Pollution, 3(3): 471-486, Summer 2017
DOI: 10.7508/pj.2017.03.0 12
Print ISSN: 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir, Email: jpoll@ut.ac.ir

Phytoremediation potential of heavy metals by two native pasture plants (Eucalyptus grandis and ailanthus altissima) assisted with AMF and fibrous minerals in contaminated mining regions

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Received: 11 Feb. 2017 Accepted: 9 Apr. 2017

ABSTRACT: The current study assesses the effect of fibrous clay minerals' amendments and arbuscular mycorrhiza incubation on heavy metal uptake and translocation in Eucalyptus grandis and Ailanthus altissima plants. For doing so, Eucalyptus and ailanthus trees have been grown in a soil sample, contaminated with heavy metal iron ore mining and collected from southern Iran. The area under study is arid, with the majority of trees being ailanthus and eucalyptus. Amounts of Cd, Pb, Zn, Cu, and Mn have initially been at toxic levels which declined after cultivation. Fibrous clay minerals have been added to soils as a natural adsorbent to adsorb heavy metals like Pb, Cd, Zn, and Mn. Accumulation of the elements in the roots and shoots has been in the following order: Cu>Zn>Mn>Cd>Pb>Fe. The organ metal concentrations have not statistically translocated from roots to shoots of plants, except for Zn and Cu whose concentrations have been significantly higher in roots. Eucalyptus is well capable of extracting elements from contaminated soils, compared to ailanthus, particularly in case of Cu and Cd. The percentage of mycorrhizal colonization proves to be more in pots with ailanthus plants grown in contaminated soil, suggesting enhanced effect of high metal concentrations on plant infection by G. mosseae. AMF assists soil remediation by enhancing the growth and retention of toxic elements by ailanthus, while no substantial change has been observed between inoculated and non-inoculated eucalyptus plants by AFM, regarding translocation of elements to plants. The possibility of increasing metal accumulation in roots is interesting for phytoremediation purposes, since most high-producing biomass plants, such as eucalyptus, retain heavy metals in roots.

Keywords: ailanthus, arbuscular mycorrhizal fungi, eucalyptus, fibrous clays, metals, phytoremediation.

INTRODUCTION

Nowadays around the world, and especially in the Middle East, rapid growth of population, industrialization of societies, inordinate mining activities, and increasingly scarce natural resources emit various pollutants, hence overburdening the environment. Pollution is the main external substance to water, air, soil, and earth to the extent that it alters physical, chemical, and/or biological quality, which is harmful in any way and form to human, plants, animals, organisms, or buildings and structures (Dabiri, 1996). In recent years, more attention has been paid to environment and contamination problems, considered a serious challenge; therefore, special

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environmental standards have been codified in various fields, compelling industries and manufacturers communities to comply with them. It is possible to cleaning up polluted sites, thanks to new technologies, which rely on eliminating pollutants. The development strategy of future remediation technologies is via researching green, environmental-friendly biological remediation, which combines remediation, in-situ remediation, based on equipped completely quick remediation, and supplying technical support of agricultural soil contamination, industrial enterprises, brown field, mining sites, etc. (Yao et al., 2012).

Among the new technologies, bioremediation is the most applicable. It is the process in which microscopic living organisms, green plants (phytoremediation), or their enzymes are used to clean contaminated environments, returning them to their original natural condition (Mani et al., 2016). The phytoremediation is the use of living green plants to fix or adsorb contaminants and clean the contaminants, reduce their risk, or disappear them, being an environmentally-sustainable and low-cost remediation technology (Daryabeigi Zand & Hoveidi, 2016). The rhizofiltration, phyto-transformation, phyto-stabilization, phyto-volatilization, phyto-extraction, and rhizosphere bioremediation are the main types of phytoremediation (Sinha et al., 2009). Association of metals with various soil phases along with element uptake by plants has been studied by some researchers. They have found that the majority of metal uptake is attributed to loosely-bonded ions (Karbassi et al., 2016). Montpetit and Lachapelle (2017) have found that knowledge is key to successful implementation of a phytoremediation plan on a contaminated site, even among those practitioners, already convinced by the technology’s merits. Reaching professionals on the ground is therefore doubly important: while it encourages acceptance, it contributes to implementation success too.

Phytoremediation has two major advantages for farmers: (1) no financial outlay to purchase chemical amendments, and (2) accrued financial or other farm-level benefits from crops grown during amelioration (Qadir et al., 2007). In addition to remedy-polluted soils, phytoremediation approaches aims at amelioration soil properties such as sodic soils improvement (Ghaly, 2002), nutrient availability status, and environment conservation in terms of C sequestration (Garg, 1998; Kaur et al., 2002). Some examples of main plants, identified with potential, for metal phytoextraction are Indian mustard (Brassica juncea) (Lim et al., 2004), Sunflower (Helianthus annus) (Adesodun et al., 2010), Rapeseed (Bi et al., 2011), Amaranthus (Amaranthus retroflexus) (Chehregani et al., 2009), Chenopodium (Chenopodium album) (Moogouei et al., 2011), and Willow (Weih & Nordh, 2002; Courchense et al., 2016).

Arbuscular Mycorrhizal Fungi (AMF) can colonize hyperaccumulator roots extensively in metal-contaminated soils and mycorrhizal hyperaccumulators produce considerably more biomass and grow faster than non-mycorrhizal plants, and form symbiotic relations between plant roots and the fungi (Ho-Man et al., 2013). Because of unlimited ability of AM fungi to survive in severely contaminated soils, mycorrhizal fungi can play a role in different processes in phytoremediation of contaminated soils, for example enhancing plants' capacity to withstand soil phytotoxicity, improving nutrition, and protecting plants against severe conditions like water shortage (Leung et al., 2010). Sorption reactions on clay surfaces can dramatically retard the release of metal ions from the geosphere (Dähn et al., 2003; Usman, 2008). In addition, preference of soil particles for different species of heavy metals varies among soil minerals and is another important factor governing bioavailability, mobility, and toxicity of these metals to plants. Despite the low CEC of palygorskite and sepiolite, they have a large capacity for adsorbing heavy metals due to their specific structure (Shirvani et al., 2006; Liang et al., 2014; Middea et al., 2013).

