Bioremediation Effect of Contaminated Soils with Copper and Lead on Sweet Basil Plants

Mayada Sabra*, Soraya Sabry, Amal Aboul Nasr***
*Dep. of Agric. Botany, Faculty of Agriculture Saba Basha, Alexandria University
**Dep. of Botany and Microbiology, Faculty of Science, Alexandria University

ABSTRACT: The effects of phosphorus amendments and arbuscular mycorrhizal (AM) fungi *Glomus intraradices* and *pseudomonas putida* on the sweet basil (*Ocimum basilicum* L.) yield, chemical composition and percent of volatile oil, and metal accumulation in plants and its availability in soil were investigated in field experiment at two seasons 2012 and 2013 under contaminated soil with Pb and Cu. The plant height, herb fresh weight, content of essential oil and shoot and root dry weights of sweet basil were increased by the application of mineral phosphorus as compared to control. Inoculation with AM fungi and bacteria reduced the metal concentration in shoot, recording a lowest value of (33.24, 18.60 mg/kg) compared to the control (46.49, 23.46 mg/kg) for Pb and Cu, respectively. Availability of Pb and Cu in soil were decreased after cultivation in all treatments compared to control. However, metal root concentration increased with the inoculation, with highest values of (30.15, 39.25 mg/kg) compared to control (22.01, 33.57mg/kg) for Pb and Cu, respectively. The content of linalool and methyl chavicol in basil oil was significantly increased in all treatments compared to control, but the interaction was not. We can thus conclude that the AM-sweet basil symbiosis and bacterial inoculation could be employed as an approach to bioremediate polluted soils and enhance the yield and maintain the quality of volatile oil of sweet basil plants.
Keywords: Arbuscular mycorrhizal fungus, *Pseudomonas*, Sweet basil, Heavy metals, Bioremediation.

INTRODUCTION

Aromatic and medicinal plants are an important source of national income and foreign currency in Egypt. Basil is one of the most important species for export among the medicinal and aromatic plants and it has a good reputation in the European countries. The area cultivated with basil in Egypt is about 4-5 thousand feddans, and the exports are more than 4000 tons per year (El-Sayed *et al*., 2003). The genus *O. cimum* comprises more than 150 species and is considered as one of the largest genera of the Lamiaceae family (Evans, 1996). *Ocimum basilicum* L. (sweet basil) is an annual herb, plants is widely used in food and oral care products; the essential oil of the plant is also used as perfumery (Bauer *et al*., 1997) and antimicrobial (Chiang, 2005). Environmental conditions and agricultural practices can significantly alter yield and chemical composition of sweet basil (Sifola and Barbieri, 2006).

Monitoring for toxic heavy metals in medicinal plants has become part of the quality control in the pharmaceutical industry as consumers demand products that are free from potentially harmful constituents (Chizzola *et al*., 2003). Phytoextraction potential of medicinal and aromatic plants grown in heavy metal polluted agricultural soils is a subject of ongoing research (Kovačik *et al*., 2009; Stancheva *et al*., 2014). In the rhizosphere, a synergism between various bacterial

genera such as *Bacillus*, *Pseudomonas*, *Arthrobacter* and *Rhizobium* has been shown to promote plant growth of various plants such as sweet basil (*Ocimum basilicum* L.) (Hemavathi *et al*., 2006). *Pseudomonas putida* has the capacity to improve growth in plants (Rodriguez *et al*., 2014) particularly in sweet basil plants (Ordookhani *et al*., 2011). .Arbuscular mycorrhizal fungi (AMF) symbiosis is formed by approximately 80% of the vascular plant species in all terrestrial biomass (Smith *et al*., 2010) which improves plant productivity (Fedderman *et al*., 2010). AM fungi can contribute plant growth, particularly in disturbed or metal polluted sites, by increasing plant access to relatively immobile minerals such as P, Zn, and Cu (Ryan and Angus 2003; Christie *et al*, 2004). In addition, they can improve soil structure (Gaur and Adholeya, 2004), stabilize metals in the soil, reduce their uptake, and thus decrease the risk of toxicity to plants growing in polluted substrates (González-Chávez *et al*., 2009).Thus, plants in symbiosis with AM fungi have the potential to take up heavy metals (HM) from an enlarged soil volume (Upadhyaya *et al*., 2010).

The aim of the present study was to examine the potency of two isolates of AMF (*Glomus intraradices*), and *Pseudomonas putida* to reduce metal accumulation in plant organs, and to evaluate how inoculation effect of mycorrhization and essential oil composition of (*Ocimum basilicum* L). cultivated in Pb and Cu contaminated soil.

MATERIALS AND METHODS

Cultivated plant: Sweet Basil (*Ocimum basilicum*) seeds were obtained from Medicinal and Aromatic Plants Department, Agricultural Research Center, Ministry of Agriculture, Egypt. Seeds were germinated in seedling trail with 209 holes containing (1:1, v: v) peat moss and Sand. In case of mycorrhizal treatment (G3, G4), the inoculant was applied to the nursery at the rate of 10% added to the mixture. Seeds were sown in the nursery on April 16th and the seedlings of 2-3 pairs of leaves were transplanted to the field at May 28th with one plant per hill.

Soil initial state analysis: Some physical and chemical properties of surface layer (0-30 cm) of the experimental field and heavy metals content (Pb and Cu) were determined according to (Page *et al*., 1982) and (Klute, 1986). The soil had the following physicochemical properties: clay loam in texture, pH (1:1 soil water suspension) 7.75 and 7.85, electrical conductivity (EC) 1.95 and 1.98 dSm^{-1}, available P (15, 14.7 mg kg^{-1}) soil, and the available Pb and Cu were (109, 100) and (90.3, 88.6) mg/ kg for the two seasons, respectively.

