J. Agric. Sci. Mansoura Univ., 30 (2): 1233 - 1247, 2005

EFFECT OF INOCULATION OF MAIZE BY ARBUSCLAR MYCORRHIZAL FUNGI ON THE AVAILABILITY OF NUTRIENTS AND BIOREMEDIATION OF HEAVY METALS IN NEWLY RECLAIMED SOILS

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ABSTRACT

The objectives of the present study were to select the best management of sewage sludge and phosphorus fertilizer for maize (*Zea mays* L.) under mycorrhizal inoculation in newly reclaimed calcareous soil and to evaluate the role of mycorrhizal fungi in combination with sewage sludge and phosphorus fertilizer on maize growth and validate the reduction of heavy metals uptake by plants against the mycorrhizal infection. Field experiment was conducted in Maryuot research station (km 37 Alexandria - Cairo desert road) on a sandy clay loam soil. Four levels of sewage-sludge (0.0, 6.0, 12.0, and 18.0 ton ha^{-1}) and three rates of phosphorus fertilizer (0.0, 36, and 72 kg P_2O_5 ha^{-1}) were applied. Some plots inoculated with mycorrhizal spores and others were without inoculation. Plant samples were collected at harvest, shoot and root dry weight; as well, shoot, root and ear leaf phosphorus concentration and heavy metal (i.e. Zinc, Cadmium, Nickel and Lead) were determined. In addition, ear yield was determined at the end of season. Shoot dry weight (SDW) showed significant increase with mycorrhizal inoculation than un-inoculation treatments under all applications of sewage sludge and phosphorus fertilizer. Root dry weight (RDW) increased with all sludge and phosphorus treatments in either un-inoculated or inoculated treatments. Shoot Dry Matter (SDM) increased with increases of sludge and phosphorus applications in both inoculated and un-inoculated treatments. MD of SDM decreased with increasing sludge applications under the three phosphorus applications. Root Dry Matter (RDM) showed, in general an increase with increasing sludge and phosphorus applications in both inoculated and un-inoculated treatments.

The maximum ear yield in un-inoculated and inoculated treatments was obtained with S_1 (6.0 ton ha^{-1} of sewage sludge) and P_2 treatments (72.0 kg P_2O_5 ha^{-1}). Shoot-P concentration increased with increases of sludge or phosphorus treatments. In general, the maximum shoot-P concentration was obtained under S_3 P_2 treatments. Root-P concentration takes the same trend of shoot-P concentration over the control treatment. The maximum root-P concentration was obtained under S_3 P_2 treatments. Increasing sludge application rates increased significantly the shoot, root and ear leaf-Zn, Cd, Ni and Pb concentrations. Also, increase of P applications decreased concentrations of Zn in shoot and ear leaf, while Cd concentration increased with P increases in inoculated treatments. Plants inoculated with AMF, have lower Zn, Cd, Ni and Pb concentrations in shoot and ear leaf but higher in root-Zn concentration. R/S ratio of Zn, Cd, Ni and Pb concertinos were higher in inoculated plants than in un-inoculated plants which means the ability of root tissues to accumulate Zn, Cd, Ni and Pb and consequently low Zn, Cd, Ni and Pb translocation from root to shoot and hence to maize grains.

Keywords: Sewage sludge, Arbuscular mycorrhiza, Heavy metals, Bioremediation.

INTRODUCTION

Beneficial plant–microbe interactions in the rhizosphere are primary determinants of plant health and soil fertility. Arbuscular mycorrhiza are the most important microbial symbioses for the majority of plants and, under conditions of P-limitation, influence plant community development, nutrient uptake, water relations and above-ground productivity. Attention is paid to the conservation of biodiversity in arbuscular mycorrhizal fungi (AMF). Examples are provided in which the ecology of AMF has been taken into account and has had an impact in landscape regeneration, horticulture, alleviation of desertification and in the bioremediation of contaminated soils. It is vital that soil scientists and agriculturalists pay due attention to the management of AMF in any schemes to increase, restore or maintain soil fertility (Jeffries *et al.*, 2003)

Sewage sludge can be used beneficially on land as a soil conditioner and fertilizer, in a variety of ways. The utilization of sewage sludge in agriculture is widely discussed. Application rates of sewage sludge are generally based on fertilizer requirements of crops. Landfill and incineration of sewage sludge deprives soils low in organic matter content of a potential source of organic material. On the one hand, the content of nutrients like nitrogen, phosphorous and organic matter should be recycled by land application but, because of contamination with pollutants, acceptance is low. Among these pollutants, the presence of substantial amounts of heavy metals is well established.

The natural background level of heavy metals depends strongly on the origin of the soil and soil characteristics, since metals occur naturally in the earth's crust. The contamination of soils with heavy metals is caused by atmospheric deposition or by direct disposal on the soil. The direct disposal of heavy metals on soil includes industrial waste disposal, impurities in fertilizers and manure, sewage sludge and pesticides containing heavy metals. The use of sewage sludge is commonly found on cultivated land (Baize *et al.*, 2001).

In the last few decades, concern for environmental contamination prompted a rethinking of the concept of utilization of sludge on land. Agricultural utilization of sewage sludge has the potential benefit of utilization of the nutrient and organic matter for crop production and soil management. Added benefits are saving in energy consumption, prevention of air pollution in the case of incineration and reduction in water contaminated in the case of ocean or lake dumping. But until recently, inadequate information existed for safe, efficient use of sludge on land for crop production (Clapp *et al.*, 1994).

The overall goal of the present research was to develop efficient sewage sludge and phosphorus fertilizer applications under inoculation with arbuscular mycorrhizal fungi. At the same time, to minimize negative environmental impacts including uptake of heavy metals by plants.

