J.Soil Sci. and Agric. Eng., Mansoura Univ., Vol. 9 (11): 549–555, 2018

Combining Mycorrhizal Inoculation and Basil Plants (*Ocimum basilicum*) for Remediating A Ni-Polluted Soil

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ABSTRACT

The current study was conducted using basil plants (*Ocimum basilicum*) combined with mycorrhizal inoculation to remediate a Ni-polluted soil. Basil plants were cultivated for 90 days under different levels of artificially Ni-pollution 0, 200, 400, 600, 800 and 1000 mg Ni kg^{-1} soil. Mycorrhiza increased fresh weight by18.27 and 47.80% and dry weight by14.79 and 46.32% for shoots and roots, respectively. Mycorrhiza increased basil potentials in extracting and immobilizing Ni and its uptake by plants increased by 53.43% and 187.59% for shoot and root respectively in presence of mycorrhiza. Mycorrhiza decreased the value of translocation factor (TF). A marked decrease in mycorrhizal infection occurred due to exposure to high level of Ni-pollution.

Keywords: Pollution – Nickel – Remediation –Mycorrhiza – Basil.

INTRODUCTION

Increasing pollution is consequence of rapid increase of population, urbanization and industrialization. Pollutants particularly heavy metals are released into the environment as a result of various anthropogenic activities (Vuckovic*et al.*, 2013). Nickel is one of the widespread heavy metals due to its importance in several industries (Kabata-pendias and Mukherjee, 2007). It is released to the environment during metal production, mining and smelting as well as through disposal of industrial sewage, agricultural drainage waters with their contents of fertilizers, and pesticides into irrigation canals (Kabata-pendias and Mukherjee, 2007; Rathor *et al.*, 2014). Combustion of fuel, diesel oil and coal are other sources (Chen *et al.*, 2009). Bioremediation is an effective strategy for soil decontamination. It is less costly, environment-friendly and easily applied (Lone *et al.*, 2008; Manshadi *et al.*, 2013). Its technology involves using plants or microorganisms to reduce, degrade, extract, stabilize or immobilize toxins of the contaminated soils (Luo, 2009; Jadia and Fulekar, 2009; Behera, 2014). Plants which have function groups such as hydroxyls, carboxyls, carbonyls, sulfhydryls, thioethers, sulfonates, amines, amides, imines, imidiazoles, phosphonates and phosphodiesters, can be used for phyto-remediation due to their tendency to complex with metal ions (Jena and Gupta, 2012). Basil plant (*Ocimum basilicum*) is one of the *Lamiaceae* family plants which have high potentials in phyto-remediation (Sarma, 2011)due to the functional groups they contain, particularly in their above-ground parts (Jena and Gupta, 2012).Using hyper-accumulating plants as host plants in association with symbiotic microorganisms can enhance the efficiency of bioremediation (Gao *et al.*, 2010; Bedini*et al.*, 2010; Upadhyaya *et al.*, 2010; Aranda *et al.*, 2013; Hassan *et al.*, 2013).The *vascular arbuscular mycorrhiza* (VAM) fungus is one of the symbiotic microorganisms which enhances plant potentials forremediating contaminated soils through its acceleration of plant growth, hence obtaining high biomass, which in turn enables removal of high amounts of pollutants (Sharma *et al.*, 2007) .It can increase plant tolerance against drought, salinity plant resistance to diseases and pathogens (Upadhyaya *et al.*, 2010; Ismail *et al.*, 2013; Iffis*et al.*, 2014).

The current study is aimed at assessing the efficiency of using basil plant combined with mycorrhiza fungi as a bio-remediation technique for Ni-polluted soils.

