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EFFECT OF AMF ON LEAD CONTAMINATED SOIL PHYTOREMEDIATION

Abdullah A. Thannoon* and Mehjin M.A. AL-Ani

Department of Environmental Technology, College of Environmental Sciences and Technology, Mosul University, Iraq.

Abstract

Under greenhouse conditions the factorial experiment was carried out, to assess the influence of Arbuscular Mycorrhizal Fungi (AMF) on lead contaminated soil phytoremediation by Maize plants. Experiment factors were inoculated with (AMF) in two levels inoculated with *Glomus mosseae* or non-inoculated, the second factor lead soil contamination in three levels (0, 1000, 2000 mg kg^{-1} Pb)). The results show that there were no mycorrhizal infection and spores noticed in roots of non-inoculated plants. Contamination with Pb significantly decreased dry weight of plant parts above and below ground, AMF infection percentage and number of spores under all levels of Pb. The contamination decreased infection percentage by 18% and 24% in contaminated soil with 1000, 2000 mg kg^{-1} Pb respectively. AMF significantly increased shoots, roots dry matter, Pb concentration in shoots and roots, Pb uptake in roots and shoots under all Pb levels. AMF increased shoot dry matter by 67%, 62% and 82% in plants grow in contaminated soil with 0, 1000, 2000 mg kg^{-1} Pb respectively. AMF increased Pb uptake in shoots by 160%, 110% and 160% in plants grow in contaminated soil with 0, 1000, 2000 mg kg^{-1} Pb respectively. Our results show that maize plants can survive and withstand under high Pb contamination condition and AMF can protected maize plants from Pb toxicity and enhance Pb uptake and phytoremediation efficiency.

Key words : AMF, contamination, soil, phytoremediation, lead.

Introduction

One of the very important environmental concern at the level of the world is heavy metal soil contamination by human activity (Ripley *et al.,* 1996). Heavy metals can get in to the human body via mouth with food and also likely via the skin if they get in touch. Some of these minerals are essential micronutrients for plant growth like Zn, Mn, Fe, Ni, and Co Cu while other heavy elements have no biological function like Cd, Pb, Hg (Marschner and Romheld, 1994). Heavy metals can remain in the soil for a long time and causing human health problems and animals, in addition, some of the heavy decrease plant development and productivity (Adriano, 1986). Lead consider one of the greatest prevalent heavy metal pollution in the soil in the world (Lambert *et al.,* 1997). Leaded gasoline is the major source of Pb soil pollution worldwide (Epstein *et al.,* 1999; McGrath *et al.,* 2001; Hovsepyan and Greipsson, 2004). Lead solubility and bioavailability are very low in the soil. The lack of lead solubility may be due to the complexes that it forms with organic matter or it adsorb on clay minerals, or on the surface of oxides, or deposited in the form of phosphates or carbonates. The two main determinants of the phytoremediation of lead-polluted soils are the lack of bioavailability in the soil and the lack of transmission from the root to the stem (Oseni *et al.,*2018). Remediation methods for heavy metals contaminated soil traditionally focus in engineering methods (Cunningham *et al.,* 1997) which are expensive and not environmentally friendly (Kertulis-Tatar *et al.,* 2006). Recently, a higher efficiency technique called phytoremediation, that utilize plants to remove or reduce contaminant in the soil, has been used. This technique has been given great scientific and economic importance (Meagher *et al.,* 2000; Diets and Schnoor, 2001; Guerinot and Salt, 2001). Phytoremediation have several advantages over traditional methods. It is a more effective, cost-effective and environmentally

