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THE ROLE OF PHOSPHORUS FERTILIZER AND SOME MICROORGANISMS ON THE GROWTH AND YIELD OF MUNGBEAN (*Vigna radiata* L. Wilczek) PLANT GROWING IN LEAD –POLLUTED SOIL

Fouda, R. A.
Agric. Botany Dept., Fac. Agric., Mansoura Univ., Mansoura, Egypt.

ABSTRACT

Polluted soil with lead at 200 ppm decreased plant growth of mungbean plants, expressed by length of shoot system, its dry weight, number of branches and leat area (cm^2) plant as well as photosynthetic pigment concentrations in the leaves in both seasons. While, lead concentrations in both root and shoot systems were increased. Lead was more concentrated in the root than shoot system.

Thickness of leaflet blade at midvein, mesophyll tissue thickness, size of the midvein vascular bundle, xylem and phloem tissues thickness as well as metaxylem vessel diameter were also decreased. Yeild and its components represented by pods number/ plant, seed weight/ plant and 100 seed weight as well as the percentages of total soluble carbohydrate, protein, N, P and K in the seeds were also decreased.

On the other hand, treatments with phosphorus, yeast and VA- mycorrhizae and their interactions increased all the above mentioned parameters in both non-polluted and polluted soils. Treatment with yeast alone as well as the interaction between P and VA-mycorrhizae were the most effective in this respect. These treatments are recommonded not only for improving plant growth and yield of mungbean plant but also for recovery the adverse effects of lead pollution.

Abbreviations: Chl. = Chlorophyll a; L = Lead; M = Mycorrhizae; Y = Yeast.

INTRODUCTION

In recent years, there is an increasing interst to study the effects of beavy metals on plant growth and development. Lead contamination of agricultural soils has become a major environmental concern over the past several decades. Accumulation of toxic levels of lead occurs, in some agricultural soils as a result of mining, smelting, motor vesicles, stationary fuel, road dust composition and traffic roads.

Heavy metals tend to accumulate in the plough layers of soils and easily absorbed by roots as well as translocated to different plant organs resulting in retardation of plant growth and yield (Khan and Frankland, 1983; Sayed, 1999 and Moftah, 2000).

Toxic concentrations of heavy metals, including lead, in the soils have an adverse effect on some physiological processes related to plant growth and development,. *i. e.*, suppression of photosynthetic activity (Krupa and Baszynski, 1995); disturbance of water relations and mineral nutrition (Siedlecka and Krupa 1996). Moreover, lead tended to have an inhibitory effect on protein synthesis (Taiz and Zeiger, 1998); carbohydrate and sugar content (Kandil, 1995). In addition, lead is associated with alternation of endogenous phytohormones and changes in the anatomical structure of plant organs (Fouda and Ramadan, 2004).

Nowadays, great attention has been focused on the possibility of using the microorganisms not only for remediation of soil contaminated with heavy metals (Fouda and Arafa, 2002; Park *et al.*, 2003), but also to improve plant growth and yield (Fouda and Arafa, 2002). The decrease of heavy metals phytotoxicity by mycorrhizae and yeast fungi has been widely demonstrated (Fouda and Arafa, 2002 and Goksungur *et al.*, 2004). These fungi play an important role in the remediation of contaminated soil by protecting the plant performance against the negative effects of heavy metals (Schutzenduble and Polle, 2002) as well as increased uptake of mineral nutrients with low mobility in soils, mainly phosphate (Diaz *et al.*, 1996 and El-Ghamriny *et al.*, 1999).

The objective of this investigation is to study the effects of lead pollulant, with or without application of phosphorus, VA-mycorrhizae and yeast fungi on growth, photosynthetic pigments as well as yield and its quality of mungbean plant. The anatomical structure of leat was also studied.

MATERIAL AND METHODS

Two pot experiments were carried out during the two successive seasons of 2001/2002 and 2002/2003 in the Expermental Farm and Laboratories of Agic. Botany Dept. Fac. of Agric., Mansoura Univ. Mansoura, Egypt. Each pot (30 cm diameter) was filled with 5 kg clay/ sand soil (1:1). The pots were divided into two sets, each set was divided into 5 groups. Each group contains 6 pots.