MATERIALS AND METHODS

A pot experiment was conducted to study the contribution of bio and phyto-remediation in a Ni-polluted soil. Basil plants (*Ocimum basilicum*) in combination with mycorrhiza (vascular arbuscular mycorrhiza "VAM") were grown on an artificially Ni-polluted soil to assess Ni removal from the soil. The experimental design was a factorial randomized complete block, in 3 replicates. Factors were as follows: (1) Ni addition: 6 treatments of 0, 200, 400, 600, 800 and 1000 mg Ni kg^{-1}soil (Ni_0, Ni_1, Ni_2, Ni_3, Ni_4 and Ni_5 respectively); the element being applied as nickel nitrate [$Ni(NO_3)_2.5H_2O$].(2) Mycorrhizal (VAM) inoculation:2 treatments no inoculation with mycorrhiza and inoculation with mycorrhiza, M_0 and M_1 respectively. Therefore the experiment involved 12 treatments. Considering 3 replicates for each treatment thus the total number of pots was 36. According to Cela and Sumner (2002) maximum allowed concentration for Ni is 150 mg Ni kg^{-1} soil.

The soil:

Soil of the experiment was taken from the 0–15-cm upper layer of an arable field Marg, Qalubiya governorate, Egypt. The soil was air dried, crushed and sieved through 2 mm sieve, then packed in PVC pots of 3 kg. Main properties of the soil were determined (Gupta, 2009), shown in Table 1. Immediately after polluting the soils with Ni, they were left for 48 hours before transplanting basil plants (*Ocimum basilicum*).

Table 1. Chemical and physical properties of the studied soil.

Soil property	Particle size distribution			Texture class	CEC ($cmol_C kg^{-1}$)	EC ($dS m^{-1}$)	pH	OM ($g kg^{-1}$)	$CaCO_3$ ($g kg^{-1}$)	Total content of Ni ($mg kg^{-1}$) [1]	Extractable Ni ($mg kg^{-1}$)[2]
	% Sand	% Silt	% Clay								
Value	58.35	17.12	24.53	Clay loam	21.42	0.8	7.2	9.33	11.26	7.64	nd[3]

1: Soil was digested according to Grimshaw, (1987).
2: DTPA extract according to Lindsay and Norvell (1978)
The atomic absorption spectrophotometer 210VGP was used for Ni measurement.
3: not detected.

Procedures of cultivation and Experimentation:

Seedlings of Basil *(Ocimum basilicum)* were soaked in VAM suspension for half an hour then transferred to soils in pots. Watering was done using tap water (no Ni was detected) as required, nutrient solution was prepared according to Douglas (1985). Plants were supplied with nutrients through foliar spray every two weeks. After 90 days of growth, plants were removed for analysis. They were rinsed with distilled water, separated into shoots and roots, dried at 70 ºC for 24 h and milled. For nickel determination samples were digested using mixture of concentrated H_2SO_4 and $HClO_4$ (Chapman and Pratt, 1961). Translocation factor (TF) represented as the ratio of Ni content in above-ground plant parts to Ni content in roots. It assesses the efficiency of Ni distribution through plant and its migration from roots to shoots (Bu-Olayan and Thomas, 2009; Radulescu *et al.*, 2013).

RESULTS AND DISCUSSION

Mycorrhizal infection (colonization):

Ni treatment decreased VAM infection and the increase in Ni concentration was associated with a progressive decrease in mycorrhizal infection (Figure 1). The highest infection occurred where no Ni (Ni_0) while the lowest one occurred in roots of plants given the highest Ni dose (Ni_5). Such decrease reflects the harmful effect of pollution to microorganisms, particularly VAM (Jamal *et al*., 2002; Shaker-Koohi, 2014). Other factors relating adverse changes in soil properties may be implicated (Giasson *et al.*, 2008).

