The Combined Use of Beneficial Soil Microorganisms Enhanced the Growth and Efficiently Reduced Lead Content in Leaves of Lettuce (*Lactuca sativa* L.) Plant under Lead Stress

Sary Hassan Mostafa Brengi and Ibrahim Ali Ahmed Abouelsaad
Horticulture Department, Faculty of Agriculture, Damanhour University, Egypt

ABSTRACT

Two pots experiments were conducted, during two successive winter seasons of 2016/2017 and 2017/2018, to test the role of biofertilizer inoculations (arbuscular mycorrhizal fungi, AMF; *Bacillus megaterium, B. megaterium*; and their mixture) in improving growth and quality of lettuce (*Lactuca sativa* L. cv. Balady) under different levels (0, 100, 200 and 300 ppm) of lead (Pb). The results illustrated that the growth and quality of lettuce were greatly affected by the escalated levels of Pb in soil. Clearly, the increased levels of Pb decreased plant growth parameters, the contents of macronutrient and micronutrient as well as photosynthetic pigments, but increased the contents of Pb in the different parts of lettuce plants. Among the tested biofertilizer inoculations, the combined inoculation (AMF + *B. megaterium*) showed superior effects for increasing plant growth, contents of nutrient and photosynthetic pigments. AMF and the combined inoculation treatment exhibited an equal efficiency for reducing the content of Pb in the old and new leaves (edible parts) of lettuce plants, while *B. megaterium* showed the best performance in reducing the content of Pb in lettuce roots. Our study suggested that the combined inoculation (AMF + *B. megaterium*) is an effective tool for improving growth and quality of lettuce in high-contaminated soil with Pb.

Keywords: Lead, Lettuce, Biofertilizer, Amf, B. megaterium, Leaves and Roots.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most common leafy vegetables worldwide and considered as a major dietary source for vitamins and nutrients with low calories (Rubatzky and Yamaguchi, 2012). Vegetable crops are the target of a wide range of pollutants that affect growth and yield. Heavy metals are among common pollutants that affect plants with a major public health risk, especially in developing countries (Flora *et al*., 2012).

Heavy metals are elements that characterized by relatively high atomic weight or atomic numbers, greater than 20, and high relative density (Raskin *et al*. 1994). Some of these metals are required in small quantities by plants such as nickel (Ni), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn) and molybdenum (Mo). However, the excessive levels of these elements in soils can become harmful for plants. Other heavy metals such as lead (Pb), cadmium (Cd) and mercury (Hg) do not have any beneficial effect the living organisms and they are very harmful for both plant and human (Chibuike and Obiora, 2014).

Lead impairs seed germination, plant growth, seedling development, cell division and chlorophyll production (Gupta *et al*., 2009; Maestri *et al*., 2010; Pourrut *et al*., 2011). This metal is widely used in many industrial processes and is existed as a contaminant in all environmental compartments (water, soil and air) (Pourrut *et al*. 2011). The prominence of environmental Pb contamination due to both its persistence and its numerous sources such as burning of fossil fuels, use of fertilizers and pesticides in agriculture, mining and smelting of metals, sewage sludge, municipal waste disposal and production of batteries (Punamiya *et al*. 2010; Chibuike and Obiora, 2014). The standard limit of Pb in vegetables is 0.3 mg/kg as reported by FAO/WHO (2014). In Egypt, high levels (higher than the allowable limit) of Pb in vegetable crops were observed in different places. For instance, Abdel-Rahman *et al* (2018) tested the levels of Pb in three common consumed vegetables (tomato, potato and cucumber) collected from four governorates (El-Faiyum, Giza, Alexandria and Cairo) with high populations. The study showed that high levels of Pb in potato and cucumber were noticed in some places. In another study by Eissa and Negim (2018), the levels of Pb in lettuce and spinach plants grown in Assiut governorate were also higher than the allowable limit. Overall, Pb is one of the greatest widespread contaminant in soils and frequently encountered by plants (Pourrut *et al*. 2011).Therefore, the development of a remediation strategy for metal polluted soils is urgent for human health and environmental conservation (Chibuike and Obiora, 2014).

Different methods of remediating metal polluted soils were investigated, that including chemical, physical and biological methods. The physical and chemical methods (e.g. Stabilization, encapsulation and soil vapor extraction) are greatly expensive and make the soil, improper for plant growth (Marques et al. 2008; Chibuike and Obiora, 2014). On the other hand, bioremediation or biological method is an environmentally friendly method because it is achieved by natural processes with less cost compared with other remediation techniques (Chibuike and Obiora, 2014).

Bioremediation of heavy metals in the polluted soils can be achieved by microorganisms or plants (Phytoremediation). During bioremediation, heavy metals transformed to become either less bioavailable or less toxic due to a change in their oxidation state (Garbisu and Alkorta, 2003). Phytoremediation is the direct use of plants for accumulation of heavy metals in the roots and shoots (Chibuike and Obiora, 2014). These plants usually possess a high ability to tolerate high levels of heavy metals that may lead to metal accumulation in the harvestable part and may result in contamination of the food chain (Marques *et al*., 2008). Therefore, alternative methods are needed.

Arbuscular mycorrhizal fungi (AMF) have been used in many remediation studies and the obtained results show that AMF employ different strategies for the remediation of heavy metal-polluted soil. For example, while some studies have shown that AMF store metals in their vacuole or chelate them in cytoplasm (Zhu *et al*. 2001; Ouziad *et al*. 2005), others reported that AMF enhanced nutrient and water acquisition for plant growth and increase the plant resistance to diseases, all are believed to support plant growth in polluted soil (Cardoso and Kuyper, 2006; Chibuike and Obiora, 2014). The inoculation with AMF decreased the content of Pb in sweet basil leaves (Elgharably and Allam, 2013), black locust leaves (Yang *et al*. 2015) and marigold. (Tabrizi *et al.* 2015).

Bacillus megaterium, generally considered soils microbe, is of particular interest as bacteria–assisted remediation of metal-polluted sites (Esringu *et al*. 2014). Saleem *et al*., (2007) reported that *B. megaterium* have the advantages of being tolerant for the high-stress environment in soil and have the capacity to resist pathogens and enhance plant growth under adverse conditions. The inoculation of tomato and garlic with *B. megaterium* increased plant growth, yield and P contents (Turan *et al*. 2007; Brengi *et al*. 2018). Also, *B. megaterium* ameliorated the negative effects of Pb toxicity in *Brassica napus* plant (Esringu *et al*. 2014) and decreased the content of Pb in the edible parts of leafy vegetables (Wang *et al*. 2006).