Experimental design: The field experiment was conducted in Abees Experimental Farm, Faculty of Agriculture, Saba Basha, 10th Village, Alexandria University, Egypt during two seasons 2012 and 2013. The experiment was carried out in a randomized split – split plot (10.5m^2, 3.5 x 3m) design in 3 replicates. Phosphorus fertilizer (0%, 75%, and 100% of the recommended dose) was the main plot, and

the two treatments un-inoculated and inoculated with *Pseudomonas putida* were randomly distributed in the sub- plot, while the three treatments with *Glomus intraradices* (control un-inoculated, G3 and G4) were arranged in the sub – sub plots.

Inoculation

Arbuscular Mycorrhizal Fungi: Two mycorrhizal isolates of *Glomus intraradices* were used in this experiment; isolate (G3) was isolated from the Experimental Station of Alexandria University at Abees (Aboul-Nasr, 1993), and isolate (G4) was obtained by Amykor Company, Germany. The inoculum consisted of expanded clay aggregates (2-4 mm in diameter, Leca) containing chlamydospores and fungus mycelium, which had been cullivated on *Tagetes erecta* L. (Aboul-Nasr, 2004). Inoculant was added at the rate of 7.0 g/per hill below basil seedling after transplanted. Control plants received the same amount of heat sterilized expanded clay.

Bacterial treatment: *Pseudomonas putida* obtained from Bio fertilization unit, Faculty of Agriculture, Ain Shams University was grown in liquid broth (LB) medium comprising of (g/L): tryptone, 10; yeast extract, 5; NaCl, 5. The pH of the medium was adjusted to 7.2–7.4 with 1 N HCl or 1 N NaoH and sterilized by autoclaving at 121 ℃ for 15 minutes. Incubation was carried at 35 ℃ for 3 days. When plants were transplanted to the field, 15 ml (1.3×10^6 viable cells/ml) of bacterial suspension were added to each hill.

Mineral fertilizers: Three different rates (zero, 75% and 100%) of the recommended dose (400 kg/fed) of mono- calcium phosphate (15.5 % P_2O_5) was used in one does at seedling transplanted for each growing seasons. Ammonium nitrate (33.5% N) was added at the recommended dose150 kg/fed (50 kg N/ fed) after 30, 60, and 90 days of cultivation. Potassium sulfate (48% K_2O) was applied to all plots after 41 days of cultivation in the rate of 100 kg/fed.

The percentage of mycorrhizal colonization: The percentage of mycorrhization was estimated three times; (first, 4 weeks from germination to confirm the colonization before transfer to the field, second, 9 weeks old 1st cut and third at 16 weeks old 2nd cut). Root samples 1 cm were cleared with 10% KOH and stained with Trypan blue (0.05%) in lactophenol to observe under microscope (Koska and Gemma, 1989). Mycorrhizal colonization was estimated by determining the percentage of length of root segments containing AM fungal structure (arbuscules, vesicles, spores) according to Biermann and Linderman, (1981).

Vegetative parameters: Ten plant samples per plot for each cut (1st and 2nd) were taken to determine the plant height (cm.) and herb fresh weight (g/plant). Five plants were air dried till constant weight and the average dry weight of root and shoot (g/plant) were calculated.

Chemical analysis

Phosphorus content: Plant powder (0.5g) was wet-digested with H_2SO_4 – H_2O_2 mixture (Lowther, 1980). Phosphorus was determined by the Vanadomolybdate yellow method (Jackson, 1967) using Millton Ray Spectronic 21 D.

Essential oil percentage: The essential oil percentage was determined in the air-dried herb according to British Pharmacopoeia (1963) by water distillation of 40 g of air dry herb for 1.5 – 2.0 hours, in order to extract the essential oil.

Essential oil analysis and its major components: The essential oils were diluted in diethyl ether (20 :1, v:v) and analyzed with GC (HP 8644) with flame ionization detector (FID) on a fused silica 132 capillary column DB-5, 25 m in length, 0.32 mm i.d., and 0.5 mm film thickness.133 Helium was used as the carrier gas with a flow rate of 1.6 ml/min; the detector 134 temperature was 260 °C, the oven temperature was programmed to increase from 130 to 260 °C at a rate of 4 °C/min. The split injector was heated at 250 °C; the split 136 ratio was 15:1. Data were processed on a DP 800 integrator. The percentage of major constituents of stander (β-caryophellene, Linalool and Methyl chavicol) was estimated by measuring the peak area of the different compounds of the chromatogram according to (Heftman, 1967) and (Gunther and Joseph, 1978). Standards of the principal components which used as reference compounds for sweet basil were obtained from Ciba Gigi, NY, USA.

Total lead and copper analysis: Heavy metals content in shoots and roots were determined by digesting the dried plant of each plot according to (Lowther, 1980). The disgusted solutions were analyzed for lead and copper using the atomic absorption spectrophotometer (A Analyst 400) (Jackson, 1967). Available lead and copper remain in soil after cultivation were measured by atomic absorption spectrophotometer model (A.A. Spectrometry Thermo Elemental Type Solar 54/2001, ser No. GE 710728) using diethylene triamina penta acetic acid (DTPA) method as described by (Page *et al*., 1982).

