

BIOREMEDIATION OF SOIL IRRIGATED FOR A LONG TIME WITH SEWAGE EFFLUENT

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ABSTRACT

Pots experiment was conducted under green house conditions to follow up the effect of fungal inoculants on remediating the heavy metal contaminated soil treated with sewage effluent for several years. Canola and clover crops (as tested crops) were cultivated in the two experimental soils (20 & 50 years of sewage effluent treatments), two species of fungi, mainly non-autogenic *Fusarium oxysporum* (F1) and *Aspirugellus paraciticus* (F2) and a mixture of them (F1+F2) were used as microbial agents lover for heavy metals. The experimental results indicated that the dry matter yields of the tested crops were significantly higher for the soil irrigated for 50 years with sewage effluent than those of 20 years. Inoculation with defined fungi has enhancement effect on metals uptake by different plant organs. This phenomenon was vigorous under soil treated with sewage effluent for 50 years as compared to those treated with effluent for 20 years, but in some cases, the concentration of Zn was reduced either in shoots or roots of the tested plants.

Keywords: Bioremediation, fungi, heavy metals, hyper-accumulator plants.

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INTRODUCTION

The remediation of soils refers to practices of either removing contaminants or converting them into less mobile species – or less bioavailability forms. Remediation methods are based on physical, chemical and biological approaches. The selection of a method is generally based on the nature of the contaminants, the soil type, the characteristics of the contaminated site (e.g., chemical and physical soil properties, and the length of the soil contamination period). Also, remediation costs and regulations of the country are involved in remediation projects (Mukherjee, 2001). Methods for remediating metal-polluted soils have been recently widely investigated and discussed (Cunningham and Berti, 2000; Iskandar, 2001 and Knox *et al.*, 2000). Several techniques, in situ and non-in situ are applied to clean up soils contaminated with trace elements. Due to the complexity of soils and the presence of multiple contaminants, only a few of these techniques have been applied successfully in practice but some of them are quite costly.

Metals are natural component in soil. Contamination, however, arises mainly from industrial activities, such as mining and

smelting, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application, and generation of municipal of waste (Blaylock & Huang, 2000 and Siegl, 2002). In addition to natural constituents, heavy metals may enter the soil via atmospheric deposits, discharge from sewage treatment plants, and application of sewage sludge as organic fertilizer or landfill material, polluted irrigation water and beneficial agricultural additives (Elsokkary, 1996).

Soils contain and receive heavy metals (such as Pb, Cd As, Se, Cu, Zn, Hg) that can be accumulated to very high contents and find their way growing plants at toxic levels, many of these metals are toxic, even in very small contents as consequence there would be a risk for ecosystems, agro-systems and health. It is suggested that knowledge of the mechanism that control the behaviour of heavy metals must be improved and can be used for risk assessment and proposition of remediation treatments (Berthelin and leyval, 2000).

Bioremediation is a process by which microorganisms, fungi, and plants degrade pollutant chemicals through utilization or transformation of the substances.

The microorganisms most responsible for bioremediation are bacteria. Bacteria are found in all environmental media, including air, water, and soil. When indigenous bacteria degrade compounds under existing subsurface conditions, the degradation is called passive bioremediation or natural attenuation. Natural attenuation is most common in the subsurface, where bacteria are plentiful and where pollutant concentrations are low enough such that the natural degradation is effective in controlling the size of a plume. (RTDF, 1996). In the same time, bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Most of bioremediation systems are run

under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules (Vidali, 2001).

Therefore, this work aimed to evaluate the role of some fungi in remediating heavy metal contaminated soils cultivated with canola and clover as tested plants and in the same time acts as hyperaccumulators.

MATERIALS AND METHODS

Pot experiment was carried out under greenhouse conditions at Soil and Water Research Department, Nuclear Research Center, Atomic Energy Authority to explore and evaluate the role of fungal inoculation on rehabilitation of heavy metals contaminated soils.

In this experiment, two tested plants i.e., canola and clover were cultivated in the two experimental soils (20 & 50 years of sewage effluent treatments), two species of fungi, mainly non-autogenic *Fusarium oxysporum* (F1) and *Aspirugellus paraciticus* (F2) and mixture of them (F1+F2) were used as microbial agents lover for heavy metals. These fungi act as

bioremediators to reduce the availability of toxic heavy metals in soil media and in the same time, to avoid the harmful effect of such metals on plants growth and offer a suitable media for plants to be grown healthy. Uninoculated control was also included.

Recommended doses of N, P and K fertilizers required were added before planting in the form of ammonium sulfate ($\text{NH}_4)_2\text{SO}_4$ (20.6% N), super phosphate Ca (HPO_4) (15.5% P_2O_5) and potassium sulfate K_2SO_4 (48% K_2O). These fertilizers were added at the rate of 40, 60 and 12 kg ha^{-1} , respectively. The experiment consisted of 48 pots divided into 2 groups i.e. 20 years and 50 years of sewage treatments for each plant. Treatments were replicated three times and statistically arranged in a completely randomized block design. The inoculation with fungi treatments was twice during the experimental time, the first was after 20 days from germination and the second after 40 days from germination.