Although microorganism- assisted remediation has been investigated, there is little information on the potential advantage of using combined microorganisms (species) for remediation of heavy metal-polluted soil. Therefore, the current study was conducted to investigate the potential use of AMT and *B. megaterium* as a tool for reducing the content of Pb in lettuce plants growing under escalated levels of Pb.

MATERIALS AND METHODS

Growing conditions and treatments

The current study was conducted at Bader, Beheira Governorate, Egypt, during the two successive winter seasons of 2016/2017 and 2017 / 2018, to test the effect of arbuscular mycorrhizal fungi (AMF), *Bacillus megaterium* (*B. megaterium*) and the mixture between them on the growth and quality of lettuce (*Lactuca sativa* L. cv. Balady) under different levels (0, 100, 200 and 300 ppm) of lead (Pb), used as lead acetate. Lettuce seedlings, purchased from a local nursery, were transplanted into plastic pots (30 cm diameter and 35 cm height), filled with 10 kg of sandy soil, and placed in the open field condition. Prior to transplanting, the physical and chemical characteristics of the used soil (Table 1) were analyzed as described by Jackson (1958) in both seasons of study.

Each experiment includes 16 treatments which are the combinations between four levels of Pb (0, 100, 200 and 300 ppm) and four treatments of biofertilizers (AMF, *B. megaterium,* AMF+ *B. megaterium* and non-biofertilized soil). The biofertilizers were applied during the transplanting of lettuce seedlings with equal doses. Additional agricultural practices were conducted when it was necessary and as recommended by the Ministry of Agriculture and Soil Reclamation for the commercial production of lettuce in sandy soil.

Table 1: The physical and chemical characteristics of experimental soil.

	First season	**Second season**
Sand (%)	47.3	46.8
Silt (%)	36.2	36.4
Clay (%)	16.5	16.8
Soil texture	**Sandy-loam**	**Sandy-loam**
pH (in water)	7.6	7.56
EC ($ds{\cdot}m^{-1}$)	0.96	0.971
Organic matter (%)	0.97	0.972
Total N (%)	0.17	0.16
Assimilable P (ppm)	10.8	10.72
Exchangeable K (ppm)	105	103
Pb (ppm)	6.32	6.84

Plant measurements

After the lettuce harvest (55 days from transplanting date), plant growth parameters (shoot fresh weight by g plant^{-1}, shoot dry weight by g plant^{-1}, plant height by cm plant^{-1} and number of leaves per plant) were recorded immediately. The lettuce roots were carefully unglued from the pots and washed three times with tap water followed by fresh distillate water. To obtain the shoot dry weight, fresh shoots samples were oven dried at 70°C for 48h and the dry samples were ground for the nutrient contents analysis. Photosynthetic pigments are estimated according to Davis (1976) using fresh tissues from lettuce leaves. Kjeldahl method was used to determine the content of N in lettuce leaves samples according to Nelson and Sommers (1982), and total P was analyzed by spectrophotometry after an open digestion according to the methods described by Jackson (1958). The contents of K, Ca, Zn, Mn, Fe and Pb in the dried lettuce tissues were estimated by the wet digestion method and analyzed using atomic absorption spectroscopy (Cottenie 1981).

Statistical analysis and experimental design

The experimental design, in the current study, was split plots in a randomized complete block design. The levels of Pb arranged in the main plots and the biofertilizers treatments were randomly placed in the sub-plots. Nine replicates (pots) for each treatment were applied with one plant for each pot. For Statistical analysis, CoStat software program (Version 6.4, Co Hort, USA, 1998–2008) was used for all the obtained data. Means were compared using least significant difference (LSD) test at $p < 0.05$.

RESULTS AND DISCUSSION

Growth of lettuce plants

The statistical analysis reveals that the lettuce growth parameters, except the number of leaves, were significantly affected by the Pb concentrations, biofertilizers inoculation and their interaction, in both seasons (Table 2). The escalated levels of Pb significantly reduce the shoot fresh and dry weights as well as plant height in comparison with the control in both seasons, where the highest level of reductions were observed with 300 ppm of Pb. The reduction in plant growth due to Pb application is in agreement with the findings of Brengi (2008) in common bean (*Phaseolus vulgaris*), Akinci *et al*., (2010) in tomato (*Solanum lycopersicum*) seedlings and Lamhamdi *et al* ., (2013) in spinach (*Spinacia oleracea*) plants. Chibuike and Obiora (2014) reported that the plant growth reduction have been attributed to both direct and indirect effects of heavy metal which sometimes results in the death of plants. Direct toxic effects include damage of plant cell structures due to oxidative stress, inhibition of cytoplasmic enzymes and inhibition of photosynthesis as well as chlorophyll synthesis (Sandalio *et al*. 2001). On the other hand, some of the indirect effect is decreasing the absorption of the essential nutrient for plant (Chibuike and Obiora 2014)

Regardless to the Pb treatments, our data showed that the applications of all the biofertilizer inoculations, significantly increased the shoot fresh and dry weights as well as plant height of lettuce, comparing with the control, in both seasons of the study (Table 2). The highest values of shoot fresh and dry weights were observed in lettuce plants treated with the combined inoculation (AMF + *B. megaterium*). These increases were estimated by 12.27 % and 10.57 % for the shoot fresh weight, with 12.9 % and 13.38 % for the shoot dry weight, over the control (without biofertilizer) in first and second seasons, respectively (Table 2). For the plant height, all bio- fertilizers inoculations revealed comparable values, except *B. megaterium* treatment which showed less value for the plant height, compared with other bio-fertilizers in only the second season. Harrier and Watson (2004) showed that the AMF, in general, increase the plant resistance to diseases, the acquisition of water and nutrients uptake, thus help for enhancing plant growth. Also, Esringu *et al*., (2014) reported that *B. megaterium* is stimulating plant growth by improving soil properties and enhancing availability of nutrients.

