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Potential of indigenous microbes as helping agents for phyto-restoration of a Pb-contaminated soil

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ABSTRACT

The aim of this study was to assess the effects of heavy metal tolerant soil microbes inoculation on growth and metal uptake of pearl millet, *Pennisetum glaucum*, couch grass,*Triticum repens* and alfalfa,*Medicago sativa* in a soil spiked (and subsequently aged) with increasing concentrations of Pb. A soil sample (soil 1) was spiked with increasing (0 to 1500 mg/kg) concentrations of Pb and incubated for a seven months period. Another soil sample with a historical background of metal contamination (soil 2), having heavy metals-resistant microbial communities, also was taken and used as inocula. The plants were grown in pots containing contaminated soils. At the end of growth period, plants shoots were harvested, washed, oven-dried, ground and analyzed for Pb. The results showed a significant reduction ($p < 0.05$) in plants yield by increasing soil Pb concentration and inoculation of stress-adapted microbes further increased this reduction. This could be attributed to the increased access of plants to the relatively immobile Pb existed in the studied calcareous soil as well as to more metal contaminant absorption caused by soil microbial activity. In general, introduction of the microbes also resulted in lower Pb uptake by the studied plants.

Keywords: *Bioconcentration, Phytoextraction, Plant growth, Soil contamination, Soil microbial activity*.

INTRODUCTION

Contamination of soils with heavy metals has been accelerated by anthropogenic activities. The threat that heavy metals pose to human and animal health is aggravated by their long-term persistence in the environment (Rajkumar *et al*., 2005). Lead (Pb), as one of the more persistent metals, was estimated to have a soil retention time of 150–5000 years. However, some specific plant species are capable of growing on such contaminated soils and accumulate significant levels of specific metals (Solhi *et al*., 2005; Soleimani *et al*., 2009). These plants are consumed by domestic animals and thus metals such as Pb enter food chains and can then cause some health effects. On the other hand, these plants provide valuable tools for reclamation of polluted soils through the "phytoremediation" technology, enhancement of soil quality and recovery and reestablishment of biotic activities (e.g., Cao *et al*., 2002).

Phytoremediation usually is a time consuming process which is mostly due to low bioavailability of metals and/or low biomass of "hyper accumulators" (Sheng and Xia, 2006). Alternative methods such as using plant growth promoting rhizobacteria (PGPRs) (Motesharezadeh and Savaghebi-Firoozabadi, 2011) and arbuscular mycorrhizal fungi (AMF) have been suggested to alleviate this problem by increasing metals bioavailability or plant biomass production (e.g., Abou-Shanab *et al*., 2006). For example, the presence of rhizosphere bacteria increased the uptake of Cd in *Brassica napus* (Sheng and Xia, 2006) and Ni in *Alyssum murale* (Abou-Shanab *et al*., 2006). In addition, bacteria producing exudates and phosphate-solubilizing bacteria are capable of stimulating plant growth (Rajkumar *et al*., 2005). AMF may stimulate phytoextraction by essentially improving plant growth and increasing the total metal uptake (Wang *et al*., 2007). AMF associated with plants play a great role in the establishment of these plants on the contaminated soils. Therefore, the application of heavy metal-solubilizing

microorganisms is a promising approach for increasing heavy metal bioavailability in heavy metal amended soils (Jiang *et al*., 2008). However, microbes which are adapted to local soil conditions could be able to stimulate plant growth better than non-indigenous species. Indigenous microbes result from long-term adaptation to soils with extreme properties (Sylvia and Williams, 1992). Therefore, inoculation of plants with indigenous and presumably stress-adopted microbes can be a potential biotechnological tool for successful restoration of degraded ecosystems (Mathur *et al*., 2007).

The aim of this study was to evaluate the potential of heavy metal tolerant soil microbes for stimulating the phytoextraction rate of Pb from contaminated soils by pearl millet, *Pennisetum glaucum*, couch grass, *Triticum repens* and alfalfa, *Medicago sativa* under greenhouse conditions.

MATERIALS AND METHODS

A soil sample belonging to typic Calcixerepts subgroup according to the USDA Soil Taxonomy was taken from Western Azerbaijan province, Iran (soil 1).