Plants were harvested after 75 days from sowing, separated into roots and shoots, and dried in the oven at 70°C for 48 hours and kept for chemical analysis. Standard

procedures were followed for soil and plant analysis as described by Page *et al.* (1982) and FAO (1980). The obtained data were subjected to ANOVA for L.S.D., as described by Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Plant Dry Matter Yield

Individual fungal (F1 and F2) inoculation gave the best values of shoot and root dry matter yields of canola and clover plants grown on soil irrigated with sewage effluent for 50 years. In the same time, the accumulation of dry matter yield (shoot and root) of canola was significantly higher than those of clover plants. This trend was irregular under 20 years of irrigation with sewage effluent (Fig. 1).

Generally, canola and clover dry matter yields were significantly higher with soil irrigated with sewage effluent for 50 years than those recorded with 20 years of irrigation with sewage effluent. It seems that the soil irrigated with sewage effluent for 50 years was more fertile than that of 20 years of irrigation with sewage effluent (Figs 1 and 2).

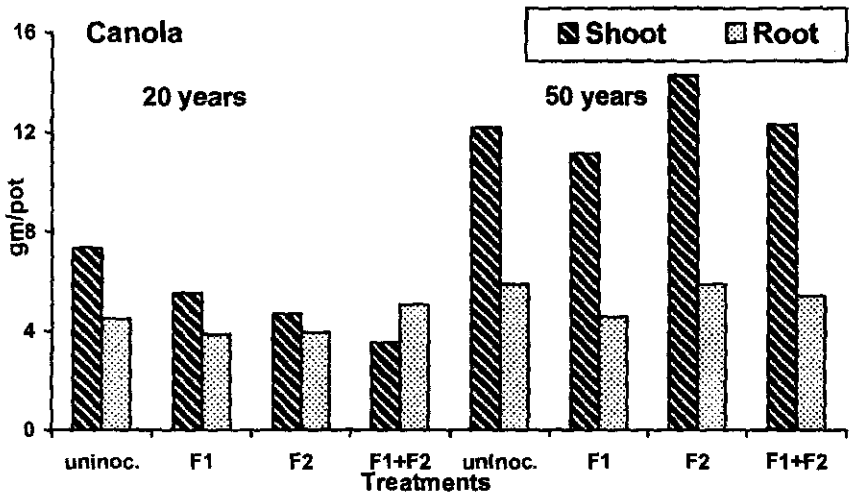


Fig. 1. Shoots and roots dry matter yield (g pot^{-1}) of canola plants grown on soil irrigated with sewage effluent for 20 and 50 years and inoculated with fungi

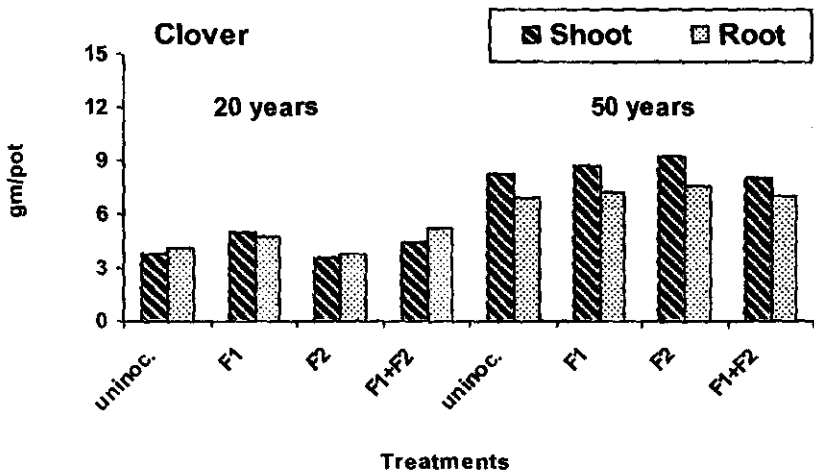


Fig. 2. Shoots and roots dry matter yield (g pot^{-1}) of clover plants grown on soil irrigated with sewage effluent for 20 and 50 years and inoculated with fungi

Effect of Fungal Inoculation on Heavy Metals Uptake

Clover

Fig. 3 indicates the effect of fungal inoculation on iron (Fe) uptake by clover plants. The obtained results showed that iron (Fe), beside the effect of clover as accumulator plant, inoculation with defined fungi had enhancement effect on Fe uptake by different plant organs. This phenomenon was vigorous under soil treated with effluent for 50 years as compared to those treated with effluent for 20 years.

Zinc uptake by clover shoots tended to reduce by fungal inoculation as compared to the uninoculated control of soil treated with effluent for 20 and 50 years (Fig. 4). Data showed that Zn uptake by either shoot or root of clover plants was frequently affected by fungal inoculation treatments. In the same time, it was higher in plants grown on soil treated with effluent sludge for 50 years comparing to those treated for 20 years. It seems that fungi could play or induce the double effect on Zn (enhancement or retard) uptake by clover plants.

Mn uptake by clover shoots as affected by sewage effluent

application and fungal inoculation was presented in Fig. 5.

It is clear that inoculation had increased Mn uptake by shoots while it decrease by roots comparing to the uninoculated control. Mn uptake by shoots and roots was higher under soil treated with effluent for 50 years than those treated for 20 years.