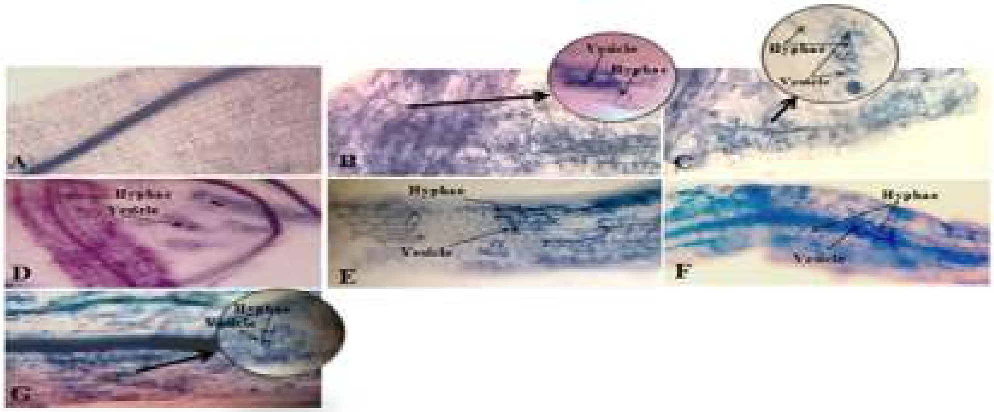

Image	Treatment	%Root Infection
A	Root without mycorrhizal inoculation or Ni-pollution (M_0 Ni_0)	0
B	Mycorrhizal inoculated root, without Ni-pollution (M_1 Ni_0)	92
C	Mycorrhizal inoculated root, Ni-pollution of 200mg kg^{-1} soil (M_1 Ni_1)	86
D	Mycorrhizal inoculated root, Ni-pollution of 400mg kg^{-1} soil (M_1 Ni_2)	82
E	Mycorrhizal inoculated root, Ni-pollution of 600mg kg^{-1} soil (M_1 Ni_3)	73
F	Mycorrhizal inoculated root, Ni-pollution of 800mg kg^{-1} soil (M_1 Ni_4)	52
G	Mycorrhizal inoculated root, Ni-pollution of 1000mg kg^{-1} soil (M_1 Ni_5)	40

Figure 1. Mycorrhizal infection in roots of Basil (*Ocimum basilicum*) at end of experiment.

Weight of plant roots and shoots:

Mycorrhizal inoculation (VAM) caused increases in the weights of shoots averaging 18.27% and 14.79%, for fresh and dry shoots respectively; and 47.80% and 46.32% for fresh and dry roots respectively as shown in Figure 2. These increases reflect the positive effect of VAM in enhancing plant growth, increasing its fresh weight, dry weight, availability of plant nutrients and the effective surface absorption area of roots (Sharma *et al.*, 2007; Motha *et al.,* 2015). Ker and Charest (2010) cultivated sunflower with inoculation of mycorrhiza in a soil treated with different rates of Ni and found that mycorrhiza inoculation significantly enhanced plant growth.

Concerning the weight of roots, the lowest weights were 8.96 and 2.11 g pot^{-1} fresh and dry weight respectively due to non-treated M_0Ni_0 (Figure 4). The treatments caused increases of up to 200.1% and 210.4% respectively due to M_1Ni_3. The lowest weights of fresh and dry shoots were 31.76 and 7.55 g pot^{-1} respectively caused by the non-treated M_0Ni_0 soil, they increased by up to 108.2% and 64.9% respectively due to M_1Ni_5.

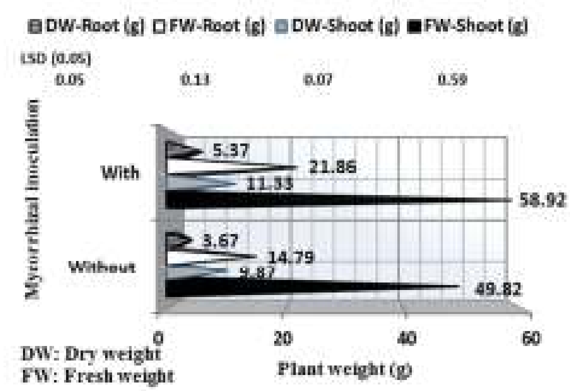

Figure 2. The main effect of mycorrhiza inoculation on fresh and dry weights of basil plants (g)