***Author for correspondence :** E-mail: abdenv2012@gmail.com

friendly method. The only drawback in this method is that it is slow and takes a long time to remove all pollutants (Laghlimi *et al.*,2015). The challenge now for botanists is to improve the plant's efficiency to remove toxic pollutants from the soil (McGrath and Zhao, 2003). Arbuscular Mycorrhiza (AM) is symbiotic relationship between plant roots and fungi, where the fungus occupies the cortex cells of their host plant roots and its external mycelium works as an extension of the root of the host. These fungi can infect 80% to 90% of plants) (Li *et al.,* 2014; Wehner *et al.,* 2010). Arbuscular Mycorrhiza Fungi (AMF) increased the absorption area of the roots to 47 times (Douds and Millner, 1999; Smith and Read, 1997). AMF developed host plant growth by increasing nutrients absorption (Wu *et al.*, 2011). AMF increased host tolerance for stress such as contamination soil with heavy metals, dryness and Plant diseases (Li *et al.,* 2014; Wehner *et al.,* 2010; Huang *et al.*, 2005(. (Akay and Karaarslan, 2011) found that AMF play a vital function in take off the toxicity of heavy metals from the soil and enhance plant growth in highly contaminated soils with heavy minerals. Therefore, (Khan *et al.,* 2014) thought it is possible for AMF to play a role in increasing the efficiency of heavy metals contaminated soil phytoremediation. The goal of this study is to investigate the effect of AMF on phytoremediation of lead contaminated soil

Materials and Methods

Experiment Design

Under greenhouse conditions the factorial experiment was carried out, to assess the impact of AMF on lead contaminated soil phytoremediation by Maize plants. The experiment was carried out in Completely Randomize Design (CRD) with three replications. Experiment's factors were Inoculation with two levels. Inoculate or non-inoculate with AMF *Glomus mosseae*. Pb contamination with three levels 0, 1000, 2000 mg kg^{-1} Pb as $Pb(NO_3)_{2..}$ Experiment treatments were

T_1: -AMF + 0 mg kg^{-1} Pb(control)

T_2: -AMF+ 1000 mg kg^{-1} Pb

T_3: -AMF+ 2000mg kg^{-1} Pb

$T_{4:}$ + AMF + 0mg kg^{-1} Pb

$T_{5:}$ + AMF + 1000 mg kg^{-1} Pb

$T_{6:}$ + AMF + + 2000mg kg^{-1} Pb

Soil preparation

The soil was collected from service layer (0-20cm) from Alrashedia area 5 km north of Mosul city (north of Iraq). The soil was passed through 2mm sieve. The physical and chemical characteristics of the soil were determined According to (Black *et al.,* 1965), Some of soil physical and chemical properties were recorded in (Table 1). The sieved soil was autoclaved in (121 C for 1 hour). The pots were filled with 3 kg sterilized soil. Adequate amount of Pb $(NO_3)_2$ aqueous solution was added according to treatments to obtain 0, 1000, 2000 mg kg^{-1} Pb concentration. Maize (*Zea mays*) seeds were service sterilized by 5% H_2O_2 for 8 minutes, and then washed five times with water. eight seeds were planted in each pot which were thinned to four and the moisture adjusted to field capacity. 50 gm of *Glomus mosseae* inoculum (spores +infected roots +soil) was put under seeds at planting. After 12 weeks from planting the plants were harvested above surface of soil. The roots samples were washed in distilled water After harvest

Inoculum preparation

The inoculum of AMF fungus *Glomus mosseae* was brought from Department of soil Sciences, University of Tikrit. The inoculum was propagated in autoclaved soil by pot culture utilizing maize plant as host plant for four months

Measurements

The root and shoots were detached and dried for 24 hrs. at 70°C. dry shoots and roots were weighed. Plants part were digested by nitro-perchloric. The lead concentration in plant parts were estimated by inductively coupled plasma atomic emission spectrometry (Mikanova *et al.*, 2001). Lead uptake was estimated for each pot as the amount of lead content of shoots and roots for 4 plants (Pb concentration of plant part x plant part dry weight). The infection rate of AM fungus was assessed by staining according to (Phillips and Hayman, 1970) and estimating infection percentage visually. Arbuscular mycorrhizal fungal spores were isolated from soil by wet sieving technique described by (Gerdemann and Nicolson, 1963).

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) and followed by Duncan's multiple range test to compare between means at $p < 0.05$.

