PHYTOREMEDIATION OF CONTAMINATED SOIL USING MYCORRHIZAE.

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ABSTRACT

The present work was conducted to study the influence of mycorrhizae (M) as a bioremediation agent for soil contaminated with lead (Pb) and cultivated with wheat or faba bean plants, and soil contaminated with cadmium (Cd) and cultivated with wheat plant. Contamination of the soil with each of Cd or Pb lead to decrease phosphorus (P) in plants leading to significant decreases in dry weight of wheat and faba bean plants. The heavy metals increased in plant parts by increasing contaminant concentration in the soil. The use of M for phytoremediation of the contaminated soil lead to more absorption of the heavy metals in plants. Thus,it significantly decreased the available heavy metals in the soil after cultivation. Also, the inoculation with M resulted in the an improvement of P absorption by plants, which caused significant increases in plant dry weights.

INTRODUCTION

The increasing heavy metals contamination, due to various human and natural activities, results in a contaminated ecosystem. Migration of contaminants into non-contaminated sites, in forms of dust or leachate through the soil and the spreading of sewage sludge are examples of events that contribute towards contamination of our ecosystem. (Khan *et al*., 2000).

Contaminated soil can be remediated by chemical, physical or biological techniques. The available techniques may be grouped into two categories:

1. Exsitu techniques, which require excavation of the contaminated soil for treatment on or off site.
2. Insitu methods, which remediate without excavation of contaminated soil.

Insitu techniques are favored over the exsitu techniques due to their lower cost and reduced impact on the ecosystem. (Khan *et al*., 2000).

Heavy metals are the main group of inorganic contaminants. Remediation of metal compounds presents a different set of problems when compared with organic contaminants. Organic compounds can be degraded while metals normally need to be physically removed or immobilized. (Khan *et al*., 2000). Insitu management of heavy metals contaminated soils can be achieved either by diluting the contaminant to safe levels by using clean soil as dilutent or immobilization of inorganic contaminants (can be achieved by increasing the soil pH by liming). The solubility of metals such as Cd, Cu, Zn and Ni are reduced due to formation of insoluble hydroxides.

The physio- chemical technologies used for soil remediation render the land useless a medium for plant growth as they also remove all biological activities. There is a need to develop suitable techniques insitu for the removal of non- volatile and non- mobile soil contaminants. (Khan *et al*., 2000). Plants that uptake heavy metals from soil offer an alternative and less expensive method, a technique termed as phytoremediation, emerging as a new tool for insitu remediation.Contaminated sites often support characteristic plant species, some of which are able to accumulate high contaminations of heavy metals in their tissue.

Mycorrhizae have been reported in plants growing on heavy metal contaminated sites, the fungi have evolved a heavy metal (HM) tolerance and they may play a role in the phytoremediation of the site. Galli *et al*. (1994) suggested that mycorrhizae can play a crucial role in protecting plant roots from heavy metals. The efficiency of protection, however, differs between distinct isolates of mycorrhizae fungi and different heavy metals. Joner and Leyval (1997) reported that extra-radical hyphae of M fungus *G. mosseae* can transport Cd from soil to subterranean clover plants growing in compartment pots, but that transfer from fungus to plant is restricted due to fungal immobilization.

The normal Cd level in soils usually ranges from 0.01 – 2.0 mg/kg,while,the critical levels ranged from 3.0 – 8.0 mg/kg as reported by Kabata and Pendias (1992). The concentrations of Cd normally encountered in the environment do not cause acute toxicity. The major hazard to human health from Cd is its chronic accumulation in the kidneys where it can cause disfunction. The concentrations in the kidney cortex may exceed 200 mg/ kg fresh weight (Alloway, 1990).

The normal Pb level in soils usually ranges from 2 – 300 mg/kg while, the critical levels ranged from 100 – 400 mg/kg as reported by Kabata and Pendias (1992).

The present work focus on how much the mycorrhizae affect the available heavy metals (cadmium and lead) in soil as a tool foe phytoremediation.

MATERIALS AND METHODS

Soil sample was collected from Agriculture Research Station, Faculty of Agriculture, Alexandria University at Abis. The sample was air dried, ground to pass 2mm sieve and thoroughly mixed before using. General soil properties were determined as follows:

Soil and Water Department, Faculty of Agriculture, Alexandria University.

