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TRANSFER OF NICKEL FROM POLLUTED SOIL TO *PISUM SATIVUM* L. AND *RAPHANUS SATIVUS* L. UNDER COMPOSTED GREEN AMENDMENT AND NATIVE SOIL MICROBES

NIVIEN ALLAM NAFADY[1], MOHAMED BAHY-EL-DIN MAZEN[1], MOHAMED MAHMOUD MOHAMED AHMED [2], OMAIMA ABDEL MONSEF [2]*

[1] Botany and Microbiology Department, Faculty of Science, Assiut University, Assiut, Egypt
[2]Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), Assiut, Egypt

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The effect of compost, inoculation with native soil microbes and their residual effects on bioavailability of nickel by peas (*Pisum sativum* L.) and radish (*Raphanus sativus* L.) grown on polluted soil were investigated in pot experiments. Plants were amendment with different compost levels (0, 0.2, 0.4, 0.6% of soil dry weight) and inoculated with different native soil microbes (4 fungal species, one bacterial species, 4 species of arbuscular mycorrhizal fungi) isolated from the polluted soil under study. Significant increases in the biomass of pea and radish plants were observed as a result of amendment application and their residual effects. The mycorrhizal dependency (MD) of pea plants was lower than of radish plants. The highest reductions of Ni levels in both plants were observed by the simultaneous applications of compost with microbes or mycorrhizal fungi to polluted soils. Soil pH increased significantly ($p < 0.05$) as a result of applying native microbes especially with arbuscular mycorrhizal fungi (AMF) alone or combined with compost. The DTPA extractability of soil Ni was significantly decreased with increasing soil pH ($p < 0.05$). The minimum transfer factor of Ni from polluted soil were 0.067 and 0.089 for pea and radish plants, respectively which were attained as a result of applying compost (0.6% of soil weight) inoculated with mycorrhizal fungi. From the results, we can conclude that the use of compost and native soil microbes as a soil remediate could be an effective strategy for soil remediation.

Key words: compost, native soil microbes, nickel, peas, radish

Nickel is an essential microelement for plants, animals, and humans, but toxic at high concentrations. Nickel adversely affects plant growth by altering different physiological and metabolic processes (Aziz *et al.* 2015). The toxic effects of nickel probably result primarily from its ability to replace other metal ions in enzymes and proteins or to bind to cellular compounds (Cempel & Nikel 2006). Excess Ni has been reported to cause leaf necrosis and chlorosis of plants (McIlveen & Negusanti 1994; Seregin & Kozhevnikova 2006). Nickel can accumulate in agricultural soils through the application of phosphate fertilisers, pesticides and other waste materials from industries like nickel-cadmium batteries, nickel electroplating, paints formulation, and vegetable fat production (Ramachandran & D'Souza 2013).

Nivien Allam Nafady, Assistant professor, Botany and Microbiology Department, Faculty of Science, Assiut University, 71515 Assiut, Egypt. E-mail: niviennafady@aun.edu.eg
Mohamed Bahy-EL-Din Mazen, Prof. Dr., Botany and Microbiology Department, Faculty of Science, Assiut University, 71515 Assiut, Egypt. E-mail: mazen941@yahoo.com
Mohamed Mahmoud Mohamed Ahmed, Prof. Dr., Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), 71717 Assiut, Egypt. E-mail: m311332@hotmail.com
Omaima Abdel Monsef (*Corresponding author), Soil, Water and Environment Research Institute (SWERI), Agricultural Research Center (ARC), 71717 Assiut, Egypt. E-mail: omaimaabdelmonsef@yahoo.com

Vegetables cultivated in soils polluted with toxic and heavy metals take up such metals and accumulate them in their edible and non-edible parts in quantities high enough to cause clinical problems both to animals and human beings. Toxic metals are known to have serious health implications, including carcinogenesis induced tumor promotion, and hence the growing consciousness about the health risks associated with environmental chemicals has brought a major shift in global concern towards prevention of heavy metal accumulation in soil, water and vegetables (Bhuiyan *et al.* 2011).

The risk of crop failure and economic losses, and decreasing human health risks from heavy metals can be reduce by using compost. Compost addition to soil alone can change the mobility and bioavailability of heavy metals in soil environment, as well as the toxic effects on plants and animals. These actions are attributed to various processes, including adsorption, complexation, precipitation, and redox reactions (Vaca-Paulin *et al.* 2006; Lagomarsino *et al.* 2011; Park *et al.* 2011; Huang *et al.* 2016).

Also, the use of microorganisms can minimize the bioavailability and biotoxicity of heavy metals (Gadd 2000; Lloyd & Lovely 2001). Another report cleared that the metal immobility in fungal-bacterial (FB) co-inoculation is governed by several mechanisms, including mass transfer of metals, biosorption, and precipitation (Bandara *et al.* 2015). Likewise, arbuscular mycorrhiza (AM) fungi can increase the tolerance of some plants to toxic metal contamination by developing the metal tolerance of the fungi themselves and binding the metals to polyphosphates within the fungal hyphae implicated (Barea *et al.* 2005; Morgan *et al.* 2005; Elgharably & Nivien 2013; Abd-Alla *et al.* 2016).

In the current investigation, peas followed by radish were grown in polluted soil with application of different levels of green compost and native soil microbes.

The objective of this work was studying the direct and residual effect of compost and native microbes and their combination on: (1) peas and radish plant growth; (2) nickel concentration in plant parts; (3) soil pH and Ni availability.

MATERIAL AND METHODS

Soil, compost and native microbes inocula

The experimental soil was collected from the polluted agriculture area near the superphosphate factory (27°N and 31°E), Assiut city, Egypt. Several samples (0–15 cm depth) were collected and bulked to give a composite sample. The soil texture was silt and classified as Typic Torrifluvents according to USDA Soil Taxonomy (2010). Soil had a pH of 6.50 (1:2 soil/water suspension). The electrical conductivity (EC), organic matter (OM) and total $CaCO_3$ were measured according to Jackson (1973) recorded 2.55 dS/m (1:2 soil water extract), 15.9, 15.2 g/kg soil, respectively. Total N (1.56 g/kg soil) was determined by Micro Kjeldahl method (Black 1965). Available P was 150 mg/kg soil (Olsen & Sommers 1982), total Ni and available Ni of soil samples were 56.75 and 0.501 mg/kg soil, respectively.

Compost heap was built alternately between corn residues, sheep manure, and peanut residues at a ratio of 70:20:10% respectively. During composting, materials were manually mixed every 15 days throughout the composting period for air circulation and temperature homogeneity. The moisture level of the heap was measured gravimetrically every week and appropriate amount of water was sprinkled onto the heap to increase the moisture content up to 60%. Compost heap reached to maturity after 4 months. Compost samples were dried at 70°C to constant weight ground. The value of pH was 8.6 determined in (1:10) [compost: water] suspension using glass electrode pH meter. The EC was 6.14 dS/m (compost water extract, 1:10). The organic matter (OM) content of the compost analysed by weight loss on ignition at 430°C for 24h was 540.9 g/kg, and total organic carbon (TOC) was 280.7 g/kg (calculated from OM) according to Navarro *et al.* (1993). The NPK contents were 20.9, 3.5 and 15.9 g/kg compost, respectively. Also, compost samples were digested using a nitric-perchloric acids mixture (HNO_3 + $HClO_4$) to determine total Ni (AOAC 1990) which recorded 2.77 mg/kg compost.