There have been few simultaneous studies on the effect of adsorbent material (fibrous clay minerals). Arbuscular Mycorrhizal Fungi (AMF), and phytoremediation by native plants of arid regions on the remediation of contaminated soils with heavy metals. Hence, this research aims at (a) assessment of the interaction between eucalyptus and tree of heaven or ailanthus plants, adapted to dry and desert climates for heavy metal phytoremediation, (b) evaluation of fibrous clay minerals in stabilizing heavy metals cadmium, lead, zinc, etc., and (c) checking symbiotic mycorrhizal fungi species in order to increase phytoremediation efficiency by plants.

MATERIALS & METHODS

Heavy-metal-polluted soil was collected from sites, around Golgohar iron ore mine, which is located south-east of Iran, 55 km from Sirjan in south-west of Kerman Province (29° 27′ N, 55° 40′ E). This site is placed at the eastern edge of Sanandaj–Sirjan geological zone, a geological unit in central and northern Iranian tectonic province (Stocklin, 1974). Rocks of the Sanandaj-Sirjan zone consist predominantly of medium- to high-grade metamorphic rocks, belonging to Precambrian age, mostly covered by Mesozoic Metasediments. Volcanic activity during Jurassic, Cretaceous, and Tertiary periods is expressed by various rock types, e.g. granite, andesite, diorite, ophiolite, and gabbro, which were emplaced into the metamorphic rock complexes (Mucke & Younessi 1994). Mining is one of Iran’s most important economic sources for nearly 900 million tons of proven ore, i.e. iron ore with the grade of 57.2% Fe. The area has a semiarid climate, with an average annual precipitation rate of 150 mm, at an altitude of 1500 to 1700 m above sea level.

Soils were sampled from the depth of 0-30 cm and 30-70 cm (plant root zone), mixed, air dried, and sieved through a 2 mm sieve. Soil properties and its constituents were analyzed by the following methods: particle size distribution via hydrometer method, saturated moisture content, Electrical Conductivity (EC), and pH in saturated paste extracted Cation Exchange Capacity (CEC) by sodium acetate 1 N at pH=8.2, OM content by wet combustion, and Calcium Carbonate Equivalent (CCE) by titration, according to Methods of Analysis for Soils in Arid and Semi-Arid Regions Handbook (Bashour & Sayegh, 2007). Available and total Fe, Mn, Zn, Cu, Cd, and Pb were extracted from soil samples with diethylene triamine penta acetic acid-DTPA (Quevauviller et al., 1998) and acid digestion (HNO3 + HF; ratio 2:3) (USEPA, 1994), respectively. Elements were determined in extracts by an AA200 Perkin Elmer Atomic Absorption Spectrophotometer (AAS). Fibrous clay minerals were used as amendments for heavy metal sorption and remediation from studied soils. Soil samples with around 75% fibrous minerals were collected from gypsic-calcic soil, belonging to Pliocene-Quaternary depositions. Quartz, cristobalite, gypsum, and calcite were present in the sample as impurities (less than 25%). X-Ray diffraction (XRD- D8 ADVANCE) and Transmission Electron Microscopy (TEM- Philips EM 300) were employed to identify fibrous clay minerals (Fig. 1). The soil samples (5000 g) were mixed thoroughly with different clays (8% or 16%). Afterwards the samples were transferred into adequate planting pots and were kept at 70-80% of their water-holding capacity at a temperature of 25°C for seven weeks. Soil samples from middle of each pot were taken after 50 days’ incubation. Air-dried samples were ground to pass a 2-mm sieve and the resultant sub samples were taken for chemical analysis. Soil pH, EC, CEC, CCE, saturation moisture content, and particle size distribution were measured in incubated samples as discussed above. Available and total Fe, Mn Zn, Cu, Cd, Pb, and Ni were extracted from the soil samples with DTPA (diethylene triamine penta acetic acid) and nitric acid, to be determined by AAS apparatus.

The study was a pot experiment, conducted at research greenhouse of Sirjan University of Technology, Sirjan, Iran. The setup was a factorial design and the treatments were replicated three times. The following treatments were studied: (1) control polluted soil; (2) soil + 8% fibrous clays (w/w); (3) soil + 16% fibrous clays (w/w); (4) soil without fungi; (5) soil + Glomus mosseae, and fungi. Mycorrhizal soil was prepared from Tooran Zist Fanavaran Company that contains at least 50 alive spores per gram of soil. The experimental pots were filled with 5000 gr contaminated soil, pre-sieved with 2 mm sieve size. Eucalyptus grandis (eucalyptus) and Ailanthus altissima Mill. Swingle (ailanthus) were germinated and the seedlings (trimester) without and with inoculum were transplanted into the contaminated soils, 2 seedlings/pot, three pots per treatment. The trials were conducted under controlled greenhouse conditions (i.e. temperature = 20–25°C and relative humidity = 60–70%) with daily watering. After 120 days, the plants were harvested, rinsed with distilled water, and dried at 60°C for 72h. Sampling of the plants was done to monitor metal uptake and soil for residual metal contents.

Translocation Factor (TF) was described as the ratio of heavy metals in plant shoot to those in plant roots, given in Equation 1 (Li et al., 2007; Malik et al., 2010).

$$\mathrm{TF} = \frac{C_{shoot}}{C_{root}} \qquad (1)$$

where C_{shoot} and C_{root} are metals concentration in plant's shoots and roots (both in mg kg^{-1}), respectively.

To investigate elements' accumulation in plant organs, Bioaccumulation Factor (BF) was calculated, using the following equation (Mani et al., 2016):

$$\mathrm{BF} = \frac{C_{root}}{C_{soil}} \quad \text{and} \quad \mathrm{BF} = \frac{C_{shoot}}{C_{soil}} \qquad (2)$$

where C_{root} is the element content (mg kg^{-1} dry weight) in roots, C_{shoot} is the metal content (mg kg^{-1} dry weight) in shoots, and C_{soil} is the total metal content (mg kg^{-1}) in the soil.