Soil reaction (pH) was measured in 1:2.5 soil:water suspension by pH- meter. Electrical conductivity was measured in the water extract of soil paste. Calcium carbonate was measured by calcimeter apparatus (Page *et al.*, 1982). Soluble cations and anions were determined in soil- paste extract (Richard, 1954). Organic carbon was determined by Walkely and Black method (Nelson and Sommers, 1982). Total nitrogen was measured by steam distillation (Bremner and Mulvaney, 1982). Texture was determined using hydrometer method according to Black (1965). Available phosphorus was measured according to the method reported by Olsen and Sommers (1982). Available cadmium and lead were measured by DTPA method as described by Page *et al.* (1982).

The characteristics of this soil were soil pH 8.20, EC 4.80 dS.m^{-1}, calcium carbonate 22.10%, organic carbon 0.80 %, available phosphorus 15.00 mg/kg, soil texture clay-loam, available cadmium 0.043 mg/kg, available lead 2.24 mg/kg, and total nitrogen 0.10%.

Seeds of Wheat (*Triticum vestium*) – Sakha 8 and Faba bean (*Vicia faba*) – Giza 716, were obtained from the Agronomy Department, Faculty of Agriculture, Alexandria University.

The wet sieving and decantation method described by Gerdemann and Nicolson (1963) was used to isolate vascular arbuscular mycorrhizae (VAM) spores from a clayey soil containing 2.87 mg/kg DTPA extractable Pb and 0.05 mg/kg DTPA extractable Cd.

Assessment of mycorrhizae infection:

The staining method of Phillips and Hayman (1970) was used for preparing root samples for microscopic observations.The gridlines intersect method of Giovannetti and Mosse (1980) was used to estimate the mycorrhizae infection %.

$$M\% = \frac{\text{No. of positive intersects}}{\text{Total number of observed intersect points}}$$

Experimental procedure:-Two experiments 2-factor were laid out in a randomized complete block design of three replicates. The different metal contents in the soil were adjusted by adding aqueous solutions of lead nitrate at the rates of 0, 50, 100, 200 and 400 mg Pb /kg soil or cadmium chloride, at the rates of 0, 2, 4, 8 and 16 mg Cd /kg soil. After carefully mixing the metal solutions with the soil on plastic sheets, this was allowed to stabilize for 30 days before using.

The experiments were started (Pb experiment) on 3[rd] January 2002, and (Cd experiment) on 15[th] February 2002. The seeds were sown in black plastic pots (30 cm diameter x 20 cm depth). 4.5 kg soil were placed above 350 g gravel, plant thinned to 4 plants per pot after germination of faba bean, and 8 plants per pot after germination of wheat. All pots were irrigated with tap water to the field capacity of the soil before planting, the moisture content of all pots were kept at 80 % field capacity by daily weighing and water was added to compensate that lost by evapo-transpiration. The recommended doses of NPK mineral fertilizers were added. All the plants were harvested after 60 days from cultivation.

Plant roots were washed thoroughly with tap water to remove the adhering soil particles. Plant roots and shoots were dried at 70 °C in an aerated oven for 48 hrs. The shoots and roots total cadmium or lead contents and uptake, shoots total phosphorus content and uptake were determined. Shoots total phosphorus was determined according to Olsen and Sommers (1982).Plant heavy metals was analyzed according to Jones (1989), the concentration of cadmium and lead were measured by Atomic Absorption spectrophotometer.

Data of the experiments were statistically analyzed using the SAS procedures (1986). The least significant difference (LSD) procedure at 0.05 probability level was used for mean comparison.

RESULTS AND DISCUSSION

Lead experiment:

Data in Table 1:A&B showed the content of available Pb or Cd in soil before cultivation of wheat or faba bean plants. The low availability of them in soil solution might be explained by the properties of the experimental soil (high pH and $CaCO_3$ content), which have a high fixing capacity for metals and consequently a low availability of Pb and Cd in soil solution, (Badry , 2005).

Table 1- A : Available lead (Pb) in the soil before cultivation of wheat and faba bean plants.

Added lead (mg/kg soil)	Available lead after stabilization (mg/kg soil)
Control	2.24
50	3.11
100	7.61
200	8.33
400	12.23

Table 1- B : Available cadmium (Cd) in the soil before cultivation of wheat plants.

