the morphobiometric traits. AMF inoculation increased the Pb and Ni contents in roots. The treatment with Pb at 220 mg kg^{-1} led to a higher stress effect than that of Ni treatment. In conclusion, the results recommend AMF inoculation as a helpful procedure to improve the growth responses and EOY of lavender in environments polluted with the tested HMs and suggest that AMF inoculation is potentially efficient in mitigating HM stress effects.

Keywords: phytoremediation; lavender; growth responses; Pb; Ni; essential oil

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1. Introduction

The ascending rate of urbanization and the industrial revolution have caused many environmental effects in and around cities. In addition, the leakage of various pollutants from different industrial origins has led to the spread of environmental contamination with various heavy metals [1]. These pollutants cause abiotic stress for plants and reduce their growth and productivity. Heavy metals (HMs) may be considered the most critical environmental pollutants [2]. The deposition of HMs such as lead (Pb), cadmium (Cd), mercury (Hg), and nickel (Ni) in the soil can affect many parameters related to plant growth [3]. One of the new and low-cost methods to remediate HM-contaminated soils is phytoremediation, which is a technology with a low destructive effect, preserving the physical, chemical, and biological characteristics of soils. Additionally, it has been undertaken due to its cheapness, simplicity, and universal acceptance [4].

Biofertilizers show remarkable advantages relative to chemical fertilizers, such as non-toxicity and improvement of physico-chemical properties of soil. Biofertilizers' specific activities and effects include increasing the root and stem growth and enhancing plant tolerance to biotic and abiotic stress factors [5]. Nowadays, arbuscular mycorrhizal fungi (AMF) are one of the most widely used biological fertilizers. They are beneficial factors in the sustainability of plant and soil systems, which coexist with the roots of more than 97% of plants and improve root expansion, nutrient uptake, shoot growth, and plant performance [6]. Therefore, plants inoculated with AMF are reliable candidates for detoxifying and removing several pollutants in ecosystems, especially in agroecosystems [7]. Mostly wild plants absorb toxic heavy metals in considerable amounts but have enough biomass production without tremendous productivity loss [8]. In this regard, Akhtar et al. [9] reported that mycorrhiza fungus increased the absorption of chromium metal and enhanced the biological yield, chlorophylls, and carbohydrate contents in tomato plants.

On the other hand, some researchers have suggested that medicinal and aromatic plants may be safe and potential candidates with an economic return to phytoremediation protocols, since their final products are free of HMs [10]. Among crops, medicinal plants have high tolerance to HM stress; therefore, they can be a suitable option for phytoremediation purposes [11,12]. Moreover, in some cases, heavy metals stimulate the biosynthesis and the accumulation of secondary metabolites in medicinal plants [13].

Lavender (*Lavandula angustifolia* L.), a perennial medicinal plant of the Lamiaceae family and native to the Mediterranean region, showed acceptable tolerance to HMs by accumulating them in the roots via the impaired movement from the roots to the aerial parts [14]. Moreover, the symbiosis of lavender with AMF improved the antioxidant status of the plant and significantly increased its essential oil content (EOC) due to the enhanced plant growth and biomass [15]. Lavender blossoms contain 3% essential oil, composed of more than a hundred different chemical compounds, such as linalyl acetate, α-bisabolol, linalool, eucalyptol, borneol, lavendulyl acetate, ocimene, camphor, and geraniol [16].

Dwivedi et al. [17] studied the effect of cadmium and lead concentrations on basil (*Ocimum tenuifolium* L.). The results showed that cadmium and lead accumulation in the roots was higher than in the shoots. In another study, Stancheva et al. [18] planted *Salvia officinalis* L. in cadmium- and lead-contaminated soils. They reported that the highest essential oil yields were produced from soils contaminated with HMs. In another study, the effects of *Funneliformis intraradice* mycorrhiza inoculation were studied on the yield, chemical composition, and metal accumulation in basil (*Ocimum basilicum* L.). The results showed that aerial parts yield, essential oil content, and root dry weight were increased with low concentrations of Cd, Pb, and Ni [3].

This study aimed to investigate the efficacy of AMF inoculation under HM stress in lavender (*Lavandula angustifolia* L.). The idea was that AMF inoculation may improve the phytoremediation of HMs and may also enhance lavender plant physiological responses.

2. Materials and Methods

2.1. Plant Materials, Growth Conditions, and Treatments

To investigate the effect of AMF inoculation on HM phytoremediation by lavender (*Lavandula angustifolia* L.), this experiment was carried out in greenhouse conditions with a temperature regime of 21 °C/25 °C night/day, 8/16 night/day photoperiod, and 70–75% relative humidity.

Lavender seeds were purchased from Pakan Bazr Seed Company (Isfahan, Iran). Their surfaces were sterilized with alcohol, and they were sown in cocopeat in seedling trays with a 4 × 4 cm cell size. The seedlings were transferred to 5 L plastic pots filled with air-dried loam sandy clay soil collected from Maragheh University Garden, Iran, at a depth of 0 to 30 cm. Before transplanting, the soil was sterilized to eliminate soilborne fungi by autoclaving for 60 min at 121 °C under 1.2 atmospheric pressure. The soil properties are presented in Table 1 [19–21]. This experiment was performed as a factorial experiment based on a completely randomized design (CRD) in 4 replications. The first factor comprised

two levels of HMs, including Pb (150 and 225 mg kg^{-1} soil from $Pb(NO_3)_2$) and Ni (220 and 330 mg kg^{-1} soil of $Ni(NO_3)_2$); these concentrations were chosen as they were two and three times higher than standard limits, according to world standards (75 and 110 mg kg^{-1} soil, for Pb and Ni, respectively). The second factor was the AMF (*Funneliformis mosseae*) application, including 5 g kg^{-1} soil without AMF. Control was without any treatments of AMF and HMs.

Table 1. The physico-chemical characteristics of the soil used for the experiment.

Soil Texture Class	Sand (%)	Silt (%)	Clay (%)	Organic Matter (%)	EC (dS m^{-1})	pH	K (mg kg^{-1})	P (mg kg^{-1})	N (%)	Pb (mg kg^{-1})	Ni (mg kg^{-1})
Loam sandy clay	55.00	15.50	29.50	1.53	1.23	7.35	494.15	8.59	0.082	0.068	0.027

The HMs dissolved in dH_2O were sprayed every day for 2 weeks on the soil, which was kept at room temperature and stirred regularly until the soil pollution became uniform. The contaminated soils were added to 5 L plastic pots. Then, three seedlings of lavender were sown in each pot. *Funneliformis mosseae* was supplied by the Biology Laboratory of the Soil and Water Research Institute, Karaj, Iran. The plants were irrigated with tap water during the growing season. At 50% flowering time, the plants were harvested and analyzed.

2.2. Root Colonization

After plant harvesting, the roots of lavender were uprooted from the soils inoculated by AMF, and subsequently were cleaned with tap water to eliminate the remaining soil particles. The fresh roots were cut into small pieces (1 cm), and cleared out in hot KOH solution (10%, w/v) for 10 min. The root pieces were rinsed with dH_2O and acidified with HCl (2%, v/v) at 25 °C for 20 min, and then stained with trypan blue (0.05%) in lactic acid (80%, v/v) for 12 h (Phillips; Koske). Finally, the samples were cleaned with dH_2O and kept in a solution including dH_2O, glycerol, and lactic acid (1:1:1, $v/v/v$) (McGonigle). The stained pieces were recognized and assessed with an Olympus microscope (BH-2). The fungus organs and hyphae that appeared blue were noted in high-quality photos. The percentage of colonization was determined by the gridline intersection method based on Giovannetti and Mosse [22] as follows:

$$\text{Percent colonization} = \frac{\text{Total infected roots}}{\text{Total noninfected roots}} \times 100 \quad (1)$$

2.3. Morphological Traits

Plant morphological traits, including plant height, number of branches, fresh weight (FW), and dry weight (DW) of shoots and roots were recorded at harvest. Shoot and root FW was calculated by an analytical scale (A&D Weighing, Tokyo, Japan) with an accuracy of 0.01 mg. The samples were dried in the oven at 75 °C for 48 h, until constant weight, and the corresponding DW of root and shoot were recorded.

2.4. Determination of Photosynthetic Pigments

Photosynthetic pigments, including chlorophyll a (Chl a) and b (Chl b), Chl a+b, and carotenoids (CARs), were determined using the Arnon method [23]. An aliquot of 0.5 g of fresh leaves was homogenized with 5 mL of 80% acetone, then centrifuged at 11,000× g for 15 min. The absorbance was recorded by spectrophotometer (UV-1800, Shimadzu, Tokyo, Japan) at three wavelengths of 470, 645, and 663 nm. The concentrations of Chl a (A), b (B), and CARs (C) were calculated as mg g^{-1} FW using the following formulas:

$$A = [12.7(A663) - 2.79(A645)] \quad (2)$$

$$B = [21.50\ (A645) - 5.10(A663)] \quad (3)$$

$$C = [1000(A470) - 1.82C_a - 85.02C_b]/198 \quad (4)$$

2.5. Total Soluble Carbohydrates (TSC) Content

To measure TSC content, 0.2 g of fresh leaf was used for extraction by 10 mL 95% ethanol for 60 min in a water bath at 80 °C, then centrifuged using a refrigerated centrifuge at 16,000× *g* for 10 min. The clear supernatant was used to measure the TSC content. Then, 500 µL phenol and 5 mL of 98% sulfuric acid were added to 1000 µL of the supernatant. The absorption was read at 483 nm with a spectrophotometer, and TSC content was recorded as mg g^{-1} FW [24].

2.6. Total Protein Content(TSP)

The standard protein of bovine serum albumin (BSA) was used to calculate total protein content using Bradford methods [25]. Fresh lavender leaves (1 g) were used for extraction in 4 mL of 50 mM phosphate buffer solution and afterward centrifuged at 11,180× *g* for 15 min. Finally, 50 µL of the extract was added to 1000 µL of Bradford 1× solution (Merck, Darmstadt, Germany), and the absorbance was measured after 5 min at 595 nm, along with standard samples. The blank was made with 50 µL of dH_2O and 1000 µL of Bradford solution.