The interaction effects on the lettuce growth parameters, for both seasons, were presented in Table 2. The highest values for shoot fresh and dry weights were observed with lettuce plants that treated with 0 ppm of Pb and the mixed inoculation (AMF + *B. megaterium*) in both seasons. However, the highest values for plant heights obtained with the lettuce plants that grown in 0 ppm of Pb and inoculated with all biofertilizer treatments. Our results showed that, in some cases, the biofertilizer inoculations under different levels of Pb exhibited improving in lettuce growth against the corresponding control (Table 2). For example, the inoculations with AMF or the mixed inoculation treatment increase shoot fresh weight, shoot dry weight and plant height under 300 ppm of Pb in comparison with the treatment of 300 ppm Pb+ no biofertilizer (corresponding control), in the first season.

The content of photosynthetic pigments

In our study, the results showed that the different levels of Pb, biofertilizer inoculations and their interaction significantly affected the photosynthetic pigments (chlorophyll a (ch a), chlorophyll b (ch b) and carotenoids) in both seasons of the study (Table 3). The increased levels of Pb significantly decreased the leaf contents of ch a, ch b and carotenoids, relative to the control treatment.

Table 2: Shoot fresh weight, shoot dry weight, number of leaves and plant height of lettuce plants as affected by lead, biofertilizer inoculations and their interaction during both seasons.

		Shoot fresh weight (g)		Shoot dry weight (g)		Plant length (cm)		Leaves number	
		First season	**Second season**	**First season**	**Second season**	**First season**	**Second season**	**First season**	**Second season**
Lead (ppm)	0	489.67	494.33	74.49	76.87	45.25	46.17	22.12	22.67
	100	377.58	378.75	58.01	58.30	42.33	41.50	19.67	20.42
	200	343.25	342.75	49.68	50.04	40.67	40.42	20.42	20.33
	300	258.17	268.25	32.43	35.78	37.92	38.83	19.33	19.83
	LSD 0.05	**8.93**	**8.47**	**1.36**	**1.00**	**1.45**	**1.09**	**NS**	**NS**
Treatments	Control	350.08	354.58	50.48	51.78	39.42	39.50	20.00	20.58
	B. megaterium	367.75	366.92	52.48	54.58	41.92	41.75	20.67	20.83
	AMF	370.40	370.50	53.23	55.91	42.50	42.67	20.42	21.08
	Mix	393.03	392.08	57.00	58.71	42.33	43.00	20.50	20.75
	LSD 0.05	**10.42**	**8.71**	**1.58**	**1.70**	**1.13**	**0.92**	**NS**	**NS**
0(ppm)	Control	456.33	468.00	72.76	72.88	44.33	45.00	22.33	23.33
	B. megaterium	477.67	477.67	73.82	74.51	46.00	46.67	21.67	22.33
	AMF	474.00	474.00	73.51	74.17	46.00	47.00	22.33	22.33
	Mix	557.67	557.67	85.87	85.91	44.67	46.00	22.33	22.67
100(ppm)	Control	358.33	365.00	55.46	55.00	41.00	40.33	19.33	20.00
	B. megaterium	380.00	378.67	57.78	58.05	42.67	41.67	20.00	20.33
	AMF	383.67	383.67	58.93	59.61	43.00	42.33	20.00	21.00
	Mix	388.33	387.67	59.85	60.55	42.67	41.67	19.33	20.33
200 (ppm)	Control	332.67	334.00	47.99	47.13	37.33	37.33	20.00	20.67
	B. megaterium	341.67	342.67	49.54	50.31	41.00	40.67	21.00	20.00
	AMF	349.33	350.67	51.25	52.55	42.00	41.00	20.00	20.67
	Mix	347.00	343.67	49.93	50.15	42.33	42.67	20.67	20.00
300 (ppm)	Control	238.00	251.33	28.71	32.12	35.00	35.33	18.33	18.33
	B. megaterium	260.67	268.67	31.80	35.46	38.00	38.00	20.00	20.67
	AMF	263.67	273.67	33.98	37.32	39.00	40.33	19.33	20.33
	Mix	270.33	279.33	35.22	38.22	39.67	41.67	19.67	20.00
LSD 0.05		**20.84**	**32.12**	**3.16**	**6.26**	**1.84**	**3.40**	**NS**	**NS**

The highest level of Pb (300 ppm) was found to induce the highest reductions in photosynthetic pigments in both seasons. These results are in line with those of Lamhamdi *et al*., (2013) who reported that the escalated levels of Pb in soil reduce the contents of photosynthetic pigment in wheat (*Triticum aestivum*) and spinach leaves. Liu *et al*., (2008) reported that, the degradation of photosynthetic pigments is a well-known symptom of lead toxicity due to the increase in chlorophyllase activity. Also, the reduction in photosynthetic pigments, under Pb stress, may result from a decreasing in the amount of thylakoids or grana and change of chlorophyll structure due to replacement of Zn, Mg, Fe and Mn by Pb (Sengar and Pandey, 1996; Akinci *et al*. 2010).

Among the treatments with different biofertilizer inoculation, the mixed inoculation (AMF + *B. megaterium*) exhibited the highest contents of (cha), (ch b) and carotenoids in lettuce leaves, with no significant differences for the values of (ch b) content which were observed with *B. megaterium* in both seasons (Table 3). The treatment with AMF showed photosynthetic pigment contents in lettuce leaves similar to the control. In our study, although the application of AMF did not show a positive effect on the pigment contents, some previous studies indicated that AMF induces accumulation of those pigments in some leafy vegetables such as lettuce (Baslam *et al*. 2011) and spinach (Khalid *et al*. 2017).

The combinations between 0 ppm of Pb and all biofertilizer inoculations exhibited comparable values for the photosynthetic pigments in both seasons (Table 3). Under Pb stress conditions, in few cases, the inoculation with biofertilizers showed positive effects on the pigments content relative to the corresponding control.

Table 3: The contents of chlorophyll a (ch a), chlorophyll b (ch b) and carotenoids in lettuce leaves as affected by lead, biofertilizer inoculations and their interaction during both seasons.

