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Contribution of microbial associations to the cadmium uptake by peppermint (*Mentha piperita*)

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ABSTRACT

Due to the high cost of conventional clean-up technologies, there is a growing interest in the use of phytoremediation i.e. the use of plants to remove, degrade or immobilize contaminants. However phytoremediation is somewhat limited by the possibility of consumption by plants from occurrence of biomagnification. Cultivation of aromatic plants is therefore recommended, as essential oil is not prone to heavy metals contamination. There is also a recent surge in research focusing on the use of plant–microbial associations to increase or decrease metal accumulation by plants. The experiment was conducted in a CRD factorial design. The factors comprised of sulfur (0, 0.5 and 1gS/kg soil), thiobacilli (Sulfur Oxidizing Bacteria, SOB) and mycorrhizal inoculation (MI). Plastic pots were treated with 50 mg/kg of cadmium using cadmium nitrate (Cd $(NO_3)_2.4H_2O$. Applications of sulfur and/or SOB in combination with MI resulted in a significant additive effect on a root's Cd uptake and root bio concentration factor. Low Teranslocation Factor showed that less Cd from the peppermint root was translocated to the shoot. Approximately all Cd remained in the residues after distillation and essential oil was free from Cd in all treatments.

Key words: Phytoremediation, Cadmium, Peppermint, Thiobacillus, Mycorrhiza, essential oil.

INTRODUCTION

Heavy metals contamination has become a serious environmental problem affecting all aspects of nature [12]. The menace posed by toxic metals needs to be addressed to protect environmental and human health. Due to the high cost of conventional clean-up technologies, there is a growing interest in the use of low cost in situ remediation method named phytoremediation i.e. the use of plants to remove, degrade or immobilize contaminants [13].

There is also a recent surge in research focusing on the use of plant–microbial associations to increase or decrease metal accumulation by plants. Plant establishment and growth in trace metal contaminated soil can undoubtedly be aided using rhizosphere microbes to reduce metal toxicity and enhance plant tolerance.

However, use of phytoremediation that results in an accumulation of toxic metals in plant organs does have some limitations [21]. Therefore the cultivation of aromatic plants is recommended because essential oil is not prone to contamination from heavy metals. In this respect peppermint, M. x piperita, is one of the most important mint species and widely cultivated for its essential oil, mostly because of its main components menthol and menthone.

Interactions between plants and associated bacteria in soils contaminated with heavy metals showed that Pb and Cd contents in the soil decreased four to fivefold and two to threefold, respectively [15]. The resulting effect of AMF inoculation on Cd accumulation was dependent on the Cd level in the soil and differed between the more Cd tolerant transgenic plants and the less tolerant non-transgenic plants [7]. Chen and Lin [2] investigated the effects of sulfur

concentration on the bioleaching of heavy metals from sediment by indigenous sulfur oxidizing bacteria. After 8 days of bioleaching, 97–99% of Cu, 96–98% of Zn, 62–68% of Mn, 73–87% of Ni and 31–50% of P were solubilized from the sediment, respectively.

The aim of this study was to assess the combined effects of mycorrhizal inoculation, Bacillus spp and sulfur on uptake of cadmium by peppermint (*Mentha piperita*) from artificially contaminated soil.

MATERIALS AND METHODS

The experiment was carried out in a completely randomized design (CRD) as a factorial with three replications. The factors comprised elemental sulfur (at a dose of 0, 0.5 and 1g/kg soil), Thiobacillus spp (applied and unused) and mycorrhizal inoculation (MI: M: mycorrhizal and NM nonmycorrhizal plants). The study was conducted in a sun-lit greenhouse of the department of horticulture, University of Kurdistan, Iran during April and August, 2010. The greenhouse daily mean temperature and relative humidity were 27±2°C and 70±5%, respectively for the period of the study.

Peppermint (*Mentha piperita*) rhizomes used for the study were procured from the Campus of Agricultural and Natural Resources, Razi University, Kermanshah, Iran one week before starting the experiment. Fingers were obtained from the rhizomes and weighed 15-20 g as planting material. Topsoil was collected from fallow land and was mixed with tiny sand in at a ratio of 2:1. The combined soil mix was sterilized by steam (at 121◦c) and used as a cultivation bed. Some physicochemical characteristics of the soil samples are presented in Table 1.