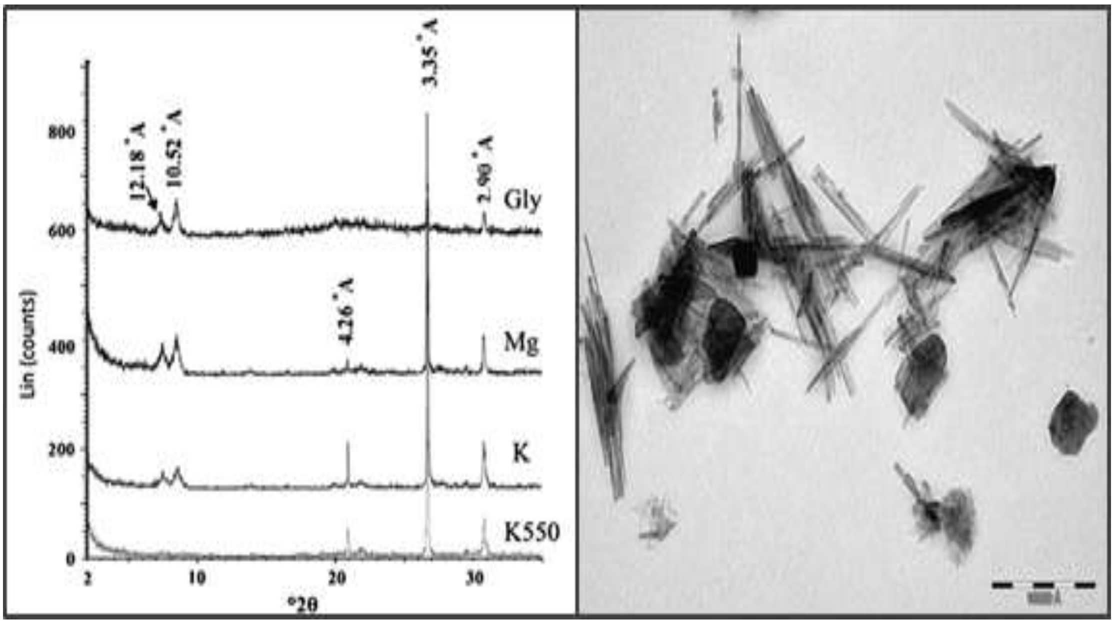

Fig. 1. X-ray diffractogram and TEM micrograph of soil used as amendment

Table 1. Physicochemical characteristics of studied soils

Treatment*	Sand %	Silt %	Clay %	Moisture Sat.%	OM %	$CaCO_3$ %	CEC $cmol_+ kg^{-1}$	pH	$EC_{sat.} dS m^{-1}$
0	84	14	2	44	1	17	7	7.3	9
8	80	17	3	46	1	19	10	7.6	11
16	75	21	4	54	1	23	12	7.9	10

*The numbers represent the added fibrous clay minerals amounts

A factorial in a completely randomized design with three replications was used in this research and the statistical analysis system software was called SAS (version 9.2). In order to compare the treatments, both analysis of variance (ANOVA) and mean separation analysis were utilized, via fisher's least significant difference (LSD) at P<0.05.

RESULTS & DISCUSSION

Table 1 shows the measured physical and chemical soil properties. As the results indicate, soil samples were classified loamy sand, based on USDA soil classification system, containing high sand and low clay percentages. After adding clay to soils, the amount of silt increased more, owing to flocculation of clay particles. Also, electrical conductivity, calcium carbonate equivalent, and pH slightly changed, which could be due to dissolved materials along with clay, not to mention the presence of lime. According to Yoo and James (2002), pH controls the solubility of metals by influencing the extent of metal-complexation with organic C-based ligands. Lead (Pb2+), for example, predominates in soil with pH below 6, and changes to the form PbOH+ (solid phase) at pH levels between six and eleven; therefore, in calcareous soils with a pH above 7, instead of total values, the available amount of elements should be considered.

The effects of heavy metals on plants, animals, and especially humans are often of high concern. The effects of Zn and Mn toxicity on humans and animals are not known (Liphadzi & Kirkham, 2006); moreover, no serious disease has been linked directly to an excessive concentration of Fe. Therefore this metal has been given no regulation limit in bio-solids by the US Environmental Protection Agency (Liphadzi & Kirkham, 2006). However, ingestion of elevated levels of Cu causes gastrointestinal distress, while long-term exposure to high Cu concentration causes liver and kidney damages (USEPA, 2002). In addition, lead impairs the nervous system, having effects on foetus, infants, and young children, resulting in a low intelligence quotient (United Nations, 1998). It is also classified as a possible human carcinogen as it can cause cancer. Cadmium is a toxic metal too that can accumulate in the human body and has a half-life greater than 10 years. Elevated levels of Cd in the body can cause kidney damages in humans (Salt et al. 1997). Other diseases associated with Cd exposure are pulmonary emphysema and bone demineralization (osteoporosis) (Bhattacharyya et al. 1988), since Cd takes the place of calcium (Ca) in bones.

