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Effect of Arbuscular Mycorrhiza on Growth and Metal Uptake of Basil and Mint Plants in Wastewater Irrigated Soil

A. Elgharably[*] and Nivien Allam[**]
[*]*Department of Soils and Water, Faculty of Agriculture and* [**]*Department of Botany and Microbiology, Faculty of Sciences, Assiut University, Assiut, Egypt.*

EFFECT of arbuscular mycorrhizal (AM) fungus on growth and uptake of heavy metals by basil (*Ocimum basilicum*) and mint (*Mentha piperita*) was assessed in a pot experiment. Pots were filled with sterile and non-sterile soil, wastewater treated over 40 years. Soil was seeded with basil or mint plants (inoculated or not with *Glomus geosporum*). After 90 days, shoot dry matter (DM) and content of N, P, Pb, Cd, Fe, Mn, Zn and Cu were determined. AM increased shoot DM of both plants. Leaf N content was not affected, but P content in basil only increased with AM inoculation. Basil accumulated 109, 0.15, 4706, 199, 156 and 33 mg, whereas mint leaves accumulated 11, 0.25, 7001, 223, 117 and 28 mg kg^{-1} of Pb, Cd, Fe, Mn, Zn and Cu, respectively. With AM concentrations of Pb, Cd, Fe, Mn, Zn and Cu in basil leaves decreased by 44, 87, 22, 18, 25 and 39%, but in mint the concentration increased by 35, 43, 13, 10, 8 and 22%, respectively. Data indicate that mint has a higher capacity than basil for metals accumulation and that AM fungi has a significant effect on metal uptake, but is dependent on the host plant.

Keywords: Bioremediation, Heavy metals, Medicinal plants, Mycorrhizal fungi, Soil contamination.

Heavy metals at high concentrations have strong toxic effects for humans and animals and are regarded as environmental pollutants (Tandy *et al*., 2006).

The use of green plants such as medicinal plants can carry residuals of pesticides or assimilate heavy metals from polluted waters and soils (Gurbisu and Alkorta, 2003). They can therefore offer an effective, environmentally non-destructive and cheap remediation method. Arab and Donia (2000) evaluated heavy metals uptake by 20 different types of spices and medicinal plants. They recorded the highest concentrations of Si and Mn in tea, of Ni and Zn in basil and of Pb in marjoram. They also showed that celery, parsley and spearmint contained the highest levels of Cd, Cu and Fe. Lowest concentrations of Pb, Cd, Ni, Sn and Mn were found in Jew's Mallow and of Fe, Zn and Cu in tea.

E-mail of the corresponding author: age@au.edu.eg

Bioremediation has gained a lot of importance recently as an alternate technology for removal of elemental pollutants in soil and water, which requires effective methods of decontamination. The potential exists for mycorrhizae to affect plant uptake of heavy metals. Arbuscular mycorrhizal fungi (AMF) efficiently contributed to the amelioration of various stresses experienced by hosting plants, including metal toxicity, oxidative stress, water stress, and effects of soil acidification (Finlay, 2008). The symbiosis of AMF with plant roots of around 80% of plant species clearly enhance the uptake of diffusion limited nutrients from the soil and AMF symbiosis increase plant growth and nutrients uptake in particular P, Zn, Mn, Cu and Fe (Liu *et al*., 2000 and Yassen *et al*., 2011). Arbuscular mycorrhizal fungi may enhance phytoremediation by reducing heavy metal stresses on plants, increasing heavy metal uptake, and affecting translocation of metals within plants. It was determined that the presence of both *Glomus claroideum* and *Glomus intraradices* enhanced the uptake and accumulation of Zn by *Solanum nigrum* (up to 83 and 49% higher Zn accumulation, respectively). AM fungi affect metal uptake by plants from soil and translocation from roots to shoots, however, mycorrhizal effects may depend on elements, plant and fungal species/ecotypes (Li and Feng, 2001). Tolerant mycorrhizal fungi may grow and solubilized toxic metal mineral better than non-tolerant strains. Metal dissolution by fungi may take place through proton-promoted or ligand-promoted mechanisms and organic acids provide both a source of protons for solubilization and metal-chelating anions to complex the metal cations (Finlay, 2008).