Added cadmium (mg/kg soil)	Available cadmium after stabilization (mg/kg soil)
Control	0.043
2	0.299
4	0.542
8	0.713
16	0.943

Mean values presented in Tables 2 and 3 indicated that amount of available Pb in the soil cultivated with faba bean or wheat decreased significantly with the mycorrhizae inoculation. Furthermore, it was clear that this available amount increased significantly with each of increasing level of added Pb to the soil. The lead concentration in faba bean shoots and roots and in wheat roots significantly increased with mycorrhizae inoculation. The same tables also showed that Pb concentration in shoots and roots of faba bean and wheat significantly increased with increasing Pb concentration. According to Kabata and Pendias (1992), the concentration of Pb in plant leaf ranged from 5 to 10 mg/kg dry weight are normal and the toxic concentration from 30 to 300 mg/kg dry weight. In Tables 2 and 3 also revealed that Pb uptake in faba bean and wheat shoots and roots significantly increased with mycorrhizae inoculation.

Table 2: Means of lead (Pb) contents in faba bean plants and its available amounts in the soil after cultivation as affected by added lead and mycorrhizae inocula (M).

Treatment		Lead				
		Concentration (mg/kg)		Uptake		Available in soil after cultivation(mg/kg)
		Shoot	Root	µg/shoot	µg /Root	
Mycorrhizal Inocula	Without (M)	2.90 b*	4.60 b	2.60 b	0.028 b	3.88 a
	With (M)	3.00 a	6.30 a	3.30 a	0.044 a	3.45 b
	LSD $_{0.05}$	0.02	0.02	0.03	0.50	0.15
Pb concentration (mg Pb/kg)	Control	2.25 e	3.18 e	2.70 b	2.77 d	1.29 d
	50	2.75 d	4.23 d	2.73 b	2.97 dc	1.77 c
	100	3.05 c	5.55 c	2.75 b	3.53 bc	3.35 b
	200	3.23 b	6.65 b	3.10 ab	3.98 ab	5.85 a
	400	3.53 a	7.70 a	3.25 a	4.72 a	6.08 a
	LSD $_{0.05}$	0.03	0.03	0.40	0.76	0.24

* = means in the same column followed by the same letters are not significantly different at 0.05 level of probability.

LSD 0.05 = least significant difference at 0.05 level of probability.

Table 3: Means of lead (Pb) contents in wheat plants and its available amounts in soil after cultivation as affected by added lead and mycorrhizae inocula.

Treatment		Lead: Concentration (mg/kg) Shoot	Concentration (mg/kg) Root	Uptake µg/shoot	Uptake µg /Root	Available in soil after cultivation(mg/kg)
Mycorrhizal inocula	Without (M)	3.30	5.70 b	1.70 b	0.70 b	4.81 a
	With (M)	0.034	6.60 a	1.90 a	1.10 a	4.16 b
	$LSD_{0.05}$	n.s.	0.05	0.10	0.10	0.06
Pb concentration (mgPb/kg)	Control	1.20 e	2.10 e	0.90 d	0.60 c	1.37 e
	50	3.40 d	4.10 d	1.80 c	0.70 c	2.07 d
	100	3.90 c	7.00 c	1.90 bc	1.00 b	5.35 c
	200	4.00 b	5.60 b	2.10 b	1.10 b	6.49 b
	400	4.20 a	9.00 a	2.30 a	1.20 a	7.18 a
	$LSD_{0.05}$	0.10	0.07	0.02	0.20	0.10

* = means in the same column followed by the same letters are not significantly different at 0.05 level of probability.

LSD 0.05 = least significant difference at 0.05 level of probability.

Mean values for whole plants, shoot and root dry weights of faba bean and wheat as affected by mycorrhizae inocula and Pb concentration are presented in Tables 4 and 5. Data indicated that mycorrhizae inocula significantly increased whole plant and shoot dry weights for both plants and root dry weight only in case of wheat plants. The mycorrhizae colonization of roots resulted in an increase in root surface area for nutrient acquisition. The extramatrical fungal hyphae can extend several centimeters in the soil and hence increase the uptake of nutrients, including heavy metals as previously explained by Khan *et al.* (2000). Furthermore, the same tables revealed that whole plant, shoot and root dry weights of faba bean and wheat significantly decreased with increasing Pb concentration. These findings are consistent with the results of Diaz *et al.* (1996), who found that increasing doses of Zn or Pb reduced logarithmically plant biomass. The results of the present study, also, indicated that lead application inhibited the plant growth in general. This may be attributed to the effect of lead on the photosynthesis, which is the key process in plant development as mentioned by Huang *et al.*, (1974).

Table 4: Means of dry weight, mycorrhizae infection (%) and phosphorus content in faba bean plants as affected by added lead (Pb) and mycorrhizae inocula.