Statistical analysis: Data were statistically analyzed by the procedure of variance to test the treatments effect on different measured parameters according to (Snedecor and Cochran, 1981). Data for the percentage of root length colonization % were analyzed using angular transformation (Steel and Torrie, 1982)

RESULTS

Mycorrhizal colonization percentage %

Mycorrhization was determined three times during every growing season before plant transplanting (4 weeks old) and two times after transplanting (9 and 16 weeks) the colonization at the 4 weeks was more than 33.3% that to confirm the colonization before transplanting. Root colonization by indigenous *G. intraradices*

(G3 and G4) were low (Fig 1), but was enhanced by the inoculation with *P. putida*. Addition of P had no effect on mycorrhization in age 9 weeks but increased significantly after 16 weeks (Table1). However, the percent of root colonization was significantly higher in the basil inoculated with *G. intraradices* G3 and G4 than the non-inoculated plants. The AM root colonization in inoculated plants ranged from 32.99 to 55.10% and the native soil AM colonized roots were 7.72 % and 11.53 % at 9 and 16 weeks, respectively. The highest percent (55.10%) of colonization was noticed in plants with 16 weeks old with G3 and *P. putida* in the presence 75% of mineral phosphorus fertilizer. Generally the mycorrhizal root colonization hadn't high value in this contaminated soil this could be attributed to the effect of heavy metals on the mycorrhizal spore germination, hyphal growth and branching.

(a) Plants with 9 weeks old **(b) Plants with 16 weeks old**

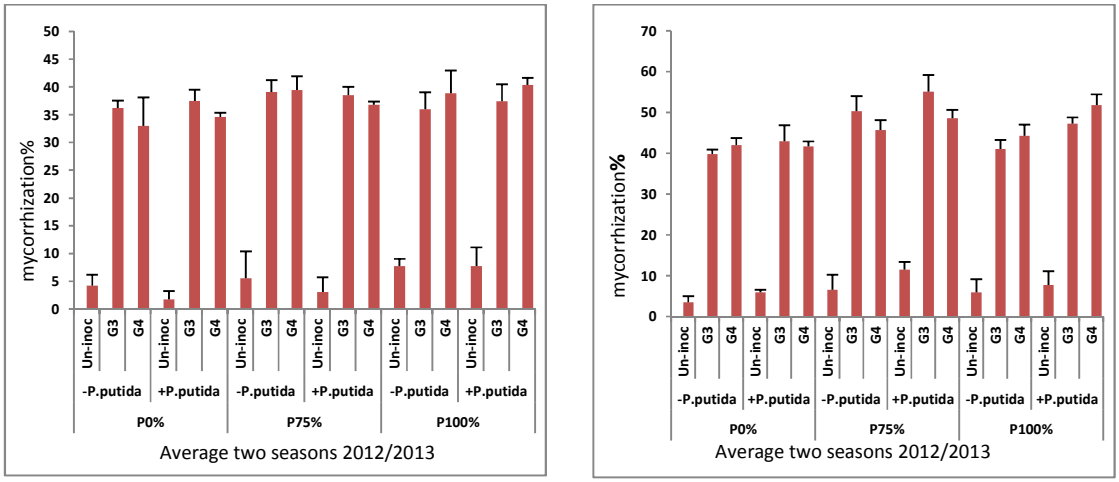

Fig (1). Effect of inoculating Ocimum *basilicum* L with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on mycorrhization after 9 and 16 weeks.

Table (1). A three-way ANOVA (LSD $_{0.05}$) showing the effect of P rates (P), *P. putida* (P.p) and *Glomus intraradices* (G.i) and their interactions.

Factors	Mycorrhization 9 weeks	Mycorrhization 16 weeks
Phosphorus (P)	3.18^{ns}	2.07^{**}
***P. putida* (P.p)**	3.87^{*}	3.22^{**}
***G. intraradices* (G.i)**	1.96^{***}	1.45^{***}
P× P.p	4.89^{ns}	4.57^{ns}
P× G.i	2.13^{**}	2.06^{***}
P.p× G.i	5.12^{ns}	5.07^{ns}
P× P.p× G.i	3.42^{ns}	6.18^{ns}

*: significant; ns: not significant

Vegetative parameters

Plant height (cm) and herb fresh weight (g/plant)

The results presented in Table (2) average values of cut one and cut two for the two seasons showed the growth responses of sweet basil grown in heavy metals polluted soil inoculated with *Pseudomonas putida* and AM fungi (G3 , G4) in the presence of different mineral phosphorus fertilizer rates. Data revealed that the plant height and herb fresh weight significantly increased in case of inoculated plants with both bacteria and mycorrhizal individually or in dual treatments, and the interaction between application of phosphorus and AM fungi had the same trend, additionally the interaction between bacteria and phosphorus or between bacteria and AMF had not affected, also the interaction between bacteria, AMF and phosphorus had the same trend except at the 2nd cut (16 weeks old).

Shoot and root dry weight (g/plant)

Sweet basil shoot and root dry weights (Table 3) responded significantly to phosphors application and AM inoculation, although the bacterial treatment had no significant impact in shoot dry weight in case of the second cut. Best results of shoots dry weight obtained with the addition of $P_{75\%}$ inoculated with *P. putida* and AM isolate G4 in the second cut. However the best results of roots dry weight (17.19 g/plant) obtained with addition $P_{100\%}$ and inoculation with bacteria and G4 in the 1st cut. Additionally no interaction could be observed in shoots or roots dry weight between the factors P nutrition and inoculation with *P. putida* while, root dry weight significantly increased in case of the interaction between the factor of inoculation with *P. putida* and AMF or the interaction between P, *P. putida* and AMF except at 1st cut for the latest interaction.

