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STUDIES ON BIOREMEDIATION OF SOILS AND PLANTS 2- IMPACTS OF ARBUSCULAR MYCORRHIZAL FUNGI AND *Pseudomonas Putida* ON DISTRIBUTION OF HEAVY METALS IN SOILS AND FABA BEAN PLANTS GROWN UNDER FIELD CONDITIONS

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ABSTRACT

Field experiment at El-Fayoum Governorate was carried out to examine the impacts of interactions between *Ps. putida* and arbuscular mycorrhizal (AM) fungi and their effects on elements distribution in soil and faba bean plant parts grown under field conditions in the presence of 25 or 50% of the recommended mineral fertilizers (NPK). The soil before cultivation was polluted with copper and zinc in total form as well as with Cu, Fe and Zn in available forms. During plant growth, the soil was polluted with the investigated elements except Cu. The best treatment to decrease the polluted soils is the inoculation of plants with AM fungi plus *Ps. putida* and fertilized with 50% NPK. All parts of plant are polluted in the two stages in case of using 25% mineral.fertilizers, while roots and leaves at mature stage under 50% mineral fertilizers, in untreated plants. The best treatment to decrease Cu pollution is the inoculation with AM fungi + 25% NPK followed by AM fungi+Ps.+50%NPK in mature stage. All plant parts in all treatments in this study are polluted with iron. The best treatment to decrease Fe pollution, is the fertilization with 50% of the recommended NPK +AM fungi+ Ps. All plant parts are polluted with Pb and the best treatment to decrease Pb pollution is using 25% NPK+ AM fungi in mature stage. The concentrations of zinc in all young plant parts are higher than 400 mg/kg then decreasing with plant growth. Using 50% NPK +AM fungi is the best treatment to decrease Zn pollution.

Keywords: Arbuscular mycorrhizal (AM) fungi–Faba bean plants- micronutrients and heavy metals-Polluted soil- *Pseudomonas putida*

INTRODUCTION

Generally, the plant growth promoted activity of the *Pseudomonas* resides in siderophores, low molecular weight compound with a high affinity for Fe_3^+ produced by these organisms. In addition, many pseudomonads produce a number of antibiotics, which also have a role in plant promoting activity (Dowling and O'Gara 1994, Thomashow and Macrodi 1997).

Heavy metals ions unclouded in the parent soil material are set free in the process of soil formation in correspondence to the rate of weathering. The further fate of the ions depends on pedological factors such as pH, humus content, redox potential as well as on external factors such as temperature, precipitations, erosion, land use practice etc. Accordingly, some elements are accumulated in the topsoil whereas others are leached out (Ernest 1991).

In order to assess heavy metals pollution of the terrestrial environment, baseline information is required on the heavy metals status of nonpolluted soils and associated vegetation. Since most of the agricultural soils and their crops in Egypt are relatively unaffected at present by the known manmade sources of heavy metals contamination, determination of their heavy metals status would provide valuable information for future assessment of soil and plant quality (Aboulroos *et al.* 1996).

The purpose of the current study is to examine the impacts of interactions between *Pseudomonas putida* and arbuscular mycorrhizal (AM) fungi and their effect on elements distribution in soil and faba bean plant parts grown under field conditions in the presence of 25 or 50% of the recommended mineral fertilizers.

MATERIALS AND METHODS

A field experiment was set up at El-Fayoum Governorate to study the impact of dual inoculation of faba bean (*Vicia faba* L.) with *Ps. putida* in combination with arbuscular mycorrhizal fungi on the accumulation of heavy metal in the different parts of plant .

Before cultivation , soil samples were taken frome surface area (0 – 30 cm) to determine the soluble , available and total elements in soils. The soluble elements in soil are extracted from a soil paste. Amonium acetate - EDTA mixture - (pH=4.65) was used to extract the available elements from soil samples after Cottenie *et al.*(1982). Aqua Regia was used to digest soil samples for total contents of the investigated trace and heavy elements (Cottenie *et al.* 1982).