Native microbes of *Aspergillus niger, A. terreus*, *Penicillium funiculosum* and *Fusarium culmorum* were isolated from the polluted site under investigation on Potato Dextrose Agar (PDA). Inoculum potential of native isolated fungi was 10^4 cfu/g. Also, *Bacillus* sp. was isolated from the soil under study and the inoculum potential was 10^6 cfu/g. The compatible mixed culture was used as *A. niger, A. terreus, P. funiculosum* and *F. culmorum* and *Bacillus* sp. (Mohammad *et al.* 2011).

Four species of native mycorrhizal fungi were isolated from the polluted soil under study. The most dominant species was extracted by wet sieving and decanting technique (Gerdemann & Nicolson 1963). The species named *Acaulospora bireticulata* F.M. Rothwell & Trappe, *Gigaspora margarita* W.N. Becker & I.R. Hall, *Glomus lamellosum* Dalpé, Koske & Tews and *Funneliformis mosseae* (Nicol. & Gerd.) Walker & Schüßler. Mycorrhizal inoculum was propagated in a mixture of sand and bulk contaminated soil (1:1v/v) using *Zea mays* L. seedlings as host plants for 2 months. The inoculum consists of spores, hyphae and colonized root fragments of which 100 gram of AM fungi inoculum was placed below seeds of the tested plant (approximately contain 10 spores/g soil). The same volume of autoclaved inoculum was added below control seeds.

Experimental design

Factorial experiment in completely randomize block design was performed in greenhouse of Assiut Agricultural Research Station, Egypt. Plastic rectangular pots (35 cm height × 26 cm width × 26 cm length) were filled with 18 kg of polluted soil under study. Four levels of dried compost (0, 0.2, 0.4 and 0.6% of soil weight as CS0, CS1, CS2, CS3) were mixed with the polluted soil and inoculated with three native microbe treatments. FB (four fungal species + one bacterial species); AMF (arbuscular mycorrhizal fungi) and FB+AMF. Five seeds of peas (*Pisum sativum* L.) were planted as a seed vegetable in each pot. Ammonium nitrate (33.5% N) and potassium sulphate (50% K_2O) were used as a mineral fertiliser of all treatments. Three replicates were used for each treatment. Pea plants were harvested after 90 days from planting.

After harvesting peas, five seeds of radish (*Raphanus sativus* L.) were planted as a root vegetable in each pot to study the residual effect of the experimental treatments without adding any compost or inoculums, only mineral fertilisation was added. Plants were maintained at a soil water potential of field capacity. Plant samples were harvested after 30 days from planting.

Soil and plant analysis

Soils samples were collected after harvesting plants pea and radish, oven dried at 40°C for 48 h and passed through a 1 mm sieve. Soil pH was measured in 1:2 (soil: water) suspensions using a glass electrode according to Mclean (1982). Soil samples were digested with nitric and hydrochloric acid mixture according to Cottenie *et al.* (1982) for measurement of total nickel and determined by Inductively Coupled Plasma Spectrometry (Ultima 2 JY Plasma). The diethylene triamine penta acetic acid extracting (0.005M DTPA, 0.1 TEA, and 0.01 M $CaCl_2$, adjusted to pH 7.3) solution (Lindsay & Norvell 1978) was employed to extract Ni as a potential indicator of plant-available heavy metals from soils.

Plant samples were washed thoroughly with running tap water and rinsed three times with deionized water. The dry weights of pea (root, shoot, peels and seeds) and radish (root and shoot) plants were recorded after drying in a forced air oven at 65°C for 48h. Half gram of each plant sample was wet digested using a nitric-perchloric acids mixture (HNO_3 + $HClO_4$) according to the procedure of Tedesco *et al.* (1995) to determined total Ni in plant tissues.

To characterize quantitatively the transfer of an element from soil to plant, the soil-plant Partition Coefficient or Transfer Factor (TF) that expresses the ratio of contaminant concentration in plant parts to concentration in dry soil is used (Cui *et al.* 2004).

Determination of mycorrhizal colonization

One-cm long pieces of roots were used to investigate the mycorrhizal colonization of peas and radish. Fresh root samples were treated with 10% KOH and the AMF stained with trypan blue (0.1%) in lactophenol (Phillips & Hayman 1970). The percentage of root colonization was determined by the grid-intersect method (Giovannetti & Mosse 1980).

Quantification of mycorrhizal dependency

The mycorrhizal dependency (MD) of plant (shoot + root) growth was determined according to Plenchette *et al.* (1983) as follow:

$$\textit{Dependency of growth} = \frac{\mathrm{M{-}NM}}{\mathrm{M}} \times 100$$

where:
M and NM refer to the dry mass of mycorrhizal and non-mycorrhizal plants, respectively.

Statistical Analysis

Results were processed and analysed using SPSS statistical analysis package for Windows®. Data is reported as mean ± standard deviation of the mean unless otherwise stated. A p-value of < 0.05 was considered significant. Two-way analysis of variance was performed (ANOVA) on the pairs of variables likely to exhibit correlation.

RESULTS AND DISCUSSION

Bioavailablability of Ni and soil pH

Effect and residual effect of compost, microbes and arbuscular mycorrhizal fungi on DTPA extractability of soil Ni and pH of pea and radish soils after harvesting was detected in Table 1. Data showed that, the DTPA extractability of soil Ni was significantly decreased with increasing soil pH ($p < 0.001$). Soil pH increased significantly ($p < 0.05$) particularly when using AMF or FB + AMF only or combined with compost and their residual effects. Results showed that the pH of radish soil were always higher than the pH of pea soil. The soil availability of Ni under compost and inoculations and their residual effects was always lower than the control. Soil cultivated with pea and treated with AMF always had the lowest amounts of DTPA-extractable Ni with all discussed compost treatments 0.904 mg/kg dry soil by reduction 32.34% at the pH (7.12). On contrast,