Results and Discussion

AMF colonized and spores Number

There is no mycorrhizal infection and spores detected in roots of non-inoculated plants. Results in Fig. 1, 2 show that Pb contamination decreased of AMF infection and number of spores in all levels of Pb. The addition of Pb decreased infection percentage by 15% and 25% in plant grow in contaminated soil with 1000 and 2000 mg kg^{-1}Pb

Table 1: Chemical and Physical Characteristic of soil.

pH	7.4
EC (µSiemens / cm)	840
Ca (mg.Kg^{-1})	101.6
Mg (mg.Kg^{-1})	12.75
K (mg.Kg^{-1})	24.18
Na (mg.Kg^{-1})	59.8
Available P (mg.Kg^{-1})	2
Sand %	5
Silt %	3
Clay %	20
Texture	Loamy

respectively and decreased spores' number by 14% and 26% respectively. Our results are consistent with various studies (Amanifar *et al.*, 2014; Vogel-Mikus *et al.*, 2006; Wang, 1990). The negative influence of heavy metals on infection rate due to negative influence of heavy metals on AMF spore germination and on hypha growth (Vogel-Mikus *et al.*, 2006; Bafeel, 2008). Our results show there is positive relationship between AMF infection rates and numbers of spores, the same relationships were noticed by (Khan *et al.*, 2014).

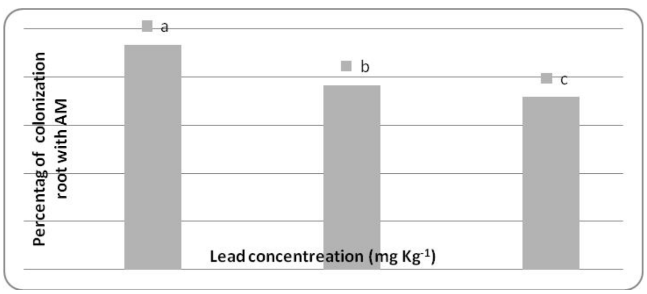

Fig. 1: Effect of lead concentration on maize root infection b colonization by AMF.

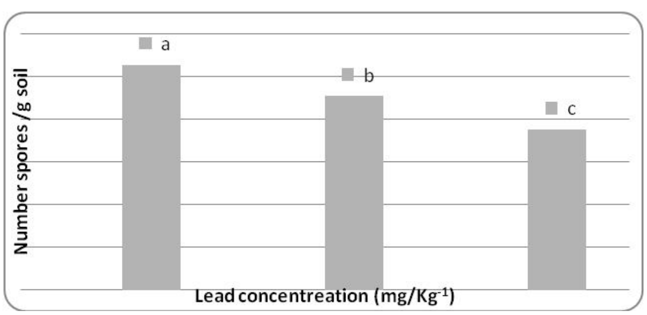

Fig. 2: Effect of lead concentration on the number of AMF spores in soil.

Dry Matter of Shoots and Roots

Results in Fig. 3, 4 show that Pb contamination decreased shoots and roots dry matter. And there is negative relationship between Pb concentration and shoots and roots dry maters. The addition of Pb decreased shoots dry matters by 30%, 63% in non- inoculated plants that grow in soil contaminated with 1000, 2000 mg kg^{-1} respectively while in inoculated plants the reduction was 32% and 56% respectively. The addition of Pb decreased roots dry matters by 26%, 58% in non- inoculated plants that grow in soil contaminated with 1000, 2000 mg kg^{-1} respectively while in inoculated plants the reduction was 22% and 53% respectively. these results are consistent with several studies (Oseni *et al.*, 2018; Ghani *et al.*, 2010) who concluded that plant growth decreased with increase Pb concentration in soil. The high concentration of Pb in soil effect on biochemical and metabolism process which associated with normal growth parameters (Miao *et al.*, 2012; Strubinska and Hanaka, 2011; Verma and Dubey, 2003). Heavy metals decreased plant growth as a result of negative influence of heavy metals on root growth (Kopyra and Gwozdz, 2003; Atici *et al.*, 2005). The high concentration of Pb effect on plant physiological process and lead toxicity inhibited enzymes activity and changed hormones states (Sharma and Dubey, 2005). Fig. 3 show AMF increased shoots dry matters in all Pb levels. Inoculation with *G. mosseae* increased shoots dry matters by 67%, 62%, 82% in plants grow in contaminated soil with 0, 1000, 2000 mg kg^{-1}Pb respectively. Fig. 4 show also AMF increased roots dry matter in all Pb levels. Inoculation with *G. mosseae* increased roots dry matter by 52%, 61%, 69% in plants