1. Heavy metal treatment:

Lead treatments were achieved by adding 500 ml aqueous solution of lead chloride at 200 ppm prepared in distilled water to each pot. After carefully mixing the heavy metal solution with the soil, it allowed to stabilize for a period of 5 days before sowing. In P treatment, phosphorus was added as calcium superphosphate (15.5% P_2O_5) at the rate of (5 g/ pot) before sowing.

2- Microorganisms:

The microorganisms used for the experiments were VA-myorrhizae (*Glomus mosseae*) and yeast extract (*Saccharomyces cerevisiae*). The mycorrhizal inoculum was obtained from Fac. of Sci., Mansoura Univ. The mycorrhizal inoculum consisting of soil with spores, hyphae and Dnion root fragments infected with *Glomus mosseae*. The inoculum was placed 3 cm below the soil surface at the rate of (10 g /pot) at the moment of sowing.

The used yeast extract was obtained from Microbiol. Dept., Fac. Agric. Mansoura Univ., multiplied in nutrient broth and centrifuged then prepared again in suspension. The inoculum was placed 3 cm below the soil surface at the rate of 10 ml yeast suspension per pot at the moment of sowing.

The second set of pots also divided into 5 groups to obtained the interactions between the above mentioned treatments.

The pots were arranged in a complete randomized block design with three replications to form the following treatments:

1- control. 2- lead 200 ppm.
3- phosphorus. 4- VA- mycorrhizae.
5- Yeast. 6- lead 200 ppm + phosphorus.

7- lead 200 ppm + VA-Mycorrhizae. 8-lead 200 ppm+Yeast.
9- lead 200 ppm+ phosphorus + VA-Mycorrhizae.
10- lead 200 ppm + phosphorus+ yeast.

3- Plant growth:

Uniform seeds of mungbean (*Vigna radiata* L. Wilczek Var. Kawmy.1) were obtained from the Agric. Research center, Dokki, Giza, Egypt. The seeds (5 per pot) were sowin on 15th May in both seasons. The pots were kept in the Agric. Farm of Agric. Bot. Dept., under a normal day/ night conditions and irrigated with tap water when required. After 15 days from sowing the plants were thinned to leave 3 uniform young plants per pot and then second dose of the microorganisms were added.

At flowering stage (55 days after sowing), length of shoot system and its fresh and dry weight (g), number of branches per plant and leat area (cm^2) per plant using Watson (1958) methods were recorded.

4- Chemical analysis:

At flowering Photosynthetic pigment concentrations (mg/g fresh weight) were determined in the 3rd compound leaf (Mackinny, 1941).Lead cations were also determined in both root and shoot systems by atomic spectrophotometers BHE 80 B).

5- Anatomical studies:

For the anatomical studies samples were taken at (Flowering) during the second season from the middle part of the midrib region of the treminal leaflet of the 3rd compound leaf. The samples were killed and fixied in FAA solution dehydrated in ethyl alcohol series, cleared in xylene and embedded in paraffin wax (52-54 Cm-P). Cross sections at 15-20 µm thick were prepared using a rotary microtome, stained in saffranin- light green combination, cleared in clove oil and mounted in canada balsam (Gerlach, 1977) and examined microscopocally.

6- Yeild and its components:

At harvesting, after 70 days from sowing, number of pods/ plant, seed weight per plant and 100 seed weight were determined. Moreover, total soluble carbohyrate percentage in the seeds (Sadasivam and Manickam, 1996) and percentages of nitrogen and phosphorus (Jackson, 1967) as well as potassium (Peterburgski, 1968) were estimated.

All the chemical analysis except photosynthetic pigments were carried out during the second season only.

Statistical analysis:

Data were statistically analyzed according to steel and Torrie (1980)

RESULTS AND DISCUSSION

Visual symptoms of lead toxicity:

Generally, two major symptoms of lead toxicity in plants were observed. The first is a severe reduction of plant growth, and the second is chlorosis in interveinal areas of leaves, being more pronounced in young leaves.