Copper (Cu) uptake by clover shoots grown on soil treated with effluent for 20 years was high content with root as compared to shoot. Copper uptake by shoots and roots of plants grown on soil treated with effluent for 50 years tended to increase with inoculation as compared to the un inoculated control. Copper accumulated in clover roots still higher than those accumulated by shoots. (Fig. 6).

Cobalt uptake by shoot and root of clover plant as affected by sewage effluent and fungal inoculation treatments was presented in (Fig. 7).

In general, it seems that cobalt was significantly accumulated and localized in the root system rather than shoots. Also, it was retarded by fungal inoculation treatments but in frequent fate.

Fig. 8 showed that cadmium uptake by shoots and roots of clover which grown on soil irrigated for 20 and 50 years with effluent.

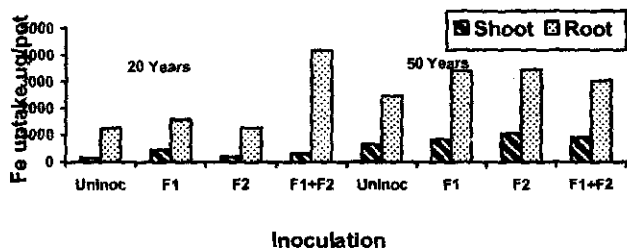


Fig. 3. Effect of fungal inoculation on Fe uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of clover plants grown on contaminated soil

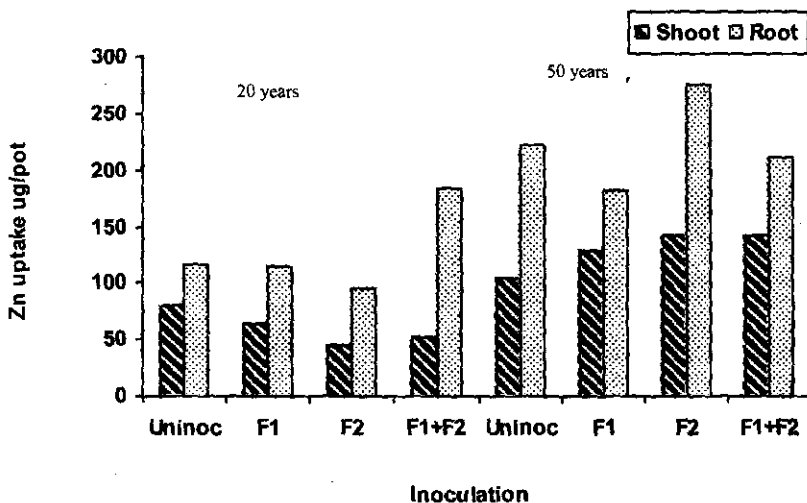


Fig. 4. Effect of fungal inoculation on Zn uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of clover plants grown on contaminated soil



Fig. 5. Effect of fungal inoculation on Mn uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of clover plants grown on contaminated soil



Fig. 6. Effect of fungal inoculation on Cu uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of clover plants grown on contaminated soil

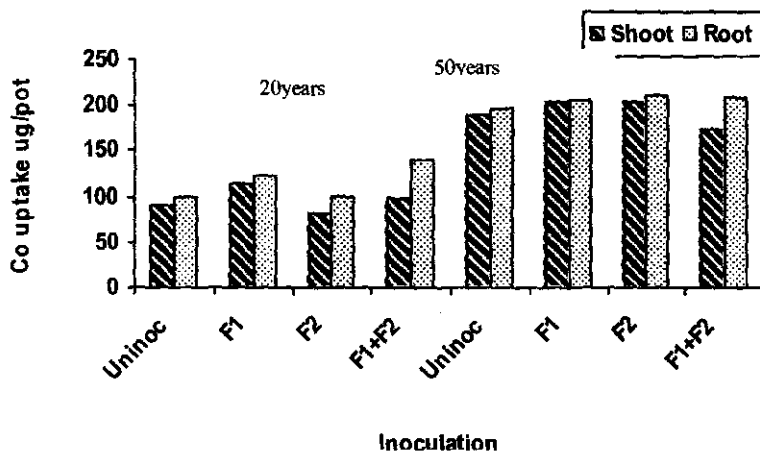


Fig. 7. Effect of fungal inoculation on Co uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of clover plants grown on contaminated soil

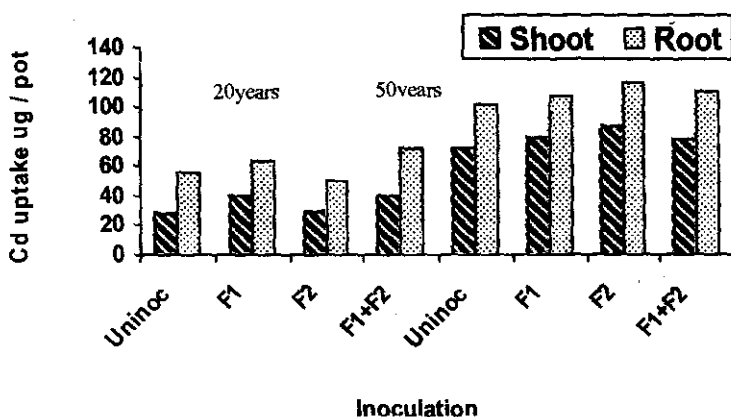


Fig. 8. Effect of fungal inoculation on Cd uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of clover plants grown on contaminated soil

It seems that the most of cadmium content was localized in the root was higher in plants grown on soil irrigated with effluent for 50 years comparing to that irrigated for 20 years. Frequent effect of fungal inoculation was noticed depending on effluent application duration.