MATERIALS AND METHODS

Experimental layout

A field experiment was carried out at Maryuot research station (km 37 Alexandria - Cairo desert road) to study the effect of inoculation with A-mycorrhiza spores, and application of sewage sludge, and phosphorus fertilizer on Maize (*Zea mays* L.), hybrid 310 variety, yield and shoot, root, and 5th leaf contents of phosphorus, zinc, nickel, cadmium, and lead.

Surface soil composite sample (0-20 cm) were collected from the experimental site, air dried and ground to pass 2 mm sieve and kept for analysis. The main physical and chemical properties of the soil were measured as described by Klute (1986) and Page *et al.* (1982) and the results obtained are given in Table (1).

Table (1): The mean chemical and physical characteristics of the used soil.

Characteristic		Mean value
pH*		7.80
EC*	dS m^{-1}	4.30
Total $CaCO_3$	%	31.70
Organic Carbon	%	0.66
Total Nitrogen	%	0.09
C/N ratio		7.33
Available Phosphorus	mg kg^{-1}	8.40
Bulk Density	Mg m^{-3}	1.55
Particle size distribution:		
Clay	%	23.20
Silt	%	21.70
Sand	%	55.10
Texture		Sandy Clay Loam

*** Measured in soil water past extract**

Split-Split statistical design of the field experiment was carried out at the experimental site Maize (*Zea mays* L) hybrid 310 variety was cultivated. The experimental layout consisted of 72 plots (four levels of sewage sludge X three rates of phosphorus fertilizer X inoculated and uninoculated with mycorrhiza X three replicates) and each plot size was 2 m x 3 m. Four levels of sewage sludge S_0, S_1, S_2 and S_3 (0, 6, 12, and 18 ton ha^{-1}) and three levels of phosphorus P_0, P_1 and P_2 (0, 36, and 72 kg P_2O_5 ha^{-1}) were applied as superphosphate fertilizer (15.5 % P_2O_5). All plots received 238 kg N ha^{-1} as ammonium nitrate (33.5 % N) in two equal doses at 21 and 40 days after sowing. Potassium fertilizer was applied to all plots after 41 days from sowing with 57 kg K_2O ha^{-1} as potassium sulfate (48 % K_2O). Some plots were inoculated with mycorrhizal spores and others were without inoculation.

Sewage sludge, used in the experiment was obtained from Station 9 N (Amryia district), Alexandria General Organization for Sanitary Drainage (AGOSD). The main characteristies of the sewage sludge analysis is given in Table (2).

Separation of mycorrhiza

Spores of mycorrhiza were isolated by wet-sieving and decantation method as described by Gerdemann and Nicolson (1963), from the soil surface of the experimental area is at Maryout research station. Mycorrhizal spores and mycelium segments were obtained by wet-sieving of soils and the soil fraction between 50 and 140 mesh was collected. The inoculums were mixed thoroughly with acid-washed sand, placed below seeds in the field experiment for inoculation.

Table (2): The main characteristic of the used composted sewage sludge.

Characteristic		Mean value
pH		6.78
EC	dS m^{-1}	5.12
Organic matter	%	39.64
Total Nitrogen	%	1.85
Total Phosphorus	%	1.02
Total Potassium	%	0.31
C / N ratio		12.4
Total heavy metals	ton kg^{-1}	
Mn		131.00
Fe		908.00
Zn		821.00
Cu		482.00
Ni		141.00
Cd		10.60
Pb		182.00
Cr		104.00

Plant sampling and preparation

The maize plants were sampled at harvest stage, divided into roots, shoots, ear leaf and grains yield The wet and dry weight of roots and shoots were measured. Roots and shoots were fully washed with tap water to remove the adhering soil particles then were washed with distilled water. The washed plant materials were dried at 80 °C for 48 hrs, weighed and cut into small pieces and ground using stainless steal mill. The root hairs were separated for determination of mycorrhizal root colonization. The staining method of Phillips and Hayman (1970) was used for preparing root samples for microscopic examination. The gridlines intersect method of Giovannetti and Mosse (1980) was used to estimate the A-mycorrhizal infection percentage.

The oven-dried plant material was wet digested by introducing 0.5 g of the oven dried plant material in 50 ml volumetric flask and digested with 2.5 ml conc. H_2SO_4 on a hotplate at approximately 270 °C. Small quantities of H_2O_2 were added repeatedly until the digest remains clear, let to cool then was diluted to 50 ml with distilled water (Cottenie, 1980). Certified reference sample was included in the analyzed. The digested samples were analyses for total phosphorus, zinc, lead, nickel and cadmium. The vanadomolybdate calorimetric method of Jackson (1967) was used to measure total P using spectrophotometer. The concentrations of trace elements (zinc, nickel, cadmium, and lead) were measured by atomic absorption spectrophotometer (Perkin Elmer 2380) in an air acetylene flame.

Mycorrhizal dependency (MD) which expresses the relative increase in plant growth due to mycorrhizal infection is calculated from the difference of SDM between mycorrhizal and un-mycorrhizal plants divided by SDM of un-mycorrhizal plants multiplied by 100.

Soil analysis

Soil paste water extract was obtained according to Page *et al.* (1982). Main soil chemical characteristics are presented in Table (1). pH and EC were measured (Page *et al.*, 1982) and total calcium carbonate ($CaCO_3$ %) was determined using the Calcimeter method (Page *et al.*, 1982). Organic carbon (OC %) was determined using Walkely and Black method (Page *et al.*, 1982). Total-N was measured using micro kjeldahl method (Page *et al.*, 1982). Available phosphorus was extracted by sodium bicarbonate and measured calorimetrically using spectrophotometer (Olsen and Sommers, 1982). The determination of particle size distribution of the soil was carried out using dry sieving for particles more than 0.025 mm in diameter and hydrometer method for particles less than 0.025 mm in diameter.

Data statistical analysis

Statistical analyses of the obtained data were carried out using ANOVA procedure and the comparison means where tested using least significant differences analysis (SAS, 1990).