Data in Table (1) indicate that contaminated soil with lead decreased significantly plant growth expressed by length of shoot system, its dry weight, number of branches and leaf area per plant in both seasons compared with control Whereas, in the unpolluted and polluted soil, application with P, VA-mycorrhizae and yeast not only increased all the above mentioned growth parameters, but also nullified the adverse effect of lead toxicity. Adding yeast was more effective in this respect. In polluted soil, the interaction between used treatments recovered the adverse effects of lead toxicity on plant growth. The interaction between P and VA-mycorrhizae was the most effective in this respect.

Table (1): Effects of lead, phosphorus, mycorrhizae and yeast and their interactions on mungbean plant growth during the two growing seasons of 2001/2002 and 2002/2003.

Characters	Shoot length (cm)		No. of branches		Leaf area/ plant		Dry weight of shoot system	
Treatment	2002	2003	2002	2003	2002	2003	2002	2003
Control	44.7	48.3	3.7	47	510.3	616.7	12.7	17.5
Lead	29.0	32.3	2.3	3.3	465.0	476.0	8.6	14.0
Phosphorus	59.7	61.3	6.7	7.3	630.3	635.0	15.8	18.2
VA-mycorrhizae	63.3	65.3	6.8	8.7	642.7	645.3	16.2	18.7
Yeast	67.0	72.7	7.3	9.0	650.3	652.3	16.6	19.2
L + P	61.3	63.3	7.7	9.3	616.3	619.7	16.2	18.6
L + M	66.0	69.0	8.0	9.5	643.0	636.3	18.3	19.3
L + Y	71.0	75.3	7.7	10.0	650.3	651.3	18.5	20.4
L + P + M	76.3	78.0	9.7	11.3	662.0	665.7	20.6	20.7
L + P + Y	77.7	82.0	9.0	9.7	655.3	661.3	19.0	20.5
L.S.D. at 5%	7.6	8.9	2.0	1.2	5.4	4.7	3.3	3.1

The deleterious effect of lead on plant growth may be attributed to retarded cell division and cell elongation through the reduction of meristem size and reduction of cell differentiation leading to decrease the number of mature cells (Kastori *et al.*, 1998 and Obroucheva *et al.*, 1998).

The stimulating effects on growth parameters of mungbean plant as a result of adding phosphorus may be attributed to an increase in cytokinins production (Bass and Kuiper 1989). Cytokinins promote cell division and cell enlargement (Arteca, 1996), beside its inbibitory effect on lead by precipitation of lead ions (Sleim, 2001).

Photosynthetic pigments:

Data in Table (2) show that polluted soil with lead ions decreased chlorophyll a, b and their total as well as carotenoid concentrations (mg/g F.W.) in the 3rd leaf of mungbean plants at flowering stage. Treatments with p, VA-mycorrhizae and yeast and their interactions not only increased significantly all photosynthetic pigments in non-polluted soil but also alleviated the adverse effect of lead on these pigments in polluted soil. The most effective treatment was yeast application as well as the interaction between p and mycorrhizae.

The reduction in photosynthetic pigment concentrations due to lead may be attributed to an inhibition of the biosynthesis of aminolevulinic acid (ALA), a precurser of chlorophyll (Thomas and Singh, 1996),to stimulation chlorophyll degradation by chlorophyllase (Abdel-Basset *et al.*, 1995) and, it can alter chlorophyll biosynthesis by inhibiting photochlorophyllide reductase through interferring the sulfhydryl site on the enzyme (Lagriffoul *et al.*, 1998).