Canola

Heavy metals, i.e. Fe; Zn; Mn; Cu; Co and Cd uptake by shoots and roots of canola plants as affected by sewage effluent application duration and fungal inoculations were presented in Table 1. Regarding the uptake of Fe by shoot and root, data showed that most of iron was localized in the root system in higher content than those accumulated by shoots under 20 years of effluent applications (Fig. 9). Similar trend was noticed, but to somewhat high extent, under the soil irrigated with effluent for 50 years. Concerning the effect of fungal inoculations on iron uptake by either shoot or root of canola plants, it was clear that the inoculation with individuals (F1 or F2 alone) had reduced the content of iron in both shoot and root of canola grown on soil treated with effluent for 20 years.

On the other hand, under the same conditions, dual inoculum induced higher accumulation of

iron in shoots but not roots. Canola grown on soil treated with effluent for 50 years reflected that although the iron uptake by shoots was enhanced by inoculation, an opposite direction was noticed with roots where the iron content significantly reduced by different inoculation treatments. In this respect, F2 fungal inoculation was superior over the others.

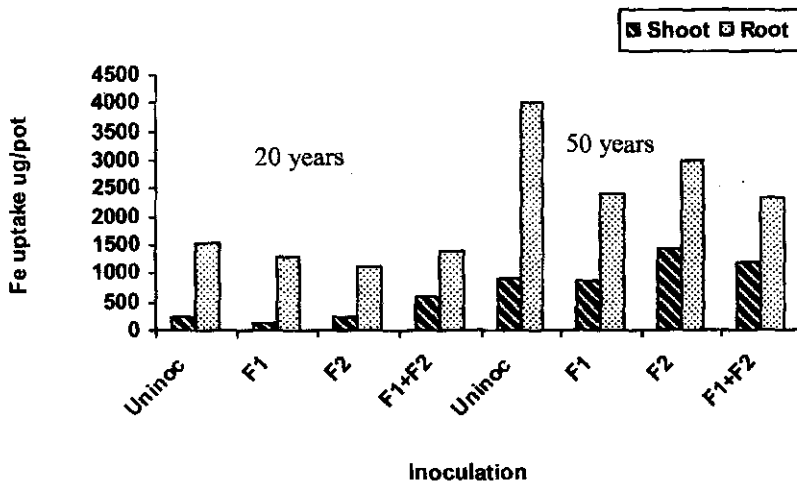
In conclusion, both effluent applications and fungal inoculation treatments have significant effects on iron uptake by canola plants. These effects were significantly varied according to interaction between treated soils and inoculation treatments.

Dealing with the uptake of Zn by canola shoots and roots, data presented in Table 1 and illustrated by (Fig. 10) showed that the inoculation treatments, in general, minimized the uptake of Zn by either shoot or root of canola plants.

Concerning Mn uptake by shoot and root of canola grown on soil treated with sewage effluent for 20 years (Table 1 and Fig. 11), data showed that Mn content of shoots and roots was frequently affected by fungal inoculation treatments according to the duration of sewage effluent irrigation. Higher reduction in Mn uptake was

Table 1. Effect of fungal inoculation on heavy metal uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of canola plants grown on contaminated soil

| Metals | Contaminated soil with sewage effluent for 20 and 50 years | | | | | | | | | | | | | | | |
|------------|--|--------|-------|--------|-------|--------|-------|--------|------------|--------|-------|--------|--------|--------|--------|--------|
| | 20 y | | | | | | | | 50 y | | | | | | | |
| | Un | | F1 | | F2 | | F1+F2 | | Un | | F1 | | F2 | | F1+F2 | |
| | Inoculated | | | | | | | | inoculated | | | | | | | |
| | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root | Shoot | Root |
| Fe | 260.1 | 1546.1 | 143.5 | 1306.3 | 247.0 | 1127.5 | 581.4 | 1380.6 | 917.3 | 4000.7 | 863.0 | 2402.1 | 1432.5 | 3008.4 | 1194.7 | 2340.0 |
| Zn | 84.1 | 101.9 | 68.3 | 89.3 | 59.5 | 80.0 | 63.5 | 87.0 | 177.8 | 192.4 | 158.1 | 105.7 | 212.0 | 155.4 | 171.9 | 152.8 |
| Mn | 276.3 | 221.7 | 217.7 | 109.4 | 203.0 | 142.4 | 214.8 | 186.9 | 425.6 | 221.7 | 408.1 | 166.4 | 593.1 | 268.0 | 390.9 | 227.8 |
| Cu | 95.8 | 120.1 | 78.6 | 91.4 | 61.5 | 88.2 | 78.0 | 95.2 | 224.7 | 198.9 | 205.7 | 127.6 | 257.4 | 150.6 | 252.7 | 142.8 |
| Co | 151.0 | 131.7 | 123.3 | 118.4 | 104.5 | 110.5 | 108.2 | 109.7 | 311.3 | 179.0 | 258.2 | 139.1 | 335.9 | 179.4 | 318.8 | 163.2 |
| Cd | 78.7 | 73.4 | 60.8 | 63.8 | 54.3 | 59.0 | 60.2 | 59.4 | 147.2 | 104.0 | 137.3 | 81.5 | 180.0 | 104.2 | 159.2 | 102.1 |
| LSD (0.05) | | Fe | | Zn | | Mn | | Cu | | Co | | Cd | | | | |
| Shoots | | 68.24 | | 20.73 | | 6.391 | | 10.51 | | 13.7 | | 17.01 | | | | |
| Roots | | 2.177 | | 14.99 | | 28.2 | | 30.78 | | 0.842 | | 0.620 | | | | |