The experimental design was a factorial organized in a randomized complete block split plot with four replications. Two levels of mineral fertilizer (50% or 25% of recommended mineral fertilizers) formed the main plot units. The subplot treatments were as follows uninoculated (control), inoculated with AMF singly or in combination with *Ps. putida*. Each sub-plot (3x3 m) consisted of four rows of plants with 30 cm between rows. The space between plots was 8 cm and between replications 1 m. Mineral fertilizers, phosphorus fertilizer, in the form of calcium superphosphate (15.5% P_2O_5) and potassium in form of potassium sulphate were broadcasted and incorporated during soil tillage, while nitrogen fertilizer in form of ammonium sulphate was added in 2 equal doses after 15 and 30 days from planting.

After two months from cultivation (20 \ 9 \ 1997) and at harvest time (26 \ 3 \ 1998), soil samples were collected from surface area to represent each soil treatments to measure the available Fe, Zn, Cu and Pb. Plant samples were divided into roots, stem and leaves at first time. At harvest time, plant samples were divided into roots, stem, leaves, flowers and pods. Therefore, all parts of plant were digested by fusion with a mixture of concentrated acids (Cotteine *et al.* 1982).

Trace elements were measured using Varian spectrAA220 atomic absorption spectrometer (AAS). Iron, copper and zinc were determined in soil and plant applying micro-sampling technique. This could overcome the matrix- and nebulization difficulties in high salt sample solutions, while lead was determined applying graphite furnace-AAS(Ramadan and Al-Ashkar 2001a, b)

RESULTS AND DISCUSSION

Soil before cultivation

Table 1 shows the total, available and soluble concentrations of Fe, Zn, Cu, and Pb in surface layers of soils (0-30 cm) before cultivation. It is clear that the percentage of available to total elements (0.36% Fe, 1.14% Zn, 8.48% Cu and 6.08% Pb) were lower.

Also, it is clear that the measured values of total and soluble copper, lead and zinc are lower than maximum tolerable concentrations (B) and the concentrations in water-soluble (D), while total copper and zinc concentrations are higher than mentioned by Ewers (1991).

The background level of extractable Cu, Pb and Zn in the non-polluted soils of Egypt is ranged from 1.86 to 2.50 *ug*/g Cu, 1.17 to 1.61 *ug*/g Pb and from 1.56 to 2.64 *ug*/g Zn (DTPA extractable), according to Aboulroos *et al* (1996). However, Follet and Lindsay (1970) reported that the amounts higher than 4.5, 1 and 0.2 *ug*/g are adequate for Fe, Zn and Cu in soil respectively.

From the data obtained, it can be said that the soil before cultivation was polluted according to Follet and Lindsay (1970), Ewers (1991) and Aboulroos *et al* (1996).

Soil after cultivation

The percentage of available Fe was increased from 144.77% to 185.89% by increasing the percentage of mineral fertilizers (50% NPK), during plant growth. Inoculation with AM fungi alone releases Fe more than inoculation in combination with AM fungi and Ps. The higher percentage of iron (181.34%) were found in case of inoculation with AM fungi and fertilizer with 25%, while the lowest percentage (32.22%)were found in case of inoculation with two microorganisms plus 50% NPK (Fig 1).

In case of adding 50% mineral fertilizers, the percentage of available Zn was increased in all treatments than that of 25% NPK; except in case of inoculation with AM fungi and Ps in combination which were decreased (13.59%). In case of adding 50% NPK, the highest percentage of Zn was found in uninoculated treatments (107.67%), while the lowest (-8.36%) was found in the uninoculated treatment fertilized with 25% NPK (Fig 1).

The available Cu was decreased in all treatments of plant growth. The highest decreased percentage of copper was found in case of inoculation with two microorganisms in combination with 50%NPK (-40.54%), while the lowest decreasing percentage was indicated in the same treatment but fertilized with 25% NPK (-6.03%). Contrary to that, the available Pb was increased in soil with plant growth. The highest percentage of available Pb (207.13%) was found in uninoculated treatment and fertilized with 50% NPK, while the lowest (64.07%) in the inoculation with AM fungi and *Ps. putida* in combination with 50% NPK (Fig 1). This is related to the different uptake rates by plants during the different growth stages, especially at harvested stage.