		Ch a (mg/100 g fresh weight)		Ch b (mg/100 g fresh weight)		carotenoids (mg/100g fresh weight)	
		First season	**Second season**	**First season**	**Second season**	**First season**	**Second season**
Lead (ppm)	0	27.84	27.73	8.49	8.46	10.19	10.15
	100	26.58	26.61	8.11	8.12	9.73	9.74
	200	24.84	24.96	7.55	7.59	9.05	9.09
	300	22.37	22.51	6.80	6.86	8.25	8.30
LSD 0.05		**0.284**	**0.43**	**0.297**	**0.98**	**0.77**	**0.139**
Treatments	Control	25.18	25.11	7.71	7.68	9.38	9.35
	B. megaterium	25.33	25.41	7.76	7.78	9.13	9.16
	AMF	25.21	25.17	7.72	7.71	9.12	9.11
	Mix	25.92	26.12	7.79	7.85	9.59	9.67
LSD 0.05		**0.338**	**0.46**	**0.054**	**0.091**	**0.287**	**0.298**
0(ppm)	Control	27.90	27.53	8.50	8.39	10.35	10.21
	B. megaterium	27.83	27.73	8.52	8.49	10.03	10.00
	AMF	27.70	27.51	8.49	8.43	10.03	9.96
	Mix	27.93	28.13	8.46	8.52	10.35	10.43
100(ppm)	Control	26.08	26.13	8.08	8.09	9.83	9.85
	B. megaterium	26.45	26.52	8.10	8.12	9.53	9.56
	AMF	26.32	26.38	8.06	8.08	9.53	9.55
	Mix	27.47	27.40	8.21	8.19	10.04	10.01
200 (ppm)	Control	24.40	24.36	7.43	7.42	9.06	9.04
	B. megaterium	24.77	25.02	7.58	7.66	8.93	9.01
	AMF	24.66	24.80	7.55	7.60	8.92	8.97
	Mix	25.53	25.66	7.63	7.67	9.29	9.34
300 (ppm)	Control	22.32	22.42	6.81	6.83	8.28	8.31
	B. megaterium	22.27	22.35	6.82	6.85	8.03	8.06
	AMF	22.16	21.98	6.79	6.73	8.02	7.95
	Mix	22.75	23.28	6.87	7.03	8.68	8.88
LSD 0.05		**1.24**	**1.699**	**0.20**	**0.34**	**1.06**	**1.099**

For instance, the application of the mixed inoculation (AMF + *B. megaterium*) enhanced the contents of (ch a) of lettuce plants submitted to 100 ppm Pb, in both seasons, comparing with the treatment of 100 ppm Pb+ no biofertilizer (corresponding control). Also, the all tested biofertilizer applications were found to increase the (ch b) content in lettuce plants that are stressed with 200 ppm Pb, relative to the treatment of 200 ppm Pb+ no biofertilizer (corresponding control), in the second season only (Table 3). However, most combinations between different levels of Pb and biofertilizer inoculations did not show any change in the content of the photosynthetic pigments against the corresponding controls.

The contents of macronutrient and micronutrient

The contents of the tested macronutrient (N, P, K and Ca) and micronutrient (Fe, Zn and Mn) in the lettuce leaves were significantly affected by the different levels of Pb, biofertilizer inoculations and their interaction, in both seasons (Table 4 and 5). Our data also showed that the increased levels of Pb significantly decreased the contents of macronutrient and micronutrient in the shoots of lettuce plants, whereas the highest reductions were observed with 300 ppm Pb (Table 4 and 5). Multiple studies showed that the macronutrient and micronutrient uptake by plants is affected by the presence of Pb in different plant species (Paivoke, 2002; Malkowski *et al*. 2002; Gopal and Rizvi, 2008; Pourrut *et al*. 2011; Lamhamdi et al. 2013). For instance, Pb exposure decreases the content of nutrients in *Spinacia oleracea* (Lamhamdi *et al*. 2013), *Raphanus sativus* (Gopal and Rizvi 2008), *Solanum lycopersicum* (Akinci *et al*. 2010), *Zea mays* (Malkowski *et al*. 2002) and *Pisum sativum* (Paivoke, 2002). Pourrut *et al*., (2011) suggested that the decreased uptake of nutrient in the presence of Pb may result from changes in physiological plant activities and/or the competition between the nutrients with atomic size similar to Pb. Sharma and Dubey (2005) reported that K ion and Pb have similar radii and these ions may compete for entry into the same potassium channels in plant root. Also, Pb effects on K-ATPase on the cell membrane cause an efflux of K from plant root (Pourrut *et al.* 2011). Pourrut *et al.*, (2011) demonstrated that Pb does not compete with nitrogen (N). However, the reduction in the content of N in plant could be induced by the reduced N assimilation process (e.g. reduced the activity of nitrate reductase enzyme), as indirect effect for Pb toxicity.

Irrespective of Pb treatments, in both seasons of the study, all biofertilizer inoculations significantly increased the contents of macronutrient and micronutrient in the lettuce shoots, except in few cases, where the content of Zn did not show any significant change with AMF or *B. megaterium* applications, only in the second season, in comparison to the control (Table 4 and 5). The data in Table (4) showed that, the highest contents of macronutrient (N, P, K and Ca) in lettuce shoots were noted with the mixed inoculation (AMF + *B. megaterium*), with no significant differences with the contents of N and K during(both seasons) compared with AMF application. The highest contents of micronutrients (Fe, Zn and Mn) in the lettuce shoots were remained in the mixed inoculation treatment except Mn with AMF in the first season, without significant differences among the contents of Mn obtained from AMF in the second season only and *B. megaterium* (second season). The mixed inoculation (AMF + *B. megaterium*) enhanced the contents of N by 16.50 % and 17.23 %, P by 19.44 % and 20.00 %, K by 11.30 % and 12.18 %, Ca by 21.11 % and 22.80 %, Fe by 22.86 % and 24.29 %, Zn by 16.53 % and 13.34 % as well as Mn by 8.69 % and 10.40 % in the first and second seasons, respectively. Previous studies showed that AMF enhanced uptake macronutrient and micronutrients such as N, P, K, Zn, Cu, Mn and Fe in plant (Liu *et al.* 2000; Berruti *et al.* 2016).

Table 4: The contents of macronutrient (N, P, K and Ca) in lettuce shoots as affected by lead, biofertilizer inoculations and their interaction during both seasons.