Samples of soil 1 were screened to pass through a 5 mm sieve before contamination. The soils were then thoroughly mixed in plastic pots with $Pb(NO_3)_2$ in powder form. For each soil, Pb salt was ground and mixed well with a small portion of soil, and this metal/soil mixture was then thoroughly mixed with a large amount of soil in order to obtain total Pb concentrations of 150, 400, 800, and 1500 mg/kg soil. The spiked soils were subsequently packed into some 5 kg plastic pots. The packed soils were incubated in a moisture regime entailing periodic wetting-drying (WD) cycles for seven months at room temperature. In each WD cycle, soils were saturated in pots (pot-saturation) and allowed to be air-dried to relatively constant moisture. To avoid leaching out of Pb, no drainage pathway was allowed. Each WD cycle lasted for 40 days. After each WD cycle, the soil was mixed thoroughly to ensure homogeneity of soil Pb.

A soil sample of alfalfa rhizosphere in vicinity of Pb, Zn-smelter from Zanjan province, Iran was also collected (soil2). This soil sample had a historical background of metal contamination. Also, it was supposed to have microbial communities with adaptability to heavy metal contamination stress and to have been used as innoculum. AM fungal spores as well as RBs were isolated, identified, and abundant fungal species found in contaminated soil (soil 2) were recorded. AM fungal spores were isolated using wet-sieving and centrifugation in sucrose solution (50%) technique (Gerdemann and Nicolson, 1963). Then fungal species were identified using synoptic keys of Raman and Mohankumar (1988).

According to the results obtained from analyzing soil 2 for abundance of AM spores and RBs, 200 g of soil 2 was added to each 1 kg of soil1 as inocula. For blank samples (samples without stress-adapted microbes), soil 2 was sterilized prior to adding to soil 1. This soil substrate was aged under a WD moisture regime for a further 3 months as described above. This soil substrate was packed in some 5 kg plastic pots in nine replicates for each treatment (each three replicates were used for each plants as discussed below).

Subsamples of the prepared soil substrate were air dried and ground to pass through a 2-mm sieve before analyzing. Some physicochemical properties and total amounts of some inherent metals (Zn, Cu, Mn, Fe, Pb, and Cd) of the soils ($\leq$2mm) extracted after Gupta (2000) are presented in Table 1.

Table 1. Some physicochemical properties of the soil used as growth medium in this study

Properties	Value
Clay (%)	28.0
Sand (%)	24.0
Silt (%)	48.0
Textural class	Clay loam
pH	7.5
OM (%)	2.6
EC (dSm^{-1})	1.0
CEC ($cmol_c kg^{-1}$)	24.7
CCE (%)	10.1
Total Zn, mg kg^{-1}	12.7
Total Cu, mg kg^{-1}	30.6
Total Mn, mg kg^{-1}	422.9
Total Fe, mg kg^{-1}	377.9
Total Cd, mg kg^{-1}	8.0
Total Pb, mg kg^{-1}	22.6

OM: organic matter, EC: electrical conductivity, CEC: cation exchangeable capacity, CCE: calcium carbonate equivalent.

Seeds of pearl millet, *Pennisetum glaucum*, couch grass, *Triticum repens* and alfalfa, *Medicago sativa* were cultured in pots containing 5 kg of Pb contaminated soils under greenhouse conditions. After four weeks, the emerged seedlings were thinned for keeping the 14 and 10 strongest seedlings for pearl millet and the 10 strongest seedlings for couch grass and alfalfa per pot.