During plant growth, the polluted soil was increased in the investigated elements; exception soil Cu much decreased in all treatments. From the data obtained, it can be concluded that the best treatment leads to decrease the polluted soils is the inoculation with AM fungi plus *Ps. putida* and fertilized with 50% NPK.

1 - Different element distribution of plant parts in the uninoculated treatments fertilized with 25% and 50% mineral fertilizers

During plant growth, the concentration of iron is increased in all plant parts. In addition, the increase of mineral fertilizers (25 to 50%), the percentage of iron increasing to 79.68% in roots, 2.05% in stem, 272.57% in leaves, 399.19% in flowers and 2.86% in seeds (Fig 2). Zinc concentrations were decreased in roots, stem and leaves in plants fertilized with 25% NPK during the plant growth, while increased in stem and leaves in case of 50% NPK. In case of 25% NPK, zinc concentrations in flowers (195.85 *u*g/g) and in seeds (89.95 *u*g/g) are higher than those 96.5 *u*g/g in flowers and 61.25 *u*g/g in seeds in case of 50% NPK (Fig 2). Copper concentrations increased in all parts of plant during plant growth except in stem when fertilized with 50% NPK. Copper concentrations were decreased in flowers and seeds in case of 50% NPK over those under 25% NPK; 35.29% and 18.17% for flowers and seeds respectively (Fig 2).

Lead concentrations decreased in most of plant parts during plant growth, namely in leaves (7.9 *u*g/g) under 25% NPK and in stem (20.6 *u*g/g) under 50% NPK. Increased percentages were 114.91% and 140.67% for flowers and seeds respectively (Fig 2).

2. Different elements distribution of plant parts inoculated with arbuscular mycorrhizal (AM) fungi and fertilized with 25% or 50% mineral fertilizers

Iron concentrations increased in all parts of faba bean plant during plant growth except in leaves (-914.5 *u*g/g) of plant inoculated with AM fungi and fertilized with 25% NPK and in stem (-1062.6 *u*g/g) in case of AM fungi plus fertilizer with 50% NPK (Fig. 3).

The use of arbuscular mycorrhizal fungi lead to increase percentages of iron plant parts at maturity in two treatments of mineral fertilizers (Fig. 3).

In all plant parts, zinc concentrations decreased during plant growth. Fig. 3 shows the decreased percentages in maturity plant parts in case of inoculation of faba bean plant with AM fungi plus fertilizers with two treatments minerals fertilizers. The inoculation plant with AM fungi increased zinc content in seeds.

In case of inoculation faba bean with AM fungi plus 25% NPK fertilizers, copper concentrations decreased in stem and leaves with plant growth and increased in stem. The percentage of copper in maturity plant parts were shown in Fig 4. Lead concentrations decreased in all plant parts with plant growth (Fig 3).

3-Elements distribution of plant parts inoculated with arbuscular mycorrhizal (AM) fungi and *Ps. putida* and fertilized with 25% or 50% mineral fertilizers

Zinc concentrations were decreased in all plant parts during plant growth. The different decreased percentages in case of inoculation faba bean with AM fungi plus *Ps. putida* and two treatments of mineral fertilizers (25% and 50%) were cleared in Fig. 4.

Fig. 4 shows copper different percentages in all plant parts. Lead concentrations decreased in roots and stem, while increased in leaves, flowers and seeds during plant growth.