Phosphorus uptake (mg/g shoot) and oil content %

Data presented in Table (4) clearly show that the P uptake significantly increased, although the interaction between all factors had no impact. Application of phosphorus with different rates increased the phosphorus uptake of sweet basil plants, in the presence of inoculants (AM and bacteria). The highest P uptake observed in plants inoculated with G4 with bacteria in 75% of phosphorus fertilizer was (0.194 mg/g shoot). Although the highest percentage increase 268.8% was clear in G4 with bacteria. On the other hand the applied treatments improved the essential oil percentage compared with untreated plants. AMF isolate G4 was the most effective treatment in increasing the oil content under contaminated soil its value was (0.47%), moreover the interaction had a significant differences between all factors. Table (4) showed that the percentage increase of oil content in plants inoculated with AM isolate G3 and G4 were (80 and 88%) , respectively more than the un-inoculated basil plants. No significant differences were observed between the duel inoculations with bacteria and phosphorus application and between mycorrhizae too.

Table (2). Effect of inoculated *Ocimum basilicum* L. with *Glomus intraradices* and *Pseudomonas putida* on plant height (cm) and herb fresh weight (g/plant) in lead and copper contaminated soil as an average of the two growing seasons 2012 and 2013

Parameter		Plant height (cm/plant)					
Phosphorus (P)	*Pseudomonas putida (P.p)*	Cutst			Cutnd		
		G. intraradices (G.i)			*G. intraradices (G.i)*		
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	22.22	37.46	39.43	23.19	37.92	43.82
	+	30.38	45.62	38.50	21.86	48.92	50.73
P_{75}	-	35.99	40.23	51.88	23.44	40.93	49.88
	+	37.52	49.39	53.81	24.33	48.08	60.13
P_{100}	-	31.91	38.26	43.47	25.98	47.67	51.17
	+	33.37	43.85	46.34	34.33	61.34	54.90
$LSD_{0.05}$							
P			2.18***			2.06***	
P.p			1.18***			3.11***	
G.i			2.14***			1.86**	
P× P.p			6.21^{ns}			7.82^{ns}	
P× G.i			1.58*			2.14**	
P.p× G.i			9.21^{ns}			9.18^{ns}	
P× P.p× G.i			5.42^{ns}			1.92*	
		Herb fresh weight(g/plant)					
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	142.21	302.58	291.75	124.03	275.04	297.09
	+	155.59	335.63	342.78	132.76	325.16	390.97
P_{75}	-	226.70	280.62	321.32	136.71	259.80	350.50
	+	177.70	326.41	360.33	165.01	398.84	381.41
P_{100}	-	152.45	374.49	446.18	198.84	405.56	504.76
	+	195.02	433.49	498.23	215.41	462.52	528.91
$LSD_{0.05}$							
P			9.45***			7.11***	
P.p			11.36*			9.28**	
G.i			5.27***			7.79***	
P× P.p			15.96^{ns}			16.02^{ns}	
P× G.i			9.32**			5.14***	
P.p× G.i			22.11^{ns}			20.41^{ns}	
P× P.p× G.i			20.62^{ns}			5.29**	

Values are means± SE, n= 10 plants,*: significant; ns: not significant

Table (3). Effect of inoculated *Ocimum basilicum* L. with *Glomus intraradices* and *Pseudomonas putida* on shoot and root dry weight (g/plant) in lead and copper contaminated soil as an average of the two growing seasons 2012 and 2013

Parameter		Shoot dry weight (g/plant)					
Phosphorus (P)	*Pseudomonas putida* (P.p)	Cutst			Cutnd		
		G. intraradices (G.i)			*G. intraradices* (G.i)		
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	13.74	18.56	25.12	19.68	24.75	30.86
	+	17.14	25.91	28.49	20.00	29.18	34.00
P_{75}	-	17.95	37.86	35.85	22.77	39.19	41.07
	+	19.41	36.21	37.70	22.36	39.31	41.43
P_{100}	-	19.30	24.46	34.03	22.42	32.31	34.57
	+	15.36	30.99	33.66	24.50	34.20	37.57
$LSD_{0.05}$							
P		2.43***			1.06***		
P.p		1.75*			4.32^{ns}		
G.i		4.27**			1.45***		
P× P.p		3.71^{ns}			4.62^{ns}		
P× G.i		2.89**			1.11***		
P.p× G.i		7.19^{ns}			9.22^{ns}		
P× P.p× G.i		5.89^{ns}			7.56^{ns}		
		Root dry weight (g/plant)					
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	9.07	10.98	10.57	9.68	11.23	11.32
	+	9.43	11.92	12.03	10.41	11.74	11.98
P_{75}	-	10.94	13.17	13.47	11.05	12.76	12.82
	+	11.23	13.98	14.60	10.97	14.13	14.37
P_{100}	-	11.50	14.47	15.35	10.55	13.74	15.12
	+	11.83	16.04	17.19	11.34	15.71	15.93
$LSD_{0.05}$							
P		0.43***			0.165***		
P.p		0.29***			0.128**		
G.i		0.25***			0.341***		
P× P.p		2.18^{ns}			2.58^{ns}		
P× G.i		0.23***			0.267***		
P.p× G.i		0.41**			0.125**		
P× P.p× G.i		2.74^{ns}			0.131**		