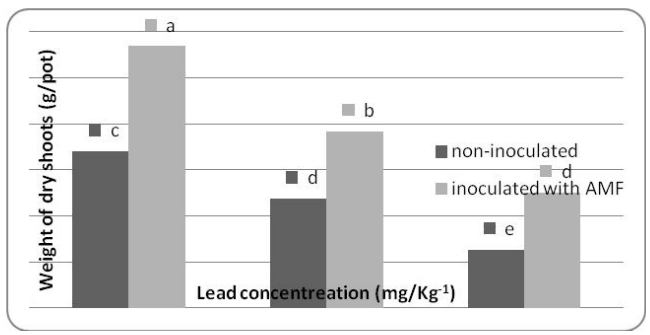

Fig. 3: Effect of AMF and lead concentration on shoots dry matter weight.

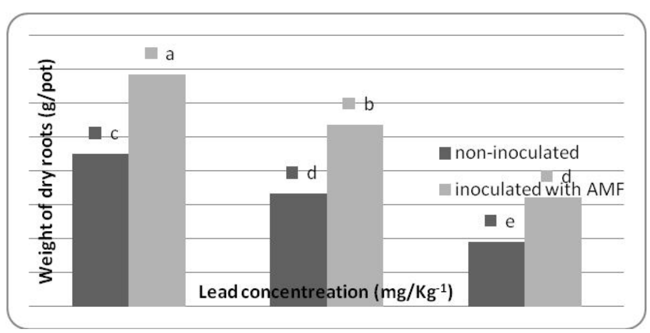

Fig. 4: Effect of AMF and lead concentration on roots dry matter weight.

grow in contaminated soil with 0, 1000, 2000 mg kg^{-1} respectively. Our results show the plants inoculated with AMF that grow in non-contaminated soil gave the higher values of shoots and roots dry matter. While the lower value was in non-inoculated plants that grow in contaminated soil with 2000 mg Kg^1. According to our results we can conclude that *Glomus mosseae* kept host plants from lead toxicity by increased plant growth. Several research groups have reached to similar results. (Bahraminia *et al.*, 2016) found that FAM increased shoots and roots dry matter of vetiver grass plants grow in Pb contaminated soil and concluded that the two mechanisms that protected plant from heavy metals toxicity are hormone production such as cytokinin and increase of water and nutrient uptake. AL-Ani, 2016 found that inoculation with AMF increased the shoots and roots dry matters for maize plants grow in zinc contaminated soil and suggested that AMF protect corn plants from zinc toxicity and enhance phytoremediation by dilution zinc concentration in plant and accumulate of zinc in roots. (Leung *et al.*, 2013) found AMF enhance growth of their host plants which grow in heavy metals contaminated soil and protect plants from heavy metals toxicity by its efficiency to extract nutrients and heavy metals and also it enhances phytoextraction and phytostabilization.

Lead concentration and uptake

Results in Fig. 5 and Fig. 6 show that Pb contamination increased Pb concentration in above and below ground plant parts of maize in both inoculated and non-inoculated with AMF and under all Pb levels. AMF increased Pb concentration in shoots by 46%, 33% and 31% in plants grow in soil contaminated with 0, 1000, 2000 mg kg^{-1}Pbrespectly. while, AMF increased Pb concentration in roots by 79%, 80% and 51% respectively. The inoculated plants with AMF that grow in contaminated soil with 2000 mg kg^{-1}had the higher Pb concentration in shoots and roots. While the non-inoculated plants that grow in non-contaminated soil had the lower value of Pb concentration in shoots and roots. Fig. 7 and 8 show that Pb contamination increased Pb uptake in shoots and roots in both inoculated and non-inoculated plants with AMF under all Pb levels. The inoculated plants with AMF and grow in contaminated soil with 2000 mg kg^{-1} had the higher Pb uptake in shoots and roots. While the non-inoculated plants that grow in non-contaminated soil had the lower value of Pb uptake in shoots and roots. AMF increased Pb uptake in shoots by 160%, 110% and 160% in plants grow in soil contaminated with 0, 1000, 2000 mg kg^{-1}respectively. while, AMF increased Pb uptake in roots by 129%, 189% and 192% respectively. Our results obviously show that maize plants can survive and withstand under high Pb contamination condition and AMF protected maize plant from Pb toxicity and increased Pb uptake and phytoremediation efficiency. Several studies found the same results (Davies *et al.*, 2001; Bafeel *et al.*, 2008; Leyval *et al.*, 1997). Inoculation with AMF enhance Pb concentration and uptake in shoots and roots of maize plants and increased translation of Pb from roots to shoots and these increasing due to ability of AMF mycelium extract and uptake the elements from a more soil volume Compared to host plant roots and this characteristic is very imported in phytoremediation of contaminated soil (Bahraminia *et al.*, 2016).(Audet and

