INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 6, p. 162-172, 2014

RESEARCH PAPER OPEN ACCESS

Lead stress differently influence survival and growth of two poplar clones in association with arbuscular mycorrhizal fungi

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Key words: Arbuscular mycorrhizae, lead, phytoremediation, poplar, soil pollution.

http://dx.doi.org/10.12692/ijb/5.6.162-172 Article published on September 20, 2014

Abstract

A greenhouse experiment was accomplished to examine whether mycorrhizal colonization, survival, growth and volume production of *Populus nigra* 62/154 and *Populus alba* 44/9 clones were influenced by metal lead (0, 100, 500 and 1000 ppm Pb soil), and whether native arbuscular mycorrhizal fungi (*Glomus mosseae* and *Glomus intraradices*) may improve establishment of poplar clones on Pb contaminated soils. Plant parameters were measured during annual growth cycle, in July and October. In *P. nigra* only stem length (SL) and volume production were significantly reduced by application of 1000 ppm Pb, and these negative effects were not reversed in plants inoculated with mycorrhizal fungi. Significant reductions in all parameters of *P. alba* occurred at 1000 ppm Pb concentration and more or less at 500 ppm Pb. Stem length (SL) and volume production of *P. alba* were improved by fungal treatments, but similar effect was not observed on other parameters. In both poplars, mycorrhizal colonization percentage (M%) of plants inoculated with mycorrhizal fungi was higher than that in non-inoculated plants. Results revealed that although *P. nigra* clone had more lead tolerance than *P. alba* clone in terms of survival, growth and volume production, however both poplars showed acceptable potential for establishing on Pb contaminated soils. Additionally in *P. alba*, fungal inoculation may improve plant growth.

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Introduction

Heavy metals are natural metallic elements with a high specific gravity (> 5 g/cm^3). These metals are ubiquitous, highly persistent and non-biodegradable (Torresday *et al.*, 2005). Various human and natural activities have contaminated ecosystems with heavy metals. Activities such as the natural weathering of rocks, controlled and uncontrolled disposal of wastes, burning of fossil fuels, the use of agricultural fertilizers, herbicides, pesticides, pigments and batteries, mining and smelting may lead to contamination of the ecosystem (Gaur and Adholeya, 2004; Abdullahi *et al.*, 2009). Heavy metals in plants play different functions that can be divided into two main groups: 1: essential (Zn, Cu, Mn, Fe and Ni), which are necessary for metabolic processes; and 2: non-essential (Cd, Pb and Hg). Lead metal is not essential for plant growth and Pb contaminated soils may result damages for plants (McLaughlin, 2001). The sensitivity of plant species to Pb is different (Arriagada *et al.*, 2005). However, for most plants, 100-500 ppm Pb in soil is toxic (Kabata-Pendias, 2004).

Since heavy metal accumulation in soil can affect ecosystem for long periods (Tabari and Salehi, 2009; Bojarczuk and Kieliszewska-Rokicka, 2010), so heavy metal contaminated soils must be subjected to remediation. In recent years, phytoremediation, the use of green plants to remove, degrade or contain xenobiotics located in soil, as a low-cost and environmentally friendly technology has received much attention, when compared to traditional physicale-chemical methods (Susarla *et al.*, 2002; Faison, 2004; Rafati *et al.*, 2011; He *et al.*, 2013). Since, entrance of heavy metals to food chain is dangerous, therefore soils contaminated with heavy metals must be forbidden from agricultural projects and exposed to remediation processes such as afforestation (Bojarczuk and Kieliszewska-Rokicka, 2010). Among tree species, poplars and willows are good candidates for heavy metal phytoremediation due to fast growth, tolerance to toxic xenobiotics and also the capacity to accumulate microelements (Pulford and Dickinson, 2005).

The plant efficiency in nutrient uptake, resistance against heavy metals and thereby growth can be improved by arbuscular mycorrhizal fungi (AMF) (Rivera-Becerril *et al.*, 2002; Kabata-Pendias, 2004). Under natural conditions the roots of most plant species have symbiosis with AMF that are important component of the soil microbial biomass (Brundrett *et al.*, 1996; Smith and Read, 1997). It has been reported that colonization by AMF is prevalent in young poplars (Khasa *et al.*, 2002).

In spite of the fact that poplars have been reported as suitable candidates among tree species for phytoremediation process (Di Baccio *et al.*, 2003), little is recognized about the potential of tolerance and establishment of poplars commonly used in Iranian poplar plantations and their mycorrhizas on heavy metal contaminated soils. Since, lead and its compounds have been often reported as common and major contaminants, and also with bioaccumulation potential (Cheremisinoff and Habib, 1982), we considered it in this study.

In phytoremediation process, tolerance and successful establishment of plants on heavy metal-contaminated soil are significant and essential factors for bioremediation of contaminants by plants and/or their associated rhizospheric microorganisms (Zalesny *et al.*, 2005). Since a method to assess the plant tolerance towards heavy metals is to measure plant survival and growth (Hunt, 1978; Borghi *et al.*, 2008), in this study special attentions were paid to: 1) evaluate the potential of tolerance and establishing of two poplar clones on soil contaminated with different concentrations of Pb in terms of survival, growth and volume production for potential use in phytoremediation systems, 2) identify the capacity of AMF for improving tolerance and establishment of host plants to Pb of soil. In reality, this work was conducted as a part of a project on phytoremediation of lead-contaminated soils via *Populus nigra* 62/154 and *Populus alba* 44/9 clones in association with AMF.