Table 1

Available Ni and pH of soils grown with peas and radish after harvesting

Compost	Inoculation	Peas		Radish	
		Available Ni [mg/kg]	pH (1:2)	Available Ni [mg/kg]	pH (1:2)
CS0	NU	1.342 ± 0.008^{k}	6.95 ± 0.04^{a}	1.140 ± 0.008^{k}	7.03 ± 0.05^{a}
	FB	1.316 ± 0.011^{ij}	6.97 ± 0.06^{a}	0.972 ± 0.006^{j}	7.05 ± 0.03^{ab}
	AMF	0.904 ± 0.012^{a}	7.12 ± 0.02^{gh}	0.840 ± 0.006^{d}	7.18 ± 0.02^{f}
	FB+AMF	1.184 ± 0.020^{g}	7.09 ± 0.01^{efgh}	0.698 ± 0.006^{a}	7.16 ± 0.01^{ef}
CS1	NU	1.336 ± 0.008^{jk}	7.00 ± 0.02^{abc}	0.928 ± 0.010^{i}	7.08 ± 0.03^{bc}
	FB	1.304 ± 0.008^{i}	6.98 ± 0.02^{ab}	0.894 ± 0.004^{h}	7.10 ± 0.02^{cd}
	AMF	0.927 ± 0.011^{b}	7.10 ± 0.01^{fgh}	0.692 ± 0.006^{a}	7.16 ± 0.03^{ef}
	FB+AMF	1.038 ± 0.014^{e}	7.10 ± 0.02^{fgh}	0.854 ± 0.007^{ef}	7.10 ± 0.02^{cd}
CS2	NU	1.194 ± 0.014^{g}	7.04 ± 0.05^{cde}	0.860 ± 0.006^{fg}	7.12 ± 0.02^{cde}
	FB	1.220 ± 0.012^{de}	7.03 ± 0.01^{bcd}	0.862 ± 0.004^{fg}	7.12 ± 0.01^{cde}
	AMF	0.938 ± 0.008^{b}	7.08 ± 0.03^{defg}	0.784 ± 0.004^{b}	7.12 ± 0.03^{cde}
	FB+AMF	1.026 ± 0.014^{de}	7.06 ± 0.03^{def}	0.844 ± 0.008^{de}	7.14 ± 0.01^{def}
CS3	NU	1.152 ± 0.008^{f}	7.05 ± 0.02^{cdef}	0.920 ± 0.006^{i}	7.09 ± 0.02^{bc}
	FB	1.194 ± 0.010^{g}	7.03 ± 0.03^{bcd}	0.884 ± 0.006^{h}	7.10 ± 0.02^{dc}
	AMF	0.962 ± 0.016^{c}	7.06 ± 0.01^{def}	0.868 ± 0.008^{g}	7.16 ± 0.01^{ef}
	FB+AMF	1.013 ± 0.007^{d}	7.14 ± 0.01^{h}	0.854 ± 0.007^{ef}	7.10 ± 0.02^{cd}
Two-way ANOVA					
CS		+++	+	+++	NS
IN		+++	+++	+++	+++
CS × IN		+++	+++	+++	+++

Mean ± SD (n=3). CS: compost amendments; NU: plants without inoculation; FB: plants inoculated with microbes; AMF: plants inoculated with arbuscular mycorrhizal fungi; FB+AMF: plants inoculated with microbes and arbuscular mycorrhizal fungi; CS: effect of compost; IN: effect of inoculation; CS × IN: the effect of interaction between compost and inoculation; One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other ($p < 0.05$) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$.

the residual effect of FB plus AMF only and CS1 plus AMF treatments attained the lowest level of DTPA extractable Ni in radish soil record 0.698 and 0.692 mg/kg dry soil, respectively at soil pH (7.16) by reduction 38.77 and 39.30%, respectively. From results, the addition of compost and inoculation has reduced the availability of Ni. At high pH, metals tend to form insoluble metal mineral phosphates and carbonates, which are less bioavailable (Rensing & Maier 2003; Sandrin & Hoffman 2007). Also, mycorrhizal plants recorded the highest values in soil pH and lowest values of DTPA-extractable Ni. These results are in agreement with Bano & Ashfaq (2013) who reported that the immobilization of some heavy metals due to the mycorrhizal association may possibly be due to a slight increase in pH in the mycorrhizosphere. Many heavy metals are immobilized due to this change in pH under conditions of high concentrations of certain metals. The beneficial effects of mycorrhiza against plant's heavy metal uptake may be associated with heavy metal solubility caused by changes in soil pH.

Plant biomass

Data in Table 2 show the impact of different fertilisation strategies on peas growth and their residual effects on radish growth. The results indicated that using different compost levels and inoculation significantly ($p < 0.05$) increased the growth measurements of pea and radish plants. Data revealed that the highest values of roots and shoots dry

T a b l e 2

Effect and residual effect of compost, microbes and arbuscular mycorrhizal fungi on biomass of peas and radish

Compost	Inoculation	Peas				Radish	
		Dry weight [g/plant]		Peels dry weight [g/plant]	Seeds dry weight [g/pant]	Dry weight [g/plant]	
		Root	Shoot			Root	Shoot
CS0	NU	0.24 ± 0.01^{a}	11.70 ± 0.36^{a}	3.36 ± 0.37^{a}	3.97 ± 0.5^{a}	1.57 ± 0.07^{a}	3.90 ± 0.48^{a}
	FB	0.28 ± 0.02^{ab}	12.57 ± 0.21^{ab}	5.29 ± 0.19^{bcd}	6.75 ± 0.81^{def}	1.87 ± 0.30^{ab}	5.02 ± 0.47^{bc}
	AMF	0.32 ± 0.04^{bc}	12.73 ± 0.70^{ab}	6.15 ± 0.54^{def}	6.92 ± 1.08^{def}	2.55 ± 0.28^{d}	5.29 ± 0.20^{cd}
	FB+AMF	0.29 ± 0.02^{ab}	11.90 ± 0.79^{a}	6.50 ± 0.70^{efg}	5.64 ± 1.14^{bcd}	2.16 ± 0.33^{bcd}	5.67 ± 0.02^{defg}
CS1	NU	0.28 ± 0.03^{ab}	13.27 ± 1.96^{abc}	4.84 ± 1.08^{bc}	5.24 ± 0.80^{abc}	2.32 ± 0.23^{cd}	5.70 ± 0.48^{defg}
	FB	0.30 ± 0.05^{ab}	12.90 ± 0.45^{ab}	4.19 ± 0.16^{ab}	5.58 ± 0.27^{bcd}	1.59 ± 0.08^{a}	5.54 ± 0.27^{cdef}
	AMF	0.35 ± 0.03^{bc}	13.78 ± 0.74^{abcd}	6.76 ± 0.55^{fg}	8.12 ± 0.48^{fg}	2.17 ± 0.30^{bcd}	6.01 ± 0.25^{fg}
	FB+AMF	0.33 ± 0.04^{bc}	14.81 ± 0.54^{bcde}	4.32 ± 0.36^{ab}	7.11 ± 0.27^{ef}	1.97 ± 0.12^{abc}	5.34 ± 0.14^{cde}
CS2	NU	0.32 ± 0.04^{bc}	15.75 ± 1.85^{de}	6.29 ± 0.69^{def}	7.23 ± 0.18^{ef}	1.92 ± 0.03^{abc}	5.55 ± 0.21^{cdef}
	FB	0.34 ± 0.03^{bc}	13.79 ± 1.01^{abcd}	7.48 ± 0.46^{g}	8.81 ± 0.94^{g}	2.12 ± 0.14^{bc}	4.61 ± 0.19^{b}
	AMF	0.38 ± 0.03^{c}	16.82 ± 1.51^{e}	6.13 ± 0.69^{def}	7.42 ± 0.86^{ef}	2.55 ± 0.40^{d}	6.18 ± 0.32^{g}
	FB+AMF	0.30 ± 0.07^{ab}	14.39 ± 1.05^{bcd}	6.89 ± 0.65^{fg}	6.22 ± 1.02^{bcde}	2.11 ± 0.19^{bc}	5.78 ± 0.35^{defg}
CS3	NU	0.30 ± 0.03^{ab}	15.58 ± 2.07^{cde}	5.21 ± 0.45^{bcd}	6.47 ± 1.10^{cde}	1.84 ± 0.09^{ab}	5.33 ± 0.49^{cd}
	FB	0.29 ± 0.04^{ab}	13.64 ± 1.33^{abcd}	6.22 ± 0.80^{def}	5.48 ± 0.62^{bcd}	2.02 ± 0.21^{bc}	4.46 ± 0.13^{b}
	AMF	0.32 ± 0.04^{bc}	15.80 ± 2.17^{de}	5.48 ± 0.50^{cde}	6.71 ± 0.81^{def}	2.35 ± 0.25^{cd}	5.96 ± 0.32^{efg}
	FB+AM	0.28 ± 0.06^{ab}	13.34 ± 0.29^{ab}	4.73 ± 0.58^{bc}	4.90 ± 0.12^{ab}	2.21 ± 0.13^{bcd}	5.89 ± 0.40^{defg}
Two-way ANOVA							
CS		+	+++	+	++	NS	+++
IN		++	+	NS	++	+++	+++
CS × IN		NS	NS	NS	+	++	+++