2.7. Essential Oil Content (EOC) and Yield (EOY)

The fresh plant samples were kept in the shade until reaching a fixed weight. The Clevenger type apparatus (British Pharmacopoeia model) extracted the essential oil during 3 h. The extracted essential oils (EOC) were dehydrated with anhydrous sodium sulfate and stored in a glass vial at 4 °C; EOY was calculated based on the dry weight of the sample per pot [26].

$$\text{Essential oil content (EOC)} = (\text{Essential oil weight}/\text{dry weight of sample}) \times 100 \quad (5)$$

$$\text{Essential oil yield (EOY)} = (\text{Total dry matter (g pot}^{-1}) \times \text{Essential oil content})/100 \quad (6)$$

2.8. Soil HM Content

Soil (1 g) was digested with 7.5 mL nitric acid, 2.5 mL hydrochloric acid, and 0.5 mL of 30% hydrogen peroxide. Soil digestion was continued until the samples became discolored and brown vapors escaped. The samples were then filtered and diluted by dH_2O in 50 mL balloons. Then, the elements' contents were measured by atomic absorption spectrometry (Shimadzu-AA6300, Tokyo, Japan) [27].

2.9. Macro- and Micronutrient Content

The extracts of fresh shoot and root samples were employed to determine K content using a flame photometer. The N content was estimated by Kjeldahl and P by the vanadate–molybdate method [28]. Furthermore, Mn, Fe, and Zn contents were recorded using an atomic absorption spectrometer (Shimadzu, UV-1800, Tokyo, Japan) [29].

2.10. Statistical Analysis

ANOVA analysis was performed using MSTAT-C version 2.1. (Michigan State Univrsity, East Lansing, MI, USA). Mean comparisons were made using the least significant difference test (LSD) at 1% and 5% probability levels. Excel software was applied to draw graphs. Heat map cluster analysis was drawn using R studio ver. 14.2.1 software (R Studio, Boston, MA, USA).

3. Results

3.1. Colonization Percentage

Microscopic images showed that the treatment with AMF significantly affected root colonization of lavender plants (Figure 1a). The minor colonization percentage was observed in 225 mg kg^{-1} of Pb treatment, while the highest was recorded in the AMF application alone (Figure 1b). The results showed that, with increasing HM levels, the

colonization of AMF decreased by up to 29.30% and 15.54% compared to control, under Pb and Ni, respectively.

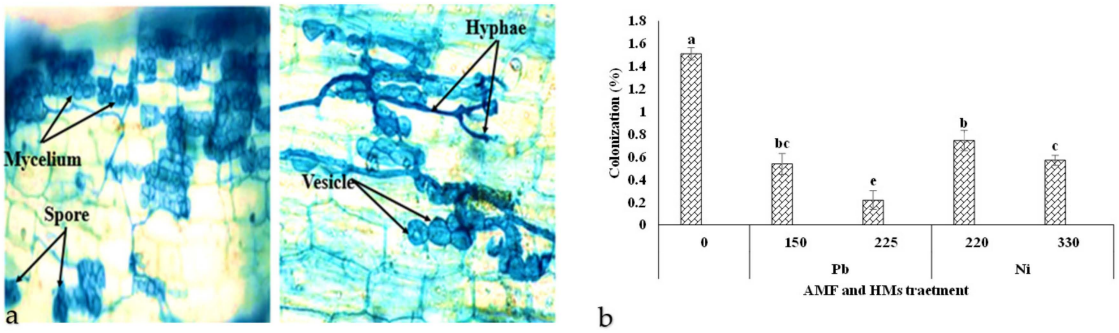

Figure 1. Microscopic images of the stained lavender roots to detect the arbuscular mycorrhiza (Funneliformis mosseae) colonization (**a**). The effect of arbuscular mycorrhizal fungi (AMF) on root colonization under Pb (mg kg^{-1} soil) and Ni (mg kg^{-1} soil) heavy metals (HMs) stress (**b**). Different letters indicate significant differences according to the LSD test at $p < 0.05$.

3.2. Morphological Traits

According to the results, the plant height and number of branches were significantly decreased by HM pollution, even with the application of AMF (Table 2). The top plant height and number of branches were recorded in AMF inoculation without HMs, and the minor data were related to 225 mg kg^{-1} of Pb without AMF inoculation. The height and number of branches of lavender plants were decreased by up to 46.28% and 33.33% under 225 mg kg^{-1} Pb in the presence and absence of AMF, respectively (Table 2).

Table 2. The effects of arbuscular mycorrhiza fungi (AMF) inoculation on the growth-related traits, essential oil content (EOC), and essential oil yield (EOY) of the lavender plant under Pb and Ni pollution.

AMF (g kg^{-1})	HMs (mg kg^{-1})		Height (cm)	Branch Number	Shoot FW (g pot^{-1})	Shoot DW (g pot^{-1})	Root FW (g pot^{-1})	Root DW (g pot^{-1})	EOC (%)	EOY (g pot^{-1})
0	Pb	0	43.90 ± 1.69 b	9 ± 0.71 bc	205.2 ±4.83 b	43.40 ± 3.01b	21.91 ± 1.50 b	4.94 ± 1.13b	0.91 ± 0.09 e	1.21 ± 0.05 c
		150	33.42 ± 1.75 d	8 ± 0.51 cd	186.6 ±4.97 d	34.89 ± 4.03 d	13.88 ± 1.24 d	2.99 ± 0.54e	1.08 ± 0.11 cd	1.14 ± 0.17 c
		225	28.75 ± 1.68 f	7 ± 0.71 d	92.7± 8.10 h	20.75 ± 8.40 h	11.69 ± 1.18 e	2.60 ± 1.35f	0.95 ± 0.06 de	0.66 ± 0.09 e
	Ni	220	30.73 ± 0.53 e	8 ± 0.48 cd	172.3± 5.17 e	30.67 ± 3.09 e	10.67 ± 0.93 ef	2.44 ± 1.01fg	1.01 ± 0.13 cde	1.09 ± 0.07 cd
		330	23.58 ± 1.79 h	7 ± 0.43 d	125.3 ± 16.39 g	23.21 ± 10.19 g	9.62 ± 1.19 f	2.19 ± 1.43 g	0.93 ± 0.05 de	0.64 ± 0.09 e
5	Pb	0	48.75 ± 0.89 a	13 ± 1.23 a	228.8 ± 15.83 a	47.36 ± 9.45 a	27.6 ± 0.70 a	5.90 ± 1.15 a	1.31 ± 0.10 b	1.80 ± 0.05 a
		150	35.1 ± 1.44 c	9.7 ± 0.56 b	206.5 ± 8.58 b	42.89 ± 5.33 b	17.05 ± 1.11 c	4.29 ± 0.95 c	1.39 ± 0.10 ab	1.49 ± 0.06 b
		225	31.88 ± 0.35e	7 ± 0.35 d	122.6 ± 5.97 g	46.44 ± 5.44 f	14.48 ± 2.06 d	3.49 ± 1.03 d	1.15 ± 0.08 c	0.91 ± 0.06 d
	Ni	220	33.63 ± 0.56 d	9 ± 0.75 bc	194.2 ± 7.37 c	37.69 ± 8.43 c	13.55 ± 1.53 d	4.26 ± 0.64 c	1.53 ± 0.10 a	1.81 ± 0.04 a
		330	26.90 ± 0.90 g	7.7 ± 0.33 d	137.8 ± 5.11 f	29.64 ± 3.05 e	12.00 ± 1.07 e	3.14 ± 0.58 de	1.07 ± 0.08 cd	0.93 ± 0.08 d
LSD at 0.05%			1.398	1.164	9.433	2.786	1.469	0.3739	0.1515	0.2041
S.O.V.		df								
HMs		4	490.229 **	19.78 **	16,836.524 **	637.45 **	248.718 **	9.116 **	0.115 **	1.008 **
AMF		1	100.806 **	22.50 **	4655.44 **	386.51 **	113.266 **	13.971 **	0.999 **	1.936 **
HMs× AMF		4	2.578 *	4.688 **	78.926 **	4.616 *	3.535 *	0.309 **	0.040 *	0.082 *
Error		30	0.937	0.650	14.129	1.324	1.035	0.069	0.011	0.20
C.V.			5.87	9.43	6.03	5.59	6.67	7.25	9.40	12.17

ns, * and ** indicate no significant difference, significant at 5% probability level and significant at 1% probability level, respectively. S.O.V. and df refer to the source of variation and degree of freedom. Different letters in columns indicate a significant difference at $p \leq 0.05$.

In the treatment with HMs, the shoot FW and DW decreased, but AMF inoculation improved the adverse effects of stress. The highest shoot FW and DW values were recorded with the inoculation of AMF in the absence of HMs, whereas the lowest shoot FW and DW were obtained under Pb of 225 mg kg^{-1} in the absence of AMF (decreased up to 54.82% and 52.18%, respectively) (Table 2).

The FW and DW of roots under HM application decreased, and this was ameliorated by AMF inoculation. The maximum values of root FW and DW were obtained from plants under AMF inoculation. In contrast, the lowest FW and DW of roots were recorded with 225 mg kg^{-1} Pb treatment without AMF, showing 56.07% and 55.61% decreases, respectively, if compared to the control (Table 2).

3.3. Essential Oil Content and Yield

The results showed that the interactions of AMF and HMs had significant effects on the EOC and EOY of lavender. The highest EOC and EOY values were obtained in plants inoculated with AMF alone and treated with 220 mg kg^{-1} of Ni. The lowest EOC and EOY values were recorded in control and the plants treated with the uppermost levels of Pb and Ni. AMF inoculation with 330 mg kg^{-1} Ni increased EOC and EOY by 18.5% and 2.96%, respectively, compared to the control (Table 2).

3.4. Photosynthetic Pigment Content

The highest contents of Chl a, b, total, and CAR were observed in the control treatment with AMF (Table 3). HMs decreased the content of the photosynthetic pigments, such that the lowest amounts of pigments were noted at 225 mg kg^{-1} Pb without AMF inoculation, recording reductions in chlorophyll a, b, total and CAR by 48.55%, 56.48%, 51.84%, and 34.33%, respectively, compared to the control. AMF inoculation ameliorated the photosynthesis pigment content under HM stress (Table 3).

