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Influence of Mycorrhizal Fungi on the Uptake of Cobalt and Nickel by Corn Plants

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THERE is an increasing interest worldwide for the maintenance of soil quality and remediation strategies for management of soil contaminated with pollutants including heavy metals. This work was carried out in pots with three compartments, a central one for root and hyphal growth and two outer ones which were accessible only for hyphae of the arbuscular mycorrhizal fungus. Mycorrhizal and nonmycorrhizal corn plants were grown on sandy soil mixed with either Co or Ni as well as Co with Ni (5, 10 and 50 mg.kg^{-1} soil).

Cobalt with mycorrhizal inoculation reduced plant growth parameters significantly compared to Ni with mycorrhizal inoculation. Such reductions for treatments of 5 ppm Co with mycorrhizal inoculation recorded 21.5, 24.4 and 10.9 % less than those of the same level from Ni with inoculated plants for plant height, shoot and root dry weights, respectively. The colonization rate was much higher in the rhizobox treated with Ni than other treatments. Results also showed that, mycorrhizal colonization significantly increased the contents of both Co and Ni in both shoots and roots of corn plants receiving either of these two elements up to 10 ppm; opposite trend were encountered using 50 ppm from the two elements. The mycorrhizal plants accumulated significantly more macro and microelements than the nonmycorrhizal plants whose nickel was less effective.

Keywords: Mycorrhiza,Cobalt, Nickel, Corn plant.

Contamination of soils with heavy metals such as Co and Ni can occur naturally or as a result of a wide range of human industrial and agricultural activities. In Egypt, total cobalt content in highly polluted soil ranged from 36 to 64 mg.Kg^{-1} due to prolonged irrigation with industrial waste water (Zohny, 2002 and Abdel-Sabour, 2003). Soils throughout the world contain Ni within the broad range of 0.2 to 450ppm (Kabata-Pendias & Pendias, 1992).

Phytoremediation utilizes biological organisms for phytoextraction through removal of plant biomass containing concentrated levels of heavy metals taken up from polluted soils. Phytoremediation is an alternative to conventional physical and chemical methods of treating contaminated soils (Salt *et al.*, 1995). Soil microorganisms are known to play a key role in the mobilization and immobilization of metal cations, thereby changing their availability to plants. Vesicular-arbuscular mycorrhiza (VAM) are soil microorganisms that establish mutual symbioses with the majority of higher plants, providing a direct physical link between soil and plant roots. Bethlenfalvay (1992) reported clear benefits for mycorrhizal symbiosis; such benefits seemed to be related to nutrient uptake and storage. Field investigations have indicated that mycorrhizal fungi can colonize plant roots extensively even in metal contaminated sites (Diaz & Honrubia,1994 and Pawlowska *et al.*, 1996). Leyval *et al.* (1997) reported that high concentrations of heavy metals in soil have an adverse effect on micro-organisms and microbial activities. Among soil microorganisms, mycorrhizal fungi are the only ones providing a direct link between soil and roots, and can therefore be of great importance in heavy metal availability and toxicity to plants.

Mycorrhiza enhances the uptake of mineral elements, especially phosphorus, through infecting plant roots. Several authors found that shoot concentrations of Zn, Cu, Pb and Cd decreased with arbuscular mycorrhizal colonization at high levels of available metals; however, at lower levels, metal uptake increased compared with non-mycorrhizal plants (Weissenhorn *et al.*,1995). This agrees with results of Heggo *et al.* (1990) who found that (VAM) increased Cd uptake in soybean when the soil concentration of Cd was low, but reduced Cd uptake when the soil Cd concentration was high. Guo *et al.* (1996) showed that (VAM) increased Cd uptake by 37% in beans and 41% in maize. Later on, Burke *et al.* (2000) found that lead uptake was increased after the application of a fungicide which reduced mycorrhizal associations, other soil microflora being also being affected (Torstensson & Wessen, 1984 and Paul *et al.*, 1989).

The aims of this work were the evaluation of (1) the influence of mycorrhiza on the phytoremediation and translocation of both Co and Ni within corn plants, and (2) the ability of mycorrhiza to modify Co and Ni effects on tissue mineral concentration.