Mean ± SD (n=9). One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other ($p < 0.05$) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$.

weight of pea plants were attained in CS2 combined with AMF treatment and its residual effect on radish plants (58.33, 43.76, 62.42 and 58.46%, respectively). The dry weight of peels and seeds increased by 122.62 and 121.91%, respectively compared with the control (CS0 and NU) when 0.4% dry compost with FB (4 fungal species + one bacterial species) were applied. This might be explained by the effect of fertilisers enrichment with beneficial strains of bacteria and fungi on increasing fertilisation in crop production (Chen 2006) by enhancing the physiology of crop plants, stimulating their growth and yielding, as well as by increasing their resistance to environmental and biotic stresses (Corte *et al.* 2013). Also application of native mycorrhizal fungi and beneficial strains of bacteria and fungi incorporated ensures their better adaptation and survival in the prevailing environmental conditions, which is an extremely important factor for their long-term effects on plants (Regvar *et al.* 2003). Two-way ANOVA indicated that both "compost" and "inoculation" factors significantly affected ($p < 0.01$) all growth measurements of peas and radish expect root dry weight of radish and peels dry weight of peas. The interaction of "compost × inoculation" factor had a non-significant effect on all growth measurements of peas except seeds dry weight while all growth measurements of radish were significantly affected ($p < 0.01$).

Mycorrhizal colonization

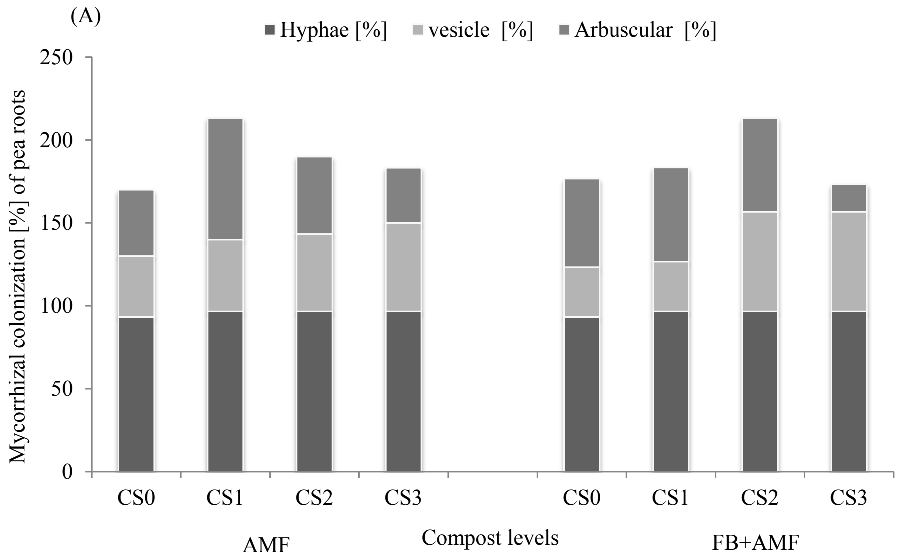
Microscopic analysis confirmed that plants of non-inoculation treatments were not colonized by AMF. There were large differences between colonization patterns in pea roots (Figure 1A). The colonized roots were occupied by intercellular, intracellular hyphae, vesicles and arbuscules. Roots of pea plants treated with CS1+AMF and CS2+FB+AMF recorded the maximum values of hyphae, vesicles and arbuscular colonization. While, the highest proportions of mycorrhizal colonization structures in radish plants were recorded with the residual effect of CS3+FB+AMF treatment (Figure 1B).

Mycorrhizal dependency for peas and radish growth

Mycorrhizal dependency (MD) is defined as the degree to which a plant is dependent on the mycorrhiza to produce maximum growth or yield at a given soil fertility level. Results given in Figure 2A, B) clearly showed that the mycorrhizal dependency (MD) for radish plants was higher than for pea plants. MD was always higher with AMF inoculation than with FB+AMF inoculation in both plants. The highest MD value of pea plants obtained from CS2 treated plants inoculated with AMF (37.22%). While the lowest MD value (17.07%) was observed in plants treated with CS3 inoculated with FB+AMF. Also, the residual effect of AMF with CS2 realized the highest value (41.02%) of MD for dry weight of radish plants. While the minimum value of MD of radish dry weight was obtained in CS1+FB+AMF (29.02%). The increase in mycorrhizal dependency (MD) values to a certain level and then decline might return to the over fertilisation. High levels of fertilisation, often occurring in intensive agriculture, have a negative effect on root growth and root colonization by mycorrhizal fungi (Smith & Read 2008).

Nickel concentration in pea and radish plants

Data in Figure 3 (A-D) and 4 (A-B) show the effect of applied compost at different levels and inoculation and their residual effects on nickel concentration in roots, shoots, peels and seeds of peas and subsequent roots and shoots of radish plants. Compost at 0.6% (CS3) inoculated with fungal-bacterial inoculum plus AMF attained highly significant reduction for nickel content by 65.60 and 71.46% in roots and seeds of peas, respectively compared with the control treatment (CS0+NU). The lowest Ni values in shoots of peas were realized in CS3+AMF and CS3+FB+AMF with reduction of 60.21% compared with the control treatment. In pea peels, there were no significant differences between most treatments. Concerning to radish plants, the lowest level of Ni in shoots of radish was observed in CS3 and AMF treatment by a reduction of 38.46%. In radish roots, the residual effect of AMF, and FB+AMF treatments recorded the minimum content of Ni when existed individually or combined with compost at any level. Also, CS3 and CS3+FB treatments attained the lowest Ni concentration in plant parts. There were no significant differences between previous treatments. The effect of applied compost levels and inoculation on decreasing Ni concentration could be arranged in descending order of: CS+FB+AMF ≥ CS+MF > CS+FB > CS. In all treatments, the nickel concentration in peas and radish organs was higher than the