Table (2): **Effects of lead, phosphorus, mycorrhizae and yeast and their interaction on photosynthic pigment concentrations (mg/g F.W.) of mungbeon plants during the two growing seasons of 2001/2002 and 2002/2003.**

Treatment	Seasons							
	2001/2002				2002/2003			
	Chl.A	Chl.B	Total chlorophyll	Total Caroti	Chl.A	Chl.B	Total chlorophyll	Total Caroti
Control	1.28	0.77	205	0.48	1.31	0.80	2.11	0.53
Lead	0.58	0.65	1.23	0.25	0.64	0.68	1.32	0.29
Phosphorus	1.73	0.88	2.61	0.46	1.75	0.92	2.67	0.49
VA-mycorrhizae	1.77	0.95	2.72	0.53	1.80	0.98	2.78	0.57
Yeast	1.83	0.99	2.82	0.68	1.84	1.12	2.96	0.74
L + P	1.78	1.10	2.88	0.35	1.82	1.20	3.02	0.41
L + M	1.86	1.23	3.09	0.45	1.90	1.28	3.31	0.48
L + Y	1.96	1.33	3.29	0.56	1.98	1.41	3.39	0.64
L + P + M	2.34	1.55	3.89	0.70	2.40	1.61	4.01	0.87
L + P + Y	2.08	1.47	3.55	0.64	2.12	1.50	3.62	0.83
L.S.D. at 5%	0.48	0.65	97	0.12	0.55	0.77	0.95	0.17

The promoting effect of P on photosynthetic pigments in non-polluted soil may be due to its providing the plant with ATP and NADPH and other compounds that play a vital role in biosynthesis of chlorophyll and other pigments (Marschner, 1995).

Moreover, the stimulating effects of P as well as mycorrhizae and yeast fungus on photosynthtic pigments may be due to their effect on increasing cytokinins content (Torelli *et al.*, 2000 and Fouda and Ramadan, , 2004). Cytokinins delaying the degradation of chlorophyll through the inhibition of chlorophyllase (Ben, 1986) and enhancing the synthesis of protein and RNA that are related with delay the aging of leaves (Natio *et al.*, 1981). Moreover, the promoting effect of these treatments on photosynthetic pigments under lead polluted soil condition may be due to their effects on reducing the concentration of lead ions (Fig. 1).

Lead concentrations:

Data illustrated in (Fig. 1) show that lead accumulated more in root than shoot system. These results may be partly due to the lower mobility of lead and partly due to restricted lead transport to shoot (Khan and Frankland 1983).

In the polluted lead soils adding p, as well as VA-mycorrhizae and yeast as bioremediants for lead ions caused a reduction in its concentrations in both root and shoot systems of mungbean plant.

The results indicate that using the yeast was more useful for lead remediation and consequently overcome the toxicity effect of lead on mungbean plant.

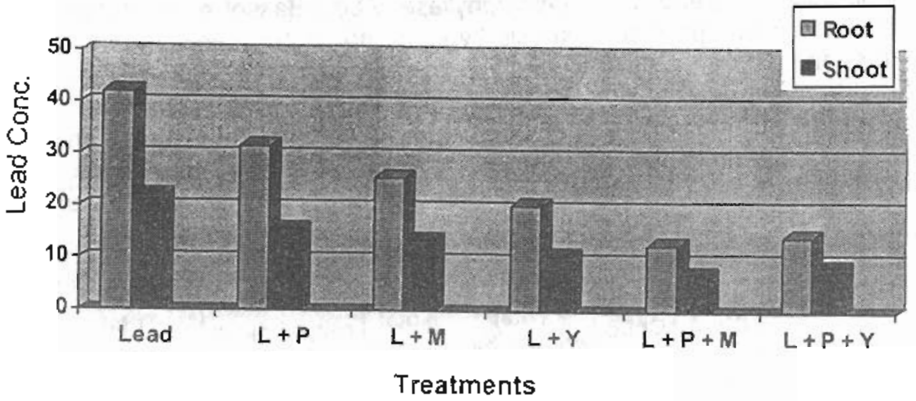

Fig. 1: Lead concentrations in both root and shoot systems (µg/g) as affected by different treatments.

On the other hand, the interaction between p and VA-mycorrhizae was most effective in this respect. These results may be attributted to that mycorrhizae fungi enhanced phosphorus availability and uptake (Diaz *et al.*, 1996).