Once the incubation period was done (adsorbent fibrous clay with soil at field capacity moisture for a duration of 7 weeks), specimens were obtained from core of each pot to determine changes in adsorption amounts by added clay. DTPA extracted of Fe, Zn, Cd, Mn, Cu, and Pb elements were measured in dried and sieved samples to compare effect of incubation by adsorbent (Fig. 2). Havlin and Soltanpour (1981) have stated that the critical level of DTPA-extracted Fe is

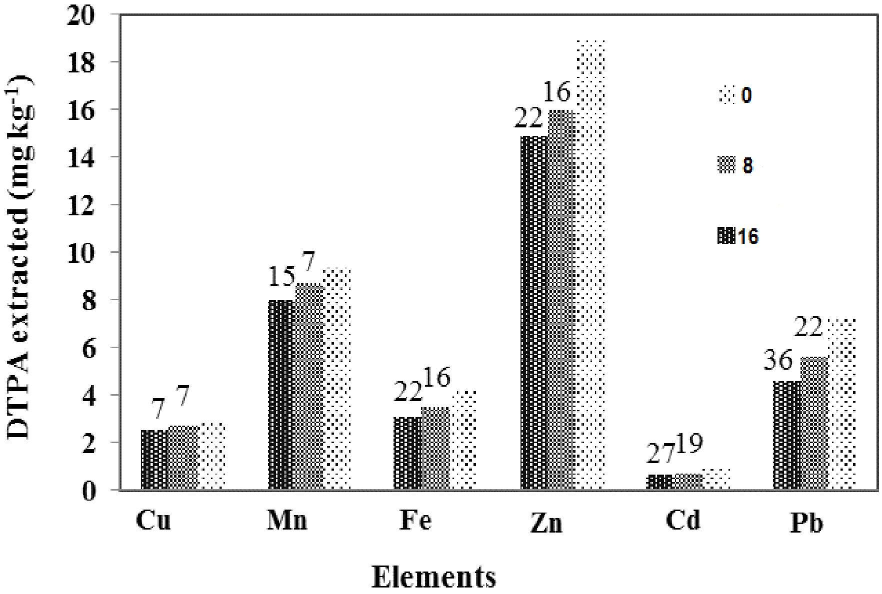

Fig. 2. DTPA of elements extracted after incubation with different amounts of clays (0%, 8%, and 17%); data labels show reducing percent of available element amount by adding clay (8% and 16%).

around 4.8 mg kg. According to Table 1, Fe is within the critical level range. Agrawal (1992) determined the critical level of DTPA-extracted Mn for wheat to be 5.5 mg kg; here, however, Mn amounts were more than critical levels in soils. Copper values are more than critical level, based on a report of Agrawal (1992) that set 0.78 mg kg as the critical levels of soils. The content of DTPA-extractable Pb and Zn from soil were below limit of human. The concentration limits of 20 mg kg^{-1} and 70 mg kg^{-1} DTPA-extractable were attributed to Pb and Zn, respectively, in order to control human risk (Winter Sydnor & Redente, 2002). Joshi et al. (2010) measured the threshold limits of DTPA-extractable Cd in soil for 10% reduction of Amaranthus, Fenugreek, Buckwheat yield as 1.3, 1.8, and 1.6 mg kg^{-1}, respectively. In the studied soils, here, DTPA-extractable Cd did not exceed 0.9 mg kg^{-1}.

Based on the results, illustrated in Figure 2, the adsorbent had a positive effect on reduction of the number of available components. Competitive sorption of nickel, cadmium, zinc, and copper on palygorskite and sepiolite silicate clay minerals has been studied by Sheikhhosseini et al. (2013), whose results confirmed that competitive sorption pattern of Ni, Cd, Zn, and Cu on the minerals varied with mineral type and the metal concentrations of the solution. Sorption of all four metals on palygorskite increased as metal concentrations in solution soared from 0 to 100 mg L^{-1}. The sequence of sorption maxima on palygorskite was Cu>Zn>Cd>Ni. In the case of sepiolite, sorption isotherms of Zn, Cd, and Ni showed an initial increase, up to metal concentrations of about 10 mg L^{-1}, but declined significantly afterwards at the presence of increasing Cu concentration. Overall, Cu and Ni were the most and the least preferentially retained metals, respectively, by both palygorskite and sepiolite minerals. The results of studied soils confirmed that sorption of elements on added fibrous clay minerals increased when the clay dosage increased. However, the changes became more prominent after adding 8% clay rather than 16% clay particles. In generals, fibrous clay minerals can effectively remove Pb and Cd from the soil, regardless of the presence of other metals. Among the metals studied, Cu showed the lowest ability to compete for sorption sites on fibrous clay minerals (Fig. 2).

Table 2. Normal and toxic concentration of elements in soil and plant (Kirkham, 1975; Alloway, 1995; Fageria et al., 2002; Zimdahi & Skogerboo, 1997)

	Pb	Cd	Zn (mg kg^{-1})	Fe	Mn	Cu
Normal Soil	10	0.06	1	200	7	2
Toxic Soil	50-100	3	70	None	1000	60
Normal Plant	0.1-12	0.1	20	50	30	5
Toxic plant	30	5	100	1000	300	20

Table 3. Interaction among plants, fungi, and added fibrous clay in soil elemental concentration

		Fungi					
		No Fungi			Mosseae		
	Clay	0	8	16	0	8	16
Eucalyptus	Pb-Total	23.7cd	18.8f	22.7ce	22.0cdef	21.5ef	22.7cde
	Pb-DTPA	7.6ab	5.6ab	6.8ab	6.7ab	5.3b	6.5ab
heaven	Pb-Total	19.9ef	29a	25c	19.9ef	20.8ef	28.3a
	Pb-DTPA	7.0ab	6.7ab	7.2ab	6.5ab	6.2ab	6.1ab
Eucalyptus	Cd-Total	1.9d	1.8d	1.9d	1.9d	2.1cd	2.1cd
	Cd-DTPA	0.56ab	0.55abc	0.58a	0.51abcd	0.45d	0.47cd
heaven	Cd-Total	2.7b	3.6a	1.9d	2.0cd	2.5bc	1.9d
	Cd-DTPA	0.47cd	0.51abcd	0.59a	0.51abcd	0.54ab	0.49bcd
Eucalyptus	Zn-Total	224cd	202g	234a	224cd	215ef	211f
	Zn-DTPA	50.5a	49ab	48.3b	50.3a	43.3c	50.6a
heaven	Zn-Total	231ab	219de	231ab	227bc	204g	203g
	Zn-DTPA	36e	39.6d	49.6ab	50.3a	43.3c	39d
Eucalyptus	Cu-Total	75.3ab	56.6cd	35.3e	74.0ab	54.3cd	56cd
	Cu-DTPA	2.0bcd	2.5a	2.53a	2.0cde	1.76def	1.7ef
heaven	Cu-Total	50.3d	75.3ab	63.6bd	60.3cd	81.7a	81.6a
	Cu-DTPA	1.7f	2.1bc	1.83def	2.0bcd	1.76def	2.23b
Eucalyptus	Fe-Total	6626e	7731b	4684f	6811de	7699b	4502f
	Fe-DTPA	2.4cd	3.1ab	3.3ab	3.6a	3.2ab	3.26ab
heaven	Fe-Total	7207cd	8256a	7127cd	7713b	7512bc	7315bc
	Fe-DTPA	2.4cd	2.8bc	3.3ab	3.6a	3.1ab	3.26ab
Eucalyptus	Mn-Total	308b	258cd	248e	224f	268c	247e
	Mn-DTPA	3.3cde	4.6a	2.73gh	3.9b	3.2def	3.5dec
heaven	Mn-Total	243e	314a	325b	222f	260c	252e
	Mn-DTPA	2.2h	2.5f	3dg	3.5b	3.2ed	4.0c