Copper is toxic, however, to many bacteria and viruses (Ernest 1991). Copper sulphate and copper oxide have been used as fungicides for many decades. Freshly added copper salts in concentration of more than 50 mg/l of soil solution reduce the growth of mycorrhiza fungi which live in symbiosis with roots (Ruegg 1989). For plants copper toxicity in virtually unknown, a situation very much like that obtaining in man because of the protective mechanisms mentioned above. Buck (1977) stated that regulatory mechanisms appear to limit the concentration of copper found in plant tissues to about 20 ppm. From the data obtained, it can be concluded that all parts of plant are polluted in two stages in case of use 25% mineral fertilizers, as well as root and leaves in mature stage in case of 50% mineral fertilizers, in untreated plants. All plant parts are polluted with copper in all treatment except the stem in two stages in all treatments, seeds in case of fertilization with 50% NPK and inoculated with AM fungi alone or/and with *Ps. putida* (Buck 1977). The best treatments are inoculated with AM fungi+25% NPK followed by AM fungi+Ps.+50%NPK in mature stage.

Iron is an essential element to physiological processes of all living organisms. It is a metal of low toxicity. Only iron concentrations exceeding 10 to 200 mg/l of nutrient solution have been found to be toxic to plants, and amounts in excess to 200 mg/day are considered toxic for man (Bowen 1979). It can be concluded that all plant parts in all treatments in this study are polluted with iron according to Bowen (1979). The best treatment is the case of fertilization with 50% NPK +AM fungi+ Ps.

In soils with natural lead concentrations (15-30 *u*g/g), only trace amounts of lead are absorbed by plants. The amount absorbed increases when the concentrations of lead in soil increases or when the binding capacity of soil for lead decreases (low organic fraction and low pH) (Ernest 1991)

It can be concluded that all plant parts are polluted with Pb according to National Academy of Science (1980), Sauerbeck (1983). The best treatment is the case of using 25% NPK+ AM fungi in mature stage.

Zinc plays an important role as an essential trace element in all living systems from bacteria to humans. The zinc content in plants is also influenced by the age and vegetation state of the plant (Erenst 1991). Usually the highest zinc content is found in young plants. During ageing, zinc concentration decreases as a result of dilution (Ernest 1991). The normal zinc concentration of plants ranges from 15 to 100 mg/kg dry weight (Ernest 1991). Values higher than 400 mg/kg are regarded as toxic (Ernest 1991 and Jones 1967). From the data in this study, it can be concluded that the concentration of zinc in all young plant parts are higher than 400 mg/kg then decreasing up with plant growth. Zinc addition using 50% NPK fertilizers +AM fungi is the best treatment.

Table 1. Comparison between the results obtained for soil samples (before cultivation) and guideline values for tolerable element concentrations to agricultural soil (ug/g) applied to Federal Republic of Germany (FRG) and Switzerland

Element	FRG*			Switzerland*		Soil samples	
	A	B	Total [C]	Water soluble [D]	Total	Water soluble [E]	Available [F]
Cu	1-20	100	50	0.7	56.74	0.039	4.81
Fe	NM	NM	NM	NM	25028.57	2.710	90.02
Pb	0.1-20	100	50	1.0	48.55	0.490	2.95
Zn	3-50	300	200	0.5	251.02	0.190	2.87

• Ewers 1991, A: Common concentration range, B: Maximum tolerable concentration, C: Extracted with concentrated HNO_3, D: Extracted with $NaNO_3$ solution, E: Soil paste extract, F: Extracted with NH_4OAC+EDTA, NM: Not mentioned