No fertilizer was applied, except that the increasing inputs of nitrogen resulting from different concentrations of $Pb(NO_3)_2$ application was taken into account and adjusted by adding appropriate amounts of urea. The plants were harvested by cutting the shoots at the soil surface at the end of growth period. Plant shoots were carefully washed with tap water to remove any adhering soil particles and rinsed twice with distilled water followed by drying at 75 °C for 72h and dry weights were recorded. For Pb analysis of plant shoots, 2.0 g aliquots of ground shoots were digested in 30 ml of HNO_3, $HClO_4$, and H_2SO_4 mixture (40:4:1) followed by 20 ml of deionized water (Gupta, 2000). The Pb concentrations in the extracts were measured by atomic absorption spectroscopy (AAS, Shimadzu 6300). In order to simultaneously test the phytotoxic effect of Pb and/or the effect of inclusion of stress-adapted microbes to soil on plant biomass production, the plants yield reductions were calculated as the relative percentage of dry biomass of a given plant in each experimental group (Y_c) to its dry biomass in the control (the treatment with no added Pb and no stress-adapted microbial community) (Y_o). This parameter was used to correct the shoot Pb concentrations of the plants for the concurrent changes in shoot biomass production as a simultaneous result of both Pb contamination and inclusion of stress-adapted microbes. Thus, the relative yield of the control treatment was regarded as 100% and the changes in relative yield as a result of Pb contamination and/or microbial inoculation were calculated when compared to the control and multiplied by the corresponding shoot Pb concentrations to normalize the shoot Pb values.

RESULTS AND DISCUSSION

Soil 1 was a non loasaline (EC = 1 dS/m), loam textured soil with 10.1% calcium carbonate equivalent. We used this soil as the main substrate to grow the plants. A preliminary sorption/desorption test indicated the strong and irreversible binding of Pb in this soil, so that ≥ 97.5% of the Pb present in the initial solutions was sorbed and more than 96% immobilized by the soil (data not shown). As indicated in Table 1, the prepared soil was a clay loam, non saline, weakly alkaline and calcareous soil with some degrees of inherent contamination with Pb. Concentration of

other metals was far below their acceptable levels in soil. The population of RBs including *Pseudomonas*, *Bacillus* and *Streptomyces* were 0.36×10^3, 0.15×10^3 and 0.23×10^3 per 10 grams of soil, respectively. Higher abundance of *Pseudomonas* bacteria (in particular fluorescent species) was found compared to *Bacillus* and *Streptomyces*. The dominant bacterial groups found in contaminated soil (soil 2) were evaluated by nutrient media and analyzed for the occurrence of main RBs including *Pseudomonas*, *Bacillus* and *Streptomyces* species (Glick, 1995). The root samples in soil 2 were fixed in FAA [6 ml of formalin (40% formaldehyde), 1 ml of glacial acetic acid, 20 ml of ethanol (96%), and 40 ml of distilled water] once upon collection (Phillips and Hayman, 1970), cleared and stained for observation of fungal structures.
Arbuscular mycorrhizal fungal spores were observed in all collected soil samples from alfalfa fields. The most abundant hairy roots effect on most abundant fungal species since the fungal colonization occurs mostly around fine hairy roots. Also, the most number of fungal spores were obtained at the end of the growing season due to the complete growth of the host plant root system. There were 1117 fungal spores on average per 50g soil sample in 3 replicates. After root clearing and staining in all studied plants, the most abundant observed fungal structures were vesicles as well as mycelia (Figure 1). The number of spores and arbuscules were low. However, these fungal structures could be observed intensively in alfalfa root samples due to its long lasting symbiosis time rather than other host plants. The results were in accordance with Muthukumar *et al* (2004) findings.

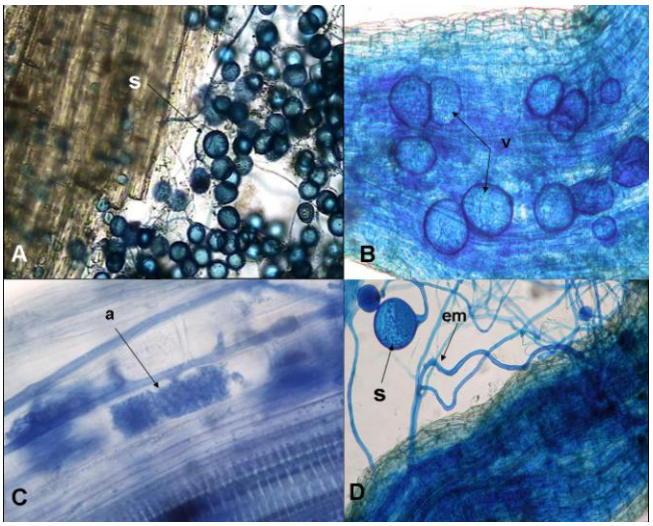

Fig 1. Colonized roots of alfalfa (A), (B), (C) and (D) by arbuscular mycorrhizal fungal after staining. Different fungal structures including vesicles (v), extraradical mycelia (em), arbuscules (a) and intraradical spores (s) could be observed.