Materials and methods

Fungi

Two native arbuscular mycorrhizal fungi (AMF) were selected in this experiment: *Glomus mosseae* (Gerd. & Nicol.) Gerdemann & Trappe and *G. intraradices* (Schenck & Smith). The AM fungi were originally isolated from an agricultural and unpolluted soil. To obtain inoculum, the native AM fungi were propagated on maize (*Zea mays* L.) for 4 months on a sterilized soil (Chellappan *et al.*, 2002). At the same time, the non-mycorrhizal inoculum was provided with the similar sterilized substrate on which maize was grown. Eventually, the AM fungal inoculum was a mixture of root-soil containing mycorrhizal root fragments of *Zea mays*, soil, hyphae and spores. On average, there were 10 and 8 spores of *G. mosseae* and *G. intraradices* per 1 g soil, respectively. A mixture of non-mycorrhizal maize roots and soil without AM fungal propagules were used for the non-mycorrhizal inoculum.

Plants

This experiment was conducted with poplar cuttings. The clones of *P. nigra* 62/154 and *P. alba* 44/9 were provided from the nursery of Research Institute of Forests and Rangelands in Karaj, Iran. These clones commonly used in Iranian poplar plantations. The homogeneously 20-cm-long cuttings of clones were collected in February and kept at 4 ^{o}C until the start of the experiment.

Soil

Since this study was simulation to a field experiment, no sterilized soil was used. So there were naturally propagules of AMF in the soil (Lingua *et al.*, 2008). A part of the soil was supplemented with different concentrations of Pb $(NO_3)_2$ (equal to 100, 500 and 1000 ppm), and the other part was not supplemented. Then, 7-L plastic pots were filled with the prepared soil. Before soil treatment, 3 samples of soil were taken and analyzed for physico-chemical characteristics. The soil texture was sandy loam (according to USDA) as indicated in Table 1.

Pot experiment

The experiment was established in a factorial completely randomized block scheme with two factors 1) plants in four levels (without fungal inoculation, inoculated with *G. mosseae*, inoculated with *G. intraradices* and inoculated with *G. mosseae* + *G. intraradices*) and 2) lead in four levels (0, 100, 500 and 1000 ppm soil). In spring (late March), after removing of the cuttings from cold storage, they were located overnight under running tap water. The cuttings were planted in the center of each pot and the fungal inoculum was posed around each cutting. Each pot received 60 g fungal inoculum including approximately 500 spores (for *G. mosseae* + *G. intraradices* treatment, equal amounts of two fungal inoculum were mixed). Control pots received the same dose of nonmycorrhizal inoculum. The prepared pots were put in a greenhouse under natural light, where temperature was fixed between 15 and 25 ^{o}C. Irrigation of the plants with tap water were done two-three times per week in accordance with the requirements. The experiment per clone was composed of 16 treatments (4 fungal treatments × 4 Pb treatments) in 3 replicates and 4 plants in each replicate. Each plant grew in its own pot.

Measurement of plant parameters

Survival and growth parameters of plants were measured in July and October. Growth was measured on the basis of stem growth (stem length (SL) and stem basal diameter (SD)). Volume production was calculated by the generalized equation $volume = diameter^2 \times height$ (Avery and Burkhart, 1994).

In October, the percentage of root colonization by AMF, on fifty 1-cm-long root segments were taken from the entire root system was assessed by the gridline intersect method (Giovannetti and Mosse, 1980) after clearing and staining of roots using the standard method of Phillips and Hayman (1970).

Statistical analyses

Factorial analysis of variance (ANOVA) was used to statistically analyze the effect of main factors (Pb and AMF treatments) and the interaction between factors. Comparison of means was accomplished by Tukey-HSD test, at $P < 0.05$ as significance level. A t-test (P

< 0.05) was applied to compare differences between two clones. Statistical analyses were performed using SPSS software.

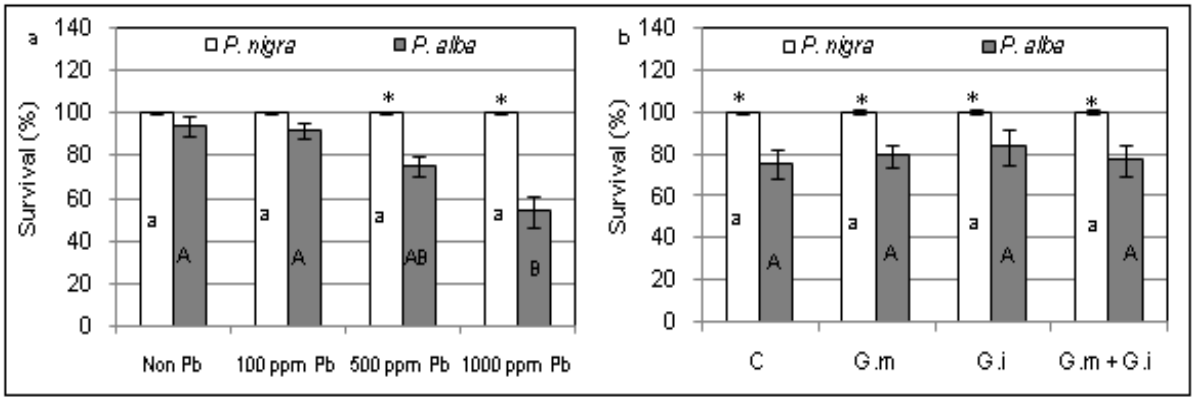
Results

The survival percentage of *P. nigra* and *P. alba* clones was recorded in July and October as follows: *P. nigra* plants showed 100% survival in all treatments; however in *P. alba* clone, the percent of plant survival ranged from 50 to 100%, plants supplied with 1000 ppm Pb, in all AMF treatments, had the least survival rate; the survival percentage of both clones was not affected by AMF treatment or interaction between two factors (Pb and AMF); in 500 and 1000 ppm Pb concentrations and all fungal treatments, *P. nigra* clone had better survival than *P. alba* clone; no change was observed in the survival percentage of both clones from July to October (Fig. 1a-b).