Material and Methods

Pots were used as to permit spatial separation of roots and hyphae growing zones in the soil (Rhizobox). Each rhizobox had three compartments, a central one for root and hyphal growth and two outer ones for hyphal growth alone (Fig.1). For compartmentation, 20 µm nylon net was used, which allows hyphae but not the roots of plants to pass through. The inner compartments of rhizoboxes were packed with fine sand which was washed with acid, followed by distilled water. The outer ones were packed with sand mixed well with cobalt, nickel or cobalt with nickel at rates of 5, 10 and 50 $mg.kg^{-1}$, respectively. The cobalt and nickel stock solutions were prepared by dissolving sulfate salts in distilled water. The rhizoboxes were vibrated during packing and the sand was packed to a height of 13 cm. The amount of sand was 5300 g in the root compartment and 4400 g in each of the outer compartments

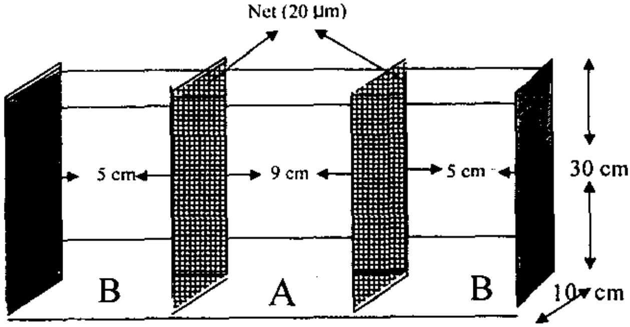

Fig.1. Details of the Rhizobox used in the experiments. A. Inner compartment (roots and hyphae); B. Outer compartments (hyphae or bulk soil).

Mycorrhyzal inoculation

A soil sample from rhizosphere of onion plants was used to prepare the mycorrhyzal inoculum. Hundred and fifty grams of the root-soil mixture were suspended in 300 ml of sterile water and shaken for one hour. The suspension was filtrered and the filtrate was collected and thereafter kept in a refrigerator to be later applied to the non-inoculated plants. Root-soil mixture was taken from the filter paper and steam-sterilized (100°) for one hour on four consecutive days to eliminate native mycorrhizal propagules. Hundred and fifty grams from either sterilized or non-sterilized soil were put in the inner compartment of the rhizobox ten cm below the surface for the treatments without or with mycorrhyza, respectively.

Five grains of corn (Zea maize L., Cv. Giza 461); were planted in the inner compartment of each rhizobox. After germination, seedlings were thinned to three plants per rhizobox. The different rhizoboxes were irrigated with 1/5 Hoagland's nutrient solution. Stock solution consists of 224, 235, 160, 62, 32, 24, 1.77, 0.27, 0.11, 0.131, 0.032, 0.05 and 1.12 mg/L of N, K, Ca, P, S, Mg, Cl, B, Mn, Zn, Cu, Mo and Fe, respectively. The pH and EC values of this solution were 6 and 0.8 dS/m, respectively. Every three days, the pots were weighed and the water content was adjusted when necessary to ensure maintenance of the soil water content at approximately 60% of the field capacity. All treatments were applied with or without mycorrhizal inoculum and there were three replicates.

The plants were harvested after six weeks of growth. The roots were carefully washed from soil with tap water and then with distilled water, dried between paper tissue, weighed and cut into segments of about 1cm length. From each pot, approximately 1g fresh roots was stained with fuchsin, and mycorrhizal colonization (colonized root length as percentage of total root length) was measured using the line-intersect method (Kormanik & McGraw, 1982). Dry weights of shoot and roots were determined after drying at 70 ° for 48 hr. For mineral nutrient analysis, plant samples were ground and about 0.5 g dry matter samples were digested in a mixture of concentrated H_2SO_4 and H_2O_2 at 400 °. N, P, K, Fe, Mn, Zn, Cu, Co and Ni were determined in the digested materials, using atomic absorption spectrophotometer.

Analysis of variance was carried out for the data using the GLM procedure of SAS (SAS Institute, 1996). The means of treatments were tested for statistically significant differences according to Duncan's Multiple Range Test (1955).

Results and Discussion

Growth parameters of corn plants

Data presented in Table 1 show the effects of different concentrations of Ni, Co and interactions between them and mycorrhizal (VAM) inoculation on some plant growth parameters (plant height, along with shoot and root dry weights). High doses of Ni and/or Co brought about larger adverse effects in the inoculated plants than in non-inoculated plants. The calculated relative decreases of plant height along with shoots and roots dry weights, when mycorrhizal inoculation was accompanied with 50 ppm from Ni and Co, were 39.7, 48.8 and 85 %, respectively compared with the same level from cobalt with nonmycorrhizal infection. At any level of added Co, compared to Ni, mycorrhizal plants growth parameters were significantly reduced. Such decreases, for the 5 ppm Co mycorrhizal plants, recorded 21.5, 24.4 and 10.9 % less than the same level of Ni inoculated micorhizal plants for plant height along with shoot and roots dry weights plant, respectively.