The capacity of medicinal plants for metals uptake seems variable. The ameliorating influence of mycorrhizae on the uptake of soil metals may be positively correlated with the degree of compatibility between the fungal strain and the plant. Therefore, work is needed to understand the relationship between mycorrhizae and heavy metals uptake by the medicinal plants. The objective of this study was to determine the effect of mycorrhizal fungi on the growth and heavy metal uptake of basil and mint in soils irrigated with wastewaters containing a variety of heavy metal.

Material and Methods

Soil

Several sub-samples (0-15 cm depth), collected from ElMadabegh area, Assiut (latitude 27°10′S, longitude 31°08′E), were bulked to give a composite sample. The soil was air-dried and sieved to ~2 mm. Soil pH and electrical conductivity (EC) were measured in 1:5 soil-water suspension after 1 hour end-over-end shaking at 25 °C. Soil Fe, Mn, Zn, Cu, Cd and Pb were extracted by diethylenetriaminepenta-acetic acid following (Lindsay and Norvell, 1978). Soil was digested with nitric and perchloric acid mixture (Baker and Amacher, 1982) for measurement of the total concentrations of Fe, Mn, Zn, Cu, Cd and Pb in soil using the atomic absorption spectrophotometer. Soil characteristics are presented in Table 1.

Mycorrhizal inoculum

The native population of arbuscular mycorrhizal fungus (AMF), *Glomus geosporum* originated from the soil of ElMadabegh area, Assiut. The single-spore-derived cultures were maintained in a pot culture using *Zea mays* L. seedlings as host plants. Fifty gram per pot of AMF inoculums were placed 3 cm below the layer of basil or mint seeds. The inoculum comprised colonized root fragment, hyphae and approximately equal to 1000 spores pot^{-1}. The inoculaum was added to pots with 3 kg soil. The control treatments received the same volume of autoclaved inoculums.

Plants

Growth of basil and mint was tested in sterilized and non-sterilized soil un-inoculated or inoculated with AMF. Five seedlings of basil (*Ocimum basilicum*) or mint (*Mentha x piperita*) were placed 1.5 cm deep in soil and thinned to 2 plants 7 days after germination. Throughout the experiment, soil moisture was maintained at 90% of the field capacity. The pots were set up in a completely randomized design with three replicates. On day 90, plant shoots were oven-dried at 70 °C to determine the dry weight. Dried shoots were ground and then digested with H_2SO_4 following the Kjeldahl method for measuring shoot N concentration. Other shoot samples were acid-digested (2:1 HNO_3:$HClO_4$ acid mixture) for measurement of P, Fe, Mn, Zn, Cu, Pb and Cd by atomic absorption spectrophotometer (AAS).

Estimation of mycorrhizal dependency and mycorrhizal colonization

Dependency of mycorrhizal shoot growth was calculated following Plenchette *et al.* (1983). Root segments of a length of 0.5-1.5 cm were boiled in 10% KOH for 5-10 minutes, then washed (4-5 X) and then immersed in 2% HCL for 15-20 min till cleared. The root segments were stained with 0.5% (w/v) Trypan blue (Brundrett, 2009). Frequency of mycorrhizas (F%), intensity of mycorrhizal colonization in root (M%) and Arbuscule frequency in roots (A%) were calculated following Trouvelot *et al*. (1986).

Statistical analysis

Data were subjected to analysis of variance using a computer software (MStat). In Tables, values are means (n=4) ± SE. In figure, error bars represent the standard error (SE).

Results

Soil physical and chemical characteristics

The physical and chemical characteristics as well as the concentrations of available and total heavy metals of the studied soils are present in Table 1. Soil of ElMadabegh area is sandy clay containing total concentrations of 123, 10, 10053, 5255, 1557 and 607 μg g^{-1} soils for total of Pb, Cd, Fe, Mn, Zn and Cu, respectively.

TABLE 1. Physical and chemical characteristics of the collected soil of Elmadabegh.

Parameter	
Clay	36
Silt	10
Sand	54
Texture	Sandy clay
$EC_{1:5}$ (dS m^{-1})	0.23 ± 0.01
$pH_{1:5}$	7.61 ± 0.09
Total N (µg g^{-1})	900 ± 27
Available P (µg g^{-1})	31.1 ± 3.1
DTPA-extractable (µg g^{-1})	
Fe	47.3 ± 2.3
Mn	148.2 ± 4.1
Zn	35.6 ± 1.7
Cu	10.1 ± 1.1
Pb	3.1 ± 0.3
Cd	3.6 ± 0.5
Total concentration (µg g^{-1})	
Fe	10053.7 ± 900.1
Mn	5255.4 ± 201.3
Zn	1557.5 ± 137.2
Cu	607.1 ± 29
Pb	123.1 ± 2.6
Cd	10.3 ± 1.5

Values are means (n=4) ± SE.