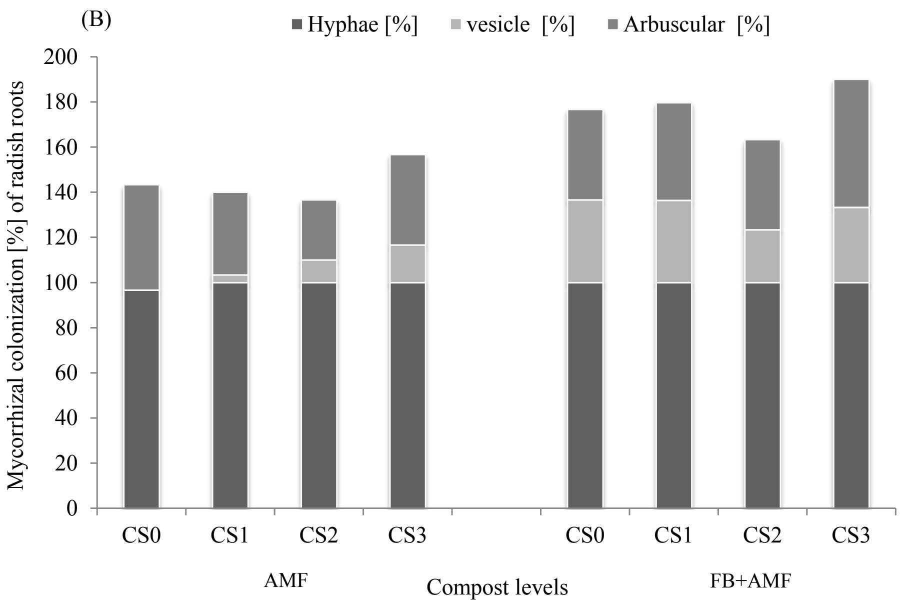


Figure 1. Percentage of mycorrhizal fungi colonized roots of (A) pea and (B) radish