The reduction in lead concentration in plants growing in polluted soils by inoculation with mycorrhizae may be attributed to its role on reducing metal uptake or root-shoot translocation caused by the sequestration of lead by polyphosphate granules in fungal vacuoles. Moreover, mycorrhizae hindered access of heavy metal to the root surface caused by the fungal sheath around the root surface (Jentschke and Godbold, 2000).

The reduction in lead concentration by using yeast as bioremediants may be attributed to accumulate lead ions on their cell walls by adsorption, complexation and precipitation as well as accumulate heavy metals in their cytoplasm (Park *et al.*, 2003). They added that the mechanism of metal ions accumulation in *saccharomyces cerevisiae* is composed of a rapid binding to netgatively charged groups on cell wall surface and penetration the heavy metal ions through the cell membrane and accumulation in the cell cytoplasm.

In this respect, Kapoor and Viraraghavan (1997) described the important role of cell wall on which ion-exchange occurs between heavy metal ions and H^+, K^+, Mg^{2+} and ca^{2+} ions on the cell wall during metal adsorption. Moreover, yeast cell walls, consisting mainly of polysaccharides, protein and lipids contains many functional groups which can bind metal ions such as carboxylate, hydroxyl, phosphate and amino groups (Goksungur *et al.*, 2004).

Leaflet anatomical structure:

Data in Table (3) and Fig. (2.B) show that contaminated soil with lead decreased the thickness of leaflet blade at the midvein and mesophyll tissue thickness as a result of decreasing thickness of palisade and spongy tissues. The size of the midvein vascular bundle was also decreased expressed by its dimensions as well as xylem and phloem tissues thickness. Moreover, metaxylem vessele diameter was also decreased.

Table (3): Some anatomical characters (um) in the terminal leaflet taken from 3rd leaf of mungbean plant as affected by lead, phosphorus, VA- mycorrhizae and yeast as well as their interaction.

Treatment	Leaflet thickness in the midrib	Mesophyll tissue thicknes	Palisade tissue thicknes	Spongy tissue thicknes	Midrib V.B. dimension		Phloem tissue thickness	Xylem tissue thickness	Metaxylem vessel diameter
					Length	width			
Control	1960	200	120	80	350	250	120	130	28
Lead 200ppm	1800	147	80	67	232	132	115	117	24
Phosphorus	2200	240	140	100	436	510	212	224	32
vA-mycorrhizae	2720	258	146	112	537	650	217	320	40
Yeast	2800	873	158	215	570	672	230	340	48
L + P	2000	233	135	98	400	463	198	202	29
L + M	2350	245	140	105	490	514	279	211	35
L + Y	2250	343	153	190	510	603	287	223	43
L + P + M	3120	404	177	227	590	615	235	355	22
L + P + Y	2950	388	165	223	575	610	228	347	53
L.S.D at 5%	27.6	16.9	15.2	13.4	15.3	13.2	6.5	6.9	1.7

L: lead P: phosporus M: Mycorrhizae Y: Yeast

Treatment with phosphorus, VA-mycorrhizae and yeast and their interactions increased all the above mentioned anatomical parameters in both non-polluted and polluted soils. Treatment with yeast alone (Fig 2D) and the interaction between P and VA-mycorrhizae (Fig 2E) were the most effective in this respect.

The inhibiting effect of lead on leaflet structure may be attributed to its effect on both cell division and cell elongation through the reduction of meristem size and decreasing the number of mature cells (Obroucheva *et al.*, 1998).

The stimulating effect of phosphorus may be attributed to the promotion of cambial activity, which produced higher amount of secondary conducting tissues (El-Shaarawi *et al*, 2004).