Shoot dry matter and mychorrizal dependency

Soil sterilization had no effect on the plant dry matter (DM). Inoculation with mycorrhizae fungi led to a significant increase in the DM with a higher increase in shoot dry weight of basil, compared to mint plant (Fig. 1).

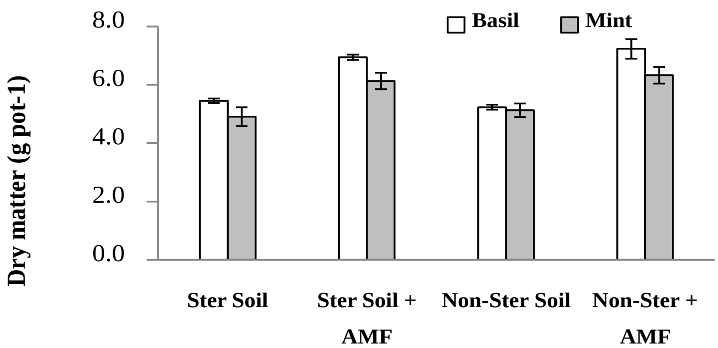

Fig. 1. Dry matter (g pot^{-1}) of basil and mint in the sterilized and non-sterilized soil with and without mycorrhizae fungi (AMF). Values are means (n=4). Error bars represent the SE.

The results given in Fig. 2 indicate that for the dry weight of basil plants the lowest value of mycorrhizal dependency (MD) was obtained from the plants grown in the sterilized soil, while the maximum value was obtained from the plants grown in non-sterilized soil. MD for dry weight of inoculated mint plants was lower than basil plants.

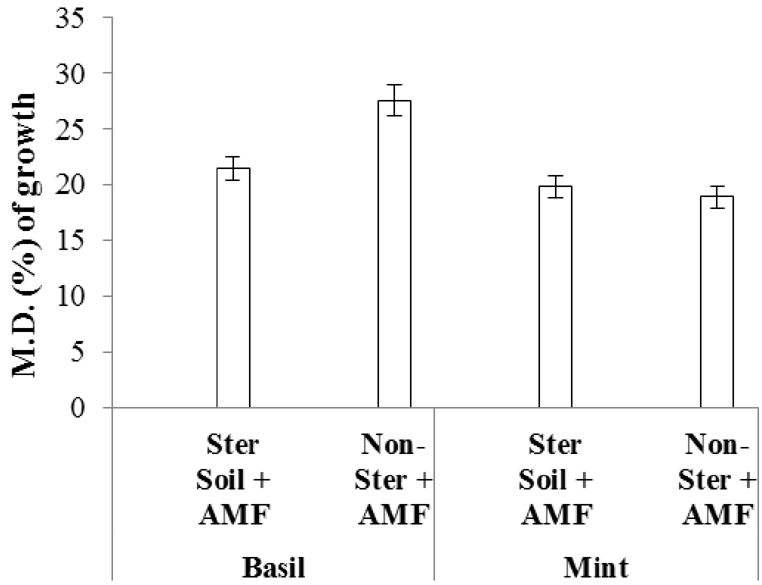

Fig. 2. Mycorrhizal dependency (M.D. %) of basil and mint growth in the sterilized and non-sterilized soil with and without mycorrhizae fungi (AMF). Values are means (n=4). Error bars represent the SE.

Shoot nutrient composition

The effect of inoculation with AMF on basil and mint shoot N, P, Zn, Cu, Fe, Mn, Cd and Pb contents were shown in Table 2. Inoculation with *G. geosporum* had no effect on the N content in basil and mint leaves, but significantly increased P content in the mint leaves only. With AMF inoculation in the sterilized soil basil leaf contents of Zn, Cu, Fe, Mn, Cd and Pb decreased by approximately 21, 37, 38, 17, 60 and 31%, whereas in the non-sterilized soil the percentage reduction reached approximately 25, 39, 22, 18, 87 and 44%, respectively. With mycorrhizae inoculation in the sterilized soil mint leave contents of Zn, Cu, Fe, Mn, Cd and Pb significantly increased by 2, 52, 25, 13, 57 and 33%, whereas in the non-sterilized soil the percentage increase reached approximately 8, 22, 13, 9, 43 and 35%, respectively.