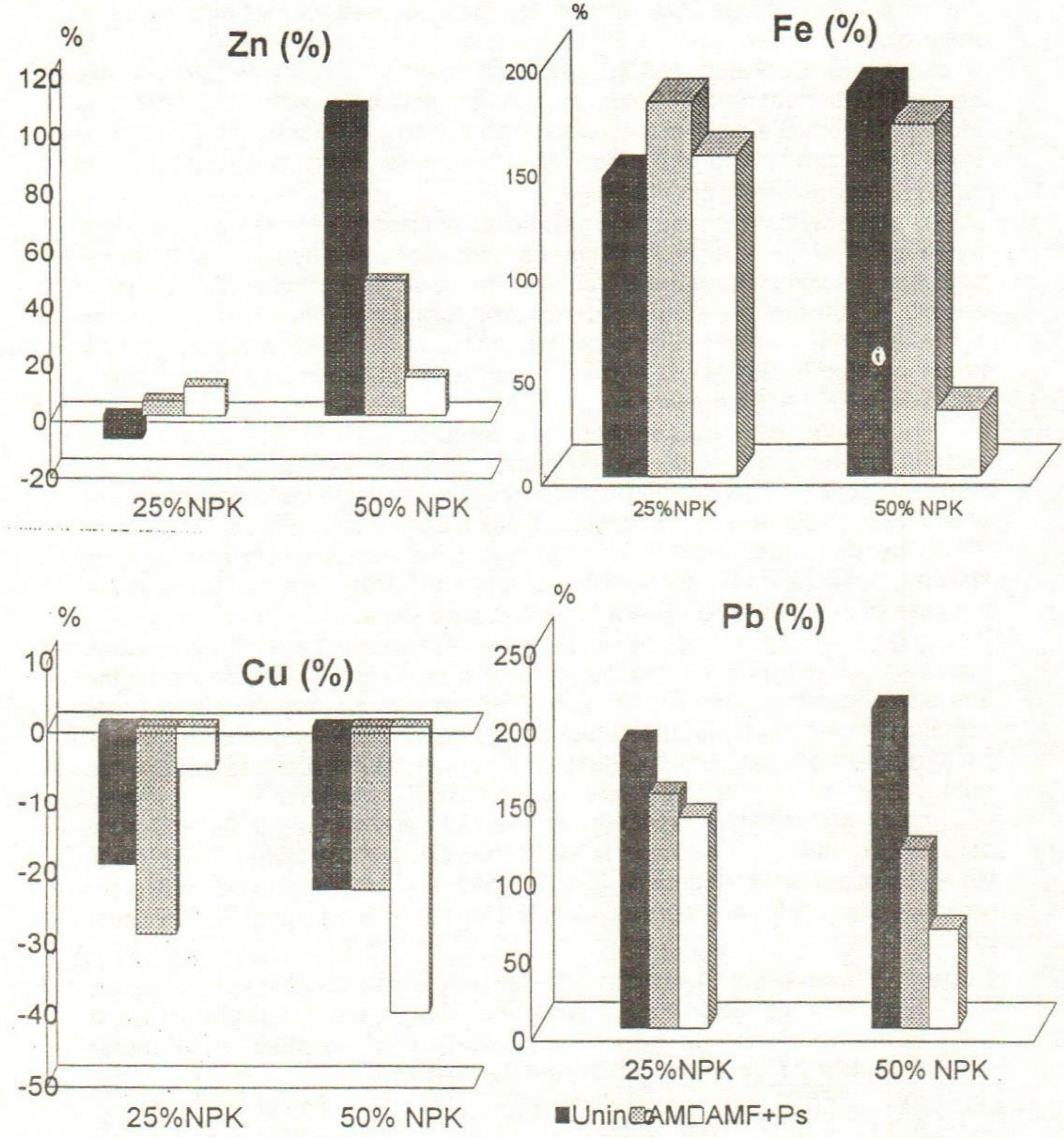

Fig. 1. The different available percentage of elements in surface soil (0-30 cm) at different treatments*

*The different available of elements % = the concentration of elements in second time – the concentration of elements in first time / concentrations in soil before cultivation X 100