The increase in leaflet thickness due to inoculation with VA-mycorrhizae fungi may be attributed to its effect on increasing both cell division and vascular cambium tissue (Berta *et al.*, 2000). Moreover, the increase in cell division and meristimatic activity due to application with phosphorus, VA-mycorrhizae and yeast are probably due to the effect of growth substances produced by these treatments, mainly, cytokinine and auxin, Bamett *et al.* (1990) and Torelli *et al.* (2000) stated that cytokinine and auxin are promote both cell division and cell elongation

Yield and its components:

It is clear from the results in Table (4) that polluted soil with lead decreased yield and its components represented by pods number/plant, seeds weight plant as well as 100 seed weights in the two growing seasons Plants grown in both non-polluted and lead polluted soil treated with phosphorus, VA-mycorrhizae and yeast had high yield. These treatments overcome the adverse effects of lead on yield parameters. Inoculation with yeast as well as the interaction between P and VA-mycorrhizae under pollution condition was most effective in this respect.

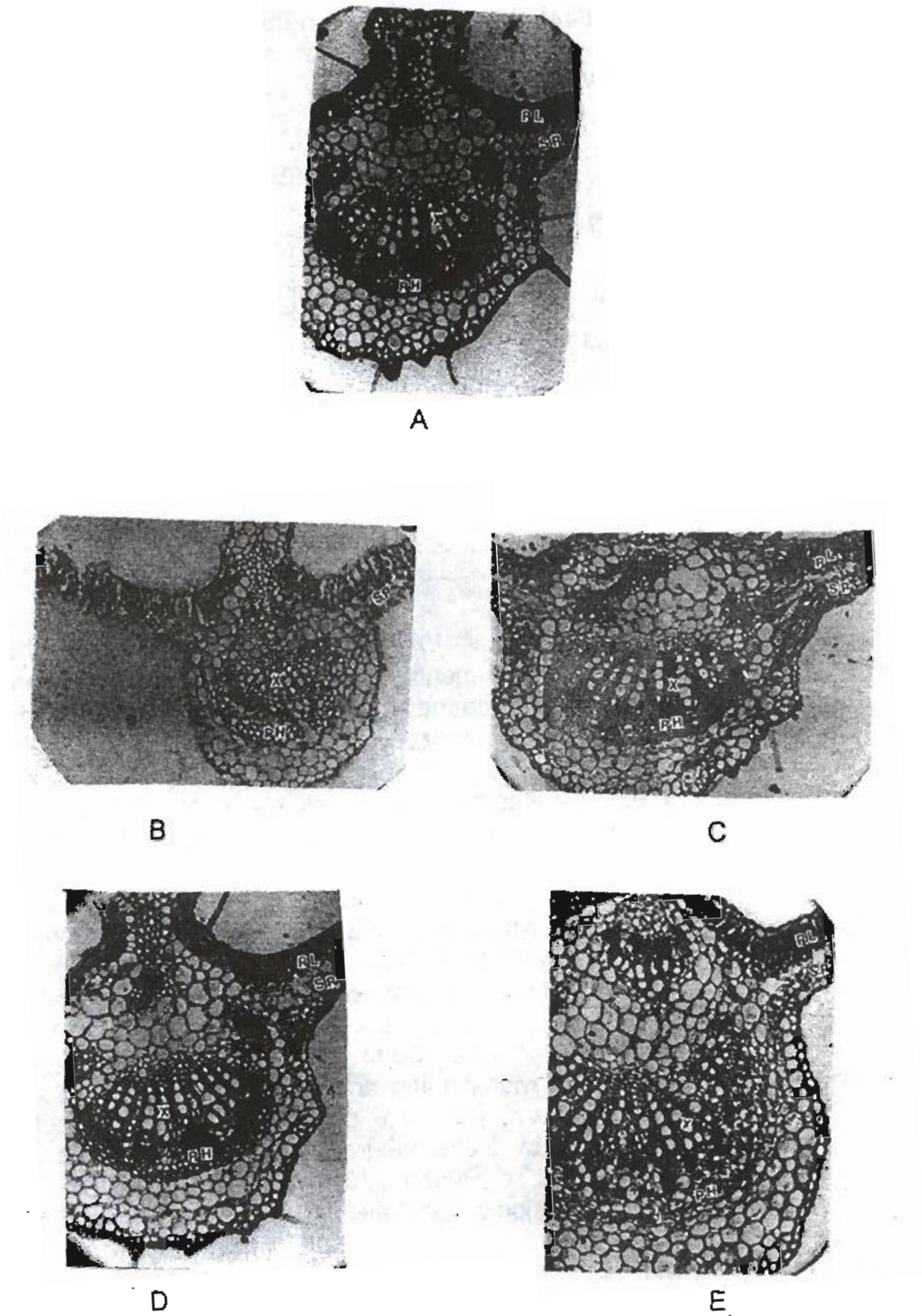

Fig. (2): cross sections of the terminal leaflet of the 3rd leaf from the mungbean plant tip as affected by lead (L) phosphorus (P), VA-mycorrhizae (M) and yeast (Y) and their interactions (Obj-x 10.0 c.x10). pl:, palisad tissue, sp:, spongy tissue, x, xylem, ph:, phloem.

A: Control. B: Lead C:L+M D: L+Y E: L + P + M.

Table (4): Effect of lead, phosphorus, mycorrhizae and yeast and their interactions on yield and its components of mungbean plant during the two growing seasons of 2001/2002 and 2002/2003.

Treatment	Season					
	2002	2003	2002	2003	2002	2003
	Pod no/ plant		Seed weight/ plant (g)		100- seed- weigh (g)	
Control	36.0	28	5.1	5 2	15 3	16.7
Lead 200 ppm	16.3	17	2.3	2.5	11.2	12.2
Phosphorus	43.0	46.3	6.0	6.7	16.5	17.3
VA-mycorrhizae	45.2	47.7	6.3	6.8	17.9	18.6
Yeast	46.0	48.3	6.7	7.1	15.7	16 2
L + P	41.0	44.0	5.9	6.0	16.3	16.5
L + M	45.8	46.6	6.1	6.3	16.7	17.0
L + Y	48.2	48.5	6.3	6.6	17.0	17.8
L + P + M	54.0	58.3	7.7	7.9	20.3	21.5
L + P + Y	50.6	51.2	6.9	7.2	18.3	19.0