Table 1. Physical and chemical properties of the soil used for the experiment.

Sand (%)	46.5
Silt (%)	34.2
Clay (%)	19.3
pH (in water)	7.4
EC (ds/m)	0.95
CEC (meq per 100gr)	14.3
Organic matter (%)	0.68
Total N (%)	0.05
Assimilable P (ppm)	10.8
Exchangeable K (ppm)	105

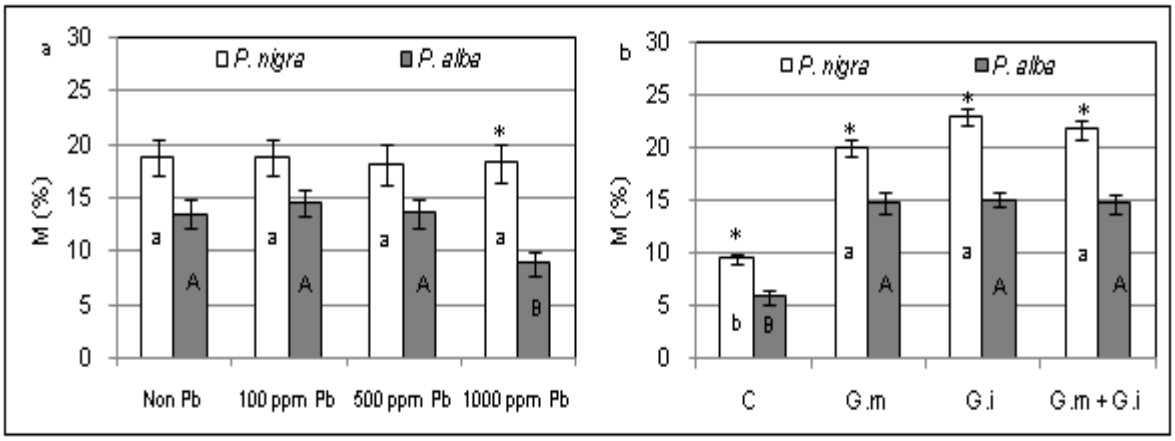
In *P. alba*, the application of 1000 ppm Pb significantly decreased the percentage of mycorrhizal colonization (M%), in all AMF treatments, but in *P. nigra*, Pb treatment had no significant effect on M% (Fig. 2a). In both poplar clones, M% of plants inoculated with fungal treatments was higher than that in non-inoculated plants, however without significant differences among the AMF treatments (Fig. 2b). As indicated in Fig. 2a-b, in *P. nigra*, M% was more extensive than *P. alba* in 1000 ppm Pb concentration and all tested AMF treatments.



ANOVA *P-value*	*P. nigra*[a]			*P. alba*		
	Pb	AMF	Pb × AMF	Pb	AMF	Pb × AMF
survival	-	-	-	0.000 *	0.252 ns	0.752 ns

Fig. 1. Survival of *P. nigra* and *P. alba* clones treated with different levels of Pb (a) and AMF (b) in July and October. C: Control; G.m: *Glomus mosseae*; G.i: *G. intraradices*; G.m+G.i: *G. mosseae* + *G. intraradices*; Bars represent standard errors; Different letters indicate significant differences in each clone; t-test shows significant differences between *P. nigra* and *P. alba* at: * $p < 0.05$; ANOVA *P values* for main effects and interactions are shown in the table (* $p < 0.05$, ns: not significant); [a] the survival of *P. nigra* plants in all treatments was 100%.

The measurements in July demonstrated that, at 1000 ppm Pb concentration, *P. nigra* plants, indicated 20 and 27% reductions of SL and volume production, respectively, compared to non Pb, in all AMF treatments (Fig. 3c-e), but these reductions in October were 16 and 22% (Fig. 4c-e). SD (Figs. 3a, 4a) did not participate in these reductions. As shown in Figs. 3 and 4, 100 and 500 ppm Pb concentrations and AMF treatments or interaction between two factors (Pb and AMF) had no influence on parameters of *P. nigra* clone.



ANOVA *P-value*	*P. nigra*			*P. alba*		
	Pb	AMF	Pb × AMF	Pb	AMF	Pb × AMF
M%	0.906ns	0.000 *	0.980 ns	0.000 *	0.000 *	0.952 ns

Fig. 2. Mycorrhizal colonization percentage (M%) of *P. nigra* and *P. alba* clones treated with different levels of Pb (a) and AMF (b) in October. C: Control; G.m: *Glomus mosseae*; G.i: *G. intraradices*; G.m+G.i: *G. mosseae* + *G. intraradices*; Bars represent standard errors; Different letters indicate significant differences in each clone; t-test shows significant differences between *P. nigra* and *P. alba* at: * $p < 0.05$; ANOVA *P values* for main effects and interactions are shown in the table (* $p < 0.05$, ns: not significant).