Figures 3 and 4 demonstrate the total element concentration in soils, before and after planting eucalyptus and ailanthus, respectively. Elements concentration decreased after cultivation of both plants, especially that of Pb, Cd, Mn, and Cu. Normally, soil incubation by mycorrhizal fungi (Glomus mosseae) and clay lessened the amounts of elements, with the exception of Fe, which did not comply with a specific trend. A noteworthy fact, regarding Cu, was that its concentration did not change by soil incubation with fibrous clay. However, cultivation of both plants and incubation with fungi varied Cu values significantly.

Based on the data in Table 2, the amounts of lead, cadmium, zinc, manganese, and copper, present in the soil, were more than normal. Adding fibrous clay reduced concentration of Pb, Cd, Mn, and Zn, unlike no-clay treatments (Figs. 3 and 4).

Concentration of the elements in plant organs was more than normal. In addition Zn and Cu in some treatments caused more toxic levels of plants in roots and shoots of eucalyptus and ailanthus (Table 2).

Table 3 presents the interaction among plants, fungi, and added fibrous clay in soil elemental concentration. The main correlations for ailanthus-cultivated treatments are as follows: DTPA-extracted amounts of Pb showed no significant relation, though it was significant among 0-0, 0-8, and 0-16 treatments, between 16-M with 8-M and also 16-M with 0-M. DTPA-extracted amounts of Cd showed a significant relation for 16-0 with 8-0 and 0-0; the total Cd amount was significant among 0-0, 8-0, and 16-0 and also between 8-M and 16-M. Total Zn amount was significant between 0-0 with 8-0 as well as between 0-M with 8-M and 0-M with 16-M. DTPA-extracted amounts of Zn and Mn along with total Mn values were significant in different clay dosages in both fungi and no fungi treatments. Total Cu amounts showed significant relations between 0-0 with 0-8, 0-M with 8-M and 0-M with 16-M, while the relation was significant between 8-M with 8-0 and 16-M- with 16-0 for DTPA-extracted Cu. Total Fe results indicated significant relation between 0-0 with 0-8, 0-8 with 0-16, 0-M with 8-M, and 8-M with 16-M treatments.

The main correlations for eucalyptus-cultivated treatments were as follows: Total Zn amounts were significant for all treatments of eucalyptus plants, except between 8-M with 16-M pots. The amount of DTPA Extracted was significant only between 0-0 with 16-0, 0-M with 8-M, and 8-M with 16-M. Total amounts of Cu were significant in different clay dosage values and no fungi treatments, while fungi treatments were not significant between 8 with 16% clay values. DTPA-extracted amounts of Cu were not significant between 8 with 16% clay dosage for both fungi and no fungi treatments. Fe and Mn total amounts were significant among different clay dosage for both fungi and no fungi treatments, while DTPA extracted Fe was only significant for 0-0, 0-8, and 0-16. DTPA-extracted values of Mn were significant among 0-0, 0-8, and 0-16 as well as 0-M, 8-M, and 16-M treatments.

Although Fe is classified as an element with a low toxicity in plants (McBride, 1994), it is potentially noxious if taken up by plants in excessive quantities. Difference between DTPA-extracted Pb and total Cd was not significant for all treatments of eucalyptus plants. Variations between 0% and 8% clay of total Pb amounts were significant in no fungi treatments, while other treatments were not so.

Bioavailability of metals declined in soil samples with high amounts of calcium carbonate, clay or oxides, and high pH values (McBride, 1994), with DTPA-extracted values of most treatments being Low (Table 3).