Table 3. The effects of arbuscular mycorrhiza fungi (AMF) inoculation on the content of photosynthetic pigments, total soluble carbohydrates (TSC), and total soluble proteins (TSP) of lavender plants under Pb and Ni treatments.

AMF (g kg^{-1})	HMs (mg kg^{-1})		Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	Total Chl (mg g^{-1} FW)	CARs (mg g^{-1} FW)	TSC (mg g^{-1} FW)	TSP (mg g^{-1} FW)
0	Pb	0	44.09 ± 2.28 b	32.94 ± 0.94 ab	77.03 ± 1.21 b	17.06 ± 0.70 b	5.81 ± 0.54 b	0.420 ± 0.01 ab
		150	34.08 ± 1.53 de	23.42 ± 2.89 g	57.50 ± 2.36 f	13.45 ± 1.57 h	4230.41 f	0.31 ± 0.02 f
		225	29.68 ± 2.59 f	21.05 ± 3.13 f	50.73 ± 6.71 g	12.70 ± 0.30 i	3.74 ± 0.37 f	0.24 ± 0.05 g
	NI	220	33.63 ± 1.09 e	28.33 ± 0.15 e	61.96 ± 1.12 e	15.58 ± 1.20 d	5160.90 cd	0.35 ± 0.02 df
		330	33.47 ± 1.22 e	26.69 ± 2.96 f	60.16 ± 3.34 e	14.04 ± 1.27 g	4.97 ± 0.77 d	0.36 ± 0.04 cd
5	Pb	0	47.28 ± 2.58 a	33.27 ± 1.68 a	80.55 ± 4.25 a	18.42 ± 0.99 a	6.30 ± 0.41 a	0.44 ± 0.01 a
		150	34.21 ± 4.63 de	29.57 ± 1.54 d	63.78 ± 6.17 e	15.41 ± 0.88 e	4600.46 e	0.33 ± 0.01 ef
		225	33.24 ± 5.59 e	26.46 ± 1.94 e	59.70 ± 6.86 e	14.71 ± 0.47 f	4.45 ±0.44 e	0.32 ± 0.03 ef
	Ni	220	40.58 ± 4.99 c	32.51 ± 0.50 bc	73.09 ± 4.51 c	16,280.96 c	5.520.11 bc	0.41 ± 0.01 ac
		330	36.21 ± 2.33 d	32.15 ± 0.52 c	68,361.91 d	15.09 ± 0.83 ef	5.07 ± 0.45 d	0.40 ± 0.02 bd
LSD at 0.05%			0.713	1.335	1.593	0.491	0.368	0.045
S.O.V.		df						
HMs		4	283.9 **	67.32 **	623.95 **	26.56 **	3.225 **	0.019 **
AMF		1	86.96 **	262.02 **	650.90 **	23.10 **	7.007 **	0.048 **
HMs × AMF		4	18.79 **	28.710 **	69.72 **	2.373 *	0.296 **	0.003 **
Error		30	1.254	0.244	1.217	0.216	0.065	0.001
C.V.			4.25	7.69	5.87	6.78	5.10	6.62

ns, * and ** indicate no significant difference, significant at 5% probability level and significant at 1% probability level, respectively. S.O.V. and df refer to the source of variation and degree of freedom. Different letters in each column indicate a significant difference at $p \leq 0.05$.

3.5. Total Soluble Carbohydrates and Total Protein Content

The co-application of HMs and AMF determined a significant impact on the TSC of lavender. The uppermost TSC content was obtained by AMF inoculation in the absence of HM treatment, whereas the lowest TSC value was recorded with 225 mg kg^{-1} of Pb treatment without AMF inoculation, showing a reduction of up to 55.31% over that of the control (Table 3).

The AMF inoculation increased TSP content in lavender under HM stress. The results showed that the highest TSP content was obtained with AMF inoculation without Pb and Ni treatment, and the lowest value was noted at 225 mg kg^{-1} of Pb without AMF

inoculation. The mentioned treatment reduced the TSP content of lavender by 76.69% compared to that of the control (Table 3).

3.6. Shoot and Root MH Contents

The results showed that Pb content was increased in the shoots with the increased amount of Pb in the soil, while AMF inoculation had a non-significant effect on the Pb content in shoots (Table 4). The highest Pb content was observed with 225 mg kg^{-1} Pb treatment, which increased up to 15.33 fold compared to that of the control (Figure 2a). AMF inoculation increased shoot Pb content by 16% compared to that of the control.

Table 4. ANOVA for the effects of arbuscular mycorrhiza fungi (AMF) inoculation × Pb and Ni stress on macro-and micronutrient contents in lavender plants.

	S.O.V.	df	Pb	Ni	Fe	Zn	Mn	Mg	K	P
Shoot	HMs	4	0.095 **	0.011 **	1333.560 **	1.672 **	1785.694 **	0.558 **	3.602 **	0.825 **
	AMF	1	0.003 **	0.004 **	90.782 *	3.428 **	259.132 **	0.142 **	0.500 **	0.402 **
	HMs + AMF	4	0.001 ^{ns}	0.002 **	17.437 ^{ns}	0.231 **	19.260 ^{ns}	0.004 ^{ns}	0.053 *	0.027 **
	Error	30	0.001	0.001	15.067	0.032	12.245	0.002	0.019	0.005
	C.V.		33.13	23.76	37.54	16.39	16.09	3.45	13.06	5.93
Root	HM	4	0.276 **	0.044 **	1.584 **	6.925 **	340.828 **	0.558 **	3.274 **	1.962 **
	AMF	1	0.003 **	0.001 **	1.351 **	1.636 **	40.240 **	0.143 **	0.729 **	0.443 **
	HMs + AMF	4	0.001 *	0.001 **	0.183 *	0.115 ^{ns}	6.067 **	0.004 ^{ns}	0.070 *	0.011 **
	Error	30	0.001	0.001	0.032	0.072	1.329	0.002	0.019	0.003
	C.V.		9.98	11.39	29.37	20.09	14.94	4.23	20.09	5.69

AMF and HMs refer to arbuscular mycorrhiza fungi and heavy metals, respectively. S.O.V. and df refer to the source of variation and degree of freedom. *, ** and ns, significant at the 5% and 1% probability levels and non-significant, respectively.

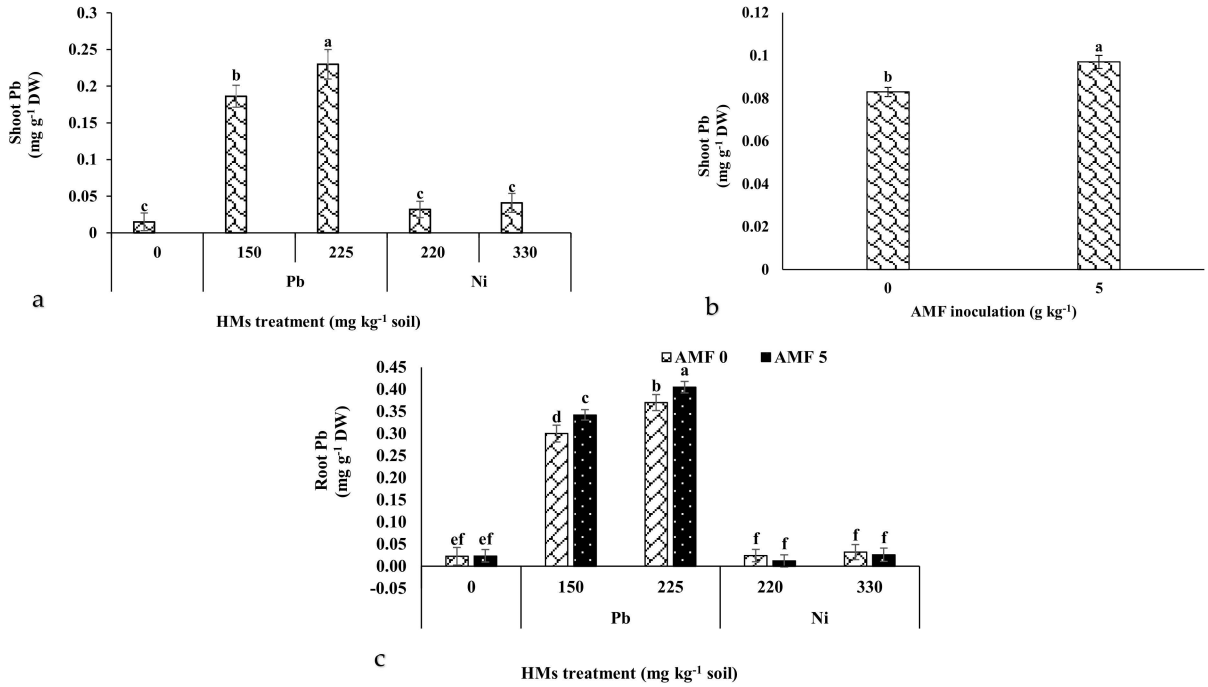

Figure 2. The effect of Pb and Ni (mg kg^{-1} soil) heavy metal (HM) stress on shoot Pb content in lavender plants (**a**) inoculated with arbuscular mycorrhizal fungi (AMF) (**b**) and root Pb content under HM stress and AMF inoculation (**c**). Different letters indicate significant differences according to the LSD test at $p < 0.05$.

The highest root Pb content was observed with 225 mg kg^{-1} Pb treatment × AMF application. The lowest values were recorded in the control with Ni treatments in the presence/absence of AMF (Figure 2c).

The results showed that the interaction effects of HM stress × AMF inoculation on Ni content were significant in shoots and roots (Table 4). The highest shoot and root Ni content was traced to the 330 mg kg^{-1} nickel treatment × AMF inoculation. In contrast, the lowest values were observed with the 225 mg kg^{-1} of Pb treatment in the absence of AMF. The 330 mg kg^{-1} of Ni treatment in the presence of AMF increased Ni uptake in roots and shoots by up to 7.9 and 10 fold compared to that in the control, respectively (Figure 3a,b).