Totally, 10 fungal species from two genera including *Glomus* (Glomeraceae, Glomerales) and *Acaulospora* (Acaulosporaceae, Diversisporales) were identified in alfalfa rhizosphere soil from Zanjan region. Nine species belonged to the genus *Glomus* including *G. fasciculatum*, *G. mosseae*, *G. intraradices, G. caledonium, G. geosporum, G. constrictum, G. versiforme, G. etunicatum and G. ambisporum*. One species belonged to the genus *Acaulospora* (*A. mellea*). The most and least abundant species were *G. fasciculatum* and *A. mellea*, respectively.
Shoot dry mass (g/pot) of the studied plants grown in a soil with different levels of Pb contamination with and without introducing soil microbial activity is presented in Table 2. These results showed that at the same levels of soil Pb, pearl millet had the highest content for shoot dry mass as compared to couch grass and alfalfa. As shown, when no Pb was added to the soil, the introduction of soil microbial activity

inoculum caused a significant decline in shoot dry mass when compared to the non treated soil. However, this reduction could be observed also in most of the treatments. This trend in treatments without adding any Pb, possibly, could be attributed to the increased access of plants to the relatively immobile inherent elements pre-existed in inoculum soil and in other treatments may be due to more metal contaminants absorption caused by adding soil microbial activity. The results obtained regarding the effect of microbial activity on biomass production of the plants are in disagreement with those of Rydlova and Vosatka, (2003) and in agreement with the results of Georgieva and Tasev, (1997), Parsad and Strzalkan, (1999), and Yell Yang *et al*. (2000).

Table 2. Shoot dry mass (g/pot) of the studied plants grown in a soil with different levels of Pb contamination with and without introducing soil microbial activity

Pb added to soil (mg kg $^{-1}$)	Shoot dry mass (g/pot)					
	pearl millet		alfalfa		couch grass	
	-M	+M	-M	+M	-M	+M
0	7.42	5.56	1.99	1.41	4.22	3.13
150	5.18	4.84	.78	0.78	3.45	2.97
400	5.29	3.60	1.53	0.94	2.49	2.62
800	5.24	4.43	1.56	0.95	3.49	2.81
1500	3.99	2.94	0.72	1.06	2.92	2.30

-M and +M: not inoculated and inoculated with heavy metal-tolerant microbes, respectively.

Pb concentrations in shoot dry biomass of the plants at different concentrations of soil Pb are presented in Table 3. Couch grass tended to take up higher Pb than alfalfa and pearl millet. The shoot Pb content of couch grass with soil microbial activity as well as without soil microbial activity increased with increasing soil Pb concentrations, but that of pearl millet and alfalfa decreased. Significant differences in the shoot Pb concentration of plants without soil microbial activity were observed at increasing concentrations of Pb. Moreover, significant differences were observed in the effects of the soil microbial activity on the shoot Pb content in Pb loaded soils (Table 3).

Table 3. Pb concentrations in shoot dry biomass of the studied plants at different concentrations of soil Pb contamination with and without introducing soil microbial activity

Pb added to soil (mg kg $^{-1}$)	Pb concentration in shoot dry biomass of plants (mg kg^{-1})					
	pearl millet		alfalfa		couch grass	
	-M	+M	-M	+M	-M	+M
0	79.04	86.9	76.2	56.9	71.8	71.8
150	105.8	66.6	86.9	72.09	78.3	79.7
400	43.6	59.9	65.5	42.1	88.7	82.1
800	49.9	48.05	54.5	50.4	86.9	95.2
1500	48.05	46.7	56.5	34.4	108.9	106.8