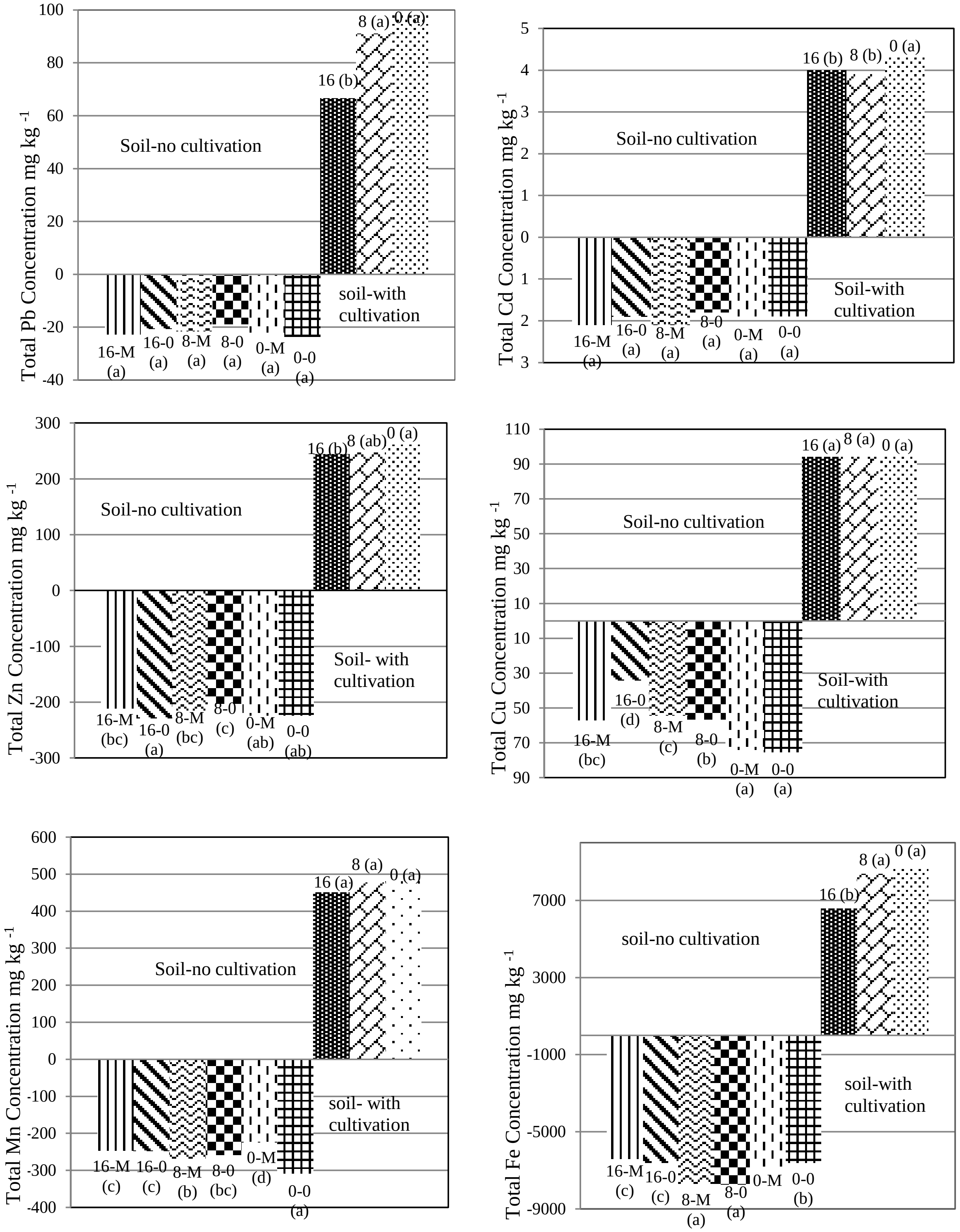

Fig. 3. Variation of total element concentration; before and after cultivation by Eucalyptus plants. Chart column labels: Numbers show clay added percentage (0, 8, and 16%) and the second labels indicate Fungi treatments (No fungi: 0, AMF: M), the small letter in parentheses stand for significant difference (LSD) at P<0.05.