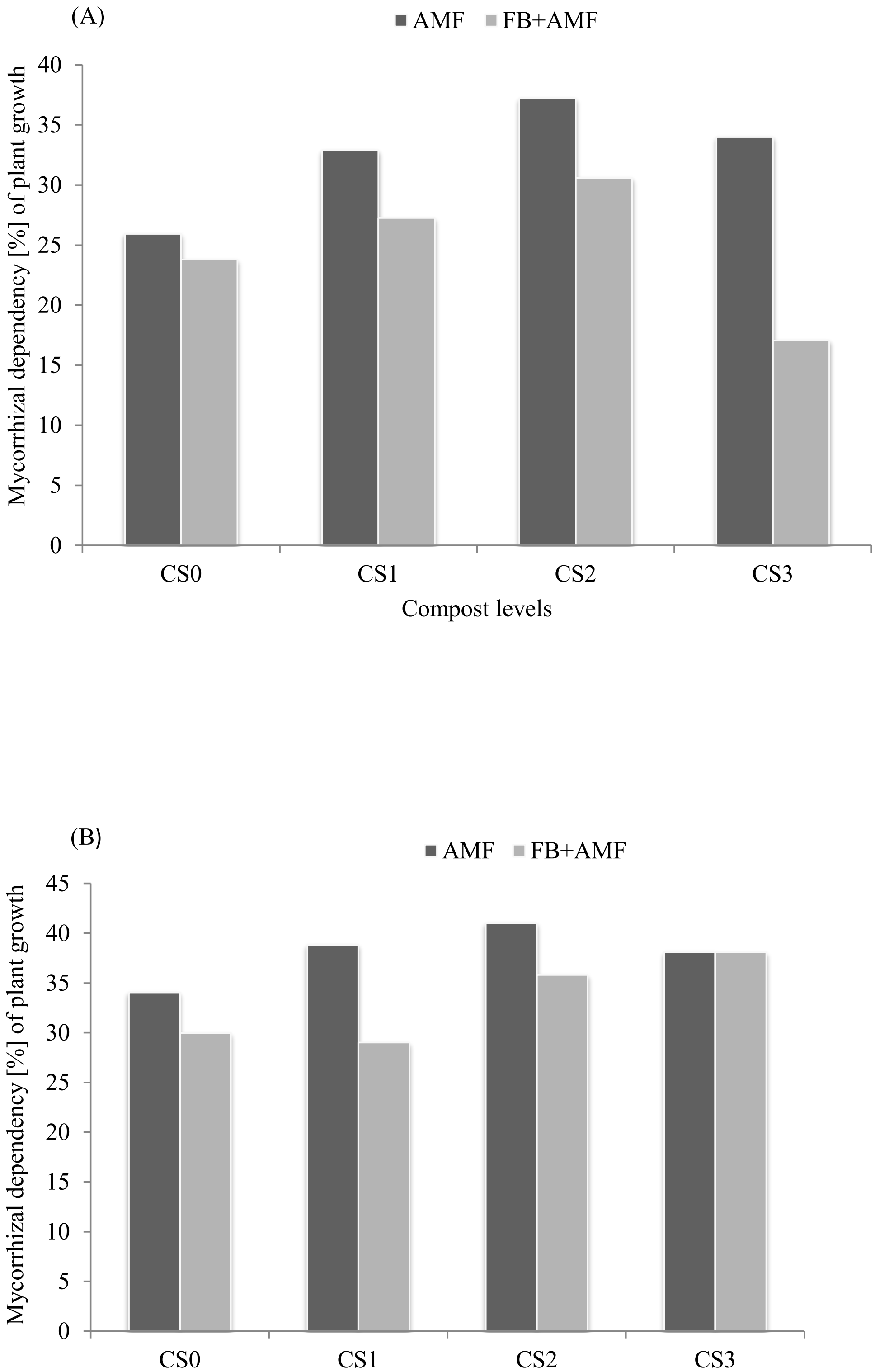
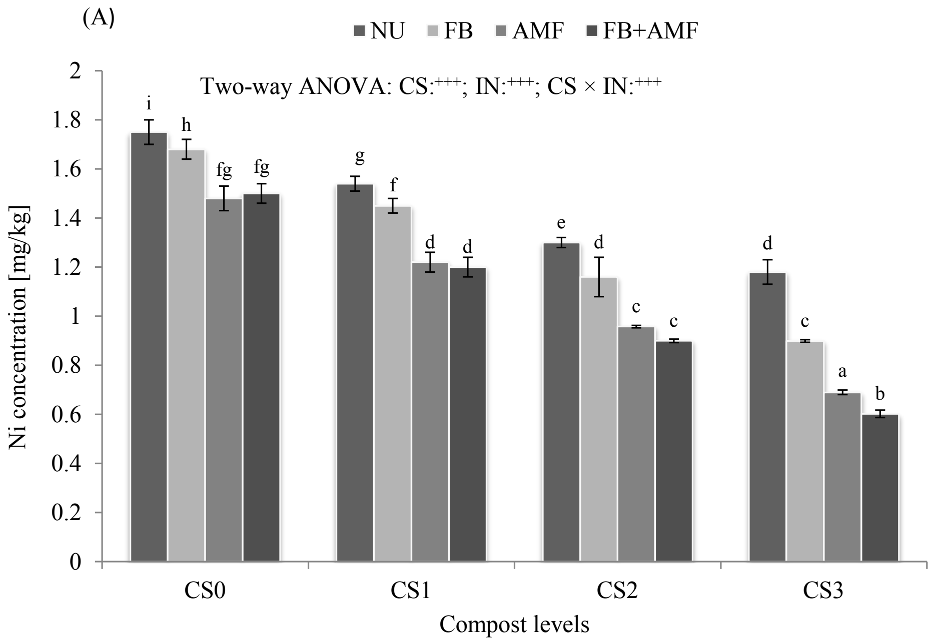
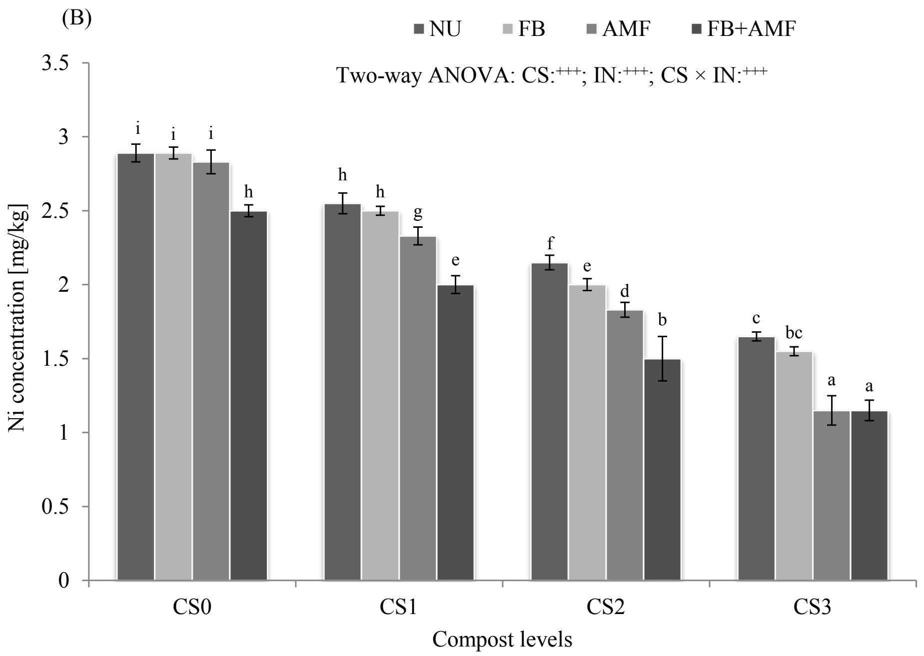
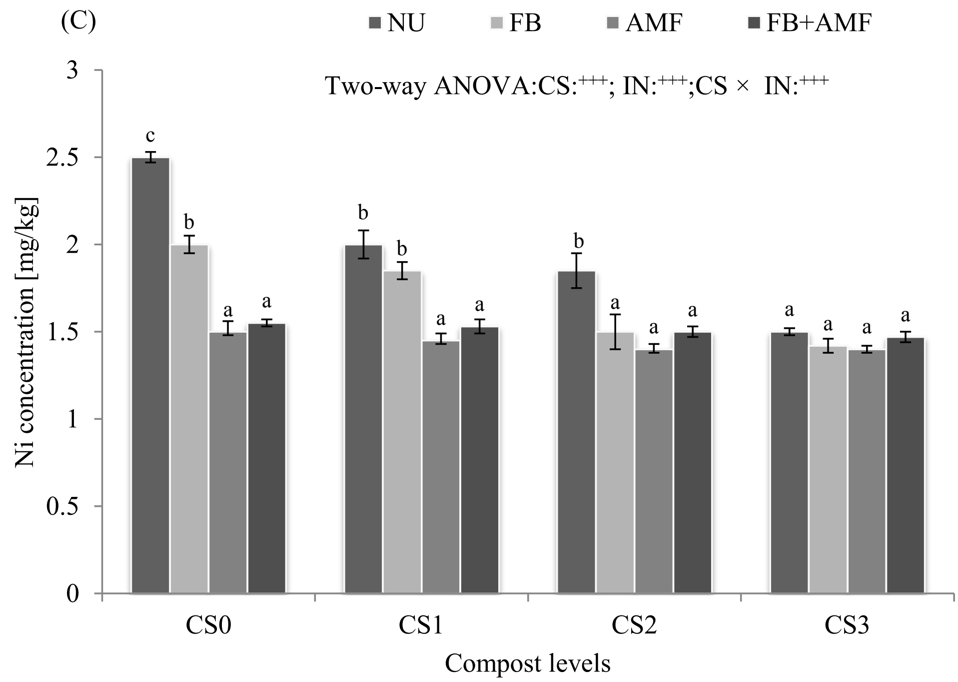


Figure 2. Effect and residual effect of compost and native microbes on mycorrhizal dependency [%] of (A) peas and (B) radish growth


(A)
NU FB AMF FB+AMF
Two-way ANOVA: CS:+++; IN:+++; CS × IN:+++
Ni concentration [mg/kg]
Compost levels
CS0
CS1
CS2
CS3


(B)
NU FB AMF FB+AMF
Two-way ANOVA: CS:+++; IN:+++; CS × IN:+++
Ni concentration [mg/kg]
Compost levels
CS0
CS1
CS2
CS3