RESULTS AND DISCUSSION

Root and Shoot Dry Weight (RDW & SDW)

Data in Table 3 showed, generally, significant increase with mycorrhizal inoculation treatment than with un-inoculation treatment under all applications of sewage sludge and phosphorus treatments. At the highest sludge application (18 ton ha^{-1}), SDWs of un-inoculated plants were 6.07, 7.11 and 7.34 ton ha^{-1} for P_0, P_1 and P_2, respectively. These treatments increased the SDW by 16.62, 36.53 and 40.44 % relative to the control treatment. SDW of inoculated plants were 6.42, 7.59 and 7.78 ton ha^{-1} for P_0, P_1 and P_2, respectively. Under these conditions the SDW increased by 23.27, 45.74 and 49.39 % relative to the control treatment. The most effective treatment causing higher increase in SDW was S_2P_2 for un-inoculated (58.7 %) and for inoculated treatment (70.2 %). These results showed that the gradual release of nutrients from sludge could be the reason of high increase of SDW in un-inoculated treatment. On the other hand, the increase of SDW in inoculated treatment may be due to the gradual release of nutrients from sludge in addition to the role of mycorrhiza in increasing availability of nutrients to the plants. The data of SDW also indicated that increasing application of sludge would reduce the role of mycorrhiza and then the SDW was decreased.

Data in Table 3 showed that RDWs were increased, generally, in all sludge and phosphorus treatments either with un-inoculated or inoculated treatments when compared to control treatment S_0P_0. The RDWs of the un-inoculated plants were increased with increasing phosphorus application from P_0, P_1 to P_2 as 1.24, 1.29 and 1.34 ton ha^{-1} for S0 treatment; 1.30, 1.59 and 1.68 ton ha^{-1} for S_1 treatment; 1.32, 1.50 and 1.53 ton ha^{-1} for S_2 treatment; and 1.36, 1.42 and 1.52 ton ha^{-1} for S_3 treatment. The RDW of the inoculated plants was increased with increasing sludge and phosphorus treatments when compared to the control treatment S_0P_0. The relative increase RDWs of un-inoculated plants were 0, 4.25 and 7.91 % while, in inoculated plants were 16.77, 18.38 and 20.8 % for P_0, P_1 and P_2 with S_0 and the same results were obtained at S_1, S_2 and S_3. This data are supported by the results of a number of workers who reported that added inoculum enhanced plant growth beyond the response to the indigenous fungal population in soils (Chandrashekara *et al.*, 1995).

Table (3): Effect of arbuscular mycorrhiza inoculation on maize growth (ton ha^{-1}) and on mycorrhizal infection percentage (%) at different sludge and phosphorus treatments.

Treatment		Un-inoculated				Inoculated			
Sludge	Phos.	Shoot	Root	R/S	Infec.	Shoot	Root	R/S	Infec.
S_0	P_0	5.21 c	1.24 c	0.24	2	6.48 c	1.45 c	0.22	10
	P_1	6.01 b	1.29 b	0.22	11	7.35 b	1.47 b	0.20	27
	P_2	6.22 a	1.34 a	0.22	9	7.47 a	1.50 a	0.20	15
	Mean	*5.81 D*	*1.29 D*	*0.22*	*7*	*7.10 D*	*1.47 D*	*0.21*	*17*
S_1	P_0	6.91 c	1.30 c	0.19	16	7.75 c	1.42 c	0.18	45
	P_1	7.05 b	1.59 b	0.23	43	7.88 b	1.73 b	0.22	52
	P_2	7.38 a	1.68 a	0.23	22	8.14 a	1.81 a	0.22	42
	Mean	*7.11 A*	*1.52 A*	*0.21*	*27*	*7.92 A*	*1.65 A*	*0.21*	*46*
S_2	P_0	6.66 c	1.32 c	0.20	28	7.22 c	1.40 c	0.19	58
	P_1	7.30 b	1.50 b	0.21	29	7.82 b	1.59 b	0.20	49
	P_2	7.66 a	1.53 a	0.20	16	8.04 a	1.61 a	0.20	40
	Mean	*7.21 B*	*1.45 C*	*0.20*	*24*	*7.69 B*	*1.53 C*	*0.20*	*49*
S_3	P_0	6.07 c	1.36 c	0.22	30	6.42 c	1.41 c	0.22	43
	P_1	7.11 b	1.42 b	0.20	29	7.59 b	1.49 b	0.20	41
	P_2	7.34 a	1.52 a	0.21	19	7.78 a	1.58 a	0.20	29
	Mean	*6.84 C*	*1.43 B*	*0.21*	*26*	*7.26 C*	*1.49 B*	*0.21*	*38*

		Shoot	Root
L.S.D.$_{0.05}$	**for inoculation**	**0.103**	**0.0151**
	for sludge	**0.027**	**0.0047**
	for phosphorus	**0.021**	**0.0046**

Root / Shoot (R/S) ratio is a very important parameter to describe the affinity or efficiency of root system for water and nutrient uptake and physiological function on shoot growth. Most of inoculation treatments has lower R/S ratio when compared to un-inoculation treatments. The higher R/S ratio means that the higher root surface area for water and nutrient uptake

(Table 3). The mycorrhizal infection percentage of maize under different sludge and phosphorus fertilizer rates (Table, 3) showed that the inoculated treatments have higher percentage of infection than un-inoculated treatments and the higher infection percentage was observed under sludge treatment S_2. In general, phosphorus treatment P_1 gave the higher percentage of infection under un-inoculated and inoculated treatments.