Treatment		Dry weight (g/plant): Whole Plant	Shoot	Root	Mycorrhizal infection(%)	Phosphorus in shoot: Concentration (mg/kg)	Uptake (mg/shoot)
Mycorrhizal Inocula	Without (M)	1.57 b*	0.92 b	0.65 a	20.90 b	616.0 b	0.60 b
	With (M)	1.81 a	1.10 a	0.71 a	75.90 a	733.0 a	0.80 a
	$LSD_{0.05}$	0.12	0.10	n.s.	6.04	47.3	0.10
Pb concentration (mg Pb/kg)	Control	2.08 a	1.23 a	0.85 a	50.50 a	855.8 a	0.106 a
	50	1.84 b	1.14 ab	0.70 b	49.50 a	715.2 b	0.082 b
	100	1.64 c	1.01 bc	0.64 b	49.50 a	681.7 b	0.069 bc
	200	1.51 dc	0.90 dc	0.61 b	47.20 a	598.2 c	0.054 c
	400	1.39 d	0.78 d	0.60 b	45.60 a	520.0 d	0.035 d
	$LSD_{0.05}$	0.19	0.15	0.13	n.s.	74.9	0.018

* = means in the same column followed by the same letters are not significantly different at 0.05 level of probability.

LSD 0.05 = least significant difference at 0.05 level of probability.

Table 5: Means of dry weight, mycorrhizae infection (%) and phosphorus content in wheat plants as affected by added lead (Pb) and mycorrhizae inocula.

	Treatment	Dry weight (g/plant) Whole Plant	Shoot	Root	Mycorrhizal infection (%)	Phosphorus in shoot Concentration (mg/kg)	Uptake (mg/shoot)
Mycorrhizal Inocula	Without (M)	0.67 b*	0.54 b	0.14 b	23.30 b	693.0 b	0.38 b
	With (M)	0.80 a	0.60 a	0.19 a	75.10 a	830.0 a	0.51 a
	LSD 0.05	0.06	0.03	0.02	6.60	53.0	0.04
Pb concentration (mg Pb/kg)	Control	1.01 a	0.73 a	0.26 a	53.90 a	909.0 a	0.67 a
	50	0.76 b	0.60 b	0.17 b	51.70 a	852.0 a	0.51 b
	100	0.69 bc	0.55 bc	0.14 bc	50.00 a	749.0 b	0.41 c
	200	0.64 c	0.50 cd	0.14 bc	47.80 a	676.0 bc	0.34 d
	400	0.59 c	0.48 d	0.12 c	42.80 a	621.0 c	0.30 d
	LSD 0.05	0.10	0.05	0.04	n.s.	84.0	0.06

* = means in the same column followed by the same letters are not significantly different at 0.05 level of probability.

LSD 0.05 = least significant difference at 0.05 level of probability.

Results in Tables 4and 5 showed that mycorrhizae infection percentages increased with the mycorrhizae inoculation. Mean values of mycorrhizae infection percentage for the same trait did not differ significantly among the tested lead concentrations, they ranged from 45.6 to 50.5% for faba bean and from 42.8 to 53.9% for wheat plants. These findings are consistent with those of Weissenhorn *et al.* (1995). They reported no significant relationship between mycorrhizae colonization and soil metai content. These results, however, are not in agreement with Gildon and Tinker (1983). They suggested that root intracellular concentrations of heavy metals may limit the spread of mycelia in vesicular-arbuscular mycorrhizae (VAM).

Shoot phosphorus concentration and uptake in faba bean and wheat plants increased significantly with mycorrhizae inocula reaching 733 mg P/kg and 0.8 mg P/shoot for faba bean and 830 mg P/kg and 0.51 mg P/shoot of wheat, for phosphorus concentration and uptake, respectively. It has been known that phosphate ions adsorbed on soil colloids are relatively immobile. Therefore, it is difficult for roots to absorb sufficient amounts of these P-ions for optimum plant growth in poor phosphate soils. The external hyphae of AM fungi can overcome this limitation by exploration of larger soil volume decreasing the distance that P-ions must diffuse to plant roots. Increasing the movement of P-ions into mycorrhizae hyphae and decreasing the threshold concentration required for absorption of P-ions stated by Bolan (1991).