TABLE 2. Concentrations of N and P (%) and Zn, Cu, Fe, Mn, Cd and Pb (µg g^{-1}) in the shoot of basil and mint as affected by mycorrhizal fungi (AMF) inoculation in the sterilized and non-sterilized soil of Elmadabegh.

Plant	Treatment	N (%)	P (%)	Zn	Cu	Fe	Mn	Cd	Pb
				µg g^{-1} dry matter					
Basil	Sterilized soil	2.70 ± 0.04	0.13 ± 0.02	129 ± 14.1	35 ± 7.7	4177 ± 339	127 ± 6.2	0.15 ± 0.03	109 ± 10.4
	Sterilized soil + AMF	2.68 ± 0.05	0.37 ± 0.03	101 ± 11.2	22 ± 4.8	2552 ± 210	105 ± 9.5	0.06 ± 0.04	75 ± 11.1
	Non-sterilized soil	2.61 ± 0.07	0.11 ± 0.01	156 ± 9.2	33 ± 5.1	4706 ± 117	199 ± 14.9	0.62 ± 0.06	45 ± 7.2
	Non-sterilized soil + AMF	2.57 ± 0.08	0.34 ± 0.01	116 ± 11.0	20 ± 6.3	3636 ± 235	161 ± 11.3	0.08 ± 0.08	25 ± 3.0
Mint	Sterilized soil	2.54 ± 0.10	0.19 ± 0.02	109 ± 2.0	18 ± 8.4	4638 ± 375	176 ± 16.5	0.09 ± 0.02	10 ± 0.4
	Sterilized soil + AMF	2.63 ± 0.07	0.18 ± 0.02	112 ± 5.1	38 ± 5.4	6250 ± 453	204 ± 11.7	0.21 ± 0.07	15 ± 2.3
	Non-sterilized soil	2.50 ± 0.10	0.16 ± 0.01	117 ± 3.1	28 ± 4.1	6066 ± 321	223 ± 11.2	0.25 ± 0.07	11 ± 0.7
	Non-sterilized soil + AMF	2.54 ± 0.02	0.16 ± 0.02	128 ± 4.2	36 ± 5.1	7001 ± 291	246 ± 9.4	0.44 ± 0.07	17 ± 0.5

Values are means (n=4) ± SE.

Data show that the metal contents in the plant tissues differed between basil and mint. Regardless of soil sterilization, uptake of N, Zn, Cu, Cd and Pb by basil leaves was higher, whereas higher amounts of P, Fe and Mn were accumulated in mint leaves.

Mycorrhizal colonization

In general, different concentration of heavy metals in contaminated soil stimulated development the frequencies of root colonization (F%), intensity of root cortex colonization (M%), and arbuscule development (A%) by *G. geosporum* at different growth stages in basil and mint plants as shown in Table 3. Microscopic assessment confirmed that plants of non-inoculation treatment were not colonized by AM. As is evident from Table 3, at vegetative stage, the highest value of F% and M% recorded in non-sterilized soil.

At flowering stage, mycorrhizal colonization increased as compared with those of vegetative stage, and F%, M% and A% recorded a maximum value in non-sterilized soil. It was observed that the highest mycorrhizal colonization was recorded at maturity stage (as compared with those of vegetative and flowering stage). The mycorrhizae of *G. geosporum* in roots of basil and mint plants consisted of arbuscules, vesicles, as well as intra- and extraradical hyphae. The arbuscules and vesicles were patchily distributed along the roots examined. The intraradical hyphae were evenly distributed and frequently formed coils.

TABLE 3. Influence of different treatments on the mycorrhizal colonization of basil and mint plants in sterilized (ster) and non-sterilized (non-ster) soils.

Growth stage	Mycorrhizal colonization (%)	Basil				Mint			
		Ster soil		Non-ster soil		Ster soil		Non-ster soil	
		NM	M	NM	M	NM	M	NM	M
Vegetative	F %	0	33.3	0	42.0	0	48.3	0	55.3
	M %	0	23.5	0	31.2	0	29.5	0	37.4
	A %	0	21.5	0	30.0	0	34.3	0	38.3
Flowering	F %	0	65.6	0	68.3	0	70.3	0	73.4
	M %	0	44.2	0	43.6	0	46.3	0	48.3
	A %	0	42.3	0	41.7	0	45.0	0	50.2
Maturity	F %	0	81.3	0	87.0	0	89.5	0	90.3
	M %	0	55.2	0	58.9	0	61.3	0	53.4
	A %	0	51.4	0	53.8	0	59.4	0	59.3

F % Frequency of mycorrhizal root segments, M% intensity of mycorrhizal colonization in root, A% Arbuscule frequency in roots, M mycorrhiza plants, NM non-mycorrhizal plants.