Table 1 Physical and chemical characteristics of field experiment location (Values represent the average of three samples)

Clay	Silt	Sand	OM	EC dS/m	pH	CEC meq/100 g	Cd	Pb
……..%.....								
20.2	29.4	50.4	0.3	1.1	7.6	11.21	No detected	No detected

Plastic pots with 4 kg of air-dried soil were treated with 50 mg/kg of Cd using cadmium nitrate (Cd $(No_3)_2.4H_2o$). The cadmium nitrate salt were dissolved in distilled water, sprayed on the soil samples, and mixed evenly.

Thiobacillus spp. including sulfur oxidizing bacteria (SOB) using a commercial product from MehrAsia Biotechnology Company (MABCo.) at a dose of 2.5 g/kg soil. Mix of *Glomus spp* were used as arbuscular mycorrhizal fungi. Then mycorrhizal inoculum was prepared by pot cultures with a host species, maize that were inoculated with the soil containing glomalean propagules (spores, hyphae, and root fragments). The inoculum was placed as a 2-cm layer at a depth of 4.5 cm in 30-cm pots filled with the steam-sterilized soil. One-week-old seedlings of maize (15 per pot) were planted in each pot. All pots were covered with aluminum foil to prevent contamination with airborne AM inoculum. Cultures were maintained under greenhouse conditions and irrigated with deionized water as needed. After 16 weeks, irrigation was discontinued then the maize roots and soil were air-dried and applied as mycorrhizal inoculums [14].

The plant samples were collected and washed three times with deionized water. Shoot and root were separated with sterilized scissors and dried at 55-60°C for 48 hours in an electric oven. The oven dried plant samples were properly ground in a mortar by pestle until homogenous and then the samples were prepared for digestion.and analyzed with an atomic absorption spectrophotometer (Varian Spectr AA 220) to determine Cd accumulation in root and in shoot. Bioconcentration Factors (BCF) for root and shoot were calculated using the equation (1).

$$BCF = \frac{\text{concentration of Cd in root or shoot (mg/kg)}}{\text{concentration of Cd in soil (mg/kg)}} \quad (1)$$

The Translocation Factor (TF) was calculated by the equation (2)

$$TF = \frac{\text{concentration of Cd in shoot (mg/kg)}}{\text{concentration of Cd in root (mg/kg)}} \quad (2)$$

To prevent loss of nutrients and Cd from the pots, plastic trays were placed under each pot and the collected leachates were put back in their respective pots. To quantify Cd in materials before starting the experiment, three peppermint rhizome indicator samples were selected randomly

RESULTS AND DISCUSSION

Quantification of Cd in the rhizomes before implementation of the treatments was not detected by the atomic absorption spectrophotometer. It was therefore supposed that the rhizomes were free from metals before implementation of the treatments.

Application of sulfur in combination with mycorrhizal inoculation resulted in a significant increase in root Cd uptake by approximately twice compared with a single application (Table 2). The highest value was obtained at 1gS/kg soil together with mycorrhizal inoculation (537.4 mg/kg root dry weight) (Table 2). The mechanisms involved in the interactions between arbuscular mycorrhizae colonization and heavy metals accumulation include a dilution effect of the toxic elements that is linked to increased growth due to P nutrition, sequestration of toxic metal in the fungal structure and the development of tolerance by the arbuscular mycorrhizae fungi [20].

Table 2: (A): Values of cadmium concentration in root and root bio concentration factor (RBCF) of cadmium of mycorrhizal (M) and non mycorrhizal (NM) peppermint plants within level of S supply. Different letters in each column indicate significant difference statistically.

level of Sulfur × Mycorrhizal Inoculation **(MI)**		cadmium concentration in root (mg/kg dry weight)	RBCF of cadmium
level of S supply (g /kg soil)	Mycorrhizal Inoculation (MI)		
0	NM	205.8d	4.11d
0	M	410.1b	8.2b
0.5	NM	227.9d	4.55d
0.5	M	439b	8.78b
1	NM	269.2c	5.38c
1	M	537.4a	10.75a

Like root, applied S at 1g/kg soil resulted in significant ($p<0.05$) Cd accumulation in shoots (Table 3). In addition, MI raised Cd accumulation in shoots significantly (Table 3). The higher heavy metal concentration in Am plants could be explained by the fact that Am infection increased plant uptake of metals by mechanisms such as enlargement of the absorbing area, volume of accessible soil, and efficient hyphal translocation [16]. However there are some different reports in respect to AMF effects on shoots. For example shoot Cd concentrations decreased in cucumber [10] and soybean [1]. Metal excluder plants prevent metal from entering their aerial parts or maintain low and constant metal concentrations over a broad range of metal concentration in the soil. The plant may alter its membrane permeability, change metal binding capacity of cell walls, or exclude more chelating substances [3].