TABLE 1. Effect of mycorrhizal inoculation along with cobalt and nickel concentrations on some growth parameters of corn plants as well as colonization rate of roots.

Treatment Conc. (ppm)	Plant height		Dry wt. of shoots		Dry wt. of roots		Root colonization	
	(cm)		g/rhizobox				(%)	
	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM
	Cobalt							
5	60.0c	38.5a	14.0e	9.19 a	1.79c	0.93a	48a	0
10	33.7b .	36.3a	7.62b	6.47 b	0.93d	0.67b	21b	0
50	19.1d	33.8b	4.34a	6.01 b	0.51e	0.62b	17b	0
	Nickel							
5	76.4e	50.9c	18.5e	11.1a	2.01c	1.07a	60c	0
10	44.7f	50.2c	10.8f	9.40a	1.17b	0.94a	43d	0
50	26.1g	49.4c	6.34d	9.36a	0.81d	0.97a	28e	0
	Co &Ni							
5	26.5g	42.3d	1.25c	2.96d	0.24f	0.74a	28f	0
10	24.2g	37.5a	1.36c	2.51d	0.15f	0.80a	20b	0
50	20.0d	33.1b	1.28c	2.50d	0.13f	0.87a	13g	0

Numbers followed by the same letter are not significantly (p <0.05) different (n=3)

Concerning the effect of different concentrations of either Ni, Co or their combination on mycorrhizal colonization rate of corn roots, data given in the same table reveal that the non-inoculated plants had no mycorrhizal colonization; the roots of the mycorhizal plants were well colonized, the colonization rate being most obvious in the rhizobox treated with Ni. Moreover, the infection rate decreased in parallel with the increased dose of the studied heavy metals especially with treatment of Ni accompanied with Co.

Contents of corn plants from Co and Ni.

Contents of Co and Ni in shoots and roots of corn plants grown in the rhizoboxes treated with different concentrations from either Co or Ni and inoculated with mycorrhiza are given in Table 2. Results show that the mycoorhizal colonization significantly increased contents of both Co and Ni in both shoot and roots of corn plants receiving either Co or Ni individually even at a rate of 10ppm. The calculated relative increases in the contents of shoots from Co or Ni for the mycorrhiza treatments recorded 11.2 or 21.7 folds over those of nonmycorrhizal infection conditions. This may be due to the delivery from the outer compartments accessible to hyphae but not roots. This finding is in accordance with the results of George *et al.* (1994) and Li *et al.* (1991) who found a high capacity of external mycorrhizal hyphae to deliver and corroborates Zn and Cu to the host plant. The contents of shoots or roots from either Co or Ni were, however, sharply decreased when using 50 ppm from either Co or Ni individually. On the other hand, the application of Co and Ni together, at all used concentrations, show a sharp decrease in contents of the two elements in both shoots and roots especially with mycoorhizal colonization. This may be due to inhibiting mycorrhizal infection as well as the absorption of both Co and Ni. Comparison between mycorrhiza and nonmycorrhiza applications showed high levels of Co and Ni in the roots of the infected plants, thereby the translocation of the elements to leaves was hindered; the high concentrations from either Co or Ni seemed to decrease the rate of mycorrhizal infection. This may suggest a preferential accumulation of these elements in root tissue at least at moderate or high supply of these elements. These results are in agreement with the findings of Dehn & Schuepp (1989) who found decreased Cd concentrations in the shoots of maize and lettuce mycorrhizal plants, regardless of the Cd contents of used soils. Generally, Ni contents in both shoots and roots were higher than those of Co for the mycorrhizal infected roots.

Contents of corn plants from macro and micronutrients.

Concentrations of macro and micronutrients in shoot and roots of the studied corn plants as affected by different concentrations applied from Co and Ni as well as mycorrhizal inoculation are shown in Table 3. Data illustrate that the myorrhizal plants accumulated significantly more macro and micronutrients than the nonmycorrhizal ones. For example, the content of iron in shoots of the Co treatment at 5, 10 and 50 ppm in the mycorrhizal plants contributed 40.3, 96.8 and 24.7 %, respectively more than nonmycorrhizal plants. This may be due to the limited diffusion of nutrient ions through soil, thus roots deplete these immobile soil nutrients just from a zone immediately surrounding the root; mycorrhizal hyphae seemed to extend into the zone of nutrient depletion and can increase the effectiveness of absorption of immobile elements by as much as 60 times (Bieleski, 1973). This is in agreement with results of Nielson & Jensen (1983) who reported that VA-mycorrhizal inoculation tends to increase the uptake of both nitrogen and potassium. Kothari *et al.* (1991) added that mycorrhizal plants often grow better than nonmycorrhizal plants due to higher P, Zn and Cu uptake.