Values are means± SE, n= 5 plant**,***: significant; ns: not significant

Table (4). Effect of inoculated *Ocimum basilicum* L. with *Glomus intraradices* and *pseudomonas putida* on phosphorus uptake (mg/g shoot) and oil content% in lead and copper contaminated soil as an average of the two growing seasons 2011/2012 and 2012/2013

Parameter		Second cut					
Phosphorus (P)	*Pseudomonas putida* (P.p)	Phosphorus uptake (mg/g DW of plant)			Oil content %		
		G. intraradices (G.i)			*G. intraradices* (G.i)		
		Un-inoc.	G3	G4	Un-inoc.	G3	G4
P_0	-	0.032	0.082	0.118	0.23	0.32	0.34
	+	0.043	0.129	0.145	0.25	0.36	0.37
	±%	-	156.3	268.8	-	39.1	47.8
	±%	-	200	237.2	-	44	48
P_{75}	-	0.051	0.101	0.175	0.25	0.45	0.47
	+	0.055	0.180	0.194	0.25	0.45	0.47
	±%	-	98.03	243.1	-	80	88
	±%	-	227.3	252.7	-	80	88
P_{100}	-	0.066	0.139	0.166	0.24	0.39	0.39
	+	0.053	0.172	0.192	0.29	0.41	0.43
	±%	-	110.6	151.5	-	62.5	62.5
	±%	-	224.5	262.3	-	41.4	48.3
$LSD_{0.05}$							
P		0.010***			0.018***		
P.p		0.009**			0.014**		
G.i		0.031**			0.018***		
P× P.p		0.21^{ns}			0.17^{ns}		
P× G.i		0.032***			0.016***		
P.p× G.i		0.31^{ns}			0.28^{ns}		
P× P.p× G.i		0.17^{ns}			0.015***		

Values are means± SE, n= 5 plants,*: significant; ns: not significant

Major oil components (Linalool and Methyl chavicol %) of sweet basil plants

Chemical characterization of essential sweet basil oil (Table 5) demonstrated that quality of oil improved on mycorrhization and bacterial treatments in the presence of phosphorus fertilizer. Linalool the major component of sweet basil affected significantly with both isolates of AM G3, G4. However the bacterial inoculation had no significant differences. Methyl Chavicol had the similar trend with inoculation; moreover the interaction showed no significant impact under contaminated soils. Data recorded in Fig (2) showed that the best results were obtained from G4 and bacterial inoculation in the presence of 100% P, it were (52.99% and 7.83%) for linalool and methyl chavicol, respectively.

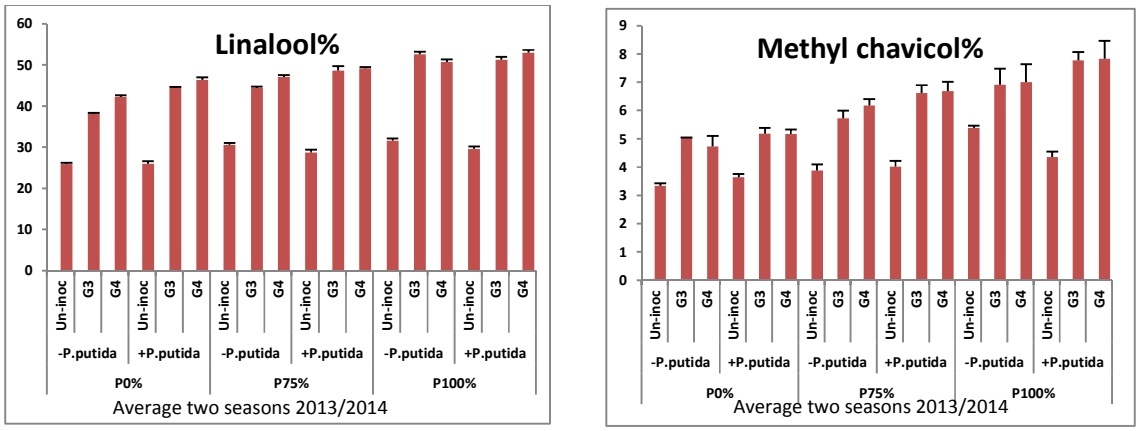

Fig (2). Effect of inoculated *Ocimum basilicum* L with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on linalool% and methyl chavicol% in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons 2012/2013

Table (5). The result of a three-way ANOVA (LSD $_{0.05}$) showing effects of the factors phosphorus rates (P), *Pseudomonas putida (P.p)* and *Glomus intraradices* (G.i), as well as the effect of their interaction.

Factors	Linalool %	Methyl chavicol%
Phosphorus (P)	3.74**	1.85***
P. putida (P.p)	2.46^{ns}	4.17^{ns}
G. intraradices (G.i)	5.22**	3.64**
P× P.p	3.15*	4.18^{ns}
P× G.i	1.27**	5.85^{ns}
P.p× G.i	5.12*	5.62^{ns}
P× P.p× G.i	4.48*	4.96^{ns}