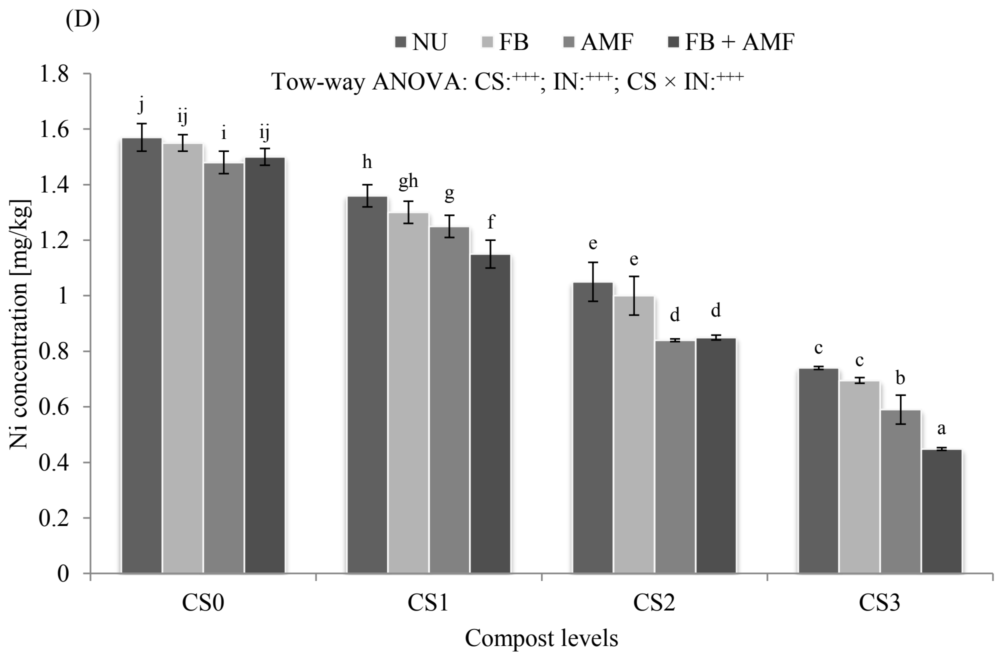
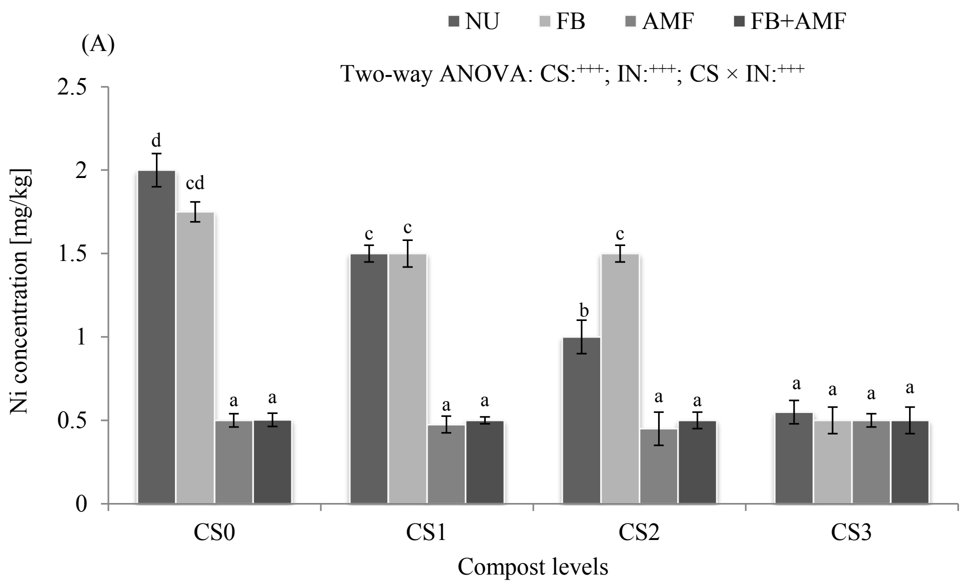


Figure 3. Ni concentration in pea plants [mg/kg, DW]. (A) roots, (B) shoots, (C) peels, (D) seeds. Mean ± SD (n=3). One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other ($p < 0.05$) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$



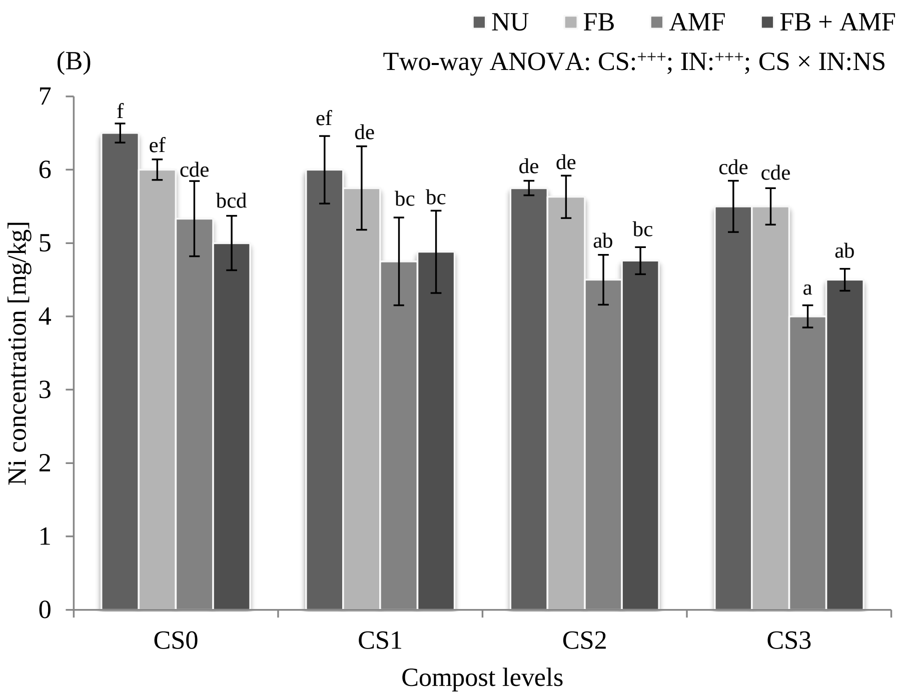
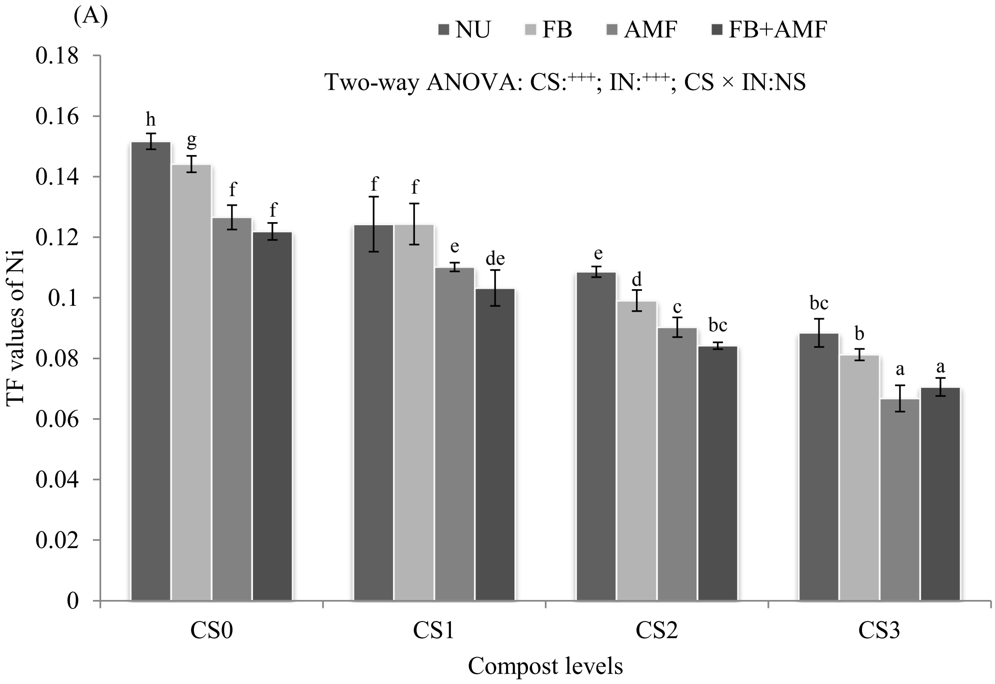