As shown in Table 3, plants maintained low and constant metal concentration over a broad range of Pb concentration in soil. It mainly restricted Pb in roots. Mycorrhizal fungal structures such as vesicules and hyphae may act as biological barriers that reduce metal Cd translocation from root to shoot (Joner *et al*., 2000; Tonin *et al*., 2001). This result indicated that plants use the metal exclusion strategy for growth on Pb contaminated soil. A metal excluder plant may alter its membrane permeability, change metal binding capacity of cell walls or exude more chelating substances (Cunningham, 1995) when grown on a metal contaminated environment. In this study, the ability of alfalfa, pearl millet and couch grass to uptake Pb was low, most likely, because of low bioavailability of Pb in soil. Correction for concurrent changes in shoot biomass production (Figure 2) further reveals that the introduction of the

heavy metals-resistant microbes lowers the Pb uptake by the studied plants. This may be a strategy applied by heavy metals-resistant microbes to better survive the plants at contaminated sites.

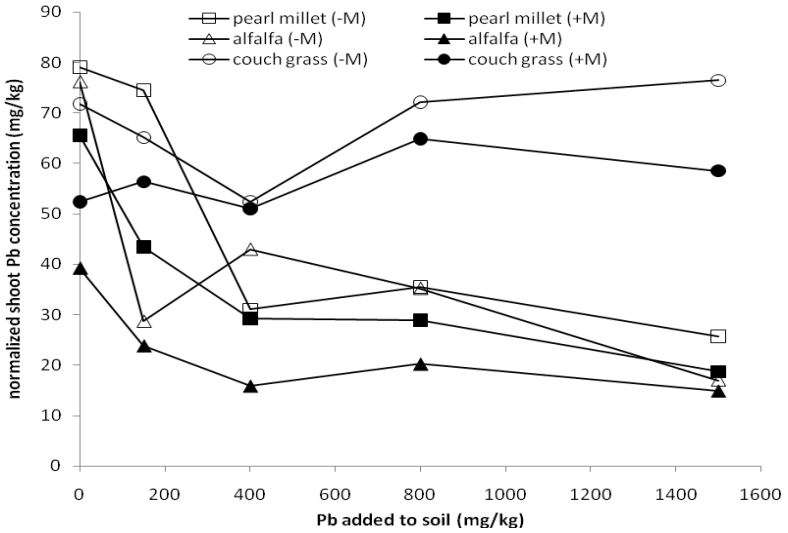

Fig 2. Shoot Pb concentrations normalized for changes in biomass relative to the non-inoculated control (no Pb addition) treatment of each plant species (-M and +M: not inoculated and inoculated with heavy metal-tolerant microbes, respectively).

Metal extraction and metal extraction ratio values of Pb for the studied plants at different concentrations of soil Pb contamination with and without introducing soil microbial activity, is presented in Table 4. For all three plants values of metal extraction for Pb were lesser than 0.6 mg Pb from each kg of soil (Table 4). Our experimental results indicated that the metal extraction values of all three plants decreased with increasing metal contamination in soil (Table 4).

Table 4. Metal extraction values of Pb for the studied plants at different concentrations of soil Pb contamination with and without introducing soil microbial activity

Pb added to soil (mg kg $^{-1}$)	Metal extraction (mg Pb from each kg of soil)					
	pearl millet		alfalfa		couch grass	
	-M	+M	-M	+M	-M	+M
0	0.142	0.101	0.33	0.178	0.56	0.48
150	0.061	0.062	0.36	0.21	0.45	0.32
400	0.136	0.077	0.10	0.11	0.34	0.21
800	0.135	0.090	0.17	0.14	0.28	0.21
1500	0.079	0.113	0.14	0.07	0.22	0.13

-M and +M: not inoculated and inoculated with heavy metal-tolerant microbes, respectively.

There were two possible reasons for the interpretation why metal extraction values decreased with increasing soil contamination. One possible reason was that the self-adjusting feature of plants plays an important role on sequestering the metals in their roots. Another possible reason is that plants could not grow well on the contaminated soils. It is especially true on the heavily contaminated soils, where plants become blasted and gradually die. In this case, because of the bad living conditions, the metal uptake ability by plants was weakened, and the metal content in plant shoots decreased. On the contrary, the corresponding metal content of soils was quite high, so the metal extraction values of plants in heavily contaminated soils significantly decreased (Wang *et al*., 2004).