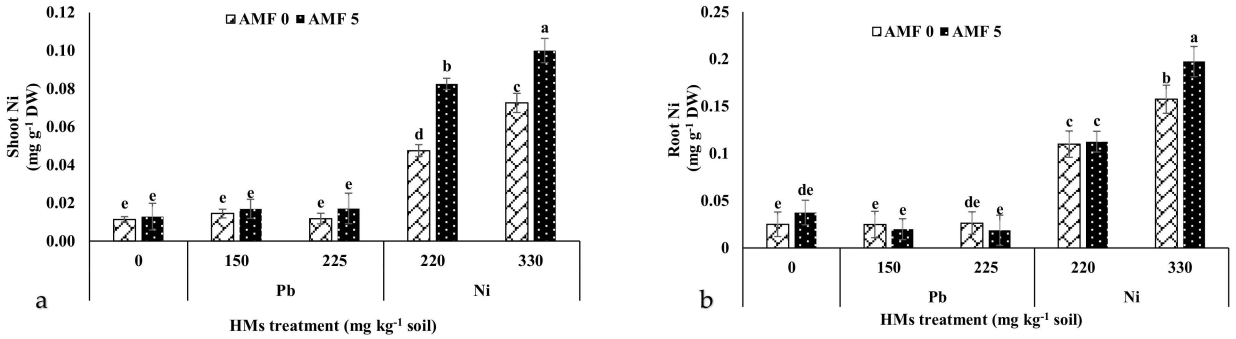

Figure 3. The effect of Pb and Ni (mg kg^{-1} soil) heavy metal (HM) stress on the shoot (**a**) and root (**b**) Ni content of lavender plants inoculated with arbuscular mycorrhizal fungi (AMF). Different letters indicate significant differences according to the LSD test at $p < 0.05$.

3.7. Shoot and Root Macronutrient Content

Variance analysis showed that the co-application of HMs and AMF significantly influenced K and P content in the lavender shoots. However, the treatments had a significant independent effect on the Mg content (Table 4). The highest P and K levels in shoots were obtained with AMF inoculation without HM stress. The shoot P and K contents increased under the 225 mg kg^{-1} of Pb treatment without AMF inoculation (by 96.65 and 57.90% compared to the control, respectively) (Figure 4a,c). Mg content was reduced owing to the increased HM stress, such that the highest values were observed in control, with the lowest was noted for the 225 mg kg^{-1} Pb treatment (Figure 4e). Moreover, AMF inoculation improved shoot Mg content compared to that with no AMF treatment (Figure 4g).

The findings showed that the root P and K contents were significantly decreased under HMs, but their content was improved using AMF inoculation. The highest P and K contents were recorded with AMF inoculation without HM stress. Still, the lowest values were recorded at 225 mg kg^{-1} Pb without AMF inoculation, which declined by 101% and 150% compared to the control, respectively (Figure 4b,d). Root Mg content was considerably decreased under Pb and Ni treatment. The highest Mg content was determined in the control, and the lowest with the 225 mg kg^{-1} of Pb treatment (Figure 4f). However, HM pollution reduced the root Mg content by 90%. AMF inoculation increased the Mg content in the roots compared to that in the control (11%) (Figure 4h).

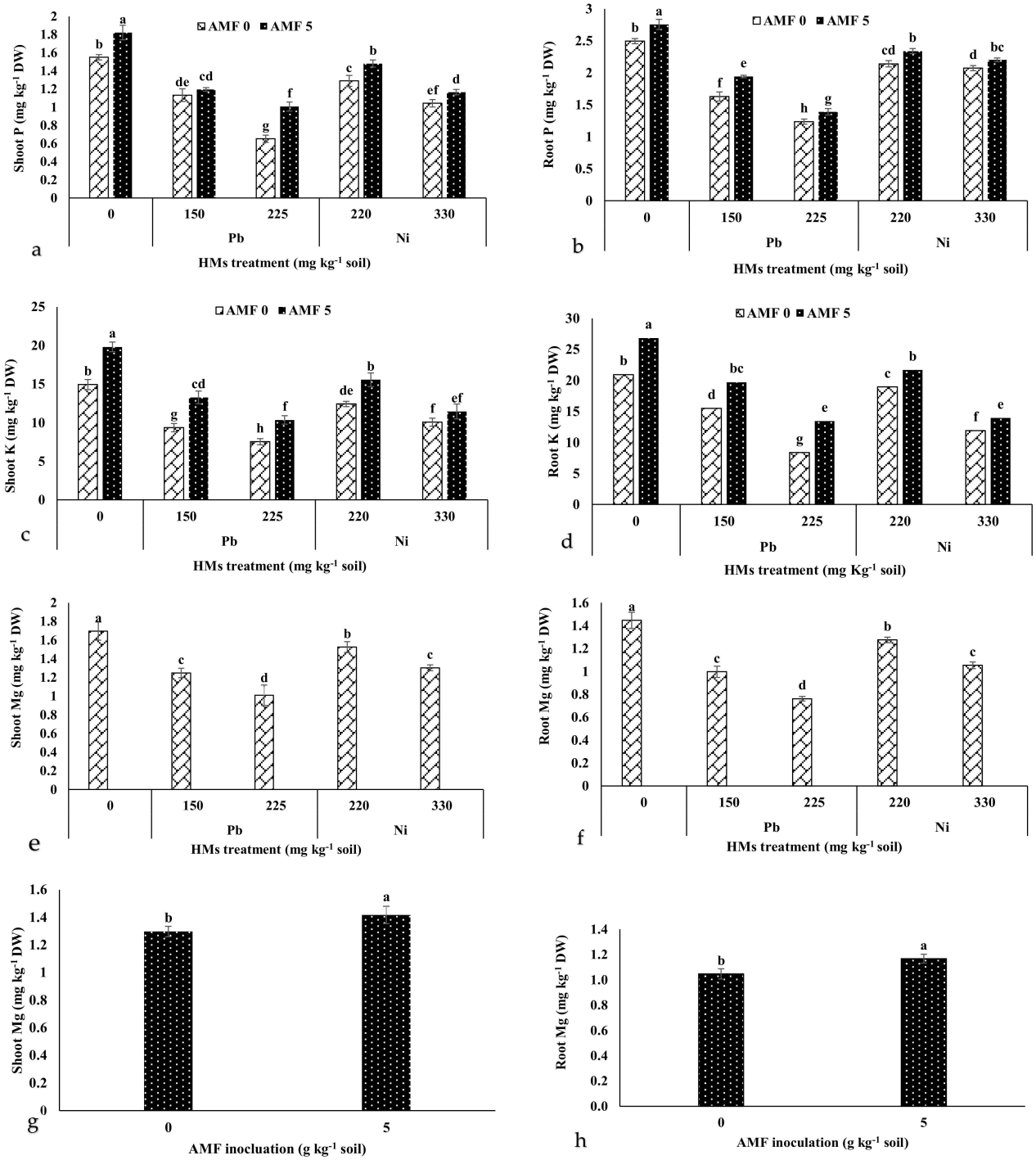

Figure 4. The effect of arbuscular mycorrhizal fungi (AMF) inoculation on the shoot P (**a**), root P (**b**), shoot K (**c**), root K (**d**), shoot Mg (**e**), and root Mg (**f**) content in lavender plant under Pb and Ni (mg kg^{-1} soil) heavy metal (HM) stress as well as the effect of AMF inoculation on the shoot (**g**) and root (**h**) Mg content in lavender. Different letters indicate significant differences according to the LSD test at $p < 0.05$.

3.8. Shoot and Root Micronutrient Content

The results revealed that HMs significantly reduced shoot Fe content, whereas AMF inoculation improved the trait (Table 4). The highest and lowest Fe contents in shoots were recorded under HM stress in control and 225 mg kg^{-1} Pb treatment, respectively (Figure 5a). Fe content in shoots decreased up to 161% under Pb treatment, while AMF inoculation improved it by 33% compared to control (Figure 5b). AMF inoculation under HM stress had

a significant effect on shoot Zn content (Table 4). So, HMs significantly decreased the shoot Zn content, but AMF inoculation ameliorated the nutrient content in the plant tissue. The highest and lowest shoot Zn contents were recorded with AMF inoculation without HMs and with 225 mg kg^{-1} of Pb without AMF inoculation, respectively (Figure 5c). Moreover, HM stress reduced the shoot Mn content, and the lowest value was observed at 225 mg kg^{-1} of Pb, while the highest was noted in the control. Mn content in plants subjected to Pb treatment was decreased by 2.79 fold (Table 4 and Figure 5e).