Table (3) showed that the mycorrhizal infection percentage of maze under different sludge and phosphorus fertilizer rates. The inoculated treatments have higher percentage of infection than un-inoculated treatments. In general, the higher infection percentage was observed in inoculated plants under sludge treatment S_2 (12 ton ha^{-1}). Nurlaeny *et al.*, (1996) found that mycorrhizal inoculation significantly increased shoot dry weight, which could be due to the role of mycorrhiza to enhance plant uptake of minerals such as P, Zn and N (Roland, 1994). On the other hand, it was reported that reestablishment of arbuscular mycorrhiza fungi is inhibited in sewage sludge amended soils (Weissenhorn *et al.*, 1995). In addition, Angle and Heckman (1986) suggested that elements often abundant in sludge, such as N and heavy metals, could reduce infection and sporulation of mycorrhizal fungi.

Shoot dry matter (SDM) generally increase with increasing sludge and phosphorus applications in both inoculated and un-inoculated treatments. At the same time, it is observed that the increase of SDM in inoculated treatments was higher than in un-inoculated treatments.

Mycorrhizal dependency (MD) of SDM was decreased with increase of sludge applications under the three phosphorus applications. At the same time, increases of the phosphorus applications also decrease the MD of SDM under four sludge applications (Figure 1).

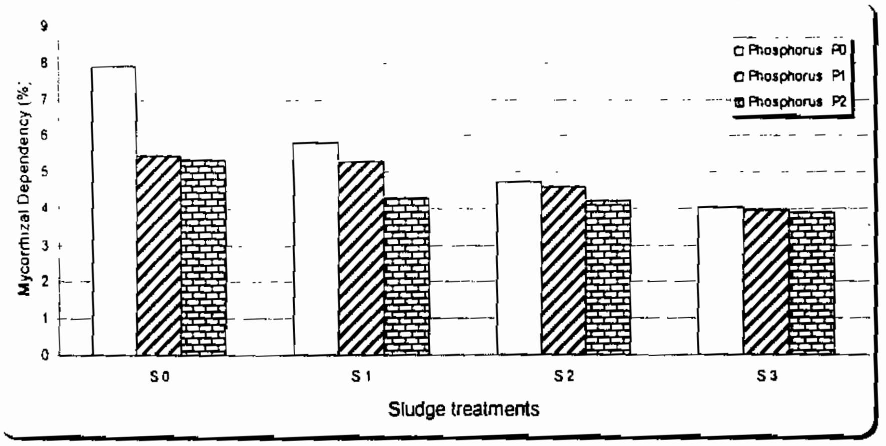

Fig. (1): Mycorrhizal dependency (MD) in shoot dry matter (%) under different sludge and phosphorus treatments.

Increasing the sludge applications from S_0 to S_1, S_2 and S_3 under the three phosphorus applications decrease the MD of SDM from 7.24 to 5.14, 4.53 and 3.97 %, respectively. In addition, increasing of phosphorus applications from P_0 to P_1 and P_2 under the four sludge applications decrease the MD of SDM from 6.38 to 4.83 and 4.44 %, respectively.

Data of root dry matter (RDM) showed, generally, an increase of RDM with increasing of sludge and phosphorus applications in both inoculated and un-inoculated treatments and it has the same trend data of SDM.

Mycorrhizal dependency (MD) of RDM was decreased with increasing sludge applications under the three doses of phosphorus applications. At the same time, increasing of the phosphorus applications also decrease the MD of RDM under the rate of four sludge applications (Figure 2). Increasing the sludge applications from S_0 to S_1, S_2 and S_3 under the three phosphorus applications decreased the MD of RDM from 5.32 to 5.20, 5.10 and 4.83 %, respectively. In addition, increasing of phosphorus applications from P_0 to P_1 and P_2 under the four sludge applications decrease the MD of RDM from 5.40 to 5.26 and 4.67 %, respectively (Figure 2).

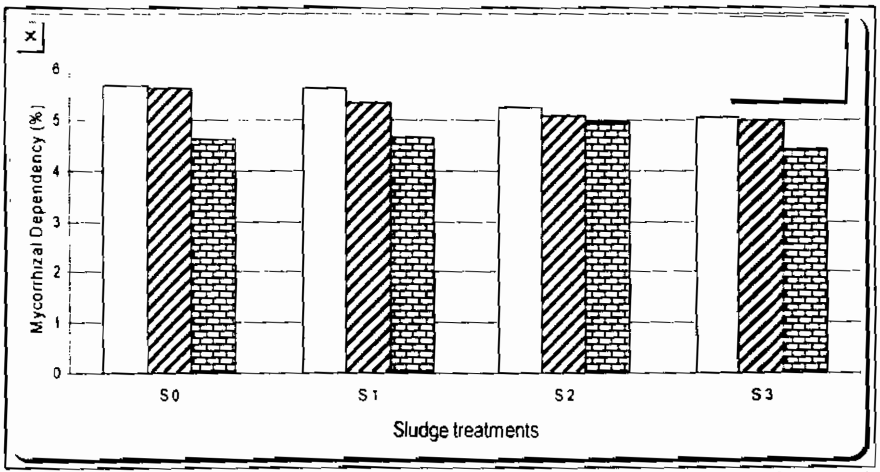

Fig. (2): **Mycorrhizal dependency (MD) in root dry matter (%) under different sludge and phosphorus treatments.**

Ear Yield

Data of ear yield as affected by sludge and phosphorus treatments are given in Table (4). There was an increase response to sludge applications and the maximum significant yield of inoculated and un-inoculated plants was obtained with S_2 treatment (12 ton ha^{-1} of sewage sludge). Decrease in sludge application decreased the ear yield. Therefore, the mean yields of un-inoculated treatments were 7.71, 8.74, 9.37 and 9.20 ton ha^{-1}; while mean yields of inoculated treatments were 8.70, 9.61, 10.16 and 9.90 ton ha^{-1}. Data also indicated that the increase of phosphorus treatments from P_0, P_1 to P_2 increased the ear yield in either the un-inoculated or the inoculated treatments (Table 4). As phosphorus increased from 0, 36 to 72 kg of P_2O_5 ha^{-1} at S_2, the yield was increased from 8.467, 9.455 and 10.186 ton ha^{-1} to 9.55, 10.121 and 10.8 ton ha^{-1} for un-inoculated and inoculated treatments, respectively. This may indicate that S_1 and P_2 treatments were enough to fulfill the plant requirements for all stages of the growing crop. Hence, the excess of sludge application rates did not contribute significantly to the maize yield production.