CONCLUSION

Inoculation with microbial communities adapted to heavy metals contamination

stress decreased the metal uptake by the studied plants. This may be a strategy applied by this kind of microbes to better survive the plants at contaminated sites.

REFERENCES

Abou-Shanab, R.A.I. Angle, J.S. Chaney, R.L. (2006) Bacterial inoculants affecting nickel uptake by Alyssum murale from low, moderate and high Ni soils. *Soil Biology and Biochemistry*, 38, 2882–2889.

Cao, X. Ma, L.Q. Chen, M. (2002) Impacts of phosphate amendments on lead biogeochemistry in a contaminated site. *Environmental Science and Technology*, 36, 5296–5304.

Cunningham, S. (1995) In Proceedings/Abstracts of the Fourteenth Annual Symposium, Current Topics in Plant Biochemistry, – Physiology, and Molecular Biology Columbia, pp. 47-48.

Georgieva, V. and Tasev, C. (1997) Growth yield, lead, zinc and cadmium content of radish, pea, and pepper plants as influenced by level of single and multiple contamination of soil. *Bulgarian Journal of Plant Physiology*, 23 (1-2), 12-23.

Gerdemann, I.W. Nicolson, T.N. (1963) Spores of mycorrhizal Endogone species extracted by wet-sieving and decanting. *Transactions of British Mycological Society*, 46, 235-244.

Glick, B.R. (1995) The enhancement of plant growth by free living bacteria. *Canadian Journal of Microbiology*, 41, 109-117.

Gupta, P.K. (2000) Soil, plant, water and fertilizer analysis. Agrobios. New Delhi. India, P. 438.

Jiang, C.y. Sheng, X.f. Qian, M. Wang, Q.y. (2008) Isolation and characterization of a heavy metal-resistant *Burkholderia* sp. from heavy metal-contaminated paddy field soil and its potential in promoting plant growth and heavy metal accumulation in metal-polluted soil. *Chemosphere,* 1-8.

Joner, E.J. Leyval, C. Briones, R. (2000) Metal binding capacity of arbuscular mycorrhizal mycelium. *Plant and Soil*, 226, 227-234.

Mathur, N.J. Singh, S. Bohra, A. Vyas, A. (2007) Arbuscular mycorrhizal fungi: a potential tool for phytoremediation. *Journal of Plant Science*, 2(2), 127-140.

Motesharezadeh, B. and Savaghebi-Firoozabadi, Gh. R. (2011). Study of the increase in phytoremediation efficiency in a nickel polluted soil by the usage of native bacteria: *Bacillus safensis* FO.036b and *Micrococcus roseus* M2. *Caspian Journal of Environmental Sciences*, 9(2), 133-143.

Muthukumar, T. Udaiyan, K. Shanmughavel, P. (2004) Mycorrhiza in sedge: an overview. Mycorrhiza, 14, 65-77.

Phillips, J.M. and Hayman, D.S. (1970) Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of British Mycological Society*, 55, 158-161.

Prasad, M.N.V. and Strzalka, K. (1999) Impact of heavy metals on photosynthesis in heavy metal stress in plants. In: Prasad , M. N. V. and Hagemeyer, J. (Eds). Springer Heidelberg, pp,117.

Rajkumar, M. Nagendran, R. Lee, K.J. Lee, W.H. Kim, S.Z. (2005) Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. *Chemosphere*, 62, 741- 748.

Raman, N. and Mohankumar, V. (1988) Techniques in Mycorrhizal Research, University of Madras, Sivakami Publications, Madras, India, p. 279.

Rydlova, J. and Vosatka, M. (2003) Effect of *Glomus Intraradices* isolated from Pb-contaminated soil on Pb uptake by *Agrostis* Capillaries is changed by its cultivation in a metal-free substrate. *Folia Geobotanica*, 38, 155–165.

Sheng, X.F. and Xia, J.J. (2006) Improvement of rape (*Brassica napus*) plant growth and cadmium uptake by cadmium-resistant bacteria. *Chemosphere*, 64, 1036–1042.