		N (%)		P (%)		K (%)		Ca (%)	
		First season	**Second season**	**First season**	**Second season**	**First season**	**Second season**	**First season**	**Second season**
Lead (ppm)	0	3.80	3.82	0.52	0.50	4.27	4.31	2.25	2.30
	100	3.39	3.43	0.44	0.41	4.05	4.07	2.09	2.05
	200	3.13	3.14	0.34	0.33	3.55	3.58	1.91	1.91
	300	2.60	2.65	0.27	0.28	3.27	3.31	1.68	1.73
LSD 0.05		**0.049**	**0.052**	**0.009**	**0.0092**	**0.046**	**0.06**	**0.023**	**0.065**
Treatments	Control	2.97	2.96	0.36	0.35	3.54	3.53	1.80	1.80
	B. megaterium	3.10	3.13	0.38	0.39	3.79	3.83	1.96	1.95
	AMF	3.37	3.42	0.39	0.40	3.91	3.96	2.01	2.02
	Mix	3.46	3.47	0.43	0.42	3.94	3.96	2.18	2.21
LSD 0.05		**0.011**	**0.06**	**0.013**	**0.008**	**0.095**	**0.07**	**0.076**	**0.07**
0(ppm)	Control	3.50	3.51	0.49	0.48	4.24	4.25	2.08	2.12
	B. megaterium	3.52	3.55	0.51	0.50	4.29	4.32	2.11	2.15
	AMF	4.04	4.08	0.52	0.53	4.32	4.32	2.30	2.36
	Mix	4.15	4.15	0.54	0.55	4.33	4.37	2.51	2.58
100(ppm)	Control	3.23	3.20	0.40	0.41	3.86	3.83	1.99	1.95
	B. megaterium	3.26	3.27	0.37	0.38	4.10	4.12	2.10	2.05
	AMF	3.55	3.59	0.43	0.44	4.08	4.13	2.12	2.09
	Mix	3.52	3.56	0.54	0.55	4.17	4.21	2.15	2.10
200 (ppm)	Control	3.07	3.01	0.29	0.28	3.11	3.05	1.59	1.60
	B. megaterium	3.09	3.13	0.35	0.36	3.65	3.70	1.96	1.94
	AMF	3.10	3.18	0.35	0.36	3.70	3.80	1.91	1.86
	Mix	3.23	3.24	0.36	0.37	3.77	3.78	2.20	2.22
300 (ppm)	Control	2.10	2.13	0.25	0.26	2.96	3.00	1.53	1.55
	B. megaterium	2.53	2.57	0.27	0.28	3.11	3.17	1.65	1.65
	AMF	2.80	2.83	0.27	0.27	3.52	3.55	1.70	1.76
	Mix	2.95	2.95	0.29	0.31	3.51	3.51	1.85	1.95
LSD 0.05		**0. 24**	**0.22**	**0.017**	**0.028**	**0.035**	**0.26**	**0.015**	**0.24**

Table 5: The contents of micronutrient (Fe, Zn and Ca) in lettuce shoots as affected by lead, biofertilizer inoculations and their interaction during both seasons.

		Fe ppm		Zn ppm		Mn ppm	
		First season	Second season	First season	Second season	First season	Second season
Lead (ppm)	0	758.72	774.80	39.32	40.23	165.22	163.28
	100	658.29	645.49	32.44	33.94	144.68	141.31
	200	581.06	577.29	30.38	30.67	138.07	138.48
	300	432.32	443.14	24.66	26.00	123.10	118.73
LSD 0.05		**13.22**	**19.36**	**0.58**	**150**	**1.69**	**8.41**
Treatments	Control	535.09	536.33	29.81	31.33	135.17	132.43
	B. megaterium	604.38	602.61	31.17	31.64	140.76	140.58
	AMF	633.49	635.18	31.08	32.38	148.22	142.59
	Mix	657.43	666.60	34.74	35.51	146.92	146.20
LSD 0.05		**2.57**	**20.85**	**0.118**	**1.62**	**0.28**	**7.05**
0(ppm)	Control	677.54	687.83	37.26	39.02	164.32	159.50
	B. megaterium	758.45	770.06	38.87	40.08	164.32	159.98
	AMF	743.62	760.04	38.23	38.23	166.09	167.10
	Mix	855.27	881.29	42.91	43.60	166.16	167.52
100(ppm)	Control	569.33	560.27	30.65	31.74	135.25	134.63
	B. megaterium	643.75	629.10	33.08	33.79	142.35	144.73
	AMF	676.46	666.37	27.80	30.02	152.66	142.39
	Mix	743.62	726.21	38.23	40.22	148.44	143.50
200 (ppm)	Control	552.08	552.20	28.75	30.63	128.60	124.85
	B. megaterium	555.05	550.47	27.53	26.59	135.40	140.56
	AMF	643.75	628.42	33.08	34.39	145.91	140.65
	Mix	573.35	578.08	32.15	31.09	142.35	147.85
300 (ppm)	Control	341.42	345.03	22.60	23.91	112.49	110.75
	B. megaterium	460.29	460.82	25.19	26.09	120.95	117.04
	AMF	470.12	485.89	25.20	26.89	128.23	120.21
	Mix	457.46	480.80	25.65	27.12	130.72	126.93
LSD 0.05		**9.49**	**76.87**	**0.44**	**5.98**	**1.017**	**25.98**

However, Konieczny and Kowalska (2017) reported that AMF decreased the contents of Cu, Fe, Mn and Mo in lettuce plants. Berruti *et al*., (2016) have demonstrated that nutrient exchange between AMF and plant depends on several factors, including the acquisition of surplus resources and environmental conditions. The increase in P and N contents was also reported in wheat after the incubation with *B. megaterium* (El-Razek and El-Sheshtawy, 2013).

Among all the combinations between the different levels of Pb and biofertilizer inoculations, our results showed that the lettuce plants grown with 0 ppm Pb and fertilized with the mixed inoculation (AMF + *B. megaterium*) exhibited the highest contents of all tested macronutrient and micronutrient in the two successive seasons, with in significant differences in some values of nutrient contents that observed with AMF or *B. megaterium* (Table 4 and 5). The data also showed that, under Pb polluted soil, the mixed inoculation (AMF + *B. megaterium*) showed superior effects for increasing the contents of macronutrient and micronutrient in the lettuce shoots, relative to the corresponding control, in both seasons. For example, the treatment with the mixed inoculation (AMF + *B. megaterium*) increased the contents of N, P, K, Ca and Fe, under the highest level of Pb (300 ppm), compared with the treatment of 300 ppm Pb+ no biofertilizer.