Mycorrhizal dependency (MD) of the ear yield indicates that the best mycorrhizal activity was achieved under S_0 sludge treatment and P_0 phosphorus treatment (Table 4). An increase of sludge applications from S_0 to S_2 increased the yield by 21.5 % and 16.8 % for un-inoculated and inoculated treatments, respectively. Accordingly, MD was decreased by 33.75 %. Also, an increase of phosphorus applications from P_0 to P_2 increased the yield by 16.7 % and 10.1 % for un-inoculated and inoculated treatments, respectively. Therefore, MD was decreased by 47.7 %.

Mycorrhizal dependency (MD) of the ear yield indicated that, the maximum mycorrhizal activity was reached under S_0 and P_0. For that reason the increase of sludge or phosphorus applications had declined the role of mycorrhiza. The depression effect of phosphorus was greater than that of sludge. In general, ear yield of inoculated treatments was higher than un-inoculated treatments. Clark (1997) found that arbuscular mycorrhizal fungi often enhance host plant growth and mineral acquisition, particularly for plant grown under low nutrient and mineral stress conditions.

Table (4): Effect of arbuscular mycorrhiza inoculation on maize grain yield (ton ha^{-1}) at different applications of sludge and phosphorus.

Treatments		Un-Inoculated		Inoculated		Mycorrhiza dependency (%)
Sludge	Phosphorus	Yield	R.I. (%)	Yield	R.I. (%)	
S_0	P_0	6.734 c	---	7.842 c	16.46	16.46
	P_1	7.892 b	17.20	8.954 b	32.97	13.46
	P_2	8.513 a	26.42	9.291 a	37.97	9.14
	mean	**7.713 D**	**21.810**	**8.696 D**	**29.131**	**13.015**
S_1	P_0	8.325 c	23.62	9.450 c	40.33	13.52
	P_1	8.731 b	29.65	9.500 b	41.08	8.81
	P_2	9.174 a	36.23	9.880 a	46.72	7.70
	mean	**8.743 C**	**29.833**	**9.610 C**	**42.709**	**10.011**
S_2	P_0	8.467 c	25.74	9.550 c	41.82	12.79
	P_1	9.455 b	40.41	10.121 b	50.30	7.04
	P_2	10.186 a	51.26	10.800 a	60.39	6.04
	mean	**9.369 A**	**39.134**	**10.157 A**	**50.834**	**8.622**
S_3	P_0	8.640 c	28.31	9.662 c	43.49	11.83
	P_1	9.316 b	38.34	9.845 b	46.20	5.68
	P_2	9.652 a	43.33	10.200 a	51.47	5.68
	mean	**9.203 B**	**36.660**	**9.902 B**	**47.052**	**7.730**

L.S.D.$_{0.05}$	for inoculation	0.104
	for sludge	0.044
	for phosphorus	0.018

Phosphorus concentration in plant

Table (5) showed that there were increases in shoot-P concentration, over control treatment, with increasing either of sludge or phosphorus applications. Increasing phosphorus treatments from P_0, P_1 to P_2 increased shoot-P concentrations in either the un-inoculated or the inoculated treatments (Table 5). In un-inoculated treatments, as sludge applications increased from S_0, S_1, S_2 to S_3, the shoot-P concentrations were 782, 996, 1078 and 1149 ton kg^{-1}, respectively. In inoculated treatments, the same increase of sludge applications increased the shoot-P concentration from 1190, 1288, 1269 and 1326 ton kg^{-1}, respectively.

Table (5) showed that root-P concentrations have the same trend of shoot-P concentrations. Data indicate that in inoculated treatments as sludge applications increased from S_0, S_1, S_2 to S_3, the root-P concentration were 619, 657, 673 and 690 ton kg^{-1}, respectively.

Mycorrhizal dependency of root-P and shoot-P concentrations indicated that MD was decreased with increasing sludge or phosphorus applications (Table 5).

Table (5): Effect of arbuscular mycorrhiza inoculation on shoot and root phosphorus content (mg kg^{-1}) at different applications of sludge and phosphorus.

Treatments		Phosphorus Content (mg kg^{-1})					
		Un-Inoculated		Inoculated		Mycorrhizal Dependency (%)	
Sludge	Phosphorus	Shoot	Root	Shoot	Root	Shoot	Root
S_0	P_0	721 c	396 c	1220 c	634 c	69.21	59.98
	P_1	766 b	421 b	1165 b	606 b	52.03	43.74
	P_2	858 a	472 a	1185 a	616 a	38.15	30.61
	mean	**782 D**	**430 D**	**1190 C**	**619 D**	**53**	**45**
S_1	P_0	894 c	474 c	1293 c	659 c	44.67	39.21
	P_1	995 b	527 b	1280 b	653 b	28.64	23.79
	P_2	1100 a	583 a	1290 a	658 a	17.27	12.85
	mean	**996 C**	**528 C**	**1288 B**	**657 C**	**30**	**25**
S_2	P_0	1013 c	567 c	1221 c	647 c	20.53	14.08
	P_1	1095 b	613 b	1296 b	687 b	18.36	12.02
	P_2	1125 a	630 a	1290 a	684 a	14.67	8.52
	mean	**1078 B**	**603 B**	**1269 B**	**673 B**	**18**	**12**
S_3	P_0	1105 c	630 c	1298 c	675 c	17.47	7.16
	P_1	1141 b	650 b	1320 b	686 b	15.72	5.57
	P_2	1200 a	684 a	1360 a	707 a	13.33	3.39
	mean	**1149 A**	**655 A**	**1326 A**	**690 A**	**16**	**5**

		Shoot	**Root**
L.S.D.$_{0.05}$	**for inoculation**	**33.19**	**13.09**
	for sludge	**7.52**	**4.56**
	for phosphorus	**1.64**	**0.89**

Ear leaf-P concentration is taken as indicator of grain-P concentration. Sludge applications, in un-inoculated treatment, from S_0 to S_1, S_2 and S_3 increased the ear leaf-P concentration by 27, 38 and 47 %, respectively (Figure 3). We can conclude that the increase of sludge application in un-inoculated treatments is more effective than in inoculated treatments. Also, increase of phosphorus application has lower effect on ear leaf-P concentration than of sludge application either with un-inoculated treatments or with inoculated treatments.