Discussion

The total concentrations of heavy metals in soil show the magnitude of contamination and thus the potential for plant metal uptake. According to Kabata-Pendias (2001), <115 μg Pb, <1.8 μg Cd, 120-21,000 μg Fe, 300-500 μg Mn, 70-400 μg Zn and 60-125 μg Cu g^{-1} soil, based on total fractions in soil, would be considered toxic to plants. Our results show that the soil under investigation is contaminated with the whole set of elements.

Our observations confirm that basil and mint plants have been growing healthy, exhibiting high tolerance and colonization of a wide range of soil metal concentrations.

The dry weight and heavy metal content of basil and mint leaves were affected by inoculation with mycorrhizal fungi (Fig. 1 and 2). The plants treated with AM fungi had higher shoot dry weight. Root colonization with mycorrhizae fungi can increase plant uptake of elements, thus increase plant growth (Chen *et al*., 2007). Van Kessel *et al.* (1975) reported that AM fungi can assimilate and translocate ammonium. In this study AM fungi had no effect on leave N content, which is not in agreement with the results of Barea and Azcon-Aguilar (1987), who found that AM fungi enhanced foliar N concentration. Our data showed that AM inoculation resulted in a significant increase in the foliar P content, especially in the leaves of basil. Basil leaves seemed P-deficient (0.13%), but with AM inoculation the P concentration increased by approximately 300%. The role of AM fungi in improving P uptake and plant growth is widely recognized (Mosse and Hayman, 1980). AM fungi increase the root surface area and thus enhance P uptake (Smith, 1980).

Leaf content of most metals was generally correlated with inoculation of AM fungi. There are two antithetical hypotheses that have been proposed for the role of AM symbiosis in the heavy metal phytoremediation (Audet and Charest, 2007). They are: (1) an increased heavy metal phytoextraction via the mycorrhizospheric action (enhanced uptake), and/or (2) reduced metal bioavailability via fungal metal-binding processes, thereby an increased metal phytostabilization.

In this study, a decrease in basil foliar metal concentration associated with AM fungi from highly-contaminated soil was noted for Pb, Cd, Fe, Mn, Zn and Cu (Table 2) which indicate that AM fungi (*Glomus geosporum*) decreased the shoot uptake of these metals. However, shoot dry matter of basil contained high metal concentrations. This suggests that the increased P concentration in the basil leaves enhanced the plant growth.

Dueck *et al.* (1986) have suggested that the mechanism of amelioration of Zn toxicity may be due to Zn adsorption on the surface of hyphae or metal adsorption to the electronegative sites in the hyphal cell wall and extrahyphal polysaccharide slime. This would effectively lower the concentration of Zn in the soil solution surrounding the roots. Bradley *et al.* (1982) further proposed that the endophyte may provide adsorptive surfaces within the cortical cells of the host roots, thus excluding metal from shoots and avoidance of metal toxicity. Mathys (1977) suggested that Zn may be chelated by malate in tolerant plants. In this study, a similar mechanism might have occurred with this and other elements, reducing metal uptake in the basil plants. AM inoculation increased metals concentrations in the shoot of mint plants (Table 2). With the healthy plant growth AM fungi may help the roots to absorb more metals from the soil and enhance metal translocation and accumulation in the shoots. Thus, the possibility of AMF use to increase the metal accumulation in the shoots is very interesting for the phytoextraction purpose, since most high producing biomass plants retain most heavy metals in the roots (Citterio *et al.*, 2005).

Plants grown in metal-enriched substrata take up metal ions in varying degrees. This uptake is largely influenced by the bioavailability of the metals which is in turn determined by both external (soil-associated) and internal (plant-associated) factors.

Outridge and Noller (1991) reported that the concentrations of heavy metals in the root tissues of freshwater macrophytes from polluted areas were usually found to contain higher concentrations of most metals compared to the aboveground parts. Fitzgerald *et al.* (2003) observed that monocotyledonous species contained higher concentrations of Pb in the roots compared to shoots. The results of this study showed that there were significant differences in the uptake of the heavy metals by the tested plants. In comparison with the ranges of metal concentrations in the mint leaves, higher concentrations of P, Zn, Cu, Cd and Pb and lower concentrations of Fe and Mn were accumulated in the basil leaves, but generally the concentration of all elements remained high in both plants (Table 2).