F1: *Fusarium oxysporum*F2: *Aspirigellus paraciticus***Fig. 9. Effect of fungal inoculation on Fe uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of canola plants grown on contaminated soil**

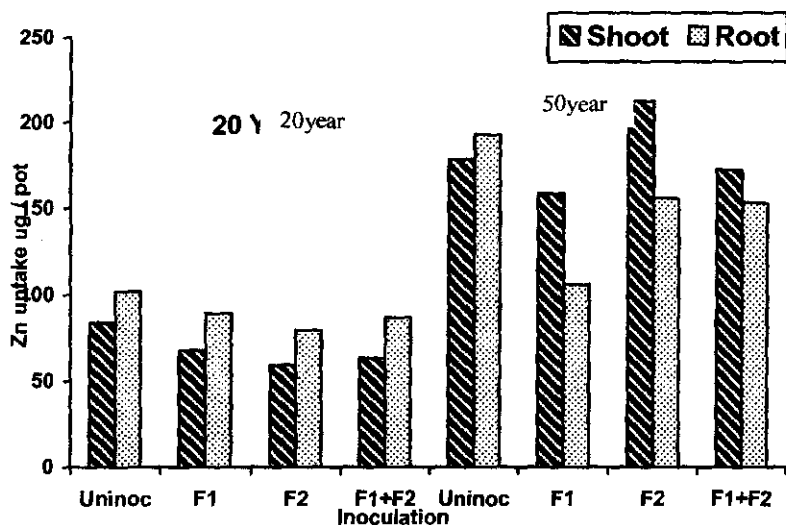


Fig. 10. Effect of fungal inoculation on Zn uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of canola plants grown on contaminated soil

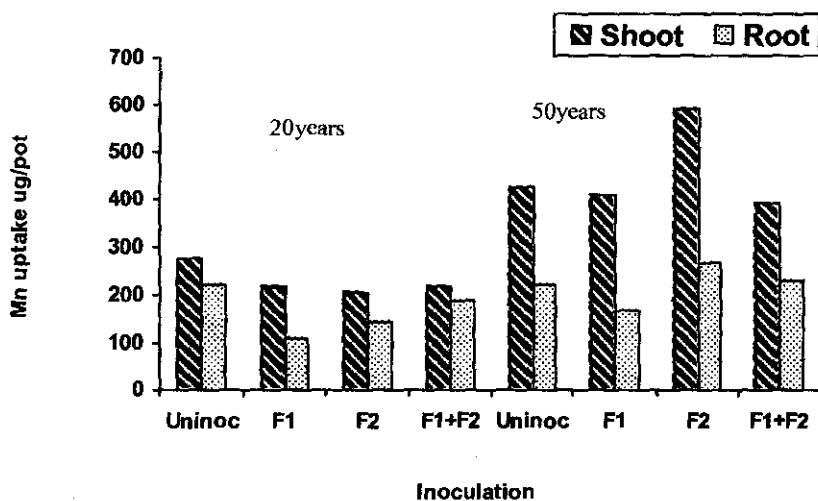


Fig. 11. Effect of fungal inoculation on Mn uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of canola plants grown on contaminated soil

noticed with 20 years of effluent irrigated soil than those recorded with soil irrigated for 50 years. Manganese, under the given conditions, has the ability to move upward from the root to the shoot system.

Data of copper (Cu) as illustrated by Fig. 12 indicated that Cu uptake by shoot and root was significantly affected, but in different directions, by fungal inoculation and in the same time frequently affected by the duration of sewage effluent irrigation.

Table 1 and Fig. 13 shows that cobalt accumulated by shoots and roots of canola grown on soil irrigated with effluent for 50 years was higher than those recorded with 20 years of irrigation. In most of the inoculation treatments, especially in case of 20 years of irrigation, Co uptake tended to decrease in both organs. It seems that the effect of inoculation treatments was dependent on the duration of sewage effluent application.

Concerning cadmium uptake by shoot and root of canola (Table 1 and Fig. 14) data showed that Cd accumulated by canola plants was higher under soil irrigated with effluent for 50 years than those

irrigated for 20 years and in the same time it localized, in most cases, in shoots comparing to roots. The role of fungal inoculation was more vigorous under soil treated with sewage effluent for 20 years than those treated for 50 years. It means that the effects of fungi were significantly correlated with effluent duration.

Data presented revealed that the heavy metals, in general, uptake by different crops (canola and clover) showed different patterns according to the fungal inoculation treatments and sewage effluent application duration in soil for each metal. Both reduction and increase in metals accumulation by crops were recognized. This indicated that the plants (phyto-) and fungi (bio-) act together as bioremediators. This phenomenon was confirmed when comparison was held between the metals concentrations in soil before cultivation and those remained in soil after crop harvest where a great reduction in metals content was detected either as total or available metals in soil. It means that bioremediation has positive and effective role in remediating such heavy metal contaminated soils.