L.S. D. at 5%	2.6	3.4	.03	0.04	2.5	2.6

The inhibitory effect of lead on the above. mentioned yield parameters may be attributed to its toxic effect on growth (Table 1) and inhibitory effect on the biosynthesis of photosynthetic pigments (Table 2), which refletced on mungbeen yield.

The enhancing effect of p, VA-mycorrhizae or yeast on mungbean yield may be due to not only its role in removing the toxic effect of lead but also to its role on producing growth substances, *i.e.*, cytokinins and auxin. These substances play an important role in promoting plant growth and removing the toxic effect of heavy metals (Fouda and Arafa, 2002).

Seed quality:

Data in Table (5) indicate that mungbean seed content of carbohydrate, protein, nitrogen, phosphorus and potassium percentages decreased significantly with lead contamination. On the other hand, treatments with phosphorus as well as mycorrhizae and yeast fungal increased carbohydrate, protein and mineral percentages in mungbean seeds under non-polluted and polluted soil with lead. Adding yeast fungi to non-polluted and polluted soil as well as the interaction between mycorrhizae and phosphorus in polluted soil with lead were the most effective in this respect.

Reduction in soluble carbohydrate percentage induced by pb^{++} treatment may be attributed to its inhibitory effect on photosynthetic activities (Fodor *et al.*, 1998) and photosynthates pigment concentration (Table 2) as well as ribulose diphosphate carboxylase (the key enzyme for carbohydrates synthesis (Stribrova *et al.*, 1986)

The increase in carbohydrate percentage due to adding phosphorus may be attributed to the promoting effect of phosphorus on synthesis of sugars and translocation rate of from leaves to the seeds (Bender *et al.*, 1986 and El-Rewaing and Galal, 2004). Moreover, Marschner (1995) stated that P is a component of RNA and DNA, therefore, it may have an important effect on biosynthesis of sugar, protein and hormones.

Table (5): Effect of lead, phosphorus as well as mycorrhizae and yeast fingi and their interaction on seed quality of mungbean plant during the second season of 2002/2003.