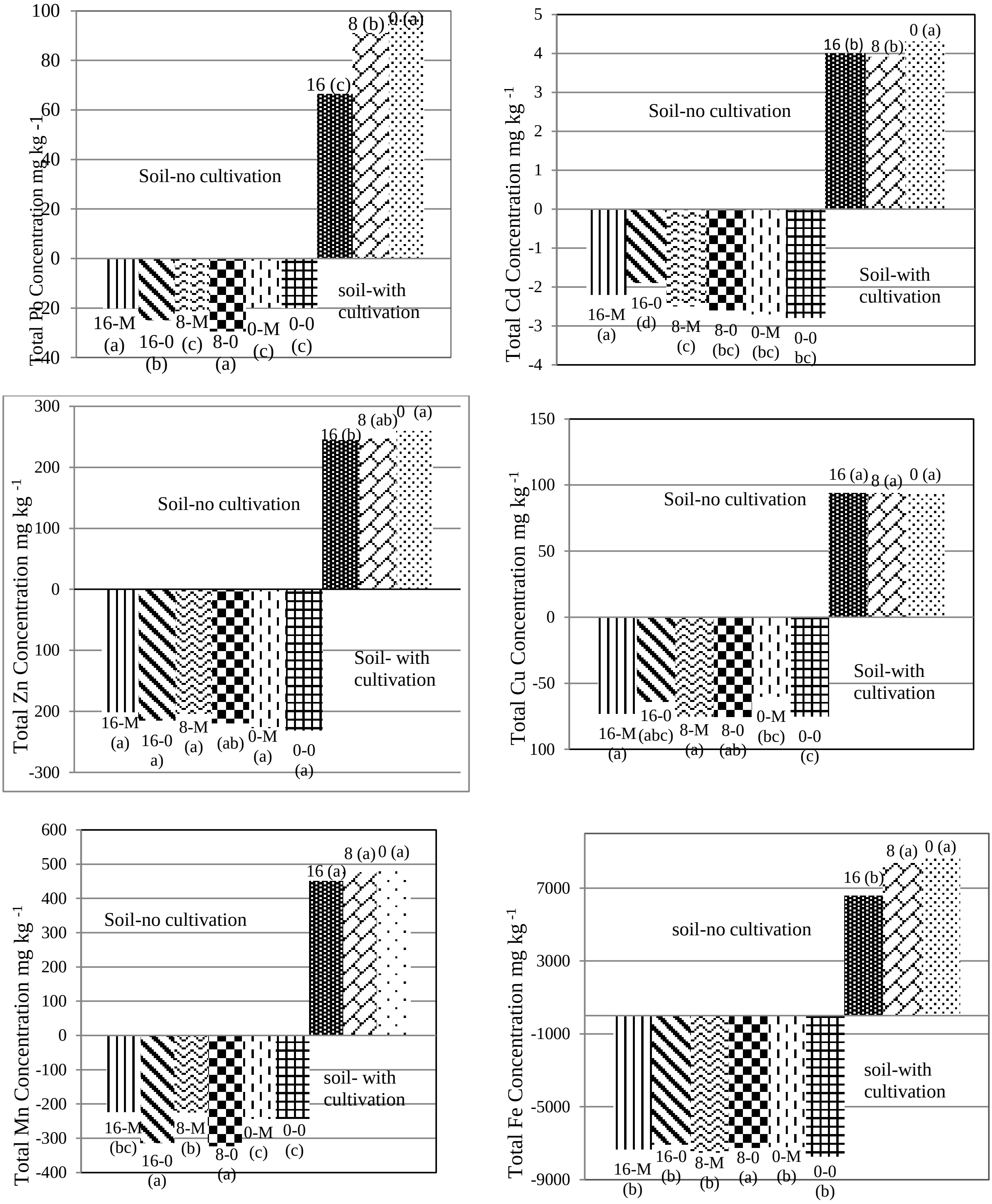

Fig. 4. Variation of total element concentration; before and after cultivation by Ailanthus plants. Chart column labels: Numbers show clay added percentage (0, 8, and 16%), the second labels indicate Fungi treatments (No fungi: 0, AMF: M), the small letter in parentheses stand for significant difference (LSD) at P<0.05

Table 4. Bioaccumulation factor of shoot and root, compared to element concentration in soil for Eucalyptus plants

Treatment	Pb	Cd	Zn	Fe	Mn	Cu
			BF shoot			
0-0	0.05	0.13	0.67	0.06	0.29	0.26
0-M	0.10	0.24	0.49	0.03	0.49	0.04
8-0	0.18	0.16	0.82	0.01	0.42	0.04
8-M	0.11	0.21	0.33	0.02	0.35	0.11
16-0	0.13	0.25	0.31	0.04	0.30	1.0
16-M	0.17	0.35	0.11	0.01	0.14	0.34
			BF root			
0-0	0.10	0.23	0.41	0.10	0.31	0.35
0-M	0.14	0.37	0.44	0.10	0.55	0.66
8-0	0.12	0.36	0.19	0.10	0.26	0.09
8-M	0.12	1.1	0.47	0.11	0.99	1.4
16-0	0.29	0.45	0.24	0.12	0.29	0.70
16-M	0.10	0.37	0.16	0.12	0.50	0.23
			TF			
0-0	0.46	0.58	1.63	0.66	0.92	0.75
0-M	0.65	0.64	1.11	0.34	0.89	0.06
8-0	1.43	0.44	4.3	0.15	1.70	0.42
8-M	0.92	0.20	0.72	0.17	0.36	0.08
16-0	0.46	0.55	1.3	0.30	1.02	1.44
16-M	2.6	0.94	0.66	0.11	0.28	1.52

Table 5. Bioaccumulation factor of shoot and root, compared to element concentration in soil for Ailanthus

Treatment	Pb	Cd	Zn	Fe	Mn	Cu
			BF shoot			
0-0	0.14	0.08	0.20	0.02	0.41	0.52
0-M	0.40	0.36	0.37	0.03	0.42	0.82
8-0	0.21	0.10	0.35	0.05	0.28	0.63
8-M	0.25	0.19	0.13	0.08	0.44	0.55
16-0	0.30	0.22	0.47	0.10	0.33	0.31
16-M	0.37	0.23	0.49	0.09	0.39	0.33
			BF root			
0-0	0.12	0.17	0.46	0.12	0.42	0.27
0-M	0.23	0.18	0.49	0.13	0.42	0.37
8-0	0.17	0.14	0.38	0.08	0.28	0.20
8-M	0.27	0.19	0.35	0.13	0.48	0.23
16-0	0.31	0.41	0.34	0.14	0.42	0.30
16-M	0.39	0.45	0.38	0.13	0.63	0.40
			TF			
0-0	1.20	0.47	0.43	0.14	0.99	2.0
0-M	1.80	2.0	0.75	0.25	1.0	2.2
8-0	1.24	0.68	0.90	0.55	1.0	3.1
8-M	0.92	1.0	0.38	0.64	0.91	2.4
16-0	0.95	0.54	1.40	0.71	0.80	1.0
16-M	0.94	0.51	1.30	0.71	0.62	0.8

Tables 4 and 6 show bioaccumulation and translocation factors for eucalyptus and ailanthus, respectively. Element accumulation in eucalyptus roots has the following sequence: Cu>Zn>Cd>Mn>Pb>Fe (Table 4). The shoot BF (Bioaccumulation factor) order for eucalyptus is Zn>Mn>Cu>Cd>Pb>Fe (Table 4) while the root BF order for ailanthus is Mn>Zn>Cu>>Pb>Cd>Fe (Table 5). Element accumulation in ailanthus shoots has the following sequence: Cu>Mn>Zn >Pb>Cd>Fe (Table 5).

It has been suggested that if BF is greater than 1, those plant species possess a potential of phytoremediation and are good accumulators for heavy metals in heavy-metal-contaminated sites (Israila et al., 2015). Based on the results of Table 4, Cu (BF=1.4) and Cd (BF=1.1) showed BF values more than 1; therefore, eucalyptus was relatively well-capable of extracting Cu and Cd. However, none of the treatments by ailanthus led to a BF greater than 1 (Table 5). Consequently, eucalyptus was more appropriate, compared to ailanthus for green space of contaminated sites.

TF>1 represents that metals were effectively translocated from root to the shoot (Zhang et al., 2002; Fayiga & Ma, 2006). Translocation from roots to shoots happened for Zn, Cu, Mn, Cd, and Pb. However, Cu and Zn translocated from root to shoot, more than all by ailanthus and eucalyptus. Plant species with slow plant growth, shallow root system, and small biomass production are generally not preferred for phytoremediation (Malik et al., 2010).

High soil pH can stabilize soil toxic elements, resulting in decreased leaching effects of soils' toxic elements. Moreover, toxic elements may also become stabilized due to high soil pH which may result in less element concentrations in the soil solution. This may restrain the absorbability of the elements from the soil solution and translocation into plant tissues (Liu et al., 2005). Studied soils were calcareous and moderately saline developed in arid climates.