The content of Pb in different parts of lettuce

The data in Table 6 indicated that, in both seasons, the contents of Pb in different parts (old leaves, new leaves and roots) of lettuce increased by the elevated Pb in soil. The treatment with 300 ppm Pb showed the highest contents of Pb in all the tested parts. Obviously, root tissues accumulated the highest level of Pb, followed by the old leaves and then the new leaves, under the different level of Pb. Such results were supported by Meyers *et al*., (2008) and Uzu *et al*., (2009) who showed that Pb is mainly accumulated in the roots of *Brassica juncea* and *Lactuca sativa*, respectively. Wierzbicka *et al*., (2007) reported that the major amount of absorbed

Pb (93 to 98 %) is stored in the plant root tissues as an insoluble form. Thus, only a lesser part of Pb is translocated from root to aerial plant parts.

The data in Table 6 also showed that all biofertilizer inoculations significantly decreased the contents of Pb in plant parts, comparing with the control (non-biofertilized soil), in both season. In both seasons of the study, the mixed inoculation (AMF + *B. megaterium*) was the most efficient treatment for reducing the contents of Pb in the old and new leaves, without in significant differences between the values that obtained with AMF. The mixed inoculation reduced the content of Pb in the old leaves by 39.26 % and 38.41 % as well as in the new leaves by 34.27 % and 33.33% in the first and second seasons, respectively. It is worth to mention that, the *B. megaterium* showed better efficiency for reducing the content of Pb in root than that of AMF and the mixed inoculation. *B. megaterium* is free-living bacteria in soil, while AMF colonize plant root (attached to root). Also, Ouziad *et al*., (2005) and Achakzai *et al*., (2012) reported that AMT reduces the Pb uptake by different strategies such as the immobilization in the external or internal hyphae, store heavy metal in their vacuole and chelate them in cytoplasm. Such differences in lifestyle and Pb reducing strategies may explain the better performance for *B. megaterium* in reducing the content of Pb in roots.

The interaction effects on Pb contents in the different parts (old leaves, new leaves and roots) of lettuce, for both seasons, were presented in Table (6). The mixed inoculation (AMF + *B. megaterium*) exhibited superior effects for decreasing the contents of Pb in the old and new leaves under 100 and 300 ppm Pb, in both growing seasons. However, the application with *B. megaterium* in the high polluted soil (300 ppm Pb) showed better performance in reducing the content of Pb in root, compared to the corresponding controls and other biofertilizer treatments.

Table 6: The contents of lead (Pb) in the different parts of lettuce as affected by lead, biofertilizer inoculations and their interaction during both seasons.

		Pb in old leaves (µg/g dm)		Pb in new leaves (µg /g dm)		Pb in roots (µg /g dm)	
		First season	Second season	First season	Second season	First season	Second season
Lead (ppm)	0	0.87	0.81	0.14	0.12	29.13	29.52
	100	3.91	3.87	0.59	0.58	387.59	383.79
	200	6.70	6.67	1.08	1.08	919.55	915.72
	300	14.68	14.79	2.55	2.57	1630.33	1646.76
LSD 0.05		**0.47**	**0.34**	**0.079**	**0.054**	**40.93**	**46.02**
Treatments	Control	8.66	8.59	1.43	1.41	924.26	925.17
	B. megaterium	6.44	6.41	1.06	1.06	558.21	555.35
	AMF	5.79	5.84	0.93	0.94	819.65	825.52
	Mix	5.26	5.29	0.94	0.94	664.48	669.73
LSD 0.05		**0.49**	**0.49**	**0.078**	**0.081**	**30.26**	**37.53**
0(ppm)	Control	1.35	1.08	0.22	0.14	32.32	32.79
	B. megaterium	0.77	0.78	0.13	0.13	24.32	24.57
	AMF	0.72	0.73	0.12	0.12	31.82	32.28
	Mix	0.62	0.63	0.10	0.10	28.07	28.43
100(ppm)	Control	5.19	5.14	0.80	0.79	541.05	536.32
	B. megaterium	3.51	3.47	0.52	0.52	270.33	266.65
	AMF	3.63	3.60	0.54	0.54	397.20	393.80
	Mix	3.29	3.25	0.48	0.48	341.77	338.39
200 (ppm)	Control	9.15	9.17	1.44	1.44	1266.32	1269.47
	B. megaterium	6.14	6.09	0.94	0.93	708.52	701.87
	AMF	6.00	5.91	0.91	0.90	952.24	938.21
	Mix	5.49	5.51	1.03	1.04	751.10	753.32
300 (ppm)	Control	18.92	18.97	3.26	3.26	1857.33	1862.12
	B. megaterium	15.34	15.30	2.67	2.66	1229.67	1228.29
	AMF	12.82	13.10	2.15	2.19	1897.33	1937.81
	Mix	11.63	11.79	2.13	2.16	1537.00	1558.80
LSD 0.05		**1.75**	**1.81**	**0.29**	**0.300**	**111.59**	**138.40**

REFERENCES

Abdel-Rahman, G.N., M.B.M. Ahmed, E.M. Saleh and A.S.M. Fouzy. (**2018**). Estimated heavy metal residues in Egyptian vegetables in comparison with previous studies and the recommended tolerable limits. J. Biol. Sci, **18(3)**;135-143.

Achakzai, A.K,K., M.O. Liasu, O.J. Popoola. (**2012**) Effect of mycorrhizal inoculation on the growth and phytoextraction of heavy metals by maize grown in oil contaminated soil. Pak J Bot **44(1)**; 221–230

Akinci, I.E., S. Akinci and K. Yilmaz.(**2010**). Response of tomato (*Solanum lycopersicum* L.) to lead toxicity: Growth, element uptake, chlorophyll and water content. African Journal of Agricultural Research, **5(6)**;416-423.