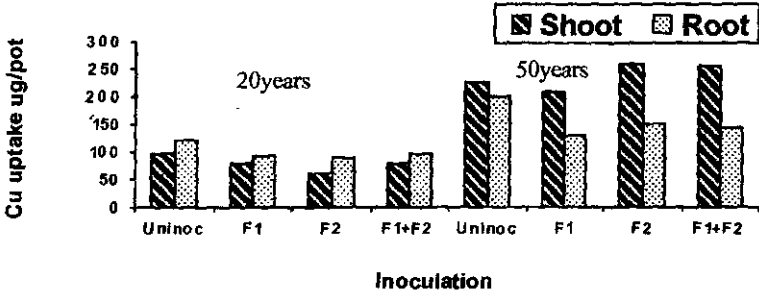


Fig. 12. Effect of fungal inoculation on Cu uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of canola plants grown on contaminated soil

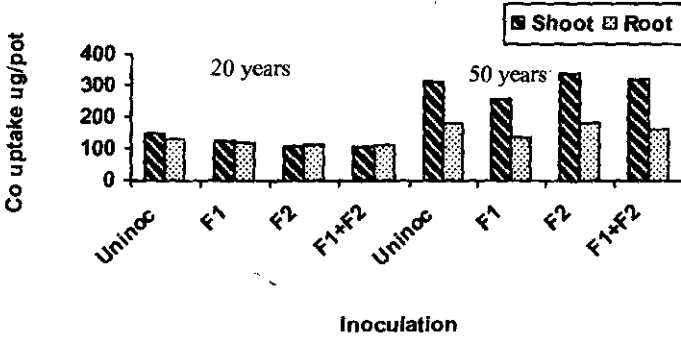


Fig. 13. Effect of fungal inoculation on Co uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of canola plants grown on contaminated soil

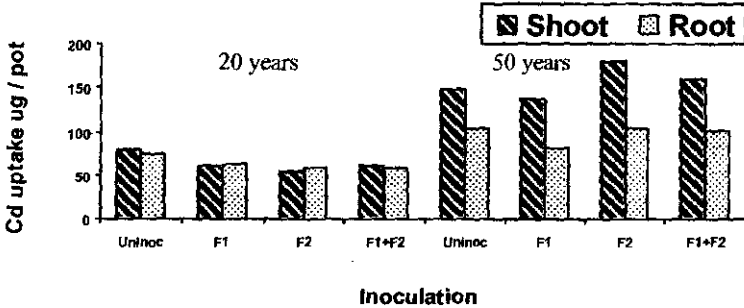


Fig. 14. Effect of fungal inoculation on Cd uptake ($\mu\text{g pot}^{-1}$) by shoots and roots of canola plants grown on contaminated soil

Recalling the data discussed in this section, it can be noticed that fungi, in general, could affect the plants grown on such heavy metals polluted soil in different ways. In this regard, the dry matter yield of both tested crops was negatively affected by fungi inoculation and this phenomenon is on line with those obtained by Abdel Aziz (2004).

Results have been suggested to use canola inoculated with *P. fluorescens* as a promising strategy to remediate metal contaminated lands. These results are, more or less in agreement with the results obtained by Ashour *et al.* (2006) indicated that the presence of Ni in irrigation water significantly promoted canola biomass. Whereas, enhancement of canola growth as a response of *Pseudomonas* inoculants was not significant. Presence of Ni at 2 mM produced plants containing more than tenfold of Ni relative to zero Ni plants.

Similarly, results with the present research are partially in agreement with Azcon *et al.* (2009) which reported that the plant growth, nutrient acquisition, metal translocation and antioxidant activities, were measured in plants growing in a heavy-metal (HM) multicontaminated soil inoculated

with selected autochthonous microorganisms, [arbuscular mycorrhizal (AM) fungus and/or plant growth promoting bacteria (PGPB)] and/or amended with an *Aspergillus niger*-treated agro waste. Also, they found that the treated agro waste on its own increased root growth by 296% and shoot growth by 504% compared with non-treated control plants. Both chemical and biological treatments, particularly when combined, enhanced plant shoot and root development. The stimulation effect on plant biomass was concomitant with increased AM colonization, P and K assimilation, and reduced metal translocation from soil to plant shoot.

Heavy Metals Remained in Soil after Harvest

The contents of heavy metals in soil after harvest of clover and canola plants were presented in Tables 3 and 4. Considering the available form of all studied microelements remained in the soil after harvest of clover Table 3 and canola Table 4 as compared to those detected in the tested soil before cultivation Table 2 we can conclude that both the plants and fungi played a significant role in absorption of considerable amounts of such microelements and in the same time, reduces its contents in the soil solution.