Treatment	Nitrogen %	Phosphorus %	Potassium %	Protein %	Carbohydrate %
Control	3.5	0.64	7.0	22.1	55.0
Lead 200ppm	2.6	0.47	5.3	16.1	44.7
Phosphorus	3.7	0.90	8 5	23.4	61.7
VA-mycorrhizae	4.0	0.81	8.6	25.4	64.3
Yeast	4.2	0.77	9 0	25.5	63.0
L + P	3.8	0.74	7.9	23.5	62.4
L + M	4.0	0.84	8.3	25.2	64.9
L + Y	4.1	0.92	8.5	25.6	65.5
L + P + M	4.3	1.1	9.6	26.8	67.7
L + P + Y	4.2	0.98	9.2	26.3	65.7
L.S.D at 5%	0.31	0.04	0.58	1 01	1.43

The reduction in protein and mineral percentages due to lead treatment may be attributed to decreased cell membrane stability as a result of changes in lipid layer in cell membranes (Sayed 1999). Moreover, its inhibitory effects on root growth and consequenttly decreased absorption surface and the absorption of minerals from the soil.

The beneficial effects of p on protein and N, P and K percentages may be attributed to the promoting effect of phosphorus on protein biosynthesis and an increase in nutrient absorption (Marschner 1995).

The increase in protein and N, P and K due to mycorrhizae and yeast fungal in both non-polluted and polluted soils may be attributed to their effect on both enhancing the protein synthesis and increasing nutrients uptake (Natio *et al.*, 1981).

In this respect, Tuladhar and Subba RAO 1985) added that yeasts increased nitrogen fixiation through their effect on increasing the number of nodules. Moreover, Subramanian and Charest (1997) suggested that mycorrhizae increased protein synthesis through its enhancing effect on N-assimilating enzymes and N-cycling as well as regulating the uptake of other essential micronutrients.

It could be concluded that treatments with phosphorus as well as mycorrhizae and yeast fungi play an important role not only in improving plant growth but also in removing the toxic effect of lead in polluted soil.

Adding yeast fungi as well as the interaction between phosphorus were the most effective in this respect.

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دور التسميد الفوسفاتى وبعض الكائنات الحية الدقيقة والتفاعل بينهم علــى نمــو ومحصول نبات فول المانج النامى فى تربة ملوثة بالرصاص

رمضان عبد المنعم فودة

قسم النبات الزراعى – كلية الزراعة – جامعة المنصورة

يهدف هذا البحث إلى دراسة تأثير الرصاص وكل من التسميد الفوسفاتي وفطريــات الميكــوريزا والخميرة والتفاعل بينهم على نمو ومحصول نبات فول المانج النامى فى تربة ملوثة بالرصــاص وإمكانيــة التغلب على التأثير الضار للرصاص باستخدام تلك المعاملات ويمكن تلخيص النتائج فيما يلي:

أدى تلوث التربة بالرصاص بتركيز ٢٠٠ جزء فى المليون إلى نقص طول المجمــوع الخضــرى ووزنه الجاف وعدد الأفرع الجانبية ومساحة الورقة وصبغات البناء الضوئى كما أدى إلــى زيــادة تركيــز الرصاص في المجموع الجذرى عنه فى المجموع الخضرى كما أدى إلى نقص سمك الوريقة فــى منطقــة العرق الوسطى والنسيج المتوسط وحجم الحزمة الوعائية للعرق الوسطى الرئيسى معبرا عنه بنقص أبعادهــا وكذلك سمك نسيجى الخشب واللحاء إلا أن المعاملة بالتسميد الفوســفاتى والتلقــيح بفطــرى الميكــوريزا أو الخميرة والتفاعل بينهم أدت إلى ليس فقط إلى تحسين نمو النبات والمحصول بل أدت إلــى ملاشــاة الأثــر الضار للرصاص وكانت أفضل المعاملات التلقيح بفطر الخميرة والتفاعل بين التسميد الفوسفاتى والميكوريزا