Soleimani, M. Hajabbasi, M.A. Afyuni, M. Charkhabi, A.H. Shariatmadari, H. (2009). Bioaccumulation of Nickel and Lead by Bermuda Grass (*Cynodon dactylon*) and Tall Fescue (*Festuca arundinacea*) from Two Contaminated Soils. *Caspian Journal of Environmental Sciences*, 7(2), 59-70.

Solhi, M. Hajabbasi, M.A. Shariatmadari, H. (2005). Heavy Metals Extraction Potential of Sunflower (*Helianthus annuus*) and Canola (*Brassica napus*). *Caspian Journal of Environmental Sciences*, 3(1), 35-42.

Sylvia, D.M. and Williams, S.E. (1992) Vesicular–arbuscular mycorrhizae and environmental stresses. In: GJ. Bethlenfalvay and RG. Linderman (eds), Mycorrhizae in Sustainable Agriculture, ASA No. 54, Madison, USA., p. 101–124.

Tonin, C. Vandenkoornhuyse, P. Joner, E.J. (2001) Assessment of arbuscular mycorrhizal fungi diversity in the rhizosphere of Viola calaminaria and effect of these fungi on heavy metal uptake by clover. *Mycorrhiza*, 10, 161-168.

Wang, F.Y. Lin, X.G. Yin, R. (2007) Role of microbial inoculation and chitosan in phytoextraction of Cu, Zn, Pb and Cd by Elsholtzia splendens – a field case. *Environment Pollution*, 147, 248–255.

Wang, X.P. Shan, X.Q. Zhang, S.Z. Wen, B. (2004) A model for evaluation of the phytoavailability of trace elements to vegetables under the field conditions. *Chemosphere,* 55, 811–822.

Yell, Y.Y. Young, J.J. Yong, S.W. Soo, S.H. and Lee, Y.S. (2000) Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of to tolerance. *Plant Physiology*, 124, 1019-1026.

کارآیی میکروب‌های بومی به عنوان عوامل کمک کننده برای بهسازی خاک آلوده به سرب با گیاهان

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چکیده

هدف از این پژوهش، بررسی تأثیر تلقیح خاک با میکروب‌های بردبار به فلزات‌سنگین بر رشد گیاه و جذب فلز به وسیله گیاهان ارزن (Millet (*Pennisetum glaucum*)، بیدگیاه (Couch grass (*Triticum repens*) و یونجه وحشی (Alfalfa (*Medicago sativa*)) در خاکی با غلظت‌های فزاینده سرب بود. یک نمونه خاک پس از افزودن غلظت‌های مختلف سرب (۰ تا ۱۵۰۰ میلی‌گرم بر کیلوگرم) به مدت تقریباً هفت ماه در معرض تناوب‌های تر و خشک شدن قرار گرفت (خاک ۱). نمونه خاک دیگری با سابقه درازمدت آلودگی (خاک ۲) حاوی سویه‌های بومی مناطق آلوده، نمونه‌برداری و برای تهیه مایه تلقیح مورد استفاده قرار گرفت. بذر یونجه وحشی، بیدگیاه و ارزن با فواصل منظم در گلدان‌های حاوی خاک تهیه شده، کشت گردید. در پایان فصل رشد، گیاهان برداشت شدند. نمونه‌های گیاهی پس از برداشت و شستشو با آب مقطر آون خشک، آسیاب و برای سرب مورد آنالیز قرار گرفتند. نتایج نشان دهنده کاهشی معنی‌دار ($p < 0.05$) در عملکرد ماده خشک گیاهان با افزایش غلظت سرب بود و تلقیح میکروب‌های بردبار این کاهش را افزایش داد که این موضوع را می‌توان به افزایش دسترسی گیاهان به سرب ذاتی موجود در خاک‌های آهکی و افزایش جذب سرب توسط گیاهان در تیمارهای دارای جمعیت میکروبی نسبت داد. در پایان، تلقیح این میکروب‌ها منجر به کاهش جذب سرب توسط گیاهان مورد مطالعه شد.

*مولف مسئول