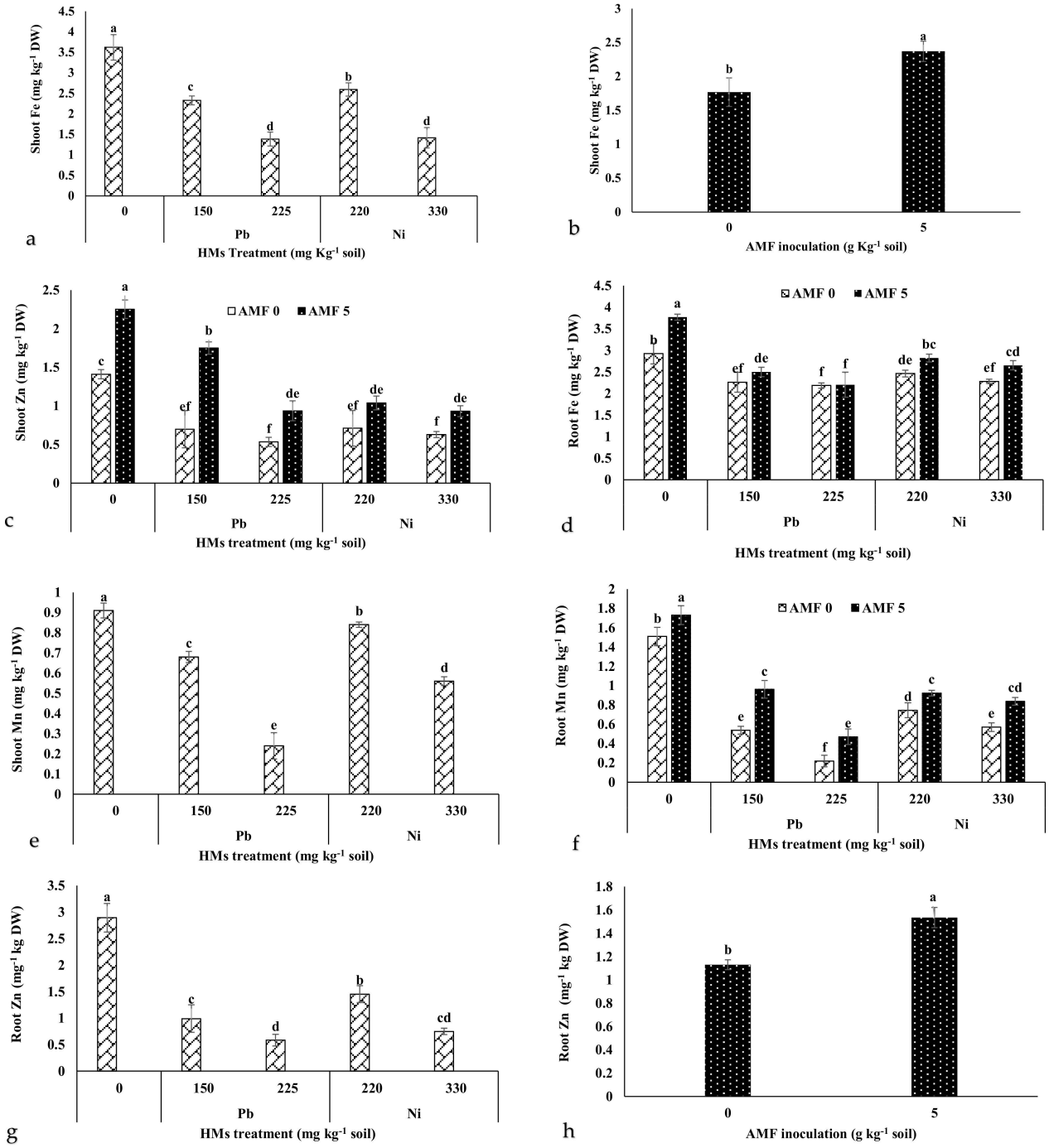

Figure 5. The effect of arbuscular mycorrhizal fungi (AMF) inoculation and Pb and Ni (mg kg^{-1} soil) heavy metal (HM) stress interactions and/or independent effects on shoot Fe content (**a**,**b**), shoot Zn (**c**), root Fe (**d**), shoot and root Mn (**e**,**f**) as well as root Zn (**g**,**h**) content in lavender plants. Different letters indicate significant differences according to the LSD test at $p < 0.05$.

Significantly reduced root Fe and Mn contents were observed in response to Pb and Ni treatments. Contrarily, AMF inoculation mitigated the adverse stress effects (Table 4). The highest and lowest root Fe and Mn contents were recorded in plants subjected to AMF inoculation in the absence of HMs and Pb (225 mg kg^{-1}) with no use of AMF, respectively (Figure 5d,f). However, the highest root Zn content was measured in plants treated with 225 mg kg^{-1} of Pb (Figure 5e). On the other hand, AMF inoculation at 5 g kg^{-1} improved soil Zn content by 35.7% over that of the control (Figure 5f).

3.9. HM Soil Decontamination

The Pb content in the soils subjected to $Pb(NO_3)_2$ was significantly increased before planting (Table 5). Hence, the highest and the lowest soil Pb contents before planting were observed with the 225 mg kg^{-1} treatment and in control (Figure 6a). After the plant harvest, Pb content alterations were significant in soils treated by AMF inoculation (Table 5). The soil subjected to AMF inoculation had lower Pb content than the soils without AMF treatment. The highest and the lowest soil Pb contents were measured in the samples with 225 mg kg^{-1} of Pb treatment without AMF and in the control, respectively (Figure 6c).

Table 5. ANOVA for the effects of arbuscular mycorrhiza fungi (AMF) inoculation under Pb and Ni stress on the soil Pb and Ni contents, before and after planting of lavender.

S.O.V.	df	Pb before Planting	Pb after Planting	Ni before Planting	Ni after Planting
HMs	4	32.207 **	19.714 **	1.584 **	0.879 **
AMF	1	0.001 ^{ns}	0.544 **	0.001 ^{ns}	0.027 **
HMs + AMF	4	0.001 ^{ns}	0.211 *	0.001 ^{ns}	0.007 **
Error	30	4.021	0.050	0.001	0.001
C.V.		6.84	13.73	4.01	5.76

AMF and HMs refer to arbuscular mycorrhiza fungi and heavy metals, respectively. S.O.V. and df refer to the source of variation and degree of freedom. *, ** and ns, significant at 5% and 1% probability levels and non-significant, respectively.

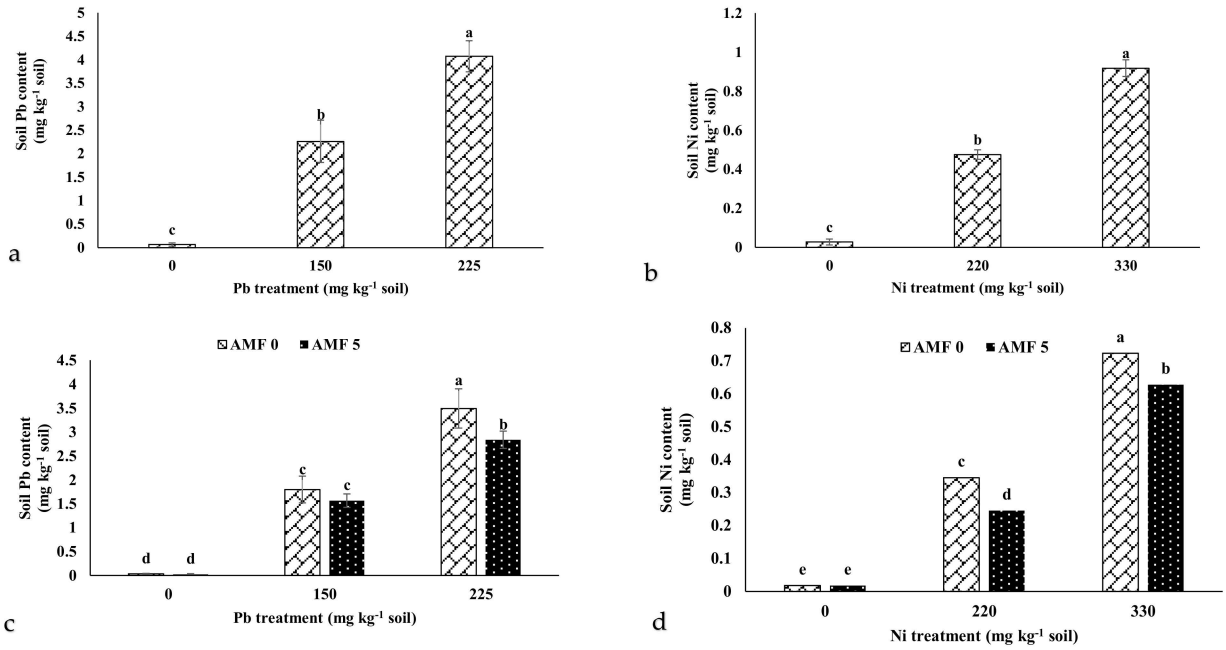

Figure 6. The effect of soil-based use of Pb and Ni and arbuscular mycorrhiza fungi (AMF) inoculation on the soil Pb content before planting (**a**) and after lavender plant harvest (**c**) as well as Ni content before planting (**b**) and after harvest (**d**) of lavender plants. Different letters indicate significant differences according to the LSD test at $p < 0.05$.

The content of Ni in the soils subjected to $Ni(NO_3)_2$ was significantly higher before planting (Table 5). Before planting, the highest and lowest soil Ni contents was observed in samples with 330 mg kg^{-1} Ni treatment and in the control, respectively (Figure 6b). The highest and lowest soil Ni contents were recorded in the samples with 330 mg kg^{-1} treatment without AMF inoculation and in the control treatment, respectively (Figure 6d).

3.10. Heat Map and Cluster Analysis

Heat map analysis (Figure 7) based on the response of lavender plants to AMF inoculation under Pb and Ni stress revealed that the attributes, including shoot and root Pb contents, had a positive response to Pb treatment. The shoot and root Ni contents showed the same response to Ni treatment. However, AMF inoculation had a promoting influence on the traits such as macro- and micronutrient contents, photosynthetic pigments, shoot and root FW and DW, plant height, EOC, and EOY.

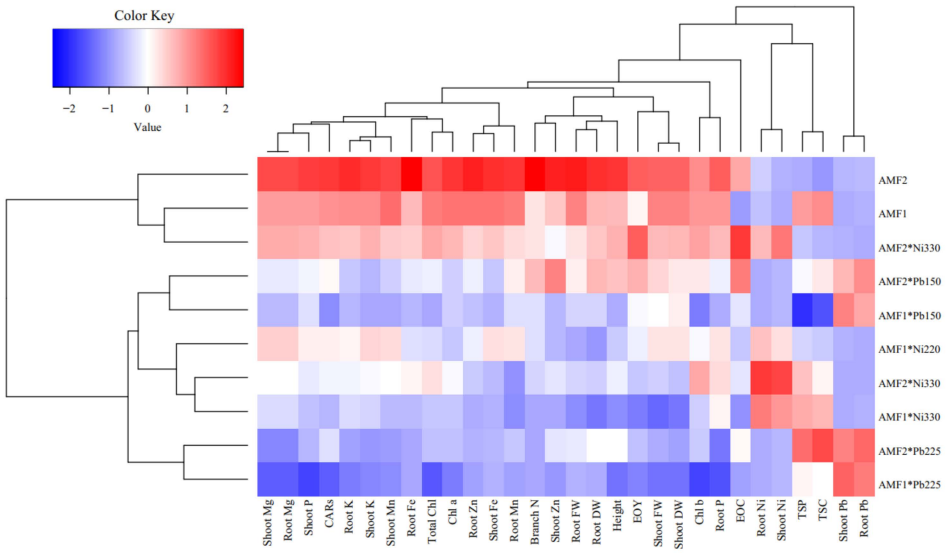

Figure 7. Heat map and cluster analysis of the growth parameters, macro- and micronutrient contents, and some biochemical changes in lavender plants exposed to HM stress and AMF inoculation. The heat map represents the shoot and root P, K, Mg, Fe, Zn, and Mn contents, branch number, plant height, essential oil content (EOC), essential oil yield (EOY), total soluble carbohydrate (TSC) content, total soluble protein (TSP) content, and the shoot and root Pb and Ni contents.