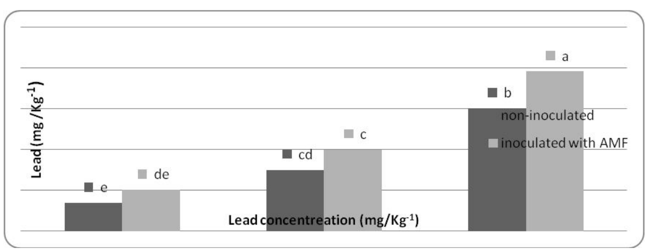

Fig. 5: Effect of AMF and lead concentration on lead concentration in Shoots.

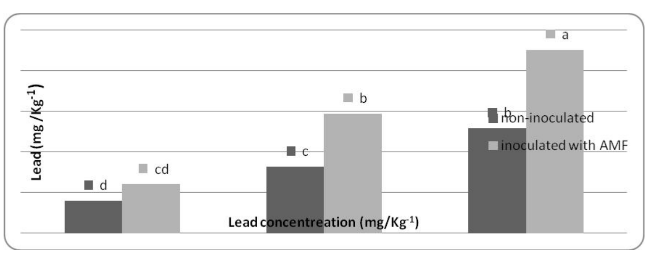

Fig. 6: Effect of AMF and lead concentration on lead concentration in Roots.

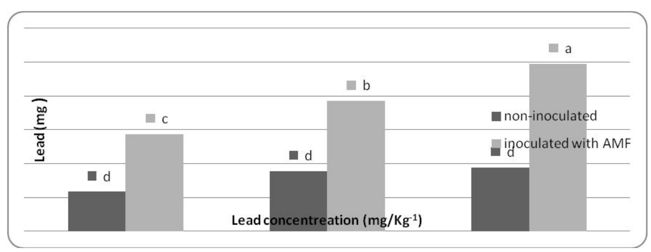

Fig. 7: Effect of AMF and lead concentration on lead uptake in shoots.

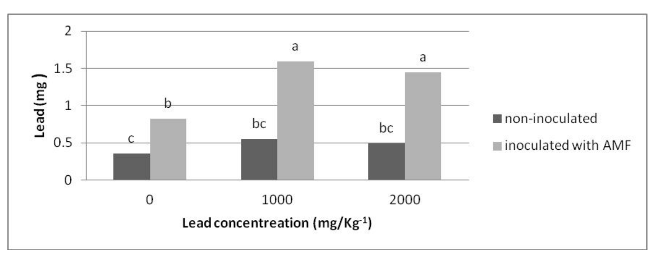

Fig. 8: Effect of AMF and lead concentration on lead uptake in roots.

Charest, 2007) concluded that the symbiotic relationship between AMF and host plant is very imported for Pb uptake from contaminated soil. AMF enhance their hosts to survive and develop host plant growth in heavy metals contaminated soil by increase extract and uptake of nutrients and protect their hosts from heavy metals toxicity by uptake heavy metals and enhance Phytostabilization and phytoextraction (Leung *et al.,* 2013). AMF facilitate their host plants to uptake toxic heavy metals and there are evidence AMF can play role in increasing of plant tolerance for heavy metals toxicity by bind toxic heavy metals with polyphosphate in AMF hyphae (Barea *et al.,* 2005; Morgan *et al.,* 2005).

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