TABLE 2. Effect of mycorrhizal inoculation along with cobalt and nickel concentrations on their content in shoots and roots of treated corn plants.

Treatment Conc. (ppm)	Cobalt				Nickel				Cobalt & Nickel							
									Cobalt				Nickel			
	Shoot		Root		Shoot		Root		Shoot		Root		Shoot		Root	
	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM
	mg.kg^{-1}															
5	2.47a	0.22d	25.70c	3.44a	13.0e	0.60d	44.2b	4.02a	1.31e	0.11d	9.41b	1.94a	9.11e	0.46d	24.9a	2.08c
10	6.12b	0.56d	56.0d	7.65b	30.6f	1.48b	76.9f	9.79b	1.02c	0.08d	3.52f	1.80a	3.67a	0.31d	11.5b	0.96d
50	0.95c	0.09d	9.98e	1.33a	4.17g	0.24d	17.1e	1.59a	0.66c	0.06d	2.17f	0.93a	2.70b	0.23d	8.44b	0.70d

Numbers followed by the same letter are not significantly ($p<0.05$) different (n=3).

TABLE 3. Effect of mycorrhizal inoculation along with cobalt and nickel concentrations on the contents of both macro and microelements in both shoots and roots of treated corn plants.

element	Cobalt						Nickel						Cobalt& Nickel					
	+VAM			-VAM			+VAM			-VAM			+VAM			-VAM		
	5	10	50	5	10	50	5	10	50	5	10	50	5	10	50	5	10	50
N																		
Shoot	40.7a	44.1a	18.4b	22.8c	23.0c	22.9c	42.4a	46.5a	32.8c	23.5c	23.1c	22.7c	45.0a	45.6a	20.2b	21.9c	23.6c	22.9c
Root	28.8a	30.1b	26.9a	20.6c	19.9c	20.2c	30.1a	29.9ab	21.9d	20.2c	19.6c	19.7c	33.0c	31.6b	24.1e	20.6c	21.23c	20.5c
P																		
Shoot	0.28a	0.5a	0.34a	0.20a	0.20a	0.20a	0.32a	0.57ab	0.34a	0.22a	0.23a	0.22a	0.34a	0.57a	0.35a	0.21a	0.22a	0.21a
Root	1.88c	2.51c	0.88b	1.37c	1.24c	1.25bc	2.04c	2.73c	0.96b	1.55c	1.56bc	1.61c	2.21c	2.95c	1.23ab	1.61c	1.63c	1.62c
K																		
Shoot	21.0a	28.3b	25.8b	15.6c	15.7c	15.1c	37.8b	31.0a	22.1c	15.9c	15.2c	15.3c	24.3c	31.1a	20.9c	15.9c	15.2c	15.5c
Root	61.7a	68.6b	52.2c	45.1d	44.8d	44.4d	60.6a	57.7a	66.9b	44.4d	45.0d	44.5d	82.8b	53.3a	48.0a	45.2d	46.8d	46.3d
Fe																		
Shoot	148a	212b	132a	106c	108c	106c	152a	209b	131a	107c	106c	107c	162a	219b	147a	1078c	107c	108c
Root	269a	271a	300b	220c	221c	221c	304d	351c	299b	226c	224c	222c	320d	320d	355d	222c	221c	223c
Mn																		
Shoot	77.9a	166.7b	36.3e	52.7d	53.4d	53.6d	85.0b	134a	45.3e	53.2d	52.3d	52.6d	89.7b	170c	44.4e	53.1d	53.8d	53.6d
Root	129a	172b	101c	66.4d	66.9d	66.7d	168f	284c	87.7e	67.5d	66.2d	67.5d	241a	278g	88.2e	67.4d	67.2d	67.9d
Cu																		
Shoot	24.7a	44.0b	24.6a	31.2c	30.1c	31.4c	27.0a	50.2d	27.8a	30.2c	30.0c	31.5c	30.8a	49.8d	19.7b	34.3b	34.3b	34.2b
Root	65.4b	92.7a	43.3c	45.2d	43.3d	45.3d	71.5a	113.7b	43.7c	45.1d	44.0d	44.2d	65.4b	75.0c	47.2c	43.5d	44.3d	43.7d
Zn																		
Shoot	51.4a	84.5b	43.3c	38.7d	37.5d	37.1d	60.9b	90.3a	59.9b	37.4d	37.1d	37.1d	59.4b	85.8c	35.9a	37.4d	37.7d	37.9d
Root	86.6b	101a	102a	61.6c	61.5c	60.4c	106a	143b	91.9d	62.3c	60.6c	62.5c	106a	129c	89.6b	64.6c	65.9c	67.0c