Ni pollution increased weight of plant, and the increase progressed with the rate of Ni (Figure 3). Average increases for fresh roots weight were 31.50, 60.99, 89.52, 85.95 and 91.53% for Ni_1, Ni_2, Ni_3, Ni_4 and Ni_5 respectively. Respective average increases for dry roots were 37.03, 66.29, 100.37, 95.56 and 104.07%. Ni pollution had the same trend with shoots (fresh and dry weights). Average increases for fresh shoots weight were26.40, 34.14, 52.33, 58.76 and 63.96%for Ni_1, Ni_2, Ni_3, Ni_4 and Ni_5 respectively. Respective average increases for dry shoots were16.48, 24.91, 33.57, 38.67 and 41.04%. Increasing plant weight due to increasing pollution levels is mainly a manifestation of increased presence of available N nutrient given in the Ni nitrate salt. The N element is essential in forming amino-acids, nucleic acids, chlorophyll and growth hormones (Leghari *et al.,* 2016), as well in promoting uptake of other nutrients (Bloom, 2015) and growth parameters (Rafiq *et al.,* 2010).

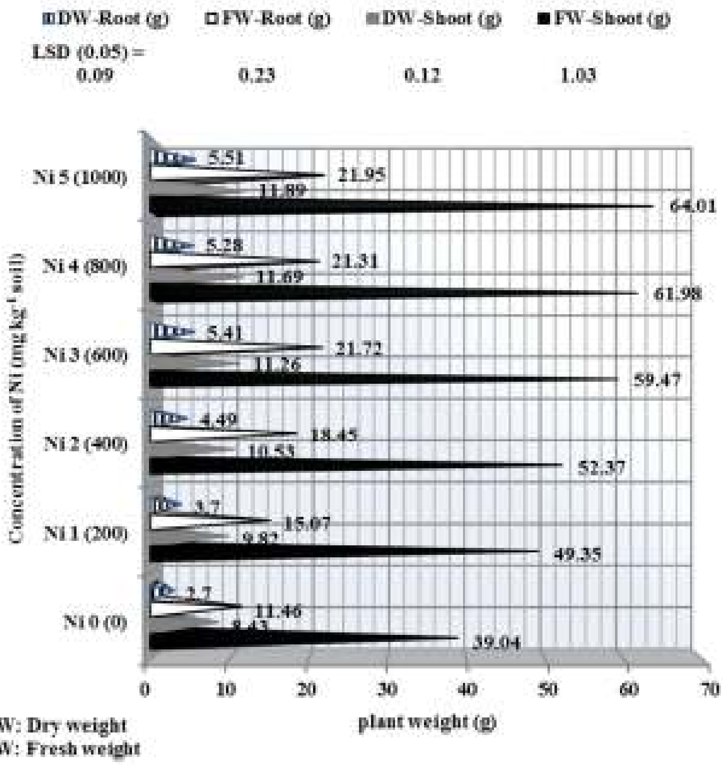

Figure 3. The main effect of applied nickel (Ni) on fresh and dry weights of basil plants (g)

There was an interaction effect caused by mycorrhizal inoculation affecting the response to Ni pollution (Figure 4). Inoculation with mycorrhizal surpassed the no inoculation treatment under different rates of Ni pollution in weights of both shoots and roots (fresh and dry). The increases in fresh weight of shoots were 45.84%, 28.68%, 16.18%, 15.62%, 11.93% and 6.85% due to Ni_0, Ni_1, Ni_2, Ni_3, Ni_4 and Ni_5 respectively. Respective increases for dry weight of shoots were 23.31%, 16.77%, 16.44%, 13.77%, 11.49% and 9.98% following a rather similar pattern of shoot fresh weight. There was no significant difference between M_1Ni_4 and M_1Ni_5 regarding shoots fresh and dry weighs, indicating that under mycorrhizal inoculation, increased Ni pollution caused no significant change in plant growth.