Furthermore, the effect of SOB increase on Cd accumulation in shoots was significant (Table 3). It demonstrated that bacteria may transform toxic heavy metals to forms that are more readily taken up into the roots. For example, bacteria could enhance Se accumulation in plants by reducing selenate to organic Se, and organoselenium forms like SeMet that are known to be taken up at faster rates into roots than inorganic forms [8].

The highest values of Cd bioconcentration for root and shoot were 10.75 and 0.78 respectively. The levels were not very high compared to other reported values in different plants such as Thlaspi caerulescens [9], however the lowest values of BCF less than 1 have also been reported. For instance in BCF 28 species of edible mushrooms were less than [4] and BCF of Cd in Osmanthus fragrans plant ranged from 0.77 to 0.27 [21]. Mane et al, [11] showed that BCF with respect to shoots was (1.24) in Triticum aestivum followed by lesser transport of Cd^{++} from the soil medium to shoots as compared to the roots that had BCF of 2.98.

Table 3: Separate effect of factors on measured traits

Treatments		Cadmium concentration (mg/Kg dry weight)			RBCF	SBCF	TF
		Root	Shoot	Residue			
Sulfur (g /kg soil)	0	307.9 c	28.43 b	27.98 b	6.16 c	0.56 b	0.093 a
	0.5	333.5 b	29.23 b	28.65 b	6.67 b	0.58 b	0.088 a
	1	403.3 a	33.09 a	32.53 a	8.06 a	0.66 a	0.081 a
SOB	unused	322.46 b	26.53 b	26.10 b	6.44 b	0.53 b	0.083 a
	applied	374.01 a	33.96 a	33.33 a	7.48 a	0.67 a	0.092 b
MI	NM	234.31 b	21.56 b	21.08 b	4.68 b	0.43 b	0.092 a
	M	462.16 a	38.93 a	38.35 a	9.24 a	0.78 a	0.083 b

*SOB: Sulfur Oxidizing Bacteria ((applied and unused), MI**: mycorrhizal inoculation** (M: mycorrhizal and NM: nonmycorrhizal), RBCF: Root Bioconcentration Factor, SBCF: Shoot Bioconcentration Factor. The sane letters show no significant difference (P< 0.05) in each column and between levels of treatments.*

Applied S at 1g/kg soil in combination with mycorrhizal inoculation caused the highest RBCF (10.75) (Table3). Although a single application of sulfur influenced the RBCF significantly (Table3), applying sulfur along with mycorrhizal inoculation had an additive effect on RBCF (Table3). For example, simultaneous applications of 0.5 1gS/kg soil and mycorrhizal inoculation increased the RBCF from 4.55 to 8.75 significantly (Table3). A significant increase in RBCF of Cd was observed following an application of sulfur oxidizing bacteria (Table 3). It is well known that adsorption of thiobacilli to sulfur particles is necessary and plays an important role in the microbial oxidation rate of sulfur. Therefore, the rate of oxidation of elemental sulfur also depends on the total surface availability of sulfur particles. An increase in sulfur concentration means an increase in the availability of total surface area of sulfur particles, which enhances the acidification rate [2].

Jankong and Visoottiviseth, [6], found that AMF infection decreased BCF of As in marigold, while there was no effect on BCF of As in melastoma.

The translocation factors of Cd with a mean value of 0.09 (Table 3) showed that on average less than 0.01 of accumulated Cd in peppermint root was translocated to the shoot, or 10 times more Cd accumulated in the roots than in the shoots. Mane et al. [11] observed that roots accumulated higher Cd content than shoot in Triticum aestivum with the highest amount of 19.82 mg/kg of Cd at 75 ppm of Cd chloride while at the same level of salt, shoots were with 13.47 mg/kg. A higher TF of Cd (1.19- 2.39) was reported with an increase of Cd in Osmanthus fragrans as phytoextraction plant by [21].