Cluster analysis and dendrograms in the heat map (Figure 7) discovered four main groups in the assessed characteristics. Group 1 contained: macro- and micronutrients, photosynthesis pigments, shoot and root FW and DW, height, EOC, and EOY; group 2 contained: shoot and root Ni; group 3 included: TSC and TSP; group 4 consisted of: the shoot and root Pb. Moreover, group 1 had a negative correlation with groups 2 and 4. In general, cluster analysis of heat maps for the AMF inoculation × HM stress showed two main groups. Group 1 contained the control, using AMF inoculation without Pb and Ni treatments and AMF inoculation × 330 mg kg^{-1} Ni. Group 2 included 150 and 225 mg kg^{-1} of Pb × AMF inoculation, 220 mg kg^{-1} of Ni in the absence of AMF, and 330 mg kg^{-1} of Ni in the presence/absence of AMF inoculation (Figure 7).

4. Discussion

In our study, AMF root colonization was reduced by increasing Pb and Ni concentrations in the soil. Our results align with those of Golubkina et al. [15] for onions and garlic. Moreover, Mahohi and Raiesi [30], in their study on three plant species (*Zea mays*, *Cynodon dactylon*, and *Stachys inflata*) under Pb contamination and symbiosis with AMF, reported that with increasing levels of HM stress, the symbiosis and colonization percentage significantly decreased.

Our results showed that Pb and Ni treatments decreased plant height and number of branches, but that by using AMF, the toxic effect of HMs was reduced. AMF inoculation improved the growth responses and morphological traits. One hypothesis is that HMs bind to the hyphal wall or central cylindrical cells of AMF. HM absorption then could be limited, leading to the improved growth, development, yield, and quality of crops under the imposed HM stress conditions [31]. Mitra et al. [32] showed that AMF treatment enhanced plant growth by modulating the hormonal pathways, which mitigated the adverse effects of HM stress. Chaturvedia et al. [33] noted that in soils contaminated with heavy metals, the height of eggplant was significantly reduced compared to the control. At the same time, plants in the AMF inoculation group grew higher than uninoculated ones. Still, in accordance with our results, they had a lower height than the controls.

Based on our results, the root and shoot FW and DW were reduced in lavender plants subjected to HM stress. Meanwhile, AMF inoculation improved those traits. Pb and Ni contamination, directly and indirectly, inhibits the physiological processes such as photosynthesis, respiration, and nutrient uptake and concomitantly impacts the plant biomass and productivity. The improvements due to AMF inoculation may be attributed to the significant increase in the availability of soil macro- and microelements, which enhance shoot yield and improve plant growth responses and yield [34]. Therefore, our results were similar to those of Daneshfar et al. [35], who stated that Pb-contaminated soil reduced plant DW, but AMF inoculation resulted in a 29.34% increase in plant DW. Additionally, Tabrizi et al. [36] reported that in marigold plants in Pb- and Cd-polluted soils, increasing concentrations of the HMs significantly decreased root and shoot growth. Still, AMF application mitigated the toxic effect by improving plant growth responses and productivity.

Our findings showed that chlorophyll content was significantly reduced by the HMs, but it was ameliorated under AMF inoculation. Increasing chlorophyll a, b, total and CARs contents in plants grown under AMF treatment could be due to the stimulation of root growth and enhanced nutrient uptake. Zhou et al. [37] reported that AMF symbiosis increased the emergence and development of hair roots and water uptake, and also improved CO_2 acquisition, chlorophyll content, and photosynthesis potential. Contrarily, the growth reduction due to HM stress could be attributed to the inhibition of enzymes responsible for chlorophyll biosynthesis. This leads to HM toxicity, which destroys the chloroplast apparatus [38]. Our findings are in line with those of Kumar [39], who observed that the amount of chlorophyll decreased in maize under cadmium stress but improved in response to AMF inoculation.

In the current study, the lavender plants subjected to the co-use of HMs and AMF attained a reasonable shoot TSC content. Since AMF inoculation increases phosphorus uptake, it improves the photosynthesis potential and enhances the biosynthesis and accumulation of carbohydrates. El-Sawah et al. [40] stated that the application of mycorrhizae improved the absorption of nutrients and TSC content. Under HM stress, the enhanced carbohydrate production helps maintain the primary metabolism of plants. Moreover, Noorani and Kafilzadeh [41] examined the effect of cadmium on safflower, which determined an increase in TSC content due to the metal stress.

Lavenders subjected to HMs had a remarkably reduced TSP content, while AMF inoculation reversed the trend. AMF inoculation, via the improvement in nutrient availability and uptake, increases the biosynthesis of amino acids in the leaves and promotes the accumulation of TSP [42]. In contrast, the decrease in TSP content with the HM treatment can be attributed to the high affinity of ROS, which eventually lead to the oxidation of proteins and to a sharp reduction in the biosynthesis and/or functioning of some structural proteins [43]. Khorshidi [44], in a study about Ni application on coriander, reported that the protein content was drastically reduced, more possibly due to the denaturation of proteins by the action of ROS.

AMF inoculation has a positive effect on the biosynthetic pathways of secondary metabolites due to the availability of elements, enhancing root formation and expansion,

and even the improvement in water potential of the rhizosphere [45]. The changes in the morphological traits of lavender plants may reflect the toxic effects of HMs on the plasma membrane, which can explain the decrease in the EOC and EOY at high concentrations of Pb and Ni. These results agreed with those of Prasad et al. [3] observed under HM stress conditions. AMF symbiosis with medicinal plants improved their growth and increased the biosynthesis of essential oil components. AMF inoculation also improved the growth of *Dracocephalum moldavica* by increasing nutrient uptake, water absorption, and synthesis of growth hormones [46].

Meanwhile, the decreased essential oil content under high levels of Pb and Ni can be explained by the decreased absorption of other nutrients, the reduction of photosynthesis potential, chlorophyll content, and leaf area, and thus by a massive reduction in the energy required for the biosynthesis of essential oils. However, the yield of lavender increased at low concentrations of these HMs. Nickel, as one of the trace elements necessary for plants in low quantities, could stimulate photosynthesis and eventually also improve the EOY [47].

According to our findings, the metal content in plant organs increased with greater concentrations of Pb and Ni in the soil. However, the HM content was not equally distributed in roots and shoots of the plant. Some reports have shown that AMF colonization generally increases the uptake and retention of HMs in the roots and reduces their transport toward the shoots [48]. Accordingly, our results showed that the root Pb and Ni contents were much higher than in the shoots. Moreover, AMF symbiosis increased HM phytoremediation in soils since mycorrhizal fungi can enhance their bioavailability for plants by strengthening the root growth and expansion, and subsequently by ameliorating the mobility of HMs [49]. In general, lavender plants inoculated with AMF had higher concentrations of Pb and Ni in their roots and shoots than the uninoculated control plants. Gonzalez et al. [50] reported that glomalin, a glycoprotein produced by AMF, binds with HMs and eventually extracts them from the soil, carrying out the phytoremediation process. Different mechanisms have been proposed concerning the plant symbiosis with AMF fungi and heavy metal swabbing. AMF acts as a direct link between soil and plant roots, so that the latter improve their phytoremediation efficiency by affecting the cleaning of HMs and increasing the plant tolerance [51]. Our results are consistent with the study by Tabrizi et al. [36], who reported a higher concentration of Pb in the roots than in the shoots. The significant differences between the concentrations of Pb in the roots and leaves suggest that a limited internal transfer of Pb from the roots to the leaves occurs. This is especially important because Pb enters the roots via the apoplastic pathway and through the calcium ion channels, accumulating mainly in the roots. The Pb transfer from the apoplastic pathway is easily accomplished by the dissolution of the element in the water and then transporting it via the vascular tissue. This mechanism prevents the transfer of Pb to the aerial parts of the plant and leads to its accumulation in the roots [52].

In our study, the concentration of Ni increased in the root along with its increasing concentration in the soil. However, AMF inoculation mediated the Ni transport to the plant tissues [53]. Ma et al. [54] confirmed our findings, showing that the Ni concentration in the roots under AMF application was higher than in the shoots. Additionally, Sayin et al. [55] stated that the application of AMF increased the HM absorption, especially the root Ni content. Our results showed that the highest Ni accumulation occurred in the root, whereas the lowest content was found in the leaves. The higher Ni accumulation in the roots reflects a tolerance mechanism at the high HM concentrations in the soil [56]. Ni content in the plant is directly related to its concentration in the soil and environment. A study by Parida et al. [57] on millet showed that the Ni content in plant tissues was directly related to the element's concentration in the plant growing medium. HM accumulation in the roots is much greater than in the aerial parts; for this reason, lavender, like many other crops, may be able to store large amounts of Ni in the roots [58].

The results showed that the plants with AMF inoculation absorbed more macro- and micronutrients than non-inoculated ones. This increment can be attributed to the secretion

of various types of siderophores and chelates, the stimulation of acid production by soil microorganisms, the release of cations from the soil particles, and then the improvement in the absorption potential of roots. In general, AMF creates an extended hyphal network, stimulates root branching and expansion, and improves plants' ability to absorb water and nutrients [59]. Alipour and Sobhanipour [60] have shown that Fe absorption was greatly improved by adding AMF and bacteria to the growth medium. Inoculation with AMF increased the absorption of Fe, P, K, Zn, and Cu compared to that of the control plants [61]. In addition, AMF inoculation has been reported to significantly improve the absorption of other nutrients, especially Mn and Mg [62]. Moreover, the enhanced phosphorus uptake in three-carbon and four-carbon plants with the use of AMF has been reported [63].