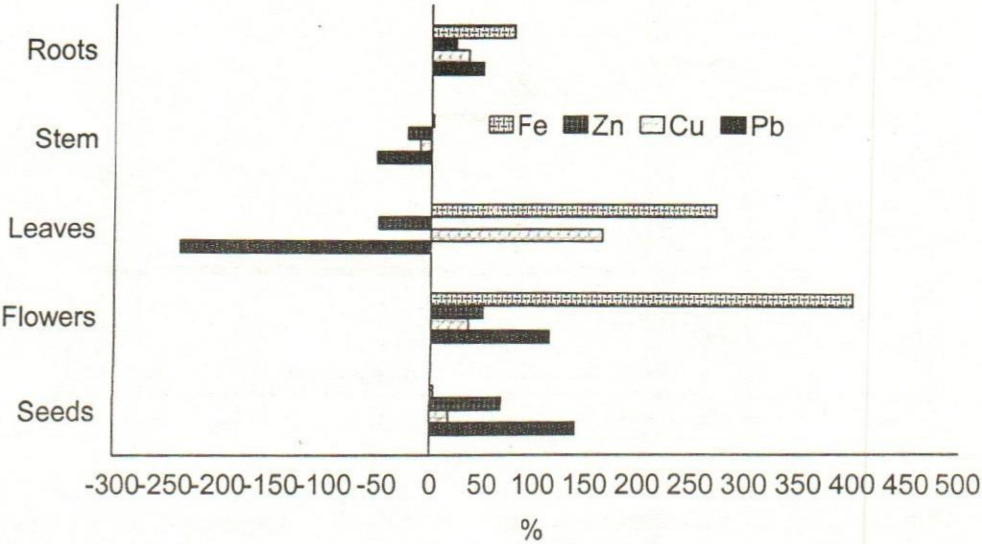

Fig. 2. Different element distribution of plant parts (%)* in the uninoculated treatments fertilized with 25 and 50% mineral fertilizers
***Increasing or decreasing percentage of elements in plant parts= concentration of elements in plant parts at second stage – concentration of elements in plant parts at first stage in case of use 50% NPK/ in case of use 25% NPK X 100**

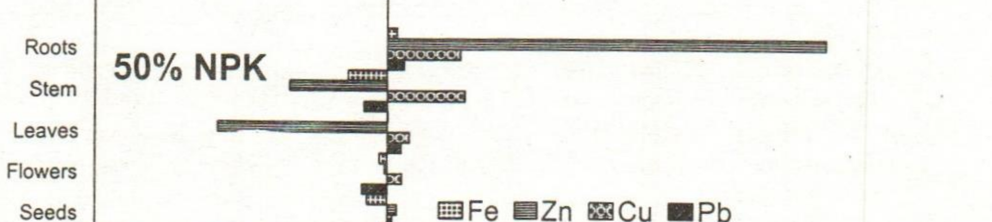

Fig. 3. Different element distribution of plant parts inoculated with AM fungi and fertilized with 25% or 50% mineral fertilizers*
***Different increasing or decreasing percentage of elements in plant parts = concentration of elements in plant parts at second stage - concentrations in plant parts at first stage/ concentrations in plant parts uninoculated with AM fungi X 100**

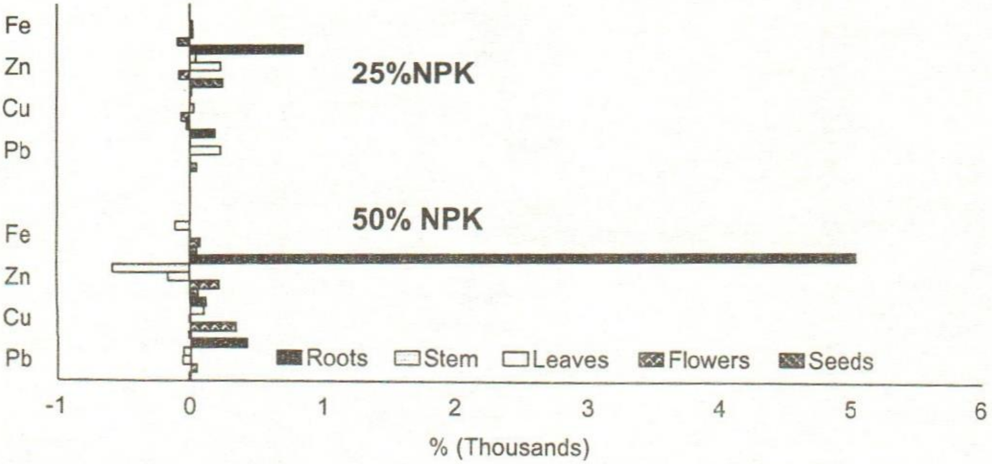

Fig. 4. Different elements distribution (%)* of plant parts inoculation with AM fungi plus *Ps. putida* and fertilized with 25 or 50% NPK