The marked effect of mycorrhizal inoculation under high pollution on fresh and dry weights of shoot could be attributed to the decreased infection of root by mycorrhizal (as shown in Figure 1) with increasing soil Ni pollution levels (Shaker-Koohi, 2014). Under mycorrhizal inoculation there was no significant difference between Ni_2 and Ni_3 in fresh weight of shoots. As for roots weight they had the same trend as shoots. The mycorrhizal inoculation gave increases in fresh weight of roots of 55.69%, 46.24%, 61.70%, 62.57%, 37.11% and 31.71%due to Ni_0, Ni_1, Ni_2, Ni_3, Ni_4 and Ni_5 respectively. Respective increases for dry weight of roots were 55.45%, 47.0%, 68.05%, 53.03%, 38.91% and 28.42%. In presence of VAM the decrease of fresh and dry weights of roots which occurred upon increasing Ni_3 to Ni_4 and Ni_5 was marked, also there was no significant difference between Ni_4 and Ni_5 (the same trend as in fresh and dry weights of shoots). The positive effect of VAM, particularly under no Ni or low Ni reflects its enhancement in utilization of nutrients, particularly NO_3-N added with Ni as nickel nitrate (Govindarajulu*et al.,* 2005) and tendency to promote root growth (Govindarajulu*et al.,* 2005 and Leghari *et al.,* 2016). Lack of favorable response to mycorrhizal inoculation under high Ni pollution could be attributed to a decrease in its infection (Figure 1) with increased soil pollution with the heavy metals (Govindarajulu *et al.,*2005, Koohi, 2014 and Leghari *et al.,* 2016).

Ni-Uptake:

The effect of using mycorrhizal inoculation on Ni-uptake by plants is shown in Figure 5. Inoculating plant roots with VAM enhanced the uptake of Ni by 51.11% and 187.59% in shoot and root respectively.

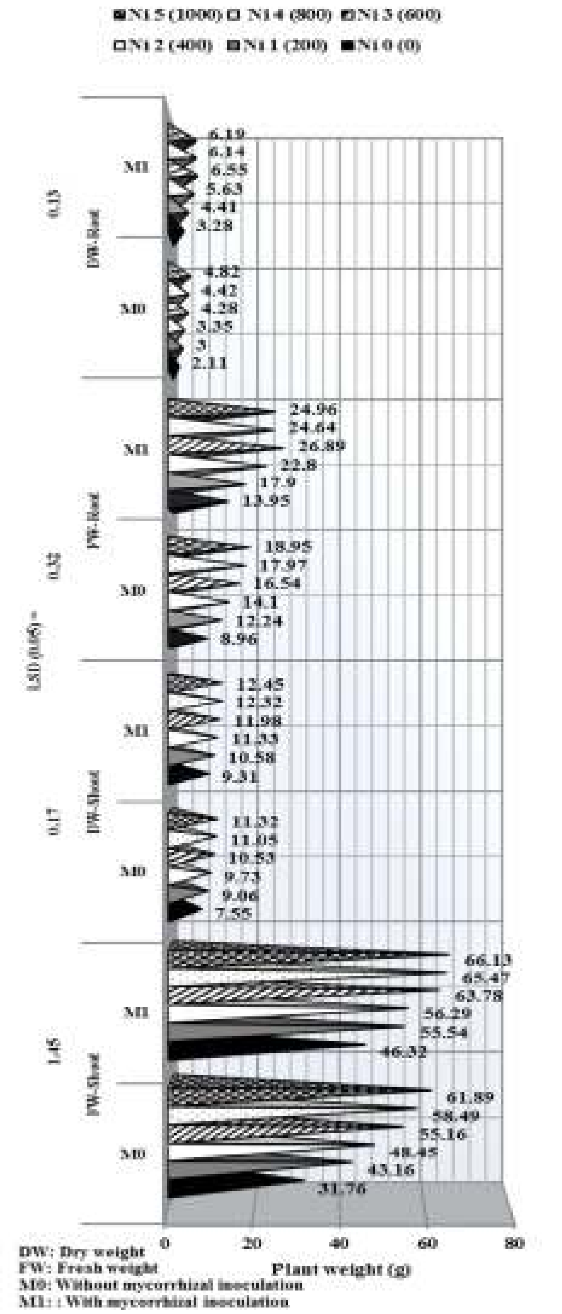

Figure 4. The effect of mycorrhizal inocultaion and applied nickel on the fresh and dry weights of basil plants (g)