Our study showed that with an increase in Pb and Ni levels in the soil, the nutrient uptake by the plants decreased, especially at the highest Pb concentration. HMs seriously impact plant metabolic processes, such as root membrane dynamics, ATPases, and other carrier functions. As known, root respiration and growth ultimately inhibit the uptake of nutrients by plants. On the other hand, HMs, directly and indirectly, interact with other nutrient elements. HMs reduce the availability and uptake of Fe in root apoplast, its uptake into the root cells, and the transfer to the aerial parts, resulting in Fe deficiency in the metabolically active tissues [64]. Cseh et al. [65] showed that the concentration of Zn in cucumber leaves decreased due to HM stress. Jayakumar et al. [66] reported that increasing levels of Pb and Co reduced Mn content in the roots and shoots. In another study, the uptake of Mg in maize decreased with higher soil concentrations of Cd [67].

5. Conclusions

The phytoremediation of heavy metals is an environmentally safe technology. Lavender can be effectively used for the remediation of contaminated soils. Results showed that arbuscular mycorrhiza *Funneliformis mosseae* allowed the phytoremediation of soils contaminated with heavy metals (Pb and Ni) and improved the growth responses in lavender. In our study, the treatment increased the yields of essential oils in lavender, and at the same time, the phytoremediation process in the soils occurred. These results could be very interesting for the agricultural and environmental fields. Moreover, since HMs predominantly accumulate in the vegetative organs, their presence in the flowering tops of lavender and the potential for essential oil contamination with HMs should be a remote occurrence, although this aspect deserves more in-depth studies.

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References

1. Harindintwali, J.D.; Zhou, J.; Yang, W.; Gu, Q.; Yu, X. Biochar-bacteria-plant partnerships: Eco-solutions for tackling heavy metal pollution. *Ecotoxicol. Environ. Saf.* **2020**, *204*, 111020. [CrossRef] [PubMed]
2. Rizwan, M.; Ali, S.; Adrees, M.; Rizvi, H.; Zia-ur-Rehman, M.; Hannan, F.; Ok, Y.S. Cadmium stress in rice: Toxic effects, tolerance mechanisms, and management: A critical review. *Environ. Sci. Pollut. Res.* **2016**, *23*, 17859–17879. [CrossRef] [PubMed]
3. Prasad, A.; Kumar, S.; Khaliq, A.; Pandey, A. Heavy metals and arbuscular mycorrhizal (AM) fungi can alter the yield and chemical composition of volatile oil of sweet basil (*Ocimum basilicum* L.). *Biol. Fertil. Soils* **2011**, *47*, 853–861. [CrossRef]

4. Favas, P.J.; Pratas, J.; Paul, M.S.; Prasad, M.N.V. Remediation of uranium-contaminated sites by phytoremediation and natural attenuation. In *Phytomanagement of Polluted Sites*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 277–300. [CrossRef]
5. Rasouli, F.; Amini, T.; Asadi, M.; Hassanpouraghdam, M.B.; Aazami, M.A.; Ercisli, S.; Mlcek, J. Growth and Antioxidant Responses of Lettuce (*Lactuca sativa* L.) to Arbuscular Mycorrhiza Inoculation and Seaweed Extract Foliar Application. *Agronomy* **2022**, *12*, 401. [CrossRef]
6. Habeeb, T.H.; Abdel-Mawgoud, M.; Yehia, R.S.; Khalil, A.M.A.; Saleh, A.M.; AbdElgawad, H. Interactive Impact of Arbuscular Mycorrhizal Fungi and Elevated CO_2 on Growth and Functional Food Value of *Thymus vulgare*. *J. Fungi* **2020**, *6*, 168. [CrossRef]
7. Pirzadah, T.B.; Malik, B.; Tahir, I.; Kumar, M.; Varma, A.; Rehman, R.U. Phytoremediation: An eco-friendly green technology for pollution prevention, control and remediation. *Soil Remediat. Plants Prospect. Chall.* **2015**, *5*, 107–126.
8. Suman, J.; Uhlik, O.; Viktorova, J.; Macek, T. Phytoextraction of heavy metals: A promising tool for clean-up of polluted environment? *Front. Plant Sci.* **2018**, *9*, 1476. [CrossRef]
9. Akhtar, O.; Kehri, H.K.; Zoomi, I. *Arbuscular mycorrhiza* and *Aspergillus terreus* inoculation along with compost amendment enhance the phytoremediation of Cr-rich technosol by *Solanum lycopersicum* under field conditions. *Ecotoxicol. Environ. Saf.* **2020**, *201*, 110869. [CrossRef]
10. Pandey, V.C.; Pandey, D.N.; Singh, N. Sustainable phytoremediation based on naturally colonizing and economically valuable plants. *J. Clean. Prod.* **2015**, *86*, 37–39. [CrossRef]
11. Palma, J.M.; Gupta, D.K.; Corpas, F.J. Metalloenzymes involved in the metabolism of reactive oxygen species and heavy metal stress. *Heavy Met. Stress Plants* **2013**, 1–17. [CrossRef]
12. Lone, M.I.; He, Z.L.; Stoffella, P.J.; Yang, X.E. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. *J. Zhejiang Univ. Sci. B* **2008**, *9*, 210–220. [CrossRef] [PubMed]
13. Manan, F.; Chai, T.T.; Abd Samad, A. Environmental pollution in Malaysia: Are medicinal plants potential phytoremediation agents. *Int. J. Sci. Technol.* **2015**, *9*, 288. [CrossRef]
14. Sierra, M.J.; Millán, R.; Esteban, E. Mercury uptake and distribution in *Lavandula stoechas* plants grown in soil from Almadén mining district (Spain). *Food. Chem. Toxicol.* **2009**, *47*, 2761–2767. [CrossRef] [PubMed]
15. Golubkina, N.; Amagova, Z.; Matsadze, V.; Zamana, S.; Tallarita, A.; Caruso, G. Effects of arbuscular mycorrhizal fungi on yield, biochemical characteristics, and elemental composition of garlic and onion under selenium supply. *Plants* **2020**, *9*, 84. [CrossRef] [PubMed]
16. Białoń, M.; Krzyśko-Łupicka, T.; Nowakowska-Bogdan, E.; Wieczorek, P.P. Chemical composition of two different lavender essential oils and their effect on facial skin microbiota. *Molecules* **2019**, *24*, 3270. [CrossRef]
17. Dwivedi, M.; Singh, A.K.; Singh, V.P.; Mishra, P.K.; Singh, S.K. Studies on different concentration of Lead (Pb) and Cadmium (Cd) on growth and accumulation in different parts of Tulsi (*Ocimum tenuifolium* L.). *Int. J. Environ. Sci.* **2012**, *2*, 1733–1741. [CrossRef]
18. Stancheva, I.; Geneva, M.; Hristozkova, M.; Markovska, Y.; Salamon, I. Antioxidant capacity of sage grown on heavy metal-polluted soil. *Russ. J. Plant Physiol.* **2010**, *57*, 799–805. [CrossRef]
19. Gee, G.W.; Or, D. Particle-size analysis. In *Methods of Soil Analysis: Part 4 Physical Methods*; Wiley: New York, NY, USA, 2002; Volume 5, pp. 255–293.
20. Buurman, P.; Van Langer, B.; Velthrost, E.J. *Manual for Soil and Water Analysis*; Backhuys Publishers: Leiden, The Netherlands, 1996; p. 314.
21. Sparks, D.L.; Page, A.L.; Helmke, P.A.; Loeppert, R.H. (Eds.) *Methods of Soil Analysis, Part 3: Chemical Methods*; John Wiley & Sons: Hoboken, NJ, USA, 2020; Volume 14.
22. Giovannetti, M.; Mosse, B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.* **1980**, *84*, 489–500. [CrossRef]
23. Arnon, D.I. Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris. *Plant Physiol.* **1949**, *24*, 1–15. [CrossRef]
24. Chen, L.; Garrett, T.J.; Varghese, J.N.; Fincher, G.B.; Høj, P.B. Crystallization and Preliminary X-ray Analysis of (1,3)-and (1,3;1,4)-β-*dcl025*; -Glucanases from Germinating Barley. *J. Mol. Biol.* **1993**, *234*, 888–889. [CrossRef]
25. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef]
26. Poshtdar, A.; Mashhadie, A.; Moradi, F.; Siadat, S.; Bakhshandeh, A. Effect of source and rate of nitrogen fertilizer on yield and water and nitrogen use efficiency of peppermint (*Mentha piperita* L.). *Iran. J. Crop Sci.* **2016**, *18*, Pe14–Pe31.
27. Kilburn, M. Aqua Regia Digestion for Soils/Sludges. Analytical methods manual. *Methods* **2003**, *H115*, 2000.
28. Tandon, H.L.S.; Cescas, M.P.; Tyner, E.H. An acid-free vanadate-molybdate reagent for the determination of total phosphorus in Soils. *Soil Sci. Soc. Am. J.* **1968**, *32*, 48–51. [CrossRef]
29. Jones, J.B. Plant tissue analysis for micronutrients. In *Micronutrients in Agriculture*; Mortvedt, J.J., Ed.; Soil Science Society America, Madison: Madison, WI, USA, 1972; pp. 319–346.
30. Mahohi, A.; Raiesi, F. The efficiency of earthworms and rhizobacteria on mycorrhizal symbiosis and growth of three plant species in a soil contaminated with Lead (Pb) metal. *J. Water Soil Conserv.* **2018**, *25*, 97–112. [CrossRef]
31. Garg, N.; Chandel, S. Role of arbuscular mycorrhizal (AM) fungi on growth, cadmium uptake, osmolyte, and phytochelatin synthesis in *Cajanus cajan* (L.) Millsp. Under NaCl and Cd stresses. *J. Plant Growth Regul.* **2012**, *31*, 292–308. [CrossRef]