One of the strategies for phytoremediation of metal-contaminated soil is phytostabilization where plants are used to minimize metal mobility in contaminated soil. Phytostabilization is useful at sites with shallow contamination and where contamination is relatively low. Phytostabilization can immobilize heavy metals through absorption and accumulation by the roots, adsorption onto roots, or precipitation within the rhizosphere. This process reduces metal mobility and leaching into the ground water, and also reduces metal bioavailability for entry into the food chain [19]. Plants that accumulate heavy metals in the roots and in the root zone are typically effective at depths of up to 24 inches (60 cm approximately) [17]. There are two different aspects of peppermint utilization; as an edible vegetable and another is chosen for its phytostabilization property on Cd. Due to the low translocation factor of Cd, edible consumption of peppermint grown in Cd contaminated soil will have lower apparent risk.

AMF has a dissimilar effect on the translocation factor of heavy metals by different plant species. Jankong and Visoottiviseth, [6] studied the effects of AMF on arsenic accumulation in silverback fern (Pityrogramma calomelanos), marigold (Tagetes erecta) and melastoma (Melastoma malabathricum). The results showed that the translocation factor value of As in fern and marigold were not significantly different between non-infected and AMF infected plants. In contrast, the translocation factor of As in melastoma was reduced by AMF infection. AMF reduced the translocation factor of Zn in Solanum nigrum [12]. They proposed that this decrease indicated that the tested AMF acted as a barrier to zinc in S. nigrum growing in a naturally contaminated matrix by immobilizing heavy metals in the roots thus restricting their translocation to stems [12]. In contrast, Lee and George [3] showed that total Cd root content and the R/S ratio for Cd was significantly increased by mycorrhizal fungal colonization at a high metal supply level. It has been suggested that AM hyphae that grow in metal contaminated soil may absorb these metals, but will not contribute to increased metal concentrations in the shoots of these plants. This supports the notion that AM fungal hyphae can differentiate between the elements in an uptake process and also have some capacity to accumulate elements themselves [3].

Approximately all Cd remained in residues after distillation and there was no significant difference between residues and shoots in respect to concentrations of Cd in all treatments (Table3). A follow up qualitative investigation showed that Cd in the essential oil of all treatments was below the detection limit of the used atomic absorption. This finding was in accordance with the results of [22], [18] and [24]. These studies have asserted the notion that essential oil was not contaminated with heavy metals therefore growing these aromatic crops in metal contaminated areas may not introduce heavy metals into the food chain and may not result in an economic penalty compared to most other edible crops. In the process of oil extraction by distillation, heavy metals remain in the extracted plant residues, limiting quantities of heavy metals in a commercial oil product.

High-value aromatic crops may well be a better alternative for heavy metal contaminated agricultural soil than woody species such as Salix and Betula or other plants like Sesbania drumondii that have been shown to hyper accumulate Pb [23]. Zheljazkov and Nielsen [24] observed that lavender could be successfully grown in highly heavy metal polluted areas without any risk of essential oil contamination. Similar results were obtained for peppermint and corn mint, where no heavy metal contamination was found in the essential oils. Despite yield reduction (up to 14%) caused by heavy metal contamination, mint still remained a very profitable crop and it could be used as a substitute for other crops [24].

The replacement of some crops in these areas with aromatic crops, grown for essential oils, might eliminate heavy metal contamination of the animal and human food chain [24]. Alternatively, Holah et al., [7] reported levels of Pb and Ni in basil essential oil and peppermint plants, which had grown on contaminated soil. While lead and nickel concentrations in the study were below the critical level of concentration in plant tissue, which was <<1 mg / L.

CONCLUSION

Results showed that Cd translocation factors were low and less than one in all treatments. In this study, the peppermint plant wasn't a hyper accumulator. However, the ability of these plants to tolerate and accumulate heavy metals may be useful for phytostabilization. The use of phytostabilization to keep metals in their current location is particularly attractive when other methods to remediate large-scale areas having low contamination are not feasible. It can be concluded that there is low risk from the toxic effects of Cd from the consumption of peppermint.

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