Table 2. Heavy metals ($\mu\text{g g}^{-1}$) content in soil before cultivation

| SOIL | Fe | | Zn | | Mn | |
|------|-------|--------|------|--------|------|--------|
| | Tot. | Avail. | Tot. | Avail. | Tot. | Avail. |
| 20 y | 10252 | 26.48 | 2562 | 27.31 | 2753 | 53.92 |
| 50 y | 10485 | 63.67 | 5100 | 64.16 | 7167 | 73.30 |
| | | Cu | | Co | | Cd |
| 20 y | 482 | 6.97 | 419 | 11.46 | 136 | 2.96 |
| 50 y | 820 | 9.33 | 477 | 11.73 | 206 | 5.61 |

Table 3. Heavy metals ($\mu\text{g g}^{-1}$) remained in soil after harvest of clover plants

| Clover | Soil irrigated for 20 years | | | | | | | | |
|-----------------|-----------------------------|--------|-----------------------------|--------|-------|--------|-------|--------|--|
| | Uninoculated | | F1 | | F2 | | F1+F2 | | |
| $\mu\text{g/g}$ | Tot. | Avail. | Tot. | Avail. | Tot. | Avail. | Tot. | Avail. | |
| Fe | 193.2 | 4.3 | 156.2 | 4.8 | 151.6 | 4.6 | 155.1 | 4.0 | |
| Zn | 6.8 | 0.2 | 6.8 | 0.3 | 6.4 | 0.2 | 6.6 | 0.2 | |
| Mn | 2.6 | 0.4 | 3.4 | 0.3 | 3.1 | 0.4 | 3.7 | 0.3 | |
| Cu | 5.8 | 0.2 | 5.4 | 0.3 | 5.9 | 0.3 | 8.4 | 0.3 | |
| Co | 14.3 | 1.5 | 14.3 | 1.2 | 14.7 | 1.4 | 10.1 | 1.0 | |
| Cd | 1.9 | 0.02 | 1.9 | 0.02 | 1.9 | 0.03 | 1.9 | 0.02 | |
| | | | Soil irrigated for 50 years | | | | | | |
| Fe | 217.7 | 12.5 | 203.0 | 10.6 | 123.8 | 11.7 | 202.4 | 10.4 | |
| Zn | 8.8 | 0.6 | 7.9 | 0.5 | 8.8 | 0.6 | 8.7 | 0.7 | |
| Mn | 5.9 | 0.5 | 5.6 | 0.5 | 6.0 | 0.6 | 6.6 | 0.5 | |
| Cu | 28.6 | 0.5 | 25.8 | 0.4 | 30.3 | 0.5 | 26.7 | 0.5 | |
| Co | 14.3 | 1.7 | 15.3 | 1.4 | 11.4 | 1.5 | 14.3 | 1.7 | |
| Cd | 1.9 | 0.04 | 1.9 | 0.03 | 2.0 | 0.1 | 1.9 | 0.1 | |
| LSD (0.05) | | Fe | Zn | Mn | Cu | Co | Cd | | |
| Total | | 53.8 | 1.49 | 1.3 | 8.4 | 2.8 | 0.18 | | |
| Available | | 0.14 | 0.16 | 0.08 | 0.23 | 0.25 | 0.23 | | |

Table 4. Heavy metals ($\mu\text{g g}^{-1}$) remained in soil after harvest of canola plants

| Canola | Soil irrigated for 20 years | | | | | | | | |
|-----------------|-----------------------------|--------|-----------------------------|--------|-------|--------|-------|--------|--|
| | Un inoculated | | F1 | | F2 | | F1+F2 | | |
| $\mu\text{g/g}$ | Tot. | Avail. | Tot. | Avail. | Tot. | Avail. | Tot. | Avail. | |
| Fe | 193.7 | 5.62 | 151.4 | 4.80 | 100.1 | 4.10 | 81.1 | 6.49 | |
| Zn | 7.2 | 0.34 | 7.0 | 0.28 | 7.0 | 0.21 | 6.1 | 0.20 | |
| Mn | 4.3 | 0.42 | 4.0 | 0.40 | 4.4 | 0.26 | 4.5 | 0.27 | |
| Cu | 6.8 | 0.36 | 6.3 | 0.31 | 6.3 | 0.30 | 6.2 | 0.29 | |
| Co | 13.3 | 1.48 | 14.8 | 1.58 | 10.5 | 1.49 | 10.4 | 1.32 | |
| Cd | 2.0 | 0.02 | 2.0 | 0.02 | 2.0 | 0.02 | 2.0 | 0.02 | |
| | | | Soil irrigated for 50 years | | | | | | |
| Fe | 178.3 | 12.54 | 141.9 | 15.32 | 168.8 | 11.47 | 209.3 | 10.32 | |
| Zn | 8.7 | 0.64 | 8.7 | 0.67 | 8.8 | 0.68 | 8.2 | 0.55 | |
| Mn | 6.7 | 0.48 | 7.2 | 0.46 | 6.2 | 1.79 | 7.0 | 0.48 | |
| Cu | 27.8 | 0.65 | 28.7 | 0.52 | 28.5 | 0.53 | 16.4 | 0.49 | |
| Co | 10.3 | 1.31 | 13.8 | 1.69 | 14.8 | 1.26 | 14.4 | 1.49 | |
| Cd | 2.0 | 0.03 | 2.0 | 0.04 | 2.0 | 0.04 | 2.0 | 0.03 | |
| LSD (0.05) | | Fe | Zn | Mn | Cu | Co | Cd | | |
| Total | | 65.3 | 1.2 | 1.29 | 4.24 | 3.35 | 0.14 | | |
| Available | | 0.22 | 0.22 | 0.23 | 0.23 | 0.22 | 0.25 | | |

F1: *Fusarium oxysporum*F2: *Aspirigellus paraciticus*

Therefore, from our viewpoint, we can accept this combination of bioremediators including phyto-plants and fungi as remediators for cleaning such polluted soils. Similar trends were noticed when considering the total content of tested microelements.

