

## **Investigating the probable consequences of super absorbent polymer and mycorrhizal fungi to reduce detrimental effects of lead on wheat (*Triticum aestivum* L.)**

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**Abstract.** In many parts of the world, agricultural use of soils is restricted due to heavy metal contamination. Absorption of heavy metals, such as (Pb), in the tissue of plants increases the plant's metabolism and causes physiological disorders or even death. In order to study the potential of super absorbent polymers (SAP) and mycorrhiza fungi application to mitigate adverse effects of lead (Pb) on wheat, a greenhouse experiment was conducted. The experiment was setup as a completely randomized design, with two treatments arranged in a factorial scheme with three levels of lead (0, 100 and 200 mg per kg soil) and four levels of SAP and mycorrhiza fungi application (without SAP and mycorrhiza fungi application, SAP application alone, mycorrhiza fungi application alone, SAP and mycorrhiza fungi application combined). The results showed that Pb significantly affected all parameters measured of wheat. The Pb-contamination caused a significantly decreasing in plant height, total dry weight per plant and total chlorophyll contents. And also, the results indicated that the combined use of superabsorbent and mycorrhiza reduced the amount of superoxide dismutase enzyme. As well as, our results show that the application of super absorbent polymer and mycorrhizal fungi seems to be a promising path to reduce detrimental effects of heavy metal pollution of agricultural soils on plant performance.

**Key words:** Wheat, lead, super absorbent polymer, mycorrhizal fungi, enzymes superoxide dismutase.

### **INTRODUCTION**

Heavy metal contamination of the environment did increase worldwide during the last years (Sheetal et al., 2016). The transfer and accumulation of heavy metals in soil-plant systems is influenced by multiple factors (Poschenrieder & Barceló, 2004; Wang et al., 2017). This is of primary concern in agricultural production due to adverse effects on crop growth and risk of crop and food chain contamination (Singh & Agrawal 2010). Consequently, attention on heavy metal induced effects increased during the last years. Removing of heavy metals from contaminated soils with traditional physical and chemical methods is inefficient and very costly (Benavides et al., 2005; Shrama & Dubey, 2005; Kinraide, 2007). Thus, efforts to develop effective and inexpensive

technologies to deal with heavy metal contamination of soils are increasing. In this context, the manipulation and use of mycorrhizal fungi was already identified as a potentially important tool to remediate copper-contaminated soils (Meier et al., 2015).

Lead (Pb) is a dangerous heavy metal pollutant in the environment, which influences the metabolic and physiological activity of living organisms. High doses of Pb likely result in metabolic disorders, growth inhibition and even death for most plant species (Shrama & Dubey, 2005). The rhizosphere is the few millimetres of soil surrounding a plant's roots that microorganisms have many biological activities there that are critically important to both plant health and soil carbon (C) transformation and stabilization (Pett-Ridge & K. Firestone 2017). The symbiosis between plants and mycorrhizal fungi is one of the most extensive bidirectional relations between plants and micro-organisms in the soil (Javaid, 2009). More than 80% of the higher plants have the ability to form arbuscular mycorrhizal associations (Garg et al., 2006). Arbuscular mycorrhizal (AM) fungi can facilitate the survival of their host plants growing on metal-contaminated land by improving nutrient acquisition, protecting them from the metal toxicity, absorbing metals, and also enhancing phytostabilization and phytoextraction (Jahromi et al., 2008; Javaid, 2009; Leung et al., 2013). These fungi reduce concentrations of heavy metals by binding these in the chitin cell wall (Hildebrandt et al., 2007) and the secretion of glomalin (Gonzalez-Chavez et al., 2004). Moreover, AM accumulate heavy metals in plant roots in non-toxic complexes (Joner & Leyval, 1997). On the other hand, the use of superabsorbent may improve physical and chemical soil properties (Kozłowski & Badora, 2007; Bai et al., 2010) and thereby already reduces the risk of heavy metal uptake by plants. Therefore, in this study the physiological and biochemical effect of mycorrhizal fungi and super absorbent polymer on different parameters on wheat were examined. By doing this, we wanted to provide experimental evidence for the potential of mycorrhizal fungi and super absorbent polymers to reduce detrimental effects of lead on wheat performance (*Triticum aestivum L.*).