Baslam, M., I. Garmendia and N. Goicoechea.(**2011**). Arbuscular mycorrhizal fungi (AMF) improved growth and nutritional quality of greenhouse-grown lettuce. Journal of agricultural and food chemistry, **59(10)**; 5504-5515.

Berruti, A., E.Lumini, R.Balestrini and V. Bianciotto .(**2016**). Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. Frontiers in microbiology, **6**;1559.

Brengi, S.H, I.A. Abouelsaad and A. H. Roshdy. (**2018**). Growth, yield and nutrient contents of garlic as affected by bio-inoculants and mineral fertilizers. Journal of Agricultural and Environmental Sciences. **17 (1)**; 1 – 19.

Brengi, S.H. (**2008**). Study of the effect of lead and some lead remediation treatments on growth, yield and chemical composition common bean, M.sc. Thesis, faculty of Agricultural, Alexandria University, Egypt.

Cardoso, I. M. and T.W. Kuyper. (**2006**). Mycorrhizas and tropical soil fertility. Agriculture, ecosystems & environment, **116(1-2)**;72-84.

Chibuike, G.U. and S.C.Obiora. (**2014**). Heavy metal polluted soils: effect on plants and bioremediation methods. Applied and Environmental Soil Science. **2014**: 1-12

Cottenie, A., (**1981**). Mobility of heavy metals in sludge amended soil. In Characterization, Treatment and Use of Sewage Sludge (pp. 251-263). Springer, Dordrecht.

CoStat, (**2008**). CoStat program, version 6.4. CoHort Software, Monterey, CA., USA.

Davis, D.J., P.A. Armond, E.L. Gross and C.J.Arntzen. (**1976**). Differentiation of chloroplast lamellae onset of cation regulation of excitation energy distribution. Archives of biochemistry and biophysics, **175(1)**; 64-70.

Eissa, M.A. and O.E. Negim.(**2018**). Heavy metals uptake and translocation by lettuce and spinach grown on a metal-contaminated soil. Journal of soil science and plant nutrition, **18(4)**;1097-1107.

Elgharably, A. and A.Nivien. (**2013**). Effect of arbuscular mycorrhiza on growth and metal uptake of basil and mint plants in wastewater irrigated soil. Egyptian Journal of Soil Science, **53**, pp.613-625

El-Razek, U.A. and A.A. El-Sheshtawy. (**2013**). Response of some wheat varieties to bio and mineral nitrogen fertilizers. Asian Journal of Crop Science, **5(2)**;200.

Esringu, A., M.Turan, A.Güneş and M.R. Karaman .(**2014**). Roles of Bacillus megaterium in remediation of boron, lead, and cadmium from contaminated soil. Communications in soil science and plant analysis, **45(13)**;1741-1759.

FAO/WHO. (**2014**). General standards for contaminants and toxins in food and feed (CODEX STAN 193-1995).

Flora, G., D.Gupta and A.Tiwari. (**2012**). Toxicity of lead: a review with recent updates. Interdisciplinary toxicology, **5(2);**47-58.

Garbisu, C. and I.Alkorta. (**2003**). Basic concepts on heavy metal soil bioremediation. European Journal of Mineral Processing and Environmental Protection, **3(1)**;58-66.

Gopal, R. and A.H.Rizvi. (2008). Excess lead alters growth, metabolism and translocation of certain nutrients in radish. Chemosphere, 70(9);1539-1544.

Gupta, D.K., F.T. Nicoloso, M.R.C. Schetinger, L.V. Rossato, L.B. Pereira, G.Y. Castro, S. Srivastava and R.D. Tripathi. (**2009**). Antioxidant defense mechanism in hydroponically grown Zea mays seedlings under moderate lead stress. Journal of Hazardous Materials, **172(1)**; 479-484.

Harrier, L.A. and C.A.Watson. (**2004**). The potential role of arbuscular mycorrhizal (AM) fungi in the bioprotection of plants against soil□borne pathogens in organic and/or other sustainable farming systems. Pest management science, **60(2)**; 149-157.

Jackson, M.L. (**1958**). Soil chemical analysis prentice Hall. Inc., Englewood Cliffs, NJ, 498.

Khalid, M., D. Hassani, M.Bilal, F. Asad and D. Huang. (**2017**). Influence of bio-fertilizer containing beneficial fungi and rhizospheric bacteria on health promoting compounds and antioxidant activity of Spinacia oleracea L. Botanical studies, **58(1)**;35.

Konieczny, A. and I. Kowalska. (**2017**). Effect of arbuscular mycorrhizal fungi on the content of zinc in lettuce grown at two phosphorus levels and an elevated zinc level in a nutrient solution. Journal of Elementology, **22(2)**;761-772.

Lamhamdi, M., O.El Galiou, A. Bakrim, J.C. Nóvoa-Muñoz, M. Arias-Estevez, A. Aarab and R.Lafont,.(**2013**). Effect of lead stress on mineral content and growth of wheat (*Triticum aestivum*) and spinach (*Spinacia oleracea*) seedlings. Saudi journal of biological sciences, **20(1)**; 29-36.

Liu, A., C. Hamel, R.I.Hamilton, B.L. Ma and D.L. Smith .(**2000**). Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) grown in soil at different P and micronutrient levels. Mycorrhiza, **9(6)**; 331-336.

Liu, D., T.Q.Li, X.F. Jin, X.E.Yang , E.Islam and Q. Mahmood. (**2008**). Lead induced changes in the growth and antioxidant metabolism of the lead accumulating and non□accumulating ecotypes of Sedum alfredii. Journal of integrative plant biology, **50(2)**;129-140.

Maestri, E., M.Marmiroli, G.Visioli and N.Marmiroli. (**2010**). Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. Environmental and Experimental Botany, **68(1)**;1-13.

Marques, A.P., R.S.Oliveira , A.O. Rangel and P.M. Castro. (**2008**). Application of manure and compost to contaminated soils and its effect on zinc accumulation by *Solanum nigrum* inoculated with arbuscular mycorrhizal fungi. Environmental Pollution, **151(3)**;608-620.

Meyers, D.E., G.J. Auchterlonie , R.I. Webb and B. Wood.(**2008**). Uptake and localisation of lead in the root system of *Brassica juncea*. Environmental Pollution, **153(2);** 323-332.