VAM has the ability to absorb and phyto-accumulate heavy metals such as Cu, Ni, Pb, and Zn (Jamal *et al.,* 2002; Al-Agely *et al.*, 2005), thus increasing the uptake by plant for such elements. Mycorrhiza can bind with metals and decrease their bioavailbility (Joner*et et al.,* 2000 and Audet and Charest, 2007). Pichardo *et al.* (2012) reported that increased uptake and translocation of heavy metals by plants is caused by inoculation of VAM, and the magnitude depends on many factors including the species of fungus, the plant, the soil and concerned element. This study shows that Ni-uptake in roots was higher than in shoots, most probably because of retention of the metal inside mycorrhizal since VAM can bind and fix heavy metals in the mycelium and cell walls (Christie *et al.* 2004). There may have been a chelating of the metal by some functional groups such as free amino, hydroxyl and carboxyl present in the soil and root system (Gadd, 1993; Shaker-Koohi, 2014). Another cause for the higher uptake of Ni in roots than shoots may be storing of the element in vacuoles of the root tissue or complexing it with amino acids or proteins (protein-complex, metallo-thionein as phytochelatins) in the cytoplasm (Pal and Rai, 2009; Upadhyaya *et al.*, 2010). Hence, mycorrhiza can play an important role in mitigating or preventing mobility of toxic metals (Galli *et al.,*1994). Ker and Charest (2010) treated sunflower with mycorrhiza using an artificially polluted soil with different rates of Ni and found that mycorrhiza significantly increased Ni uptake by plant.

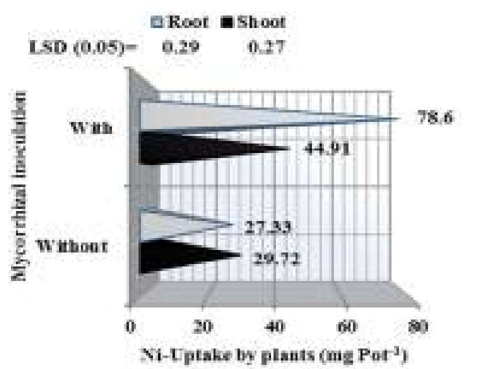

Figure 5. The main effect of mycorrhiza inoculation on the Ni-uptake by basil plants (mg Pot^{-1})

Increasing pollution with Ni had increased its uptake by plant as demonstrated in Figure 6. The increased uptake associated with increased pollution with Ni is a direct outcome of increased plant growth (Figures 2 to 4). There was no Ni uptake in treatments not polluted with Ni. Among the Ni-addition treatments Ni_1 gave the lowest Ni-uptake of 23.14 mg pot^{-1} in shoots and25.58 mg pot^{-1} in roots. The highest Ni-uptake of 60.25 mg $pot^{-1}$by shoots, and87.07mg $pot^{-1}$by roots, were obtained byNi_5dose of Ni pollution. Sadiq (1985) contaminated 16 calcareous soils with heavy metals and cultivated with maize (*Zea mays*), and found that uptake of heavy metals was in the following descending order: Cd>Pb>Ni. Ker and Charest (2010) studied the effect of adding different rates of Ni (as $NiCl_2.6H_2O$) to a soil; the rates were 0, 100, 200 and 400 mg Ni kg^{-1}; soils were cultivated with sunflower in presence and absence of mycorrhiza. Ni uptake by all parts of the plant increased with increasing the rate of Ni added to soil, the highest rate of Ni added to the soil (400 mg Ni kg^{-1} soil) showed the highest Ni uptake by plant.