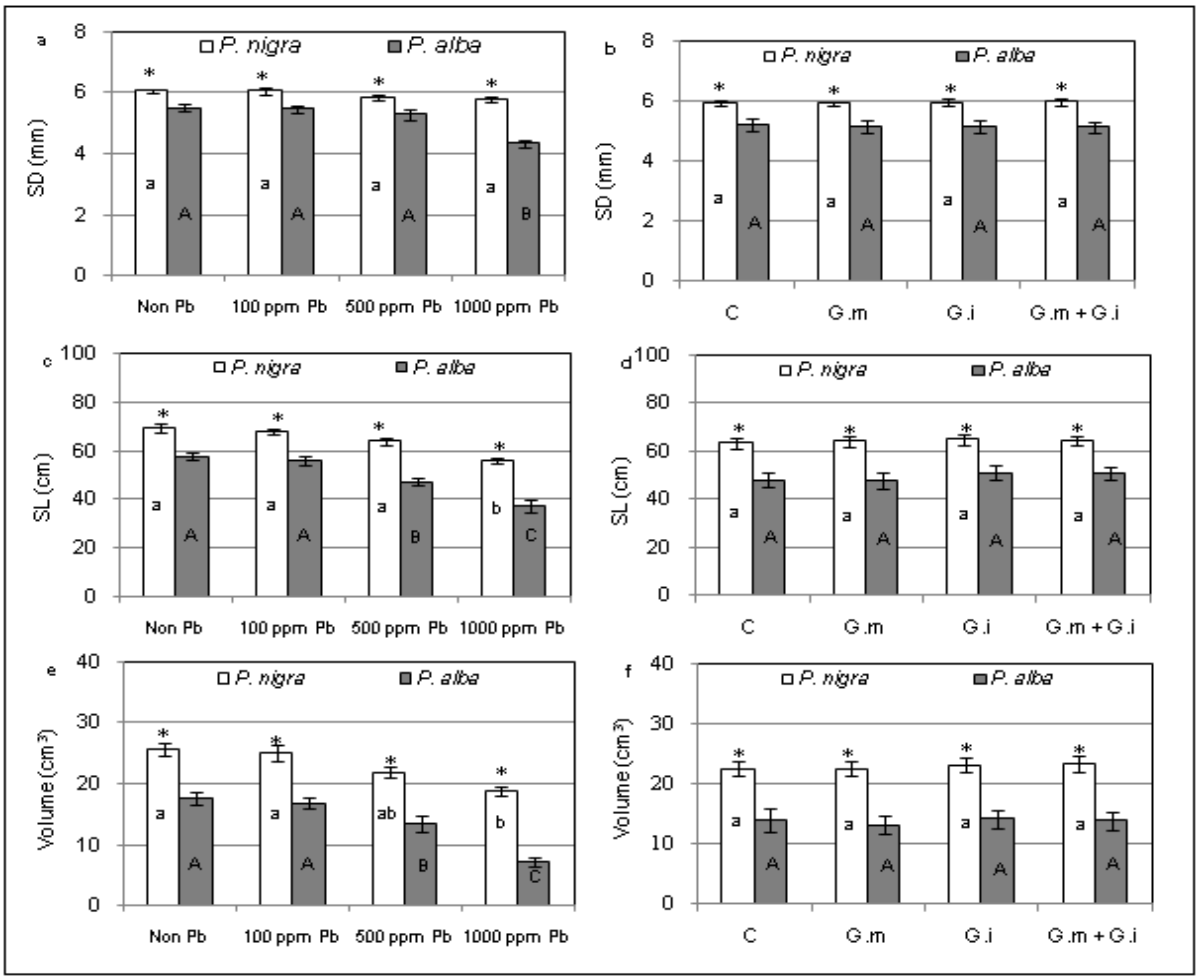
Significant decreases in SD (Figs. 3a, 4a), SL (Figs. 3c, 4c) and volume (Figs. 3e, 4e) of *P. alba* plants were observed as a consequence of 1000 ppm Pb treatment compared with non Pb treatment. In July, SD, SL and volume were, respectively, 21, 35 and 59% less than those in non Pb, and in October, these reductions were 15, 28 and 47%. This trend was observed in all tested AMF treatments. The concentration of 500 ppm Pb only reduced SL and volume in July (Fig. 3c-e), and 100 ppm Pb concentration had no effect on measured parameters. In October, AMF treatments had a significant positive influence on SL and volume production of *P. alba*, in polluted and non-polluted soils (Fig. 3d-f), however the same effect did not observe on SD or in July.

Based on the t-test results, in all Pb and AMF treatments, growth and volume production of *P. nigra* clone were greater than *P. alba* clone (Figs. 3, 4).

Discussion

One of the primary parameters for evaluation of plant performance in heavy metal-contaminated substrates is plant survival rate (Tanvir and Siddiqui, 2010). In accordance with the observations in July and October, we found that in each treatment all plants of *P. nigra* survived up to 100%, however in *P. alba*, it ranged from 50 to 100%. Plants treated with 1000 ppm Pb showed a 41% reduction compared with control, in all AMF treatments (Fig. 1). Different survival percentages of poplar clones grown on contaminated soils with heavy metals during the previous surveys were observed. For example, 100% survival of *P. deltoides* plants on soil contaminated with cadmium (Cd) (Tanvir and Siddiqui, 2010), 0-80% survival of clones of *P. alba* and *P. nigra* on

polluted soil with Cu and Zn (Castiglione *et al.*, 2009; Gamalero *et al.*, 2012) and 2.5% survival of poplars grown on heavy zinc contaminated soils (Schnoor, 2000) were reported. As for the broad genetic diversity of poplars (Aravanopoulos *et al.*, 1999), this variability of survival in various poplar clones and species could be anticipated. As in heavy metal contaminated soil, survival rate of approximately 30% can be considered as promising for practical objectives (Castiglione *et al.*, 2009), so the both clones can be regarded for potential use in phytoremediation systems.