***Different increasing or decreasing percentage of elements = concentration of elements in plant parts at second stage- concentrations in plant parts at first stage/ concentrations in plant parts uninoculated with AM fungi or AM fungi+*Ps. putida* X 100**

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دراسات على المعالجة الحيوية فى الارض والنباتات
٢- تأثير فطريات الميكرويزا الداخلية وبكتريا*Ps. putida* على توزيع العناصر الثقيلة فى الارض ونباتات الفول البلدى النامية تحت ظروف الحقل

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تم إجراء تجربة حقلية لدراسة تأثير العلاقة المزدوجة بين فطريات الميكـوريزا الداخليـة و*Ps. putida* وتأثيرها علي توزيع العناصر الثقيلة فى التربة وأجزاء نبات الفول البلدى النامى تحت ظروف الحقل. فى وجـود التسميد المعدنى (نيتروجين:فوسفور:بوتاسيوم) بنسبة ٢٥ او ٥٠% من الكميات الموصى بها.

وقد وجد أن الارضى قبل الزراعة ملوثة بكل من النحاس والزنك الكلى وكذلك بكل من النحـاس والحديـد والزنك الميسرين. وخلال نمو النبات زاد التلوث بهذه العناصر فى التربة ما عدا النحاس الذى نقصت كميتة فى كل المعاملات. وقد وجد أن احسن معاملة تؤدى الى تقليل التلوث بهذه العناصر فى التربة هى تلقيح النباتات بفطريـات الميكوريزا و *Ps. putida* فى وجود التسميد المعدنى بنسبة ٥٠% من الكميات الموصى بها.

وأظهرت النتائج زيادة تركيز الفوسفور فى سيقان وازهار الفول البلدى فى معاملة التلقيح بفطريات الميكوريزا على الرغم من عدم وجود زيادة معنوية فى تركيز الفوسفور فى الجذور مقارنة بالمعاملة غير الملقحـة. وادى التلقـيح المزدوج بكل من الفطر والبكتريا الى نقص تركيز الفوسفور فى سيقان وازهار الفول البلدى كنتيجة لزيادة النمو.

فى النباتات غير الملقحة وجد أن كل اجزاء الفول البلدى ملوثة فى كل من مرحلة الإزهار والنضـج مـع وجـود التسميد المعدنى بنسبة ٢٥% وكذلك الجذور والاوراق فى مرحلة النضج مع التسميد المعدنى بنسبة ٥٠% وكانـت احسن المعاملات التى تؤدى الى تقليل التلوث بالنحاس هى حالة التلقيح بفطريات الميكوريزا مع التسـميد ب ٢٥% من الاسمدة المعدنية يليها التلقيح المزدوج بكل من الفطر والبكتريا مع التسميد المعدنى ب ٥٠% فى مرحلة النضج.

كذلك وجد تلوث بعنصر الحديد فى كل اجزاء نباتات الفول البلدى تحت ظروف التجربة وكانت احسن المعاملات التى تؤدى الى تقليل التلوث بالحديد فى حالة التسميد ب ٥٠% من الاسمدة المعدنية مع التلقيح المـزدوج بكل من الفطر والبكتريا. ووجد كذلك تلوث بعنصر الرصاص فى كل اجزاء نبات الفول البلـدى وكانـت احسـن معاملة تؤدى الى تقليل التلوث بالرصاص فى حالة التسميد ب ٢٥% من الاسمدة المعدنية مع التلقيح المزدوج بكـل من الفطر والبكتريا فى مرحلة النضج. أما تركيز الزنك فوجد انه مرتفع فى أجزاء النباتات الصغيرة ثم قلـت مـع نمو النباتات وكانت احسن معاملة لتقليل التلوث هى التلقيح بفطريات الميكرويزا مع التسميد ب ٥٠% من الاسـمدة المعدنية.