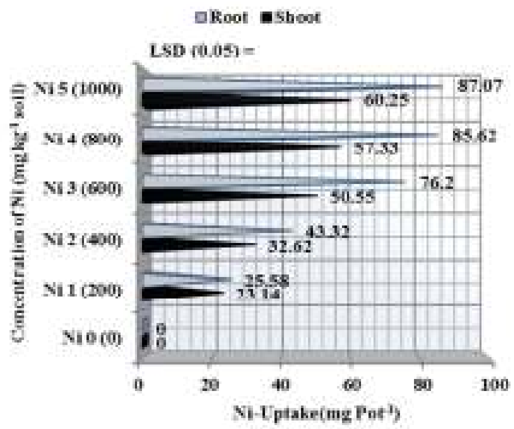

Figure 6. The main effect of applied nickel (Ni) on the Ni-uptake by basil plants (mg Pot^{-1})

There was an interaction effect caused by mycorrhizal inoculation affecting the response to Ni pollution (Figure 7). Ni-uptake by plants increased significantly due to mycorrhizal inoculation. In presence of mycorrhizal inoculation, Ni-uptake by shoots recorded increases of 74.7%, 89.0%, 72.3%, 48.0% and 16.8%, under Ni_1, Ni_2, Ni_3, Ni_4 and Ni_5 respectively. Comparable increases in roots were236.9%, 306.7%, 245.5%, 194.9%, and 102.2%respectively. The highest Ni-uptake by shoot is 68.44 mg pot^{-1} due to M_1Ni_4 treatment followed by a significant decrease 64.94mg pot^{-1} due to M_1Ni_5 treatment. The highest Ni-uptake by root is 127.87mg pot^{-1} due to M_1Ni_4 followed by a significant decrease 116.53mg pot^{1} due to M_1Ni_5. A decreased VAM infection must have had occurred causing such a decrease (Figure 1). Panwar *et al.* (2002) cultivated two species of Indian mustard in a soil contaminated with different rates of Ni and found that Ni uptake in both plants (all parts of them) increased with increasing Ni applied to soil.

Effect of treatments on the transfer factor (TF):

Results in Figure 8 show the effect of mycorrhizal inoculation and Ni pollution on the translocation factor (TF) which assesses the mobility of the metal within the plant (Radulescu *et al.*, 2013; Bu-Olayan and Thomas, 2009). If the TF value exceeds 1 , this indicates that the plant is a perfect choice in phyto-extraction accumulating more in its shoots than in its roots , thus enabling the removal (or decreasing the contents) of the pollutant from the rhizosphere system and the soil (Yoon *et al.*, 2006). The TF values obtained in the current study are less than 1 indicating that the basil plants could be a very suitable choice in phyto-immobilization or phyto-stabilization (Nirola *et al.*, 2015). There were relatively lower TF values in treatments having VAM than in those inoculated without VAM. This reflects mycorrhiza ability to immobilize and fix the element (Shaker-Koohi, 2014).