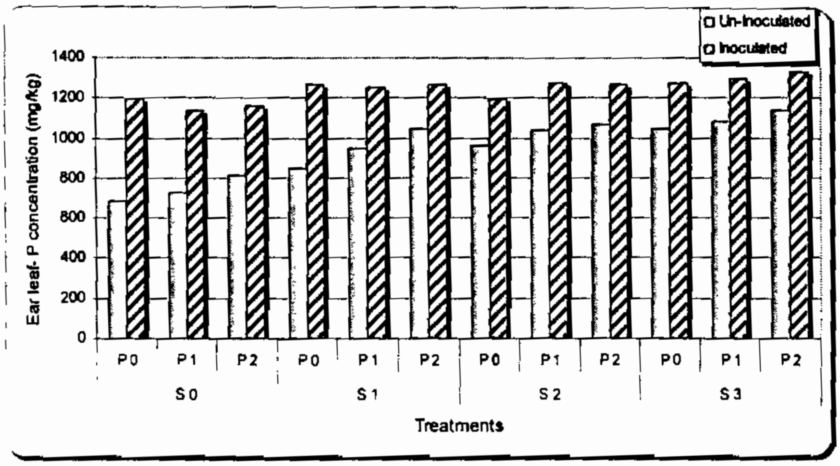

Fig. (3): Ear leaf-P concentration as responses to mycorrhizal inoculation, sludge and phosphorus treatments

Data of shoot-P, root-P and ear leaf-P concentration indicating that the higher MD was observed under treatments S_0 and P_0. The MD decreased with increasing sludge and phosphorus applications (Figure 4).

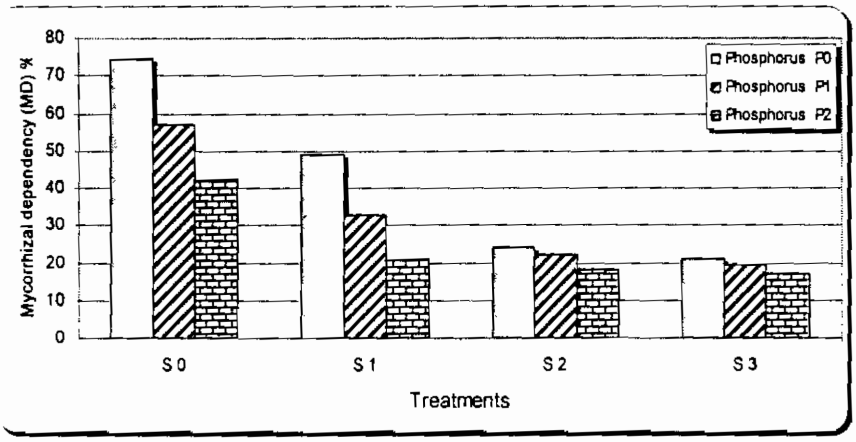

Fig. (4): Mycorrhizal dependency of ear leaf-P concentration as responses to mycorrhizal inoculation, sludge and phosphorus treatments

Heavy metals concentrations in plant

Shoot, root and ear leaf Zinc, Cadmium concentrations are given in Table (6), whereas Shoot, root and ear leaf Nickel and Lead concentrations are illustrated in Table (7). Increasing sludge applications increased significantly the shoot, root and ear leaf heavy metals concentrations. In contrast, increasing phosphorus applications which decreased the concentration of heavy metals in all plant parts. At the same time, plants inoculated with AM has lower concentrations of heavy metals in shoot, root and ear leaf.

Table (6): Zinc and Cadmium concentrations (mg kg^{-1}) as responses to mycorrhizal inoculation, sludge and phosphorus treatments.

Treatments	Zinc (Zn)				Cadmium(Cd)			
	Shoot	Root	R / S	Ear leaf	Shoot	Root	R / S	Ear leaf
Mycorrhiza Inoculation								
Uninoculated	15.61 a	15.49 b	0.99	16.33 a	1.55 a	1.75 b	1.13	1.56 a
Inoculated	14.32 b	17.27 a	1.21	15.96 b	1.46 b	1.86 a	1.27	1.50 b
LSD$_{0.05}$	0.158	0.223		0.045	0.008	0.016		0.004
Sludge								
S_0	11.22 d	13.79 d	1.23	14.18 d	1.12 d	1.36 d	1.21	1.21 d
S_1	14.68 c	16 53 c	1.13	16.02 c	1.21 c	1.62 c	1.34	1.24 c
S_2	15.73 b	16.80 b	1.07	16.20 b	1.54 b	1.87 b	1.21	1.54 b
S_3	18.23 a	18 39 a	1.01	18.18 a	2.15 a	2.35 a	1.09	2.13 a
LSD$_{0.05}$	0.183	0.123		0.103	0.029	0.027		0.028
Phosphorus								
P_0	15.25 a	16.79 a	16 00	16.71 a	1 18 c	1.41 c	1 19	1.20 c
P_1	15 24 a	16.57 b	1.09	16.39 b	1.52 b	1.86 b	1 22	1.54 b
P_2	14.40 b	15.77 c	1.10	15.34 c	1.82 a	2.12 a	1.16	1.58 a
LSD$_{0.05}$	0.025	0 025		0.029	0.01	0.011		0.011

Table (7): Nickel and Lead concentrations (mg kg^{-1}) as responses to mycorrhizal inoculation, sludge and phosphorus treatments.