*: significant; ns: not significant

Analysis of heavy metals
Lead concentration mg/kg in shoots and roots

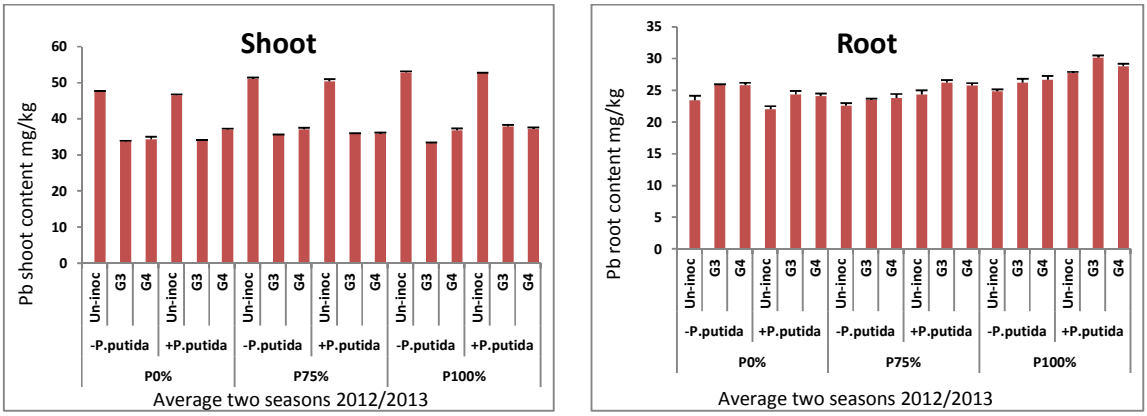

Fig (3). Effect of inoculated *Ocimum basilicum* L with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on Pb content (mg/kg) in shoot and root in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons 2012/2013

Table (6). The result of a three-way ANOVA (LSD $_{0.05}$) showing effects of the factors phosphorus rates (P), *Pseudomonas putida (P.p)* and *Glomus intraradices* (G.i) as well as the effect of their interaction.

Factors	Pb shoot content (mg/kg)	Pb root content (mg/kg)
Phosphorus (P)	2.54***	1.23*
P. putida (P.p)	1.31*	3.15^{ns}
G. intraradices (G.i)	2.14**	1.06**
P× P.p	1.15*	2.84^{ns}
P× G.i	0.85**	0.87*
P.p× G.i	0.94*	0.93**
P× P.p× G.i	1.02*	1.74*

*: significant; ns: not significant

Copper concentration mg/kg in shoots and roots

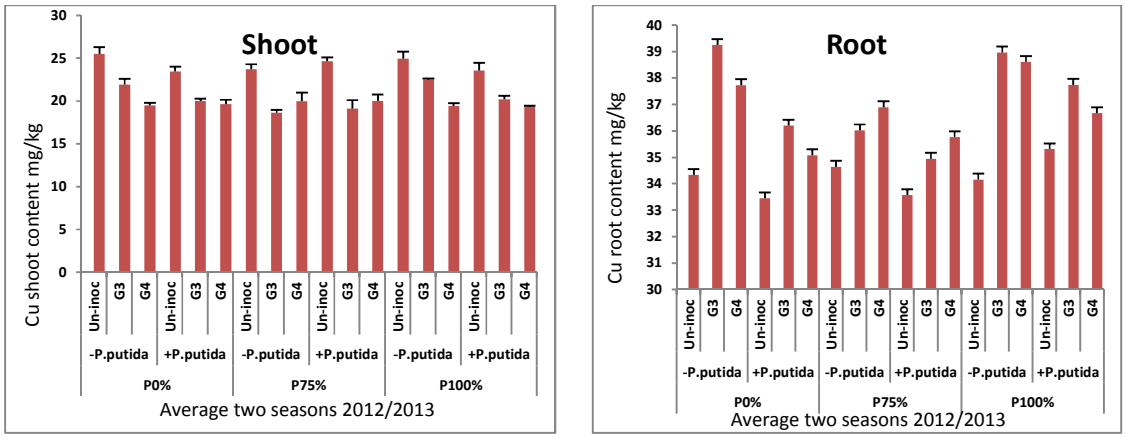

Fig (4). Effect of inoculated (*Ocimum basilicum* L) with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on Cu shoot and root concentration in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons 2012/2013

Table (7). The result of a three-way ANOVA (LSD $_{0.05}$) showing effects of the factors phosphorus rates (P), *Pseudomonas putida (P.p)* and *Glomus intraradices* (G.i) as well as the effect of their interaction.

Factors	Cu shoot content (mg/kg)	Cu root content (mg/kg)
Phosphorus (P)	2.18***	1.38***
***P. putida* (P.p)**	1.67*	0.99*
***G. intraradices* (G.i)**	2.41**	2.83**
P× P.p	3.81^{ns}	3.14^{ns}
P× G.i	2.04*	1.77**
P.p× G.i	2.89^{ns}	2.08*
P× P.p× G.i	3.79^{ns}	3.87^{ns}

*: significant; ns: not significant

Lead and copper available in soil after cultivation

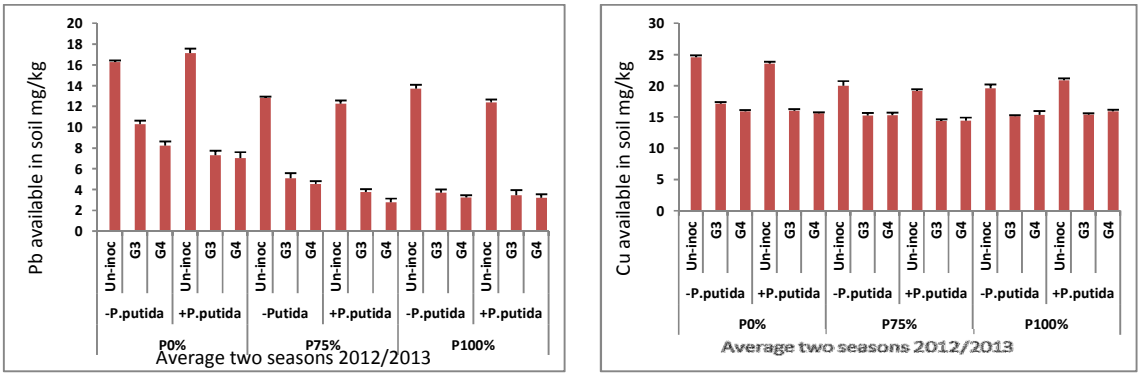

Fig (5). Effect of inoculated (*Ocimum basilicum* L) with *Glomus intraradices* (G3, G4) and *Pseudomonas putida* on Pb and Cu available in soil after cultivation (mg/kg) in the presence of different mineral phosphorus fertilizer rates as an average of the two growing seasons 2012/2013