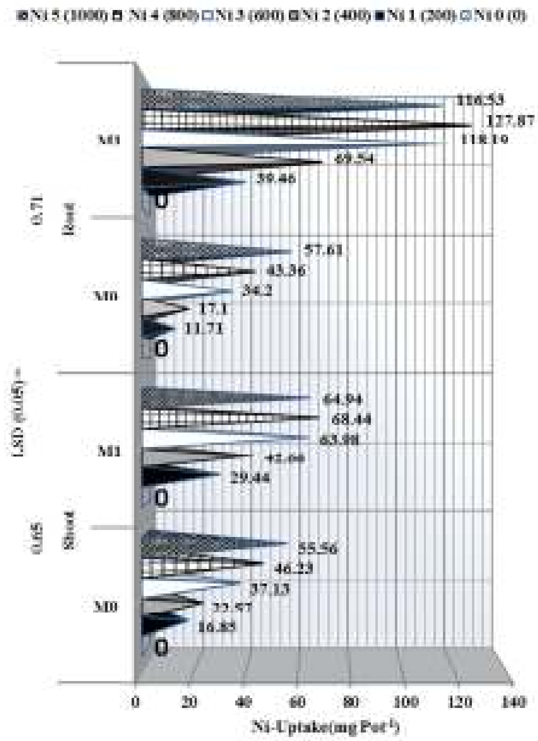

Figure 7. The effect of mycorrhizal inocultaion and applied nickel on the Ni-uptake by basil plants (mg Pot^{-1})

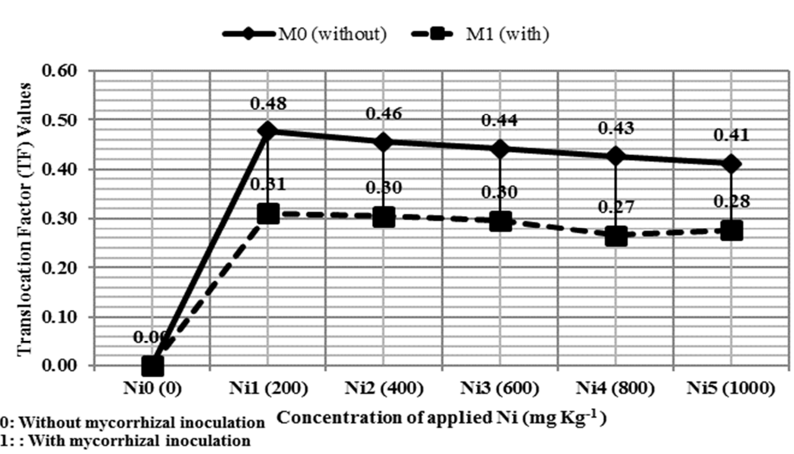

Figure 8. The effect of mycorrhizal inoculation and applied Ni on the values of transfer factor (TF)

CONCLUSION

Mycorrhizal inoculation had a considerable effect on raising the ability of basil plants to absorb and stabilize Ni in the polluted soil through various routes such as increasing plant growth, plant weights and surface area of absorption by roots. Increasing the accumulation and uptake of Ni, particularly by plant roots, and mitigating its mobility to shoots can occur. Despite the mycorrhizal efficiency in remediating Ni-polluted soils, there was a marked decrease in mycorrhizal infection due to the exposure to high level of Ni-pollution.

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دمج التلقيح بالميكروريزا مع نبات الريحان (*Ocimum basilicum*) لمعالجة ارض ملوثة بالنيكل

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تم استخدام نبات الريحان (*Ocimum basilicum*) بالتكافل مع التلقيح الميكوريزي فى معالجه تربة ملوثه بالنيكل, زرع النبات لمده 90 يوم تحت مستويات مختلفه من التلوث الصناعى بالنيكل 0 , 200 , 400 , 600 , 800 و 1000 ملليجرام Ni كجم$^{-1}$ تربه. قدرت أوزان النباتات و الممتص من النيكل فى وجود و غياب التلقيح الميكوريزى و حدثت زيادة في الاوزان الطازجه للنباتات تحت ظروف التلقيح بالميكوريزا بمعدل 18.27% , 47.80 % كذلك زياده فى الاوزان الجافه بمعدل 14.79% , 46.32% لكل من المجموع الخضرى , المجموع الجذرى على التوالى. أحدث التلقيح الميكوريزى زياده في قدرة النبات على استخلاص و تقييد عنصر النيكل حيث أن الكميه الممتصه من عنصر النيكل زادت بمقدار 53.43% , 187.59% للمجموع الخضرى و الجذرى على التوالي بسبب التلقيح الميكوريزى والذي أدى الى نقص فى قيم معامل الانتقال (TF). لوحظ انخفاض في الإصابة بالميكوريزا عند التلوث العالى من النيكل.