Treatments	Nickel (Ni)				Lead (Pb)			
	Shoot	Root	R / S	Ear leaf	Shoot	Root	R / S	Ear leaf
Mycorrhiza Inoculation								
Uninoculated	1.25 a	1.27 b	1.02	1.24 a	2.04 a	2.10 b	1.03	2.03 a
Inoculated	1.18 b	1.33 a	1.13	1.18 b	1.96 b	2.18 a	1.11	1.97 b
LSD$_{0.05}$	0.012	0.009		0.004	0.011	0.011		0.015
Sludge								
S_0	1.00 d	1.10 d	1.10	0.99 d	1.71 d	1.83 d	1.07	1.67 d
S_1	1.14 c	1 24 c	1.09	1.16 c	1.85 c	1.98 c	1.07	1.88 c
S_2	1.26 b	1.33 b	1.06	1.25 b	2.11 b	2.26 b	1.07	2.13 b
S_3	1.45 a	1.54 a	1.06	1.45 a	2.32 a	2.48 a	1.07	2.31 a
LSD$_{0.05}$	0 012	0.012		0.011	0.017	0.02		0.019
Phosphorus								
P_0	1.18 c	1.28 c	1.08	1.19 c	1.96 c	2.12 b	1.08	1.97 c
P_1	1.22 b	1.31 a	1.07	1.21 b	2.01 b	2.15 a	1.07	1.99 b
P_2	1.24 a	1.30 b	1.05	1.24 a	2.04 a	2.15 a	1.05	2.02 a
LSD$_{0.05}$	0.002	0 001		0.001	0.002	0.002		0.001

Metals R/S ratio was higher in inoculated plants than in un-inoculated plants at low rates of sludge and phosphorus applications but at higher rate applications no differences in R/S ratio were found between un-inoculated and inoculated treatments. These data elucidate that the ability of root tissues to accumulate heavy metals at low applications of either sludge or phosphorus and consequently low heavy metals translocation from root to shoot and hence to maize grains. On the other hand at higher applications of either sludge or phosphorus, more heavy metals will arrive to the grains (Tables 6, 7).

Khan *et al.* (2000) found that very little translocation of heavy metals absorbed by mycorrhizal maize seedlings grown in contaminated soil, to the shoot. Turnau (1998) studied the location of heavy metals within the fungal mycelium and mycorrhizal roots of *Euphorbia cyparissias* from Zn contaminated wastes and found higher concentrations of heavy metals as crystalloids deposited within the fungal mycelium and cortical cells of mycorrhizal roots.

Mycorrhizal symbiosis increased plant size, shoot and root dry weight ten fold than the non-inoculated plants. Inoculation with native AMF also benefited plant growth, N fixation and P acquisition by plants. The results support the general conclusion that the introduction of target indigenous species of plants, associated with a managed community of microbial symbionts, is a successful biotechnological tool to aid the recovery of desertified ecosystems, suggesting that this represents the initial steps in the restoration of a self-sustaining ecosystem (Requena *et al.*, 2001).

REFERENCES

Angle J.S. and J.R. Heckman (1986). Effect of soil pH and sewage sludge on VA mychorrhizal infection of soybeans. Plant and Soil. 93:437-441.

Baize, D.; G. Biodoglio; S. Cornu and D. Brus. (2001). Heavy metals (Trace elements) and organic matter content of European soils. Project Report, Institut National de la Recherche Agronomique, Center de Recherches de Bordeaux, Aquitaine.

Chandrashekara C.P.; V.P. Patil and M.N. Sreenivasa (1995). VA-mycorrhiza mediated P effect on growth and yield of sunflower (Helianthus annuus L.) at different P Levels. Plant and Soil. 176: 325-328.

Clapp C.E.; W.E. Larson and R.H. Dowdy (1994). Sewage Sludge: Land Utilization and the Environment. SSSA Miscellaneous publication. Madison, WI, USA.

Clark R.B. (1997). Arbuscular mycorrhizal adaptation, spore germination, root colonization, and host plant growth and mineral acquisition at low pH. Plant and Soil. 192: 15-22.

Cottenie A. (1980). Soil and plant testing as a basis of fertilizer recommendations. F.A.O. No. (38/2). Method of Plant Analysis. Wet destruction. pp: 94-95.

Gerdemann J.W. and T.H. Nicolson (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 64:235-244.

Giovannetti M. and B. Mosse (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in root. New Phytol. 84:489-500.

Jackson M L. (1967). "Soil Chemical Analysis". Printica Hall, Inc. Englewood cliffs, New Jerrsey. p. 134-182.

Jeffries P.; S Gianinazzi; S. Perotto, K. Turnau and J. Barea (2003). The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol. Fertil. Soils. 37:1–16.

Khan A.G.; C. Kuek, T.M. Chaudhry; C.S. Khoo and W.J. Hayes (2000). Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere. 41: 197-207.

Klute A. (1986). "Method of Soil Analysis", Part 1, 2nd ed., Agron. Monograph 9, ASA and SSSA, Madison, Wisconsin USA.

Nurlaeny N.; H.Marshner and E. Gearoge (1996). Effect of liming and mycorrhizal colonization on soil phosphate depletion and phosphate uptake by maize (Zea mays L) grown in two tropical acid soils. Plant and soil. 181:275-285.

Olsen S.R. and L.E. Sommers (1982). Phosphorus. In: A.L. Page *et al.* (ed). Methods of Soil Analysis, Part2. Agronomy 9:403-430.

Page A.L.; R.H. Miller and D.R. Keeney (eds) (1982). Methods of Soil Analysis. Part 2. American Society of Agronomy, Madison, Wisconsin, USA.