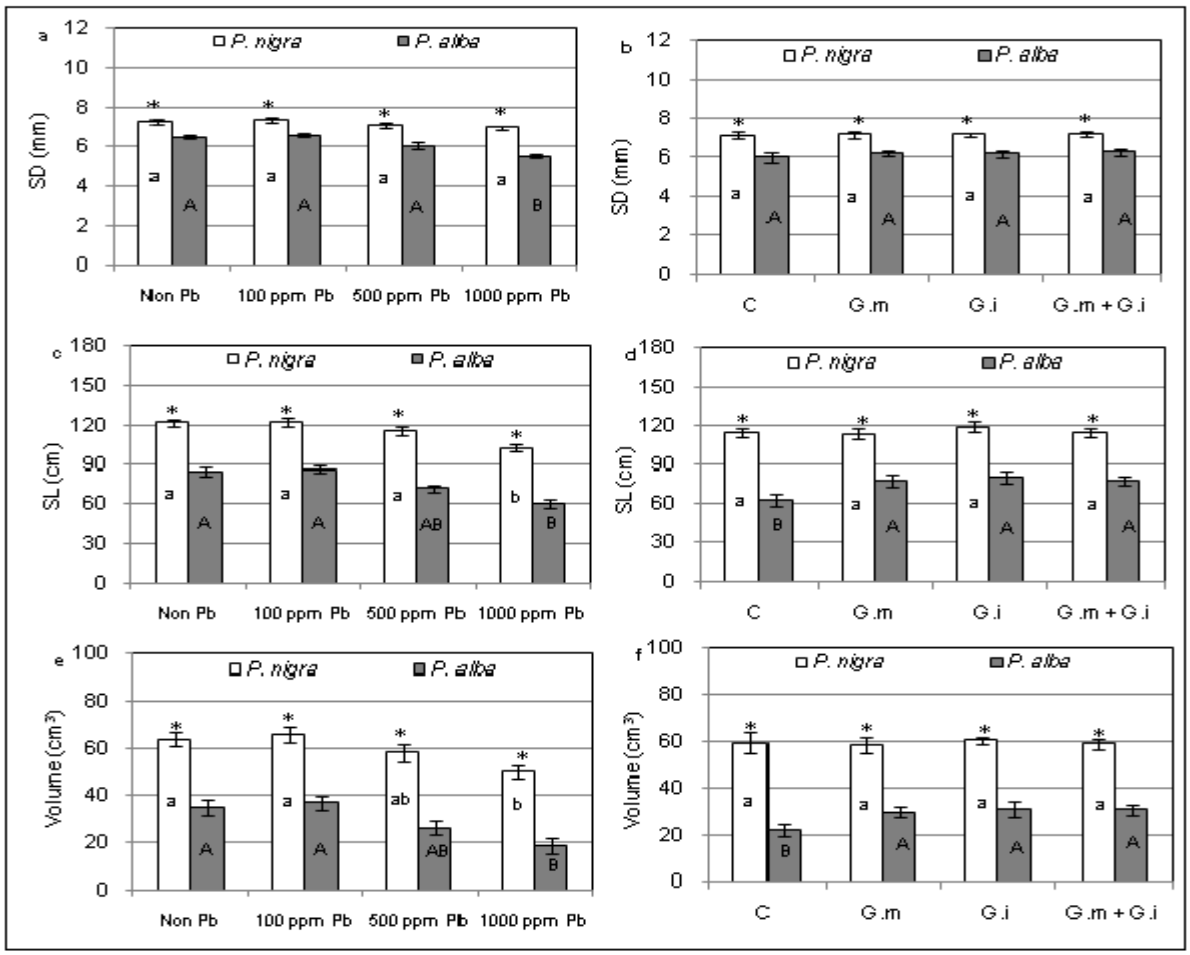


ANOVA *P-value*	*P. nigra*			*P. alba*		
	Pb	AMF	Pb × AMF	Pb	AMF	Pb × AMF
SD	0.152ns	0.979ns	0.997 ns	0.000 *	0.982 ns	0.992 ns
SL	0.000 *	0.914ns	0.998 ns	0.000 *	0.525 ns	0.992 ns
Volume	0.000 *	0.949ns	0.999 ns	0.000 *	0.921 ns	0.999 ns

Fig. 3. Stem diameter (SD) (a-b), stem length (SL) (c-d) and volume (e-f) of *P. nigra* and *P. alba* clones treated with different levels of Pb and AMF in July. C: Control; G.m: *Glomus mosseae*; G.i: *G. intraradices*; G.m+G.i: *G. mosseae* + *G. intraradices*; Bars represent standard errors; Different letters indicate significant differences in each clone; t-test shows significant differences between *P. nigra* and *P. alba* at * $p < 0.05$; ANOVA *P values* for main effects and interactions are shown in the table (* $p < 0.05$, ns: not significant).

Two poplar clones reached a greater M% when inoculated with AMF than control (Fig. 2b), suggesting that fungal inoculation could conquer the lack of natural mycorrhization (Turnau, 1998). As shown in Fig. 2b, there was no significant difference in M% by three AMF experimental treatments

consisted of either individual AM fungi (*G. mosseae* and *G. intraradices*) or their combinations (*G. mosseae* + *G. intraradices*). In *P. alba*, M% was significantly lower in 1000 ppm Pb treatment compared with control. On the contrary, M% of *P. nigra*, which was higher than *P. alba* in some treatments, showed no significant differences among Pb treatments (Fig. 2). In literature, the positive, negative or neutral influences of soil heavy metals on mycorrhizal colonization, in fact, has been described. For instance, in the survey of Cicatelli *et al.* (2010) M% of *p. alba* was not affected by the presence of Zn + Cu in soil. While, a higher root mycorrhizal colonization of some clones of *P.* × *euramericana* and the reduction of root colonization of *P. alba* clone in soils contaminated with heavy metals were reported by Taka`cs *et al.* (2005) and Lingua *et al.* (2008), respectively. The mycorrhizal colonization rate of two poplar clones is in agreement with previous data concerning poplars (Taka`cs *et al.*, 2005; Lingua *et al.*, 2008; Quoreshi and Khasa, 2008; Cicatelli *et al.*, 2010).