32. Mitra, D.; Djebaili, R.; Pellegrini, M.; Mahakur, B.; Sarker, A.; Chaudhary, P.; Mohapatra, P.K.D. Arbuscular mycorrhizal symbiosis: Plant growth improvement and induction of resistance under stressful conditions. *J. Plant Nutr.* **2021**, *44*, 1993–2028. [CrossRef]
33. Chaturvedia, R.; Favasb, P.; Pratasc, J.; Varuna, M.; Paul, M.S. Assessment of edibility and effect of arbuscular mycorrhizal fungi on *Solanum melongena* L. grown under heavy metal (loid) contaminated soil. *Ecotoxicol. Environ. Saf.* **2018**, *148*, 318–326. [CrossRef]
34. Krpata, D.; Fitz, W.; Peintner, U.; Langer, I.; Schweiger, P. Bioconcentration of zinc and cadmium in ectomycorrhizal fungi and associated aspen trees as affected by level of pollution. *Environ. Pollut.* **2009**, *157*, 280–286. [CrossRef]
35. Daneshfar, A.H.; AliAsgharzad, N.; Ostan, S.; Khoshroo, B. The role of rhizophagus irregularis to alleviate Pb absorption by sunflower. *J. Agric. Sci. Sustain. Prod.* **2018**, *28*, 37–50.
36. Tabrizi, L.; Mohammadi, S.; Delshad, M.; Moteshare Zadeh, B. Effect of arbuscular mycorrhizal fungi on yield and phytoremediation performance of pot marigold (*Calendula officinalis* L.) under heavy metals stress. *Int. J. Phytoremediat.* **2015**, *17*, 1244–1252. [CrossRef] [PubMed]
37. Zhou, Y.; Tang, L.; Zeng, G.; Zhang, C.; Zhang, Y.; Xie, X. Current progress in biosensors for heavy metal ions based on DNAzymes/DNA molecules functionalized nanostructures: A review. *Sens. Actuators B Chem.* **2016**, *223*, 280–294. [CrossRef]
38. Tripathi, A.K.; Gupta, M.K.; Verma, N.; Sinha, S.; Bhushan, A. Antioxidative and biochemical responses in *Dalbergia sissoo* Roxb seedlings growing under cobalt and lead stress. *Indian J. For.* **2016**, *39*, 31–36.
39. Kumar, P.P. Impact of Polyamines and Mycorrhiza on Chlorophyll Substance of Maize Grown under Cadmium Toxicity. *Int. J. Curr. Microbiol. Appl. Sci.* **2018**, *7*, 1635–1639. [CrossRef]
40. El-Sawah, A.M.; El-Keblawy, A.; Ali, D.F.I.; Ibrahim, H.M.; El-Sheikh, M.A.; Sharma, A.; Sheteiwy, M.S. Arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria enhance soil key enzymes, plant growth, seed yield, and qualitative attributes of guar. *Agriculture* **2021**, *11*, 194. [CrossRef]
41. Noorani, A.H.; Kafilzadeh, F. The effect of cadmium toxicity on growth, soluble sugars, photosynthetic pigments and some of enzymes in safflower (*Carthamus tinctorius* L.). *Iran. J. Biol.* **2011**, *24*, 858–867.
42. Ram, R.D.; Kodandaramaiah, J.; Reddy, M.P.; Katiyar, R.S.; Rahmathulla, V.K. Effect of VAM fungi and bacterial biofertilizers on mulberry leaf quality and silkworm cocoon characters under semiarid conditions. *Casp. J. Environ. Sci.* **2007**, *2*, 167–178.
43. Khudsar, T.; Katiyar, R.S.; Iqbal, M. Cadmium-induced changes in leaf epidermes, photosynthetic rate and pigment concentrations in Cajanus Cajan. *Biol. Plant.* **2001**, *44*, 59–64. [CrossRef]
44. Khorshidi, M. Influence of nickel on the indices of growth and content of photosynthetic pigments, protein, soluble sugar, and proline and nickel accumulation in coriander. *Appl. Res. Plant Ecophysiol.* **2016**, *2*, 59–74.
45. Kapoor, R.; Bhatnagar, A.K. Attenuation of cadmium toxicity in mycorrhizal celery (*Apium graveolens* L.). *World J. Microbiol. Biotechnol.* **2007**, *23*, 1083–1089. [CrossRef]
46. Fadaee, E.; Parvizi, Y.; Gerdakane, M.; Khan-Ahmadi, M. The effects of mycorhiza (*Glomus mosseae* and *Glomus intraradiceae*) and phosphorus on growth and phytochemical traits of *Dracocephalum moldavica* L. under drought stress. *J. Med. Plants* **2018**, *17*, 100–112.
47. Pande, P.; Anwar, M.; Chand, S.; Yadav, V.K.; Patra, D.D. Optimal level of iron and zinc in relation to its influence on herb yield and production of essential oil in menthol mint. *Commun. Soil Sci. Plant Anal.* **2007**, *38*, 561–578. [CrossRef]
48. Huang, X.; Ho, S.H.; Zhu, S.; Ma, F.; Wu, J.; Yang, J.; Wang, L. Adaptive response of arbuscular mycorrhizal symbiosis to accumulation of elements and translocation in Phragmites australis affected by cadmium stress. *J. Environ. Manag.* **2017**, *197*, 448–455. [CrossRef] [PubMed]
49. Karimi, R.; Solhi, S.; Salehi, M.; Solhi, M.; Mollahosaini, H. Effects of Cd, Pb and Ni on growth and macronutrient contents of *Vicia faba* L. and *Brassica arvensis* L. *Int. J. Agron. Plant Prod.* **2013**, *4*, 739–744.
50. Gonzalez-Chavez, M.C.; Carrillo-Gonzalez, R.; Wright, S.F.; Nichols, K.A. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. *Environ. Pollut.* **2004**, *130*, 317–323. [CrossRef] [PubMed]
51. Gaur, A.; Adholeya, A. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. *Curr. Sci.* **2004**, *86*, 528–534.
52. Gardea-Torresdey, J.L.; Peralta-Videa, J.R.; De La Rosa, G.; Parsons, J.G. Phytoremediation of heavy metals and study of the metal coordination by X-ray absorption spectroscopy. *Coord. Chem. Rev.* **2005**, *249*, 1797–1810. [CrossRef]
53. Shanker, A.K.; Ravichandran, V.; Pathmanabhan, G. Phytoaccumulation of chromium by some multipurpose-tree seedlings. *Agrofor. Syst.* **2005**, *64*, 83–87. [CrossRef]
54. Ma, Y.; Rajkumar, M.; Zhang, C.; Freitas, H. Inoculation of Brassica oxyrrhina with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. *J. Hazard. Mater.* **2016**, *320*, 36–44. [CrossRef]
55. Sayın, F.E.; Khalvati, M.A.; Erdinçler, A. Effects of Sewage Sludge Application and Arbuscular Mycorrhizal Fungi (*G. mosseae* and *G. intraradices*) Interactions on the Heavy Metal Phytoremediation in Chrome Mine Tailings. *Front. Plant Sci.* **2019**, *112*, 217–224.
56. Brunetti, G.; Farrag, K.; Rovira, P.S.; Nigro, F.; Senesi, N. Greenhouse and field studies on Cr, Cu, Pb and Zn phytoextraction by *Brassica napus* from contaminated soils in the Apulia region, Southern Italy. *Geoderma* **2011**, *160*, 517–523. [CrossRef]
57. Parida, B.K.; Chhibba, I.M.; Nayyar, V.K. Influence of nickel-contaminated soils on fenugreek (*Trigonella corniculata* L.) growth and mineral composition. *Sci. Hortic.* **2003**, *98*, 113–119. [CrossRef]
58. Fuentes, F.; Montes, J.; Fernandez, L.M. Total quality management, strategic orientation and organizational performance: The case of Spanish companies. *Total Qual. Manag. Bus. Excell.* **2006**, *17*, 303–323. [CrossRef]

59. Wu, Q.S.; Liu, C.Y.; Zhang, D.J.; Zou, Y.N.; He, X.H.; Wu, Q.H. Mycorrhiza alters the profile of root hairs in trifoliate orange. *Mycorrhiza* **2016**, *26*, 237–247. [CrossRef] [PubMed]
60. Alipour, Z.T.; Sobhanipour, A. The effect of *Thiobacillus* and *Pseudomonas fluorescent* inoculation on maize growth and Fe uptake. *Ann. Biol. Res.* **2012**, *3*, 1661–1666.
61. Askari, A.; Ardakani, M.R.; Vazan, S.; Paknejad, F.; Hosseini, Y. The effect of mycorrhizal symbiosis and seed priming on the amount of chlorophyll index and absorption of nutrients under drought stress in sesame plant under field conditions. *Appl. Ecol. Environ. Res.* **2018**, *16*, 335–357. [CrossRef]
62. Babajide, P.A.; Fagbola, O. Growth, yield and nutrient uptakes of sesame (*Sesamum indicum* L.) as influenced by biofertilizer inoculants. *Int. J. Curr. Microbiol. Appl. Sci.* **2014**, *3*, 859–879.
63. Frew, A. Arbuscular mycorrhizal fungal diversity increases growth and phosphorus uptake in C3 and C4 crop plants. *Soil Biol. Biochem.* **2019**, *135*, 248–250. [CrossRef]
64. Tafvizi, M.; Motesharezadeh, B.; Savaghebi, G.R. Investigating the effects of lead contamination and foliar application of iron on some physiological characteristics in two forage corn (*Zea mays* L.) hybrids in calcareous soil. *Iran. J. Field Crop Sci.* **2014**, *45*, 213–226.
65. Cseh, E.; Fodor, F.; Varga, A.; Záray, G. Effect of lead treatment on the distribution of essential elements in cucumber. *J. Plant Nutr.* **2000**, *23*, 1095–1105. [CrossRef]
66. Jayakumar, K.; Jaleel, C.A.; Vijayarengan, P. Changes in growth, biochemical constituents, and antioxidant potentials in radish (*Raphanus sativus* L.) under cobalt stress. *Turk. J. Biol.* **2007**, *31*, 127–136.
67. Ciećko, Z.; Kalembasa, S.; Wyszkowski, M.; Rolka, E. Effect of soil contamination by cadmium on potassium uptake by plants. *Pol. J. Environ. Stud.* **2004**, *13*, 333–337.