This relationship could be interpreted in terms of the chemical specification of the metals in the soil and competition between metal ions and protons at the plant–soil–water interface. Compared to mint, P had significant negative relationships with Fe and Mn and positive relationships with Pb, Cd, Zn and Cu contents in basil and mint leaves.

High P levels in soil may decrease Zn, Fe and Cu availability and uptake by plants due to chemical reactions in the rhizosphere where P has a strong tendency to absorb metals (Kabata-Pendias, 2001). However, in this study, compared to mint, shoot biomass of basil was greater, which might was attached with a greater root biomass (data not available) that were able to colonize and take up more Pb, Cd, Zn and Cu and translocate it to the aboveground parts.

The results presented here suggest that the metal-tolerating strategy is widely evolved and exists in plant species when they grow in metal-contaminated areas.

Conclusion

Results of this study indicated that mycorrizal fungi *(Glomus geosporum)* are highly effective in decreasing and increasing plant uptake of heavy metals. The plant species examined in this study grew very well and propagated quickly without fertilization which would be a great advantage in the remediation of the soils of ElMadabegh area without fertilization. According to the different capacities of metal uptake, plants able to accumulate relatively high metal concentrations in the aboveground tissues could be good candidates for phytoextraction. According to the present results as shown in Table 2, mint plants would be good choices for extracting Fe and Mn and basil plants for extracting Cd, Pb, Zn and Cu from the heavy metal contaminated soils.

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أثر الميكوريزا على نمو نباتات الريحان والنعناع وأمتصاص العناصر فى الأراضى المروية بمياه الصرف الصحى

أحمد الغرابلى* **و نيفين علام****
*قسم الأراضى والمياه ـ كلية الزراعة و** قسم الميكروبيولوجى والنبات ـ كلية العلوم ـ جامعة أسيوط ـ أسيوط ـ مصر.

أقيمت تجربة لمدة 90 يوم داخل الصوبة لتقييم أثر فطر الميكوريزا على نمو نباتات الريحان والنعناع وأمتصاصهما للعناصر الثقيلة فى أراضى رويت بمياه الصرف الصحى على مدار 40 عام. وقد ملئت الأصص ب 3 كيلوجرام من تربة (معقمة وغير معقمة) جمعت من منطقة المدابغ فى أسيوط وهى تربة زرعت بمحاصيل مختلفة رويت بمياه الصرف الصحى على مدار 40 عام و تم زراعة 5 شتلات من نباتات الريحان أو النعناع فى الأصص وريها بمياه مقطره. وعند الحصاد تم تقدير الوزن الجاف للسيقان والأوراق ومحتواهما الكلى من عناصر النيتروجين والفوسفور والرصاص والكادميوم والحديد والمنجنيز والزنك والنحاس.

وقد أدى التلقيح بالميكوريزا الى زيادة الوزن الجاف لكل من الريحان والنعناع وقد تبين أن التركيز المتراكم فى الأوراق والسقيان من الرصاص والكادميوم والحديد والمنجنيز والزنك والنحاس فى الريحان هو 109 و 0,15 و 4706 و 199 و156 و 33 مليجرام /كيلوجرام نبات وفى النعناع 11 و 0,25 و 7001 و 223 و117 و 28 مليجرام /كيلوجرام نبات على التوالى. وقد أدى التلقيح بالميكوريزا الى زيادة تركيز الرصاص والكادميوم والحديد والمنجنيز والزنك والنحاس فى أوراق وسيقان الريحان بمعدل 44 و 87 و 22 و 18 و 25 و 39٪ والى نقصها فى أوراق وسيقان النعناع بمعدل 35 و 43 و 13 و 10 و 8 و22٪ على التوالى.

وتدل النتائج أنه مقارنة بنباتات الريحان فان نباتات النعناع لها قدرة عالية على تخزين العناصر فى أوراقها وسيقانها كما أن التلقيح بالميكوريزا له تأثير أيجابى على قدرة النباتات العطرية على امتصاص العناصر من التربة الملوثة بالعناصر الثقيلة ولكن ذلك يتوقف على نوع النبات.