ANOVA *P-value*	*P. nigra*			*P. alba*		
	Pb	AMF	Pb × AMF	Pb	AMF	Pb × AMF
SD	0.278ns	0.996ns	0.995 ns	0.000 *	0.568 ns	0.955 ns
SL	0.001 *	0.725ns	0.998 ns	0.000 *	0.042 *	0.998 ns
Volume	0.003 *	0.974ns	0.995 ns	0.000 *	0.028 *	0.963 ns

Fig. 4. Stem diameter (SD) (a-b), stem length (SL) (c-d) and volume (e-f) of *P. nigra* and *P. alba* clones treated with different levels of Pb and AMF in October. C: Control; G.m: *Glomus mosseae*; G.i: *G. intraradices*; G.m+G.i: *G. mosseae* + *G. intraradices*; Bars represent standard errors; Different letters indicate significant differences in each clone; t-test shows significant differences between *P. nigra* and *P. alba* at * $p < 0.05$; ANOVA *P values* ($p < 0.05$) for main effects and interactions are shown in the table (* $p < 0.05$, ns: not significant).

The stem growth (SD and SL) and volume production of *P. alba* plants subjected to 1000 ppm Pb (in July and October) and SL and volume in 500 ppm Pb concentration (in July) were significantly lower than those to control. However in *P. nigra* clone, the influences of Pb on growth parameters were less (Figs. 3a-c-e, 4-a-c-e). In fact, although growth reduction in metal stressed plants is one of the important responses (Adriaensen *et al.*, 2003; Gu *et al.*, 2007), however, since plant growth on polluted soil is related to plant tolerance to heavy metals (Borghi *et al.*, 2008), so plants respond differently to heavy metals in terms of their tolerance. The variable degrees of plant growth and tolerance were previously reported regarding poplars growing on heavy metal polluted soils. For example, Borghi *et al.* (2008) indicated that in comparison with *P. alba*, *P. x canadensis* was more tolerant to high Cu. Lingua *et al.* (2008) reported that while in *P. alba* Zn reduced all growth parameters, in *P. nigra* the effects of Zn on growth were less; Gu *et al.* (2007) demonstrated that tolerance of four *Populus* cultivars as judged by growth reactions to various Cd concentrations is different.

AMF treatments had no significant effects on measured parameters of both poplar clones in July (Fig. 3b-d-f). In October, fungal treatments exhibited significant positive influences on SL and volume of *P. alba* plants in polluted and non-polluted soils, however plant parameters of *P. nigra* remained unaffected relative to AMF treatments (Fig. 4b-d-f). In general, improvement of plant growth and tolerance by AM symbiosis in polluted soils have been reported (Wang *et al.*, 2005; Ouahmane *et al.*, 2007; Bissonnette *et al.*, 2010); however, plant protection by AM fungi to toxicity of heavy metals relies on many variables including plant species, type of microorganism, heavy metal concentration, growth conditions, soil properties, plant physiological status and age, root system and species or clonal sensitivity (Arriagada *et al.*, 2005; Mrnka *et al.*, 2012). For example, Lingua *et al.* (2008) reported that in *P. alba* clone, the growth reduction induced by Zn treatment alleviated by AMF inoculation, while no similar advantageous influence was detected in *P. nigra* clone. Mrnka *et al.* (2012) found that *Hebeloma mesophaeum* fungus increased height and biomass of *Salix alba* in a polluted soil (Cd, Pb and Zn), while in the same condition *G. intraradices* significantly reduced height of *P. nigra* clone.

Conclusion

Based on results of this study, it is concluded that whereas in *P. alba* plants Pb significantly reduced survival, growth parameters and volume production, in *P. nigra* plants the influences were less intense as they included only SL and volume. In fact, at 1000 ppm Pb concentration, survival rate of *P. nigra* clone was dramatically greater than *P. alba*. Also, at both times, in all tested treatments, *P. nigra* produced significantly higher volume than *P. alba*. In reality, volume production of *P. nigra* clone was up to three-fold more than *P. alba* clone, in 1000 ppm Pb concentration. Since, tolerance to one heavy metal may lead to decrease in plant sensitivity to other toxic metals (Bojarczuk, 2004), it is possible to conclude that *P. nigra* will be more metal stress tolerant plant than *P. alba*. Generally, salicaceae family show good tolerance to heavy metals, however there are inter- and intra-specific variations (Mrnka *et al.*, 2012). Although, *P. nigra* clone proved to be more tolerant to Pb than *P. alba* clone, however survival and growth of both poplar clones demonstrated that their establishment on soils contaminated with Pb could be successful. Plants of two clones showed no symptoms of phytotoxicity under any Pb treatment. Nevertheless the positive effect of fungal inoculation was only observed on some parameters of *P. alba* (SL and volume), however, to fully evaluate the AMF potential in phytoremediation, it is essential to survey the influences of AMF colonization on heavy metal translocation and accumulation in organs of plant, too.

Acknowledgment

Financial support of this work was provided by Tarbiat Modares University (TMU) Research Foundations. Authors are thankful to Forestry Department of TMU for scientific assistance of this

research and Agricultural Faculty of TMU for its help throughout the experimental work.

References

Abdullahi MS, Uzairu A, Okunolam OJ. 2009. Quantitative determination of heavy metal concentration in onion leaves. International Journal of Environmental Research **3,** 271-274.