Phillips J.M. and D.S. Hayman (1970). Improved procedures for clearing and staining parasitic and Vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br, Mycol. Soc. 55:158-161.

Requena N.; E. Perez-Solis; C. Azcn-Aguilar; P. Jeffries and J.M. Barea. (2001) Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Appl. Environ Microbiol. 67:495–498

Roland F.B.E. (1994). Effect of indigenous arbuscular mychorrhizal endophytes on the development of six wild plants colonizing a semi-arid area in south-east Spain. New Phytol. 127:115-121.

SAS (1990). Statistical Analysis System Institute. SAS/STAT Users Guide. Cary, NC, USA.

Turnau K. (1998). Heavy metal content and localization in mycorrhizal Euphorbia cyparissias from zinc wastes in Southern Poland. Acta Soc. Bot. 67: 105–113.

Weissenhorn I.; C. Leyval; G Belgy and J. Berthelin (1995). Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. Mycorrhiza. 5:245-252.

تأثير تلقيح الذرة بفطريات الميكورهيزا الداخلية علي تيسر العناصر الغذائية والمعالجة البيولوجية للمعادن الثقيلة في الأراضي حديثة الاستصلاح

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تعتبر العلاقة التعاونية التى تنشا بين فطر الميكورهيزا والنبات من العلاقات الهامة وخاصة تحت ظروف محدودية الفوسفور في الأرض، فهى تعمل على تحفيز نمو النبات وزيادة امتصاص العناصر الغذائية والمياه وبالتالى تؤدى إلى زيادة الإنتاجية، وفى نفس الوقت يكمن استخدامها كمعالج حيوى للأرضى الملوثة بالعناصر الثقيلة. ولقد اتجهت الأنظار فى الأونة الأخيرة إلى استخدام الحماة كمصدر للتسميد العضوى وكمحسن للأراضى، إلا انه يعيبها احتوانها على تركيزات من العناصر الثقيلة مما يقلل من استخدامها على نطاق واسع ولهذا كان الهدف من البحث اختيار افضل إدارة لكل من الحماة والسماد الفوسفورى لنباتات الذرة الملقحة بفطر الميكورهيزا والمزروعة فى الأراضى حديثة الاستصلاح وكذا تقييم دور فطر الميكورهيزا فى وجود كل من الحماة والسماد الفوسفورى على نمو نبات الذرة.

أجريت تجربة حقلية فى محطة بحوث مريوط – مركز بحوث الصحراء و تم استخدام أربعة مستويات من الحماة (صفر، ٦ ، ١٢ و ١٨ طن/هكتار) وثلاثة مستويات من السماد الفوسفورى (صفر ، ٣٦ و ٧٢ كجم P_2O_5 /هكتار) ، نصف الوحدات التجريبية تم تلقيحها بفطر الميكورهيزا والنصف الأخر غير ملقح.

جمعت عينات نباتية عند الحصاد لتقدير مكونات الإنتاج وكذلك محصول الكيزان، وأيضا تقدير تركيز عنصر الفوسفور والعناصر الثقيلة (زنك ، نحاس ، كادميوم ، نيكل ، رصاص) .

أوضحت النتائج زيادة معنوية في الوزن الجاف للمجموع الخضرى فى حالة النباتات الملقحة بالميكورهيزا عن الغير ملقحة تحت جميع الإضافات من الحماة والسماد الفوسفورى وافضل المعاملات التى أدت إلي زيادة فى الوزن الجاف هي المعاملات S_2P_2 و S_1P_2 لكل من المعاملات الغير ملقحة والملقحة ، كما لوحظ أن الزيادة فى إضافة الحماة والسماد الفسفورى أدت إلي انخفاض دور الميكورهيزا.

أعلى محصول للكيزان ظهر تحت المعاملة S_1 (٦ طن/هكتار حماة) و P_2 (٧٢ كجم P_2O_5 / هكتار) فى كل من المعاملات الغير ملقحة والملقحة حيث كان (١٠,١٩ و ١٠,٨٠ طن/هكتار) أى بمعدل زيادة مقداره ٥١,٣ % و ٦٠,٤% عن معاملة الكنترول.

كما اوضحت النتائج أن تركيز الفوسفور فى المجموع الخضرى يزداد بزيادة الحماة من S_0 إلى S_3 وعموما فان أعلى تركيز للفوسفور فى المجموع الخضرى لوحظ تحت المعاملة P_2 S_3 و تركيز الفوسفور والاعتمادية على الميكوريزا فى كل من الجذر وورقة الكوز اخذا نفس الاتجاه السابق فى المجموع الخضرى وكانت أفضل معاملة هى P_2 S_3.

توضح نتائج تركيز العناصر الثقيلة انه بزيادة الحمأة المضافة يزداد تركيز كل من الزنك – الكادميوم – النيكل –الرصاص في المجموع الخضرى والجذر وورقة الكوز وفى نفس الوقت توضح النتائج أن المعاملات الملقحة تحتوى تركيز أقل من المعاملات الغير ملقحة.

ويلاحظ أن النباتات الملقحة احتوت على تركيزات أقل في الغير ملقحة فى العناصر الثقيلة الأربعة سواء فى المجموع الخضرى أو ورقة الكوز ولكن احتوت على تركيزات أكبر فى الجذر وعليه يقل تركيز هذه العناصر فى محصول الكيزان والحبوب. كما ان النسبة بين تركيز العنصر فى الجذر على تركيز العنصر فى المجموع الخضرى فى كل العناصر الثقيلة المقدرة أكبر فى النباتات الملقحة عن الغير ملقحة وهذه النتائج متوافقة مع نتائج الاعتمادية على الميكورهيزا والتى توضح قدرة أنسجة الجذر على تراكم العناصر الثقيلة وعليه تقلل من انتقالها إلى المجموع الخضرى ومنها إلى الكيزان و إلى الحبوب .