Results showed that most of plant pots accumulated higher concentration of Pb, Cu, and Zn in plants. The normal level in shoots of plants for Pb, Cu, and Zn as given by Zu et al. (2004) are 5, 10 and 100 mg/kg. Zn and Cu are essential to plant growth, needed in small (micro) quantities; however, their excessive concentration in plant tissues may cause toxic symptoms. Cu concentration above 40 mg/kg for dry matter could induce toxicity in plants and cause toxic effects in animals (i.e. sheep) feeding on them (Annenkov, 1982).

The interest of palygorskite and sepiolite minerals in environmental applications lies in their special sorptive properties. Absorption and adsorption are two properties related to the surface area of clay minerals (Galan, 1996; Moreira et al., 2017). García-Sánchez et al. (1999) studied the heavy-metal adsorption capacity of various minerals in order to evaluate their potential ability to reduce metal mobility and bioavailability as well sa their possible application for remediation of polluted soils in the Guadiamar valley, SW Spain. In case of the sepiolite from Orera deposit, the maximum retention capacity, obtained for Cd, was 8.3 mg g^{-1}, followed by Cu (6.9 mg g^{-1}), and finally Zn (5.7 mg g^{-1}). The sorption and retention of studied elements by fibrous clay minerals after seven weeks of incubation, which reduced DTPA concentration, was in the following sequence: Pb> Cd>Zn≈Fe>Mn>Cu.

Arbuscular Mycorrhizal Fungi (AMF) organizes symbiotic relations with 80–90% land plant roots in forest ecosystems, rangeland, and agricultural lands (Brundrett, 2002; Candido et al., 2015). It is obvious that AMF is an important constituent of natural environments, able to influence the development of plant community composition and ecosystem process. The phytoremediation of damaged lands and the course of plant succession in such ecosystems may be extremely influenced by inoculation with AM fungi and their associate rhizobacteria (Khan, 2005; 2006). Mohammad et al. (2004) reported improved growth of wheat in a field, containing low levels of phosphorus and a low population of indigenous AMF, when inoculated with commercially-produced sheared-root inoculum of Glomus intraradices, indicating that the introduced AMF can compete with indigenous AMF and benefit plant growth. AM fungi can facilitate the survival of their host plants, growing on metal-contaminated land by enhancing their nutrient acquisition, protecting them from the metal toxicity, absorbing metals, and enhancing phytostabilization and phytoextraction. Such information may be useful to develop phytoremediation program at metal-contaminated sites (Ho-Man et al., 2013). Mycorrhizal plants are more efficient than non-mycorrhizal ones in the acquisition of micro-nutrients such as copper, iron, manganese, and zinc, when available at low concentrations. However, the effects of AM fungi on toxic metal uptake and accumulation of their hosts varied considerably. This inconsistency seems to arise from the fact that the effects of the AM fungi varied, depending on the fungal inoculum source, the plant and metal species, and the soil abiotic conditions (Ho-Man et al., 2013).

Distribution of absorbed metals, within the plant, was related to the type of heavy metal and plant. In plants, grown in non-inoculated soil by mycorrhizal fungi, the greater part of absorbed heavy metals was found in shoots with no significant difference, determined between inoculated and non-inoculated plants of eucalyptus. On the contrary, ailanthus plants, grown in contaminated soil, accumulated the highest amount of metal in root and shoot organs of plants that were grown in pots, inoculated by Fungi. In this soil, mycorrhization significantly enhanced ailanthus' translocation of most heavy metals from soil to plants.

CONCLUSION

The present study was designed to assess three remediation strategies for the treatment of a soil co-contaminated by moderate levels of heavy metals (Pb, Cd, Cu, Zn, and Mn), measuring metal adsorption by fibrous clay minerals, phytoremediation, and MAF partnership in soil remediation. Soils, contaminated with Pb, Cd, Zn, Cu, and Mn, collected from mining sites of an arid region (Iron Ore Mine, south east Iran) to address the capability of dominant tress in green space for phytoremediation by enhancing through fibrous clay minerals and arbuscular mycorrhizal fungus Glomus mosseae. Fibrous clay minerals have a wide range of industrial application, especially sorption. Soils incubated with the mentioned minerals to remediate soils. Comparison of elements' adsorption from soil by clays with dosages of 0 to 16% showed that by increasing the clay via adding 8% clay, rather than 8 to 16% clay, element adsorption of the soils reduced due to the saturation of adsorption sites after adding 8% fibrous clay minerals. AM fungi can facilitate the survival of their host plants, growing on metal-contaminated land, by enhancing their nutrient acquisition, protecting them from metal toxicity, absorbing metals, and enhancing phytostabilization and phytoextraction. In our experimental condition, eucalyptus growth did not change prominently in inoculated and non-inoculated pots, which may demonstrate a weak and non-symbiotic relation of fungi with eucalyptus roots, suggesting a significant negative effect of high metal concentrations on plant infection by G. mosseae. On the other hand, ailanthus growth declined in non-inoculated plants. This reduction was related to the degree of mycorrhization as well as the symbiotic relations with ailanthus, enhanced metal absorption, and growith in contaminated soils. In general, fibrous minerals by immobilization and reducing available element concentration, Arbuscular Mychoriza Fungi by bioaugmentation, and rosemary by phytoremediation (phytostab - ilization and phytoextraction), were assisted in remediation of soils.

All told, phytoremediation played a substantial role in reduction and remediation of soils, mostly by phytoextraction; AMF and fibrous clay minerals assisted soil remediation by promotion of growth and retention of toxic elements, respectively. Eucalyptus was well capable of extracting Cu and Cd; however, none of treatments by ailanthus led to a BF greater than 1. Consequently, eucalyptus was more appropriate than ailanthus for the green space of contaminated sites. The results of this study also indicated that there is an increasing need for further research on mechanisms, whereby such plants can survive in contaminated soils. Furthermore, studies are needed to determine the growth performance, biomass production, and metal accumulation of these species in metal-contaminated soils for their better management and conservation.

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