Adriaensen K, van der Lelie D, Van Laere A, Vangronsveld J, Colpaert JV. 2003. A zinc-adapted fungus protects pines from zinc stress. New Phytologist **161,** 549-555. http://dx.doi.org/10.1046/j.1469-8137.2003.00941.x

Aravanopoulos FA, Kim KH, Zsuffa L. 1999. Genetic diversity of superior Salix clones selected for intensive forestry plantations. Biomass and Bioenergy **16,** 249-255. http://dx.doi.org/10.1016/S0961-9534(98)00013-0

Arriagada CA, Herrera MA, Ocampo JA. 2005. Contribution of arbuscular mycorrhizal and saprobe fungi to the tolerance of *Eucalyptus globulus* to Pb. Water, Air and Soil Pollution **166,** 31-47. http://dx.doi.org/10.1007/s11270-005-7711-z

Avery TE, Burkhart HE. 1994. Forest Measurements. McGraw Hill Series in Forest Resources, New York, McGraw Hill. 4th edn. 408 P.

Bissonnette L, St-Arnaud M, Labrecque M. 2010. Phytoextraction of heavy metals by two Salicaceae clones in symbiosis with arbuscular mycorrhizal fungi during the second year of a field trial. Plant and Soil **332,** 55-67. http://dx.doi.org/10.1007/s11104-009-0273-x

Bojarczuk K. 2004. Effect of toxic metals on the development of poplar (*P. tremula* L. × *P. alba* L.) cultured in vitro. Polish Journal of Environmental Studies **13,** 115-120.

Bojarczuk K, Kieliszewska-Rokicka B. 2010. Effect of ectomycorrhiza on Cu and Pb accumulation in leaves and roots of silver birch (*Betula pendula* Roth.) seedlings grown in metal-contaminated soil. Water, Air and Soil Pollution **207,** 227-240. http://dx.doi.org/10.1007/s11270-009-0131-8

Borghi M, Tognetti R, Monteforti G, Sebastiani L. 2008. Responses of two poplar species *(Populus alba* and *Populus x canadensis*) to high copper concentrations. Environmental and Experimental Botany **62,** 290-299. http://dx.doi.org/10.1016/j.envexpbot.2007.10.001

Brundrett M, Bougher N, Grove T, Malajczuk N. 1996. Working with Mycorrhizas in Forestry and agriculture. ACIAR, Canberra, 374 p.

Castiglione S, Todeschini V, Franchin C, Torrigiani P, Gastaldi D, Cicatelli A, Rinaudo C, Berta G, Biondi S, Lingua G. 2009. Clonal differences in survival capacity, copper and zinc accumulation, and correlation with leaf polyamine levels in poplar: A large-scale field trial on heavily polluted soil. Environmental Pollution **157,** 2108-2117. http://dx.doi.org/10.1016/j.envpol.2009.02.011

Chellappan P, Anitha Christy SA, Mahadevan A. 2002. Multiplication of arbuscular mycorrhizal fungi on roots, In: Mukerji K G, Manoharachary C, Chaloma B P, (eds), Techniques in mycorrhizal studies, Kluwer, Derdrecht, 285-297.

Cheremisinoff PN, Habib YH. 1982. Cadmium, lead, mercury: a plenary account for water pollution, occurrence, toxicity and detection. Water & Sewage Works **119,** 73-83.

Cicatelli A, Lingua G, Todeschini V, Biondi S, Torrigiani P, Castiglione S. 2010. Arbuscular mycorrhizal fungi restore normal growth in a white poplar clone grown on heavy metal-contaminated soil, and this is associated with upregulation of foliar metallothionein and polyamine biosynthetic gene expression. Annals of Botany **106,** 791-802. http://dx.doi.org/10.1093/aob/mcq170

Di Baccio D, Tognetti R, Sebastiani L, Vitagliano C. 2003. Responses of *Populus deltoides* × *Populus nigra* (*Populus* × *euramericana*) clone I-214 to high zinc concentrations. New Phytologist **159,** 443-452. http://dx.doi.org/10.1046/j.1469-8137.2003.00818.x

Faison BD. 2004. Biological treatment of metallic pollutants. In: Sing A, Ward O P (Eds.), Applied Bioremediation and Phytoremediation. Soil Biology Series. Springer, Berlin, 81e114 P.

Gamalero E, Cesaro P, Cicatelli A, Todeschini V, Musso C, Castiglione S, Fabiani A, Lingua G. 2012. Poplar clones of different sizes, grown on a heavy metal polluted site, are associated with microbial populations of varying composition. Science of the Total Environment **425,** 262-270. http://dx.doi.org/10.1016/j.scitotenv.2012.03.012

Gaur A, Adholeya A. 2004. Prospects of arbuscular mycorrhizal fungi in phytoremediation of heavy metal contaminated soils. Current Science **86,** 528-534.

Giovannetti M, Mossem B. 1980. An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. New Phytologist **84**, 498-500. http://dx.doi.org/10.1111/j.14698137.1980.tb04556.x

Gu J, Qi L, Jiang W, Liu D. 2007. Cadmium accumulation and its effects on growth and gas exchange in four *Populus* cultivars. Acta Biologica Cracoviensia Series Botanica **49,** 7-14.

He J, Ma C, Ma Y, Li H, Kang J, Liu T, Polle A, Peng C, Luo Z. 2013. Cadmium tolerance in six poplar species. Environmental Science and Pollution Research **20,** 163-174. http://dx.doi.org/10.1007/s11356-012-1008-8

Hunt R. 1978. Plant Growth Analysis. Camelot Press Ltd., Southampton, UK.