Concerning the significant effect of Pb concentration on P concentration and uptake in faba bean and wheat shoots are presented in Tables 4and 5. Data indicates that both parameters decreased significantly with each increase in Pb concentration from the control to 400 mg Pb/kg added. These findings are consistent with the results of Hasset *et al.* (1976) and Hahne and Kroontje (1973), They found that increasing phosphorus anions reduced the uptake of lead by plants. Furthermore, many investigators had provided conclusive evidence for the ability of P to immobilize dissolved Pb in contaminated soils through precipitation of fluropyromorphite ($Pb_{10}(PO_4)_6(F)_2$), pyromorphite ($Pb_3(PO_4)_2$), hydroxypyromorphite ($Pb_{10}(PO_4)_6(OH)_2$) and chloropyromorphite ($Pb_{10}(PO_4)_6(Cl)_2$), as reported by Bolan *et al.* (2003).

Cadmium experiment:

Mean values presented in Table 6 indicated that amounts of available Cd after cultivation of soil decreased significantly with the mycorrhizae inoculation, reaching 0.26 mg/kg.The amount of available Cd increased significantly with each increased level of added Cd to the soil, (Badry. 2005).

The cadmium concentration in plant shoots and roots significantly increased with mycorrhizae inoculation (Table 6). Also, the Cd uptake by shoots significantly increased with the mycorrhizae inoculation, but the cadmium (Cd) uptake by roots did not differ significantly with mycorrhizae inoculation. The root colonization by mycorrhizae had increased the root surface area for nutrient acquisition. The extramatrical fungal hyphae can extend several centimeters in the soil and hence increase the uptake of nutrients, including heavy metals as mentioned by Khan *et al.* (2000). The Cd concentration and uptake in shoots and roots significantly increased with each increase of Cd concentration in soil. These results are in agreement with those reported by Jakson (1990), who found linear relationship between total soil Cd and plant Cd content.

The whole plant and shoot dry weights significantly increased with the mycorrhizae inoculation (Table 7). While the root dry weight did not differ significantly with mycorrhizae inoculation. This may be due to the beneficial effects of arbuscular mycorrhizae hyphae on plant growth, the increase of root surface area by growth of mycorrhizae hyphae associated with roots into soil and, consequently, the absorption of nutrients by hyphae and their translocation to plant, mobilizing sparingly available nutrients and releasing of chelating compounds or exoenzymes.

The same Table 7 also indicated that all the previous traits (plant, shoot and root dry weights) significantly decreased with each increase of Cd concentration. These indicated that cadmium application inhibited the plant metabolism in general. This may be attributed to the effect of cadmium on the photosynthesis, which is the key process in plant development as mentioned by Huang *et al*, (1974), also, Bolan *et al*, (2003) reported that dry matter yield decreased with increasing level of Cd application, indicating the phytotoxic effect of Cd.

Table 6: Means of cadmium content (Cd) in wheat plants and its available amounts in the soil after cultivation as affected by added cadmium and mycorrhizae inocula.

Treatment		Cadmium Concentration (mg/kg) Shoot	Concentration (mg/kg) Root	Uptake µg /shoot	Uptake µg /root	Available in the soil after cultivation(mg/kg)
Mycorrhizal Inocula	Without (M)	0.50 b*	1.77 b	0.10 b	0. 19 a	0.31 a
	With (M)	1.74 a	2.58 a	0.37a	0. 27 a	0.26 b
	LSD 0.05	0.14	0.43	0.05	0. 09	0. 001
Cd concentration (mg Cd/kg)	Control	0.14 d	0.19 d	0.06 c	0. 07 c	0.04 e
	2	0.26 d	0.53 cd	0.09 c	0. 07 c	0.17 d
	4	0.53 c	0.92 c	0.14 c	0. 10 c	0.25 c
	8	1.52 b	3.30 b	0. 35 b	0. 36 b	0.36 b
	16	3.19 a	5.94 a	0. 55 a	0. 55 a	0.63 a
	LSD 0.05	0. 22	0. 68	0. 08	0. 14	0.001

* = means in the same column followed by the same letters are not significantly different at 0.05 level of probability.
LSD 0.05 = least significant difference at 0.05 level of probability.

Table 7: Means of dry weight, mycorrhizae infection (%) and phosphorus content in wheat plants as affected by added cadmium (Cd) and mycorrhizae inocula.