Nelson, D.W. and L. Sommers. (**1982**). Total carbon, organic carbon, and organic matter 1. Methods of soil analysis. Part 2. Chemical and microbiological properties, (methods of soil an2); 539-579.

Päivöke, A.E., (**2002**). Soil lead alters phytase activity and mineral nutrient balance of Pisum sativum. Environmental and Experimental Botany. **48(1)**;61-73.

Pourrut, B., M. Shahid,, C. Dumat, P.Winterton and E. Pinelli. (**2011**). Lead uptake, toxicity, and detoxification in plants. In Reviews of Environmental Contamination and Toxicology Volume **213** (pp. 113-136). Springer, New York, NY.

Punamiya, P., R. Datta, D. Sarkar, S. Barber , M. Patel and P. Das. (**2010**). Symbiotic role of Glomus mosseae in phytoextraction of lead in vetiver grass (*Chrysopogon zizanioides* L.). Journal of hazardous materials, **177**(1-3);465-474.

Ouziad, F., U. Hildebrandt , E. Schmelzer and H. Bothe. (**2005**) Differential gene expressions in arbus cular mycorrhizal-colonized tomato grown under heavy metal stress. J Plant Physiol **162**; 634–649.

Raskin, I., P.N. Kumar, S. Dushenkovand D.E. Salt. (**1994**). Bioconcentration of heavy metals by plants. Current Opinion in biotechnology, **5(3)**; 285-290.

Rubatzky, V.E. and M. Yamaguchi .(**2012**). World vegetables: principles, production, and nutritive values. Springer Science & Business Media.

Saleem, M., M. Arshad, S. Hussain, and A.S. Bhatti. (**2007**). Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. Journal of industrial microbiology & biotechnology, **34(10)**; 635-648.

Sandalio, L.M., H.C. Dalurzo, M.Gomez, M.C. Romero□Puertas and L.A. Del Rio .(**2001**). Cadmium□induced changes in the growth and oxidative metabolism of pea plants. Journal of experimental botany, **52(364)**;2115-2126.

Sengar, R.S. and M. Pandey. (**1996**). Inhibition of chlorophyll biosynthesis by lead in greening *Pisum sativum* leaf segments. Biologia plantarum, **38(3)**;459-462.

Sharma, P. and R.S. Dubey. (**2005**). Lead toxicity in plants. Brazilian journal of plant physiology, **17(1)**; 35-52.

Tabrizi, L., S. Mohammadi, M. Delshad and B. Moteshare Zadeh. (**2015**). Effect of arbuscular mycorrhizal fungi on yield and phytoremediation performance of pot marigold (*Calendula officinalis* L.) under heavy metals stress. International journal of phytoremediation, **17(12)**; 1244-1252.

Turan, M., N. Ataoglu and F. Sahin. (**2007**). Effects of Bacillus FS-3 on growth of tomato (*Lycopersicon esculentum* L.) plants and availability of phosphorus in soil. Plant Soil and Environment, **53(2)**;58.

Uzu, G., S. Sobanska,, Y. Aliouane, P. Pradere and C. Dumat. (**2009**). Study of lead phytoavailability for atmospheric industrial micronic and sub-micronic particles in relation with lead speciation. Environmental Pollution, **157(4)**;1178-1185.

Wang, G., M.Y. Su, Y.H. Chen , F.F. Lin , D. Luo and S.F. Gao. (**2006**). Transfer characteristics of cadmium and lead from soil to the edible parts of six vegetable species in southeastern China. Environmental Pollution, **144(1)**; 127-135.

Wierzbicka, M.H., E. Przedpełska, R. Ruzik, L.Ouerdane, K. Połeć-Pawlak, M. Jarosz, J. Szpunar, and A. Szakiel. (**2007**). Comparison of the toxicity and distribution of cadmium and lead in plant cells. Protoplasma, **231(1-2)**, p.99.

Yang, Y., Y. Song, H.V. Scheller, A. Ghosh, Y.Ban, H. Chen and M. Tang. (**2015**). Community structure of arbuscular mycorrhizal fungi associated with Robinia pseudoacacia in uncontaminated and heavy metal contaminated soils. Soil Biology and Biochemistry. **86**;146-158.

Zhu, Y., P. Christie and A.S. Laidlaw. (**2001**). Uptake of Zn by arbuscular mycorrhizal white clover from Zn-contaminated soil. Chemosphere, **42(2)**,; 193-199.

الملخص العربى

الاستخدام المشترك للكائنات الحية الدقيقة المفيدة في التربة لزيادة النمو وتقليل محتوى الرصاص بكفاءة في أوراق الخس تحت اجهاد التلوث بالرصاص

سارى حسن مصطفى برنجى، ابراهيم على احمد ابو السعد

قسم البساتين- كلية الزراعة- جامعة دمنهور- مصر

تم اجراء تجربتي في اصص خلال الموسم الشتوي لعامي ٢٠١٦ – ٢٠١٧ و٢٠١٧ – ٢٠١٨ لدراسة مدي قدرة التسميد الحيوي بالميكوريزا والباسلس ميجاتريم والخليط بينهما علي تحسين نمو وجودة نبات الخس تحت مستويات مختلفة من الرصاص(٠، ١٠٠، ٢٠٠ و٣٠٠ جزء في المليون). اوضحت نتائج الدراسة ان النمو والجودة انخفضا بشدة بزيادة مستويات الرصاص في التربة حيث انخفضت مقاييس النمو الخضري ومحتوي الاوراق من العناصر الكبري والصغري كذلك صبغات البناء الضوئي لكن زاد محتوي الرصاص في الاجزاء المختلفة من النبات. كما بينت النتائج ان الخليط بين فطريات الميكوريزا وبكتريا باسلس ميجاتريم قد ادت الي زيادة نمو النبات ومحتواه من العناصر وصبغات البناء الضوئي وان التلقيح الحيوي بالميكوريزا قد خفض محتوي الاوراق من الرصاص لكن البكتريا خفضت محتوي الجذور ايضا. من الدراسة يتضح ان الخليط بين فطريات الميكوريزا وبكتريا باسلس ميجاتريم كانت طريقة فعالة في تحسين نمو وجودة نبات الخس كذلك تقليل محتواه من الرصاص تحت ظروف النمو في بيئة ملوثة بالرصاص.