Kabata-Pendias A. 2004. Soil-plant transfer of trace elements-an environmental issue. Geoderma **122,** 143-149. http://dx.doi.org/10.1016/j.geoderma.2004.01.004

Khasa PD, Chakravarty P, Robertson A, Thomas BR, Dancik BP. 2002. The mycorrhizal status of selected poplar clones introduced in Alberta. Biomass and Bioenergy **22,** 99-104. http://dx.doi.org/10.1016/S0961-9534(01)00072-1

Lingua G, Franchin C, Todeschini V, Castiglione S, Biondi S, Burlando B, Parravicini V, Torrigiani P, Berta G. 2008. Arbuscular mycorrhizal fungi differentially affect the response to high zinc concentrations of two registered poplar clones. Environmental Pollution **153,** 137-147. http://dx.doi.org/10.1016/j.envpol.2007.07.012

McLaughlin MJ. 2001. 'Bioavailability of metals to terrestrial plants', in H. E. Allen (ed.), Bioavailability of Metals in Terrestrial Ecosystems. Importance of Partitioning for Bioavailability to Invertebrates, Microbes and Plants, SETAC Press, Pensacola, FL, . 39-68 P.

Mrnka L, Kuchár M, Cieslarová Z, Matějka P, Száková J, Tlustoš P, Vosátka M. 2012. Effects of endo- and ectomycorrhizal fungi on physiological parameters and heavy metals accumulation of two species from the family salicaceae. Water, Air and Soil Pollution **223,** 399-410. http://dx.doi.org/10.1007/s11270-011-0868-8

Ouahmane L, Hafidi M, Thioulouse J, Ducousso M, Kisa M, Prin Y, Galiana A, Boumezzough A, Duponnois R. 2007. Improvement of *Cupressus atlantica* Gaussen growth by inoculation with native arbuscular mycorrhizal fungi. Journal of Applied Microbiology **103,** 683-690. http://dx.doi.org/10.1111/j.1365-2672.2007.03296.x

Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British

Mycological Society **55,** 158-161. http://dx.doi.org/10.1016/S0007-1536(70)80110-3

Pulford ID, Dickinson NM. 2005. Phytoremediation technologies using trees. In: Prassad MNV, Naidu R [eds.], Trace elements in the environment, 375-395. CRC Press, New York.

Quoreshi AM, Khasa DP. 2008. Effectiveness of mycorrhizal inoculation in the nursery on root colonization, growth, and nutrient uptake of aspen and balsam poplar. Biomass and Bioenergy **32,** 381-391. http://dx.doi.org/10.1016/j.biombioe.2007.10.010

Rafati M, Khorasani N, Moattar F, Shirvany A, Moraghebi F, Hosseinzadeh S. 2011. Phytoremediation Potential of *Populus Alba* and *Morus alba* for Cadmium, Chromuim and Nickel Absorption from Polluted Soil. International Journal of Environmental Research **5,** 961-970.

Rivera-Becerril F, Calantzis C, Turnau K, Caussanel JP, Belimov AA, Gianinazzi S, Strasser RJ, Gianinazzi-Pearson V. 2002. Cadmium accumulation and buffering of cadmium induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes. Journal of Experimental Botany **53,** 1177-1185.

Schnoor JL. 2000. Phytostabilization of metals using hybrid poplar trees. In: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of Toxic Metals. Using Plants to Clean Up the Environment. John Wiley & Sons, New York, 133-150 P.

Smith SE, Read DJ. 1997. Mycorrhizal Symbioses, second ed. Academic Press, San Diego, USA.

Susarla S, Medina VF, McCutcheon SC. 2002. Phytoremediation: An ecological solution to organic chemical contamination. Ecological Engineering **18,** 647-658. http://dx.doi.org/10.1016/S0925-8574(02)00026-5

Tabari M, Salehi A. 2009. Long-term impact of municipal sewage irrigation on treated soil and black locust trees in a semi-arid suburban area of Iran. Journal of Environmental Sciences **21,** 1438-1445. http://dx.doi.org/10.1016/S1001-0742(08)62437-7

Taka`cs T, Radimszky L, Ne´meth T. 2005. The arbuscular mycorrhizal status of poplar clones selected for phytoremediation of soils contaminated with heavy metals. Zeitschrift fuer Naturforschung Section C Journal of Biosciences **60,** 357-361.

Tanvir MA, Siddiqui MT. 2010. Growth performance and cadmium (Cd) uptake by *Populus deltoides* as irrigated by urban wastewater. Pakistan Journal of Agricultural Sciences **47,** 235-240.

Torresday JL, Videa JRP, Rosa GD, Parsons J. 2005. Phytoremediatoin of heavy metals and study of the metal coordination by X- ray absorption spectroscopy. Coordination Chemistry Reviews **249,** 1797-1810. http://dx.doi.org/10.1016/j.ccr.2005.01.001

Turnau K. 1998. Heavy metal content and localization in mycorrhizal *Euphorbia cyparissias* from zinc wastes in southern Poland. Acta Societatis Botanicorum Poloniae **67,** 105-113. http://dx.doi.org/10.5586/asbp.1998.014

Wang FY, Lin X, Yin R. 2005. Heavy metal uptake by arbuscular mycorrhizas of *Elsholtzia splendens* and the potential for phytoremediation of contaminated soil. Plant and Soil **269,** 225-232. http://dx.doi.org/10.1007/s11104-004-0517-8

Zalesny Jr RS, Bauer EO, Hall RB, Zalesny JA, Kunzman J, Rog CJ, Riemenschneider DE. 2005. Clonal variation in survival and growth of hybrid poplar and willow in an in situ trial on soils heavily contaminated with petroleum hydrocarbons. International Journal of Phytoremediation **7,** 177-197. http://dx.doi.org/10.1080/16226510500214632