Figure 4. Ni concentration in radish plants [mg/kg, DW]. (A) roots and (B) shoots. Mean ± SD (n=3). One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other ($p < 0.05$) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$



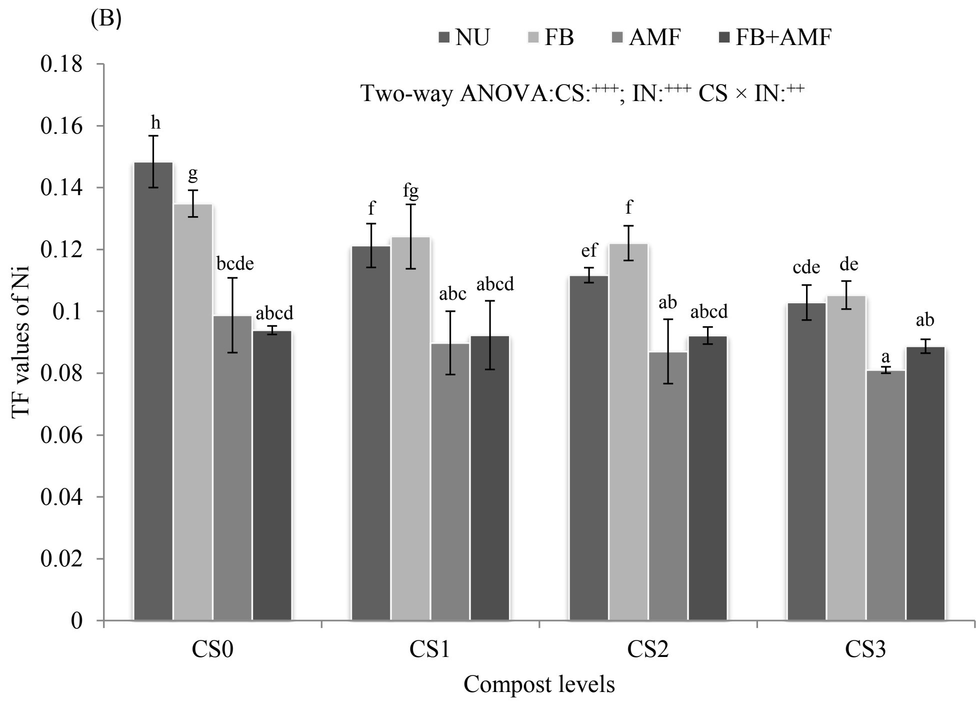


Figure 5. Transfer factor (TF) values of Ni in total plants of (A) pea and (B) radish. Mean ± SD (n=3). One-way ANOVA was performed for amendment treatments; Means for each parameter with different letters are significantly different from each other ($p < 0.05$) according to the Duncan test; Two-way ANOVA was performed to determine the effect of compost, inoculation and their interaction on polluted soil. NS not significant; $^{+}p < 0.05$; $^{++}p < 0.01$; $^{+++}p < 0.001$

maximum permissible level (0.01 mg/kg) recommended by WHO/FAO (2007). In general, results indicated that combination between compost and native microbes positively decreased Ni concentration in all plant organs compared to compost only and the control one. Several previous studies found that compost is a promising strategy to immobilize heavy metals in soils through changing the physicochemical property of soils and reacting with heavy metals (Liu *et al.* 2009; Bolan *et al.* 2014). Compost mainly immobilizes heavy metals through its humus substance, microorganisms, and inorganic components. Abundant humus substances in compost contain a large number of organic functional groups, such as carboxyl, carbonyl, and phenols, which can bind metal ions through complexation (Caporale *et al.* 2013; Tsang *et al.* 2014). The effectiveness of the fungal – bacterial inoculation on the reduction of Ni concentration may be explained on the basis of that microbes mobilize the heavy metals from the contaminated sites by leaching, chelation, methylation and redox transformation of toxic metals. Heavy metals can never be destroyed completely, but the process transforms their oxidation state or organic complex, so that they become water-soluble, less toxic and precipitated (Garbisu & Alkorta 2001). The positive decline of Ni in mycorrhizal plants of peas and radish may be due to the interaction effects between compost and AM fungi. Many research studies reported about the effect of AMF in improving plant tolerance to Ni stress which explained by decreasing metal accumulation and translocation to shoots (e.g., Vivas *et al.* 2006; Amir *et al.* 2013). As well as, Hildebrandt *et al.* (2007) suggested that AMF could filter out toxic metals by accumulating them in their mycelia. Two-way ANOVA revealed that the factor "compost" and "inoculation" had an extremely significant ($p < 0.001$) effect on Ni concentration for all organs of peas and radish. Although, the interaction of "compost × inoculation" factor had an extremely significant ($p < 0.001$) effect on Ni concentration for all organs of peas and radish but affected non-significantly on Ni content in the shoot of radish.

Transfer factor (TF) from soil to plants

Soil to plant transfer of heavy metals is the major pathway of human exposure to metal contamination (Jolly *et al.* 2013). Results in Figure 5A, B illustrate the transfer factor (TF) of Ni from polluted soil to pea and radish plants. Results observed that the TF values of different treatments varied from 0.067 to 0.152 for Ni by pea plants while varied from 0.089 to 0.148 for Ni by radish plants. The data showed that TF decreased significantly ($p < 0.001$) by increasing compost rates and combination between compost and inoculation. The minimum TF of Ni (0.067 and 0.089) was attained as a result of applying CS3 inoculated with AMF and its residual effect as a soil remediate polluted soil to peas and radish respectively. The seed vegetable (peas) is found to show a lower transfer factor for Ni than in root vegetable (radish). It was observed that TF for Ni in peas and radish less than 1. This means that these plants are not good bioaccumulators for Ni. The effect of applied compost levels and inoculation on decreasing TF values of Ni for peas and radish plants could be arranged in descending order of: CS+AMF ≥ CS+BF+AMF > CS+BF > CS. Two-way ANOVA indicated that the factor "compost" and "inoculation" extremely significantly ($p < 0.001$) affected on TF of Ni for the two tested plants but the factor interaction of "compost × inoculation" significantly ($p < 0.01$) affected Ni TF for radish plants and non-significant affected of Ni for pea plants.

CONCLUSIONS

The present study suggests that the combined application of compost and native microbes (*Bacillius* sp., *Aspergillus niger, A. terreus*, *Penicillium funiculosum, Fusarium culmorum Acaulospora bireticulata*, *Gigaspora margarita*, *Glomus lamellosum*, *Funneliformis mosseae*) was more effective in decreasing the availability of Ni in polluted soil. Also, our results indicated that compost with inoculation and their residual effects positively decreased Ni concentration in all plant organs compared to compost only and the control one. This could be important bio-resource for efficient bioinoculant development to enhance the tolerance of vegetables plants to heavy metal stress. Our findings indicate that compost and inoculation affected significantly the growth measurements of peas and radish. The current study recommends the use of compost and native microbes to provide a competitive advantage for remediation and pay attention concerning the industrial dusts and air fumes which contain toxic heavy metal to avoid their hazards impacts on environment.

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