Treatment		Dry weight (g/plant) Whole Plant	Dry weight (g/plant) Shoot	Dry weight (g/plant) Root	Mycorrhizal infection(%)	Phosphorus in shoot Concentration (mg/kg)	Phosphorus in shoot Uptake (mg/shoot)
Mycorrhizal inocula	Without (M)	0.34 b*	0.23 b	0.112 a	18.21 b	530.5 b	0.13 b
	With (M)	0.43 a	0.30 a	0.126 a	77.68 a	647.9 a	0.20 a
	LSD 0.05	0.03	0.02	0.018	6.45	48.5	0.02
Cd concentration (mg Cd/kg)	Control	0.59 a	0.43 a	0.16 a	49.95 a	710.2 a	0.307 a
	2	0.42 b	0.29 b	0.12 b	48.90 a	612.3 b	0.183 b
	4	0.37 bc	0.26 b	0.11 b	47.17 a	567.8 bc	0.147 c
	8	0.32 c	0.22 c	0.10 bc	47.10 a	536.3 bc	0.115 d
	16	0.24 d	0.16 d	0.08 c	46.62 a	519.3 c	0.085 d
	LSD 0.05	0.05	0.04	0.029	10.20	76.7	0.031

* = means in the same column followed by the same letters are not significantly different at 0.05 level of probability.
LSD 0.05 = least significant difference at 0.05 level of probability.

The mycorrhizae infection percentage significantly increased with the mycorrhizae inoculation (Table 7). The same table also indicated that the root colonization by mycorrhizae fungi was not affected by any of the cadmium concentration. This finding is in consistence with the results of Weissenhorn *et al.* (1995), they did not find relationship between mycorrhizae colonization and metal content in soil, however, it was not in agreement with that of Gildon and Tinker (1983), they suggested that root intracellular concentrations of heavy metals may limit the spread of mycelia in vesicular-arbuscular mycorrhizae (VAM).

The mean values of P concentration (mg/kg) and uptake (mg/shoot) in shoots of wheat plants as affected by cadmium concentration added and mycorrhizae inocula significantly increased with the mycorrhizae inoculation. Phosphorus concentration and uptake in shoot significantly decreased with each increase of Cd concentration. The addition of phosphate as KH_2PO_4 increased the pH, negative charge and Cd adsorption by the soil, so that the Cd concentration in the plant tissues decreased with increasing the rate of phosphate addition (Bolan *et al.* 2003).

Guided with the previous results a general recommendation could emphasize that in the soils contaminated with high concentrations of cadmium, advised is not to be inoculated with mycorrhizae fungi, because it was found to enhance cadmium accumulation in plant shoots and roots and so the photosynthesis rate is decreased. Therefore, in soil contaminated with heavy metals, it is recommended to do the bioremediation using some other natural agents, such as *Lygeum spartum* L. (Poaceae) and *Anthyllis cytisoides* L. (Leguminosae), as stated by Diaz *et al.* (1996) that both are plants naturally growing in heavy metal contaminated soils.

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الملخص العربى

المعالجة الحيوية بالفطريات للأرض الملوثة بالمعادن الثقيلة

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أدت زيادة تركيز المعادن الثقيلة بالأرض نتيجة للنشاط الإنساني و الطبيعة الى تلوث النظام البيئى الذى تعتبر الأرض جزء منة، و قد تم إجراء تجارب لدراسة المعالجة الحيوية بتلقيح نباتات بالفطريات الجذرية حيث زرعت فى أرض ملوثة بالمعادن الثقيلة (الكادميوم أو الرصاص). تم زراعة الأرض الملوثة بالرصاص بالقمح أو الفول البلدي. بينما زرعت الأرض الملوثة بالكادميوم بنبات القمح.

ووجد أن زيادة تركيز كل من الكادميوم أو الرصاص بالأرض أدت الى نقص أمتصاص الفوسفور من الأرض و بالتالى حدث نقص معنوي فى وزن النبات الجاف ، وكذلك أدت الى زيادة تركيز المعادن الثقيلة فى النبات عموما .

وأدى استخدام المعالجة الحيوية بواسطة الفطريات الجذرية الى نقص معنوي فى التركيز المتاح من هدين العنصرين فى الأرض بعد الزراعة وبالتالى حدثت زيادة معنوية فى تركيزهما فى النبات، كما أدى ذلك لتحسين امتصاص الفوسفور و بالتالى حدثت زيادة معنوية فى وزن النبات الجاف.

اى أن المعالجة الحيوية للأرض تعتبر فعالة وبتكاليف بسيطة، ولكن يفضل استخدام نباتات لا تؤكل وذات علاقة تعاونية ناجحة مع الفطريات الجذرية وتنمو طبيعيا فى وجود المعادن الثقيلة.