Table (8). The result of a three-way ANOVA (LSD $_{0.05}$) showing effects of the factors phosphorus rates (P) *Pseudomonas putida (P.p)* and *Glomus intraradices* (G.i) as well as the effect of their interaction.

Factors	Pb available in soil (mg/kg)	Cu available in soil (mg/kg)
Phosphorus (P)	2.13***	2.18**
***P. putida* (P.p)**	1.21*	1.64*
***G. intraradices* (G.i)**	1.67**	3.14***
P× P.p	4.31^{ns}	5.48^{ns}
P× G.i	0.92**	2.87**
P.p× G.i	1.35*	3.66^{ns}
P× P.p× G.i	5.71^{ns}	4.18^{ns}

*: significant; ns: not significant

Data presented in Figure (3, 5) showed the effect of mycorrhizal and bacterial inoculum in the presence of different phosphorus rates on Pb shoot and root content (mg/kg). From the analysis of variance (ANOVA) presented in Tables (6, 8) it was clear that Pb concentrations in sweet basil shoots, were highly significant affected by bacterial and mycorrhizal inoculation as well as the interaction between them. On the other hand Pb concentrations in shoots were increased by increasing phosphorus application and were decreased by the inoculation with AM isolate G3 or G4 and *P. putida*. Shoots of inoculated plants with G3 had the lowest value of Pb (33.24 mg/kg) showed in treatments with $P_{100\%}$ without bacteria. Opposite results were obtained in roots, lead concentration was higher in the inoculated plants than un-inoculated one.

Low levels of Pb were measured in the control basil roots compered to treated plants. Available lead residues in the soil were lower as a result of inoculation with both bacteria and AM mycorrhizal isolate G4 and G3 then the inoculation with bacteria alone. Significant reduction of Pb compared with the controls was observed in both soil and shoot of basil inoculated G3 and G4 isolate. However the highest value of soil available Pb (17.18 mg/kg) was observed in the absences of fungi and with bacteria inoculation and not amended with phosphorus fertilizer. It is clear also, that the AM isolate G4 was more effective mostly than G3 in reducing Pb value in soil. In the same time, there was a significant difference between the Pb values over all treatment for the soil after cropping except the interaction between P*Pp, P*Gi and Pp*Gi Table (6, 8). The same trend was observed in copper concentration copper metal accumulation in shoot and a root tissue of sweet basil was significantly affected by application of AM inoculation Fig. (4, 5). It was accumulated in very low concentration in shoot tissue (< 25 mg/kg) and soil (<12 mg/kg) as compared to accumulation in control plants. Inoculation significantly increased the concentration of Cu in roots than shoots under all phosphorus fertilizer rates. It is clear that the treatments reduce the soil available Cu compared to non-treated plants Table (4, 5).

DISCUSSION

The present study demonstrates that colonization of basil plant root by the AM fungus *G. intraradices* was significantly affected by the application of different phosphorus fertilizer rates and inoculation with *P. putida*. Non-inoculated sweet basil plants showed >10.0% root colonization due to native mycorrhizae in soil and the percent root colonization was significantly enhanced by plant inoculation with AM fungi and bacteria under heavy metals (Pb and Cu) contaminated soil. Similar results were reported by (Banni and Faituri, 2013) who observed that *Glomus* spp. treated plants had higher mycorrhizal colonization rates than other inoculation-treated plants. The data of the present work also showed that shoot and root of basil plant biomass increased in inoculated plant compared to control. Positive effects of mycorrhization on the growth of essential-oil-containing plants were reported with *Ocimum basilicum* L. (Khaosaad *et al*., 2006) and *Salvia officinalis* L. (Geneva *et al*., 2010). The outcome of the plant-AM fungal association is metal-specific and depends on bioavailability of metals in soil and on both plant and AM species (Sudova and Vosatka, 2007).

AM fungal inoculation increased shoot yield, content of essential oil, and root yield of sweet basil probably due to the increase of nutrient uptake (Marschner and (Dell, 1994). (Banni and Faituri, 2013) reported that the two AM fungi species significantly increased the root and shoot dry weights and this species was more effective than non-mycorrhizal treatment in protection the maize plants against Cu toxicity. (Vinutha, 2005) observed increased shoot and root growth weight, biomass and essential oil content of *Ocimum* spp. when inoculated with *Glomus fasciculatum*, *Azotobacter chroococcum* and *A. awamori*. (Hemavathi *et al*., 2006) found similar observations in *Ocimum basilicum*, where plant growth increased after inoculation with *G. fasciculatum*, *Pseudomonas fluorescens* and *Bacillus*

megaterium. In another study, (Ordookhani *et al*., 2011) found an increase in shoot, root dry weight, N, P and potassium (K) content and essential oils in *Ocimum basilicum* inoculated with PGPR *Pseudomonas putida* and *Azotobacter chroococcum* additionally. (Ordookhani, 2011) reported that *P. putida* as PGPR had the capacity to increase *Ocimum basilicum* microelement contents and significant differences between PGPR treatments on essential oil, Fe, Zn, Mn and Cu contents compered to control.