The contents of different elements in shoots and roots tended to decrease with increasing concentration of either Co or Ni treatment In general, the contents of different elements were significantly influenced by concentration of either Co or Ni. This may be related to higher Co or Ni in mycorrhizal roots sufficient to protect the shoots from element accumulation. Effects of nickel treatment on the contents of different elements were most distinct when used with mycorrhizal inoculation; mycorrhiza treatments with 5 ppm from Ni gave values of N, P, K, Fe, Mn, Cu and Zn contents in shoots of corn plants equivalent to 4.11, 14.29, 80.30, 2.28, 9.20, 9.53 and 18.41%, respectively more than given by the same level from cobalt treatment. This may be due to increasing Co content in the soil; hyphae may act partly as a barrier for uptake of different elements and may have reduced the transport of different elements into the plant (Denny & ridge, 1995). Except for nitrogen, the contents of different elements in roots were usually higher than in shoots. On the other hand, the interaction between Co and Ni, at the low levels, were not significantly effective on contents of different elements in shoots and roots of corn plants. With high levels (50ppm) from Co accompanied with Ni, contents of different elements in shoots and roots of corn plants were sharply decreased.

As a conclusion, mycorrhiza transported large amounts from Co and Ni to the corn plants when these elements were used individually; such transport was negligible when Co and Ni were present together. In fact mycorrhizal infection enables most of Co and Ni to be retained by roots, allowing less heavy metal translocation to leaves. The filtering property of mycorrhiza may contribute to the efforts introduced to overcome hazards of high levels of heavy metals in soils which are a direct linkage with the food chain. These results may be of ecological significance, in particular because of natural presence of these together in soil.

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تأثير استخدام فطر الميكوريزا على امتصاص نبات الذرة لكل من الكوبالت والنيكل

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أجرى هذا البحث بهدف دراسة تأثير التلقيح بفطر الميكوريزا على سلوك عنصري النيكل والكوبالت في الأرض الملوثة بهما وأمتصاصهما بواسطة نبات الذرة. ولذلك تم استخدام أصص مقسمة إلى ثلاث أقسام (Rhizobox) بحيث تنمو جذور النبات وهيفات الفطر معا في الجزء الأوسط وتنمو هيفات الفطر فقط في الجزئيين الخارجيين. تم زراعة نبات الذرة في رمل مغسول ومعامل بأي من الكوبالت أو النيكل أو الاثنين معا في معاملة واحدة بتركيزات ٥، ١٠، ٥٠ ملجم/ كجم تربة في الأقسام الخارجية للأصيص وتم التلقيح بفطر الميكوريزا مع الري باستخدام محلول مغذى (١/٥هوجلاند).

أوضحت النتائج مايلى:

أدى التلقيح بفطر الميكوريزا مع إضافة الكوبالت إلى نقص في طول النباتات والوزن الجاف لكل من المجموع الجذري والخضري عند جميع التركيزات المستخدمة، فعلى سبيل المثال عند استخدام ٥ ملجم كوبالت/ كجم تربة أنخفض كل من طول النبات والوزن الجاف لكل من المجموع الجذري والخضري بمقدار ٢١,٢، ٢٤,٤، ١٠,٩% على الترتيب مقارنة بنفس التركيز من النيكل. أتضح أيضا أن الإصابة بفطر الميكوريزا قد زاد في جذور النباتات مع إضافة النيكل مقارنة بالكوبالت، كما أدى التلقيح بفطر الميكوريزا إلى زيادة محتوى النبات من الكوبالت والنيكل والعناصر الغذائية الأخرى بحيث كان تركيز تلك العناصر في الجموع الجذري أكبر من المجموع الخضري خاصة عند إضافة أي من الكوبالت أو النيكل كلا على حدة حتى تركيز ١٠مللجم / كجم تربة. أخيرا أدى استخدام الكوبالت والنيكل معا في معاملة واحدة أو زيادة تركيز هما إلى ٥٠ ملجم /كجم تربة إلى نقص محتوى النبات من تلك العناصر، مع زيادة محتوى نبات الذرة من العناصر المختلفة زيادة معنوية عند استخدام النيكل مقارنة باستخدام الكوبالت مع فطر الميكوريزا.

مما سبق تشير النتائج المتحصل عليها إلى أهمية استخدام فطر الميكوريزا في الأراضي الملوثة لتقليل امتصاص العناصر الثقيلة ويمكن اعتبارها طريقة تطبيقية واعدة في زراعة الأراضي الملوثة بالعناصر الثقيلة.