Gadd 2004 stated that the presence of metals in soils, and their potential input in food chain, always raise the questions relating to land use and need for reclamation. Total concentrations of heavy metals as well as their bioavailability should be taken into consideration in planning a reclamation strategy for contaminated lands. Various reclamation methods are generally based on two opposite schemes aimed either to remove metals from soil or to effectively stabilize them by decreasing their mobility and availability. Both strategies have to consider the complex knowledge about biotic and non-biotic factors that may affect metal solubility and change their speciation. Alternatively, metal stabilization may be in many cases good enough to eliminate serious environmental risk. Direct and indirect antagonistic actions of nonpathogenic *Fusarium oxysporum* strains (including nutrient competition, antibiosis, and the induction of localized and

systemic plant defenses responses) are responsible for the control of *Fusarium* wilts in suppressive soils from different areas in the world (Fravel, Olivain and Alabouvette 2003).

Rhizospheric microorganisms may interact symbiotically with roots to enhance the potential for metal uptake. In addition, some microorganisms may excrete organic compounds which increase bioavailability, and facilitate root absorption of essential metals, such as Fe (Crowley *et al.*, 1991) and Mn (Barber and Lee, 1974) as well as nonessential metals, such as Cd (Salt *et al.*, 1995). Soil microorganisms can also directly influence metal solubility by altering their chemical properties. (Blake *et al.*, 1993 and Park *et al.*, 1999).

On the other hand, Sayer and Gadd (2001) demonstrated that citric and oxalic acid and the *A. niger* culture filtrates can bind Co^{2+} and Zn^{2+} and in some cases, the culture filtrates were more efficient than commercial organic acids. Gluconic acid did not bind Co^{2+} or Zn^{2+} under the conditions used in this study. The presence of insoluble metal phosphates in the growth medium was found to markedly influence the production of organic acids and, while large concentrations of gluconic acid

were produced in the presence of $\text{CO}_3(\text{PO}_4)_2$, the culture filtrates was unable to bind Zn^{2+} . The production of oxalic acid by *A. niger* when grown in the presence of $\text{Zn}_3(\text{PO}_4)_2$ led to the precipitation of insoluble zinc oxalate, a phenomenon with implications for metal tolerance and toxicity.

In the context of bioremediation, Gadd (2004) stated that solubilization of metal contaminants provides a means of removal from solid matrices, such as soils, sediments, dumps and other solid industrial wastes. Alternatively, immobilization processes may enable metals to be transformed in situ and are particularly applicable to removing metals from aqueous solution. This contribution will outline selected microbiological processes which are of significance in determining metal mobility and which have actual and potential application in bioremediation of metal pollution. These include autotrophic and heterotrophic leaching mechanisms, reductive precipitation, sulfate reduction and metal sulfide precipitation.

In conclusion, it can be said that the mechanisms of fungal solubilization and immobilization of metal (loid)s and related substances are of potential for

bioremediation. While biosorption has received little development in an industrial context, work on metal leaching from contaminated matrices such as soil, metal (loid) transformation and bioprecipitation shows promise of development as well as providing fundamental scientific insights into metal-microbe interactions. In addition to the biotechnological significance, it should be emphasized that this work also provides understanding of the roles of fungi in affecting metal mobility and transfer between different biotic and abiotic locations and their importance in the bio-cleaning of metal (loid) in the environment (Gadd 2001).

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المعالجة الحيوية للأراضي المروية بمياه الصرف الصحي لفترات طويلة

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أجريت تجربة أصص في الصوبة لمتابعة أثر التلقيح الفطري على المعالجة الحيوية للمعادن الثقيلة للأرض الملوثة بمياه الصرف الصحي لعدد من السنين. تم زراعة محاصيل الكانولا والبرسيم كمحاصيل التجربة المحبة للمعادن الثقيلة في نوعين من الأراضي المروية بمياه الصرف الصحي لمدة ٢٠ ، ٥٠ سنة مع التلقيح الفطري بواسطة فيوسيريام اوكسيسيريام (F١)، اسبيريجلاس (F٢) بالإضافة الى معاملة خليط بينهما (F١+F٢). أشارت نتائج التجربة الى زيادة معنوية في وزن المادة الجافة لمحاصيل التجربة وذلك في حالة الري لمدة ٥٠ سنة بمياه الصرف الصحي مقارنة بالأرض المعاملة لمدة ٢٠ سنة. كذلك وجد أن التلقيح الفطري أدى الى تحسن ملموس على امتصاص المعادن الثقيلة باختلاف أجزاء النباتات. واختلفت هذه الظاهرة في حالة الأراضي المعاملة بمياه الصرف الصحي لمدة ٥٠ سنة مقارنة بالأراضي المعاملة لمدة ٢٠ سنة ولكن وجد اختلاف في حالة تركيز بعض المعادن (الزنك) في سيقان أو جذور النباتات المعاملة.