Relatively little is known about the effects of AM colonization on the accumulation of active phytochemicals in shoots of medicinal plants, which are often the harvest products. However, it was reported that *Glomus mosseae* directly increased the essential oil content in shoots in two of three tested oregano genotypes *Origanum* sp grown on industrially Cd and Pb polluted soil (Khaosaad *et al.,* 2006; and Hristozkova *et al.,* 2015). Similar results were obtained by (Copetta *et al*., 2006) in studies with *Ocimum basilicum* L. Our results are in an agreement with the reports of (Prasad *et al*., 2011) that the AM fungal inoculation maintained the level of linalool, methyl chavicol, in sweet basil oil, which were either increased or decreased by the application of heavy metals. (Ordookhani *et al*., 2011) showed that sweet basil inoculated with *P*. *putida* increase oil yield compared to control.

The arbuscular mycorrhiza (AM) fungi can increase plant uptake of nutrients especially relatively immobile elements such as P, Zn and Cu (Ryan and Angus, 2003), and consequently, they increase root and shoot biomass and improve plant growth. It has been indicated that AM fungi can colonize plant roots in metal contaminated soil (Vogel-Mikus and Marjana., 2006), while their effects on metal uptake by plants are conflicting. (Zhang *et al*., 2010) reported that AMF increased lead accumulation in the roots. The same trend was observed in case of inoculation with isolate G3+*P. putida* under $P_{100\%}$ rate in our results. The most important finding of the present research is that AMF can keep metal concentrations in aromatic plants at low levels and alleviate harmful effects caused by heavy metal pollution. The inoculation with the AMF strains of EEZ 54 and EEZ 55, isolated from a place with naturally high levels of metals, reduced the Pb concentration in marjoram shoots and roots (Hristozkova *et a.l,* 2015). The effects of mycorrhizal colonization on remediation of contaminated soils depend on the plant–fungus–heavy metal combination and are influenced by soil chemical and physical conditions (Siddiqui and Pichtel., 2008). According to (Azcon *et al*., 2010), AMF symbiosis may contribute to phytoremediation via strategies such as HM sequestration or accumulation, keeping metal concentrations in the plants below critical values and improving plant growth and nutrition (Pawlowska and Charvat ., 2004). Prasad et al, (2011), demonstrated that AM species could be effective in protecting sweet basil exposed to high levels of metals. Mycorrhizal fungi are reported to protect plants from the toxic effects of high external concentration of several metals, possibly by binding the metals in their hyphae or by reducing the translocation of metals to the plants tops (Mozafar *et al*., 2002). The higher AM-plant metal content of roots could be attributed to fungal metal binding and sequestration in intraradical hyphae, and these metal forms are not bioavailable to

plants (Christie *et al*., 2004). Plant under study accumulated more copper in roots but large reductions in shoots; this is in agreement with Banni and Faituri, (2013) that the comparisons of the two AM fungal species indicate that the AM fungal represented by *Glomus* spp (mixed) can benefit against potentially toxic Cu and therefore play a role in bioremediation of Cu-contaminated soils. Such results underline the importance of indigenous AM fungi, which are presumably more adapted to heavy metals.

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الملخص العربي

تأثير المعالجة الحيوية للأراضي الملوثة بالنحاس والرصاص علي نباتات الريحان

ميادة صبره* وثريا صبري وآمال أبو النصر***

* قسم النبات الزراعي– كلية الزراعة – سابا باشا– جامعة الإسكندرية

** قسم النبات والميكروبيولوجي– كلية العلوم – جامعة الإسكندرية

أجريت تجربة هذا البحث في مزرعة كلية الزراعة (سابا باشا) بأبيس خلال موسمي ٢٠١٢ و٢٠١٣ وذلك بإضافة سماد الفوسفور بثلاث معدلات (صفر %، ٧٥ % ، ١٠٠%) من الكميه الموصي بها والتلقيح بكل من فطر الميكوريزا (*Glomus intraradices*) وبكتيريا (*Pseudomonas putida*) على نبات الريحان وذلك لدراسة المعالجة الحيوية للأراضي الملوثة بكل من عنصري الرصاص والنحاس وأوضحت النتائج أن هناك زيادة معنوية في كل من (طول النبات ، كمية المحصول ، النسبة المئوية للزيت والمواد الفعالة في زيت الريحان ، الوزن الجاف للسيقان والجذور) للنباتات المعاملة بالمقارنة بالنباتات غير المعاملة وأدى التلقيح بكل من فطر الميكوريزا والبكتيريا إلى إنخفاض نسبة عنصري الرصاص والنحاس في المجموع الخضري والتربة حيث سجلت أقل نسبة للمجموع الخضري وهي (٣٣,٢٤ ، ١٨,٦٠ مجم / كجم) لكل من عنصري الرصاص والنحاس علي التوالي بالمقارنه بالنباتات غير المعاملة فكانت (٤٦,٤٩ ، ٢٣,٤٦ مجم/كجم) علي التوالي ، علي الجانب الآخر إتضح زيادة لتركيز عنصري الرصاص والنحاس في المجموع الجذري مع إضافة المعاملات وبلغت (٣٠,١٥ ، ٣٩,٢٥ مجم / كجم) بالمقارنة بالكنترول (٢٢,٠١ ، ٣٣,٥٧ مجم/كجم) علي التوالي.