## MATERIALS AND METHODS

The experiment was conducted in a greenhouse in Varamin in 2016. The study was designed as a two-factorial, completely randomized experiment. The first factor was lead concentration in the soil (Pb) ( $k = 3$ ; 0, 100 and 200 mg per kg of soil) and the second factor were different combinations of super absorbent polymer and mycorrhizal fungi ( $k = 4$ ; no super absorbent and no mycorrhiza = control, only super absorbent, only mycorrhiza, simultaneous application of super absorbent and mycorrhiza). The soil of the experimental site was a clay loam one, with a montmorillonite clay type, low in nitrogen (0.06–0.07%), low in organic matter (0.56–0.60), and alkaline in reaction with pH of 7.2 and  $E_c = 0.66 \text{ dS m}^{-1}$ . The soil texture was sandy loam, with 10% of neutralizing substances. Super absorbent polymer used in this study was a hydrophilic polymer (SUPERAB-A200) produced by the Rahab Resin Co. Ltd., under license of the Iran Polymer and Petrochemical Institute. It is a white granular powder with a 90% active ingredient, 75–1,000  $\mu\text{m}$  particle size, and  $0.60 \text{ g cm}^{-3}$  bulk density, which swells to form a gel in water. All factor combinations were replicated three times. Soils were treated in a steam autoclave at a temperature of 121 °C and a pressure of 20 atmospheres for one hour to delete all spores and fungal propagules in the soil. Lead concentrations were selected according to the tentative map of soil pollution by heavy metals and the range

of lead concentration in contaminated soils of Iran (Instrument Manual Digesdahl Digestion Apparatus, 1999). The mycorrhizal inoculant fungi *Glomus intraradices* (producer of biotech company Turan, Semnan, Iran) was used for mycorrhizal inoculation. For this purpose, pots were filled with 100 g soil and in each pot 250 mycorrhizal propagules were added. Fifteen surface sterilized seeds of wheat (*Triticum aestivum* L c.v *SW*) were planted in the pots. In case of more than 5 emerged plants per pot excess seedlings were removed. Pots were regularly watered. During the growing season number of plants per pot was reduced to three. These plants were used to measure fitness traits during the growing season and at the end of the experiment. First, height of the plants was measured and averaged across three plants per pot. At the end of the experiment, biomass was assessed by drying these three plants for 48 hours at 70 °C in an oven. The chlorophyll content was measured with a spectrophotometer (Arnon, 1949). In order to evaluate the activity of superoxide dismutase Giannopolitis method was used (Ries, 1977). Therefore, 2.0 g of a sample were added in 3 mL of buffer containing 1.0 mM EDTA pH 7.8 with HEPES-KOH. The resulting product was centrifuged at around 15,000 rpm for 15 minutes at a temperature of 4 °C and the supernatant was used for measuring the activity of superoxide dismutase reaction mixture containing 50 mM KOH buffer at pH 8/7 with EDTA-HEPES 1.0 mM, 50 mM sodium carbonate at pH 2/10, L-methionine 12 mM, Nitro Blue Tetrazolium 75 mM, 1 mM and 200 mL extract the enzyme was riboflavin. The samples were exposed for 15 minutes, after which the absorption at a wavelength of 560 nm was measured using a spectrophotometer. A test tube containing the reaction mixture to a fermentation extract was used as a control. One unit of SOD activity was defined as the amount of enzyme that caused 50% inhibition of photochemical reduction of nitro blue tetrazolium. To determine the concentration of lead in the shoots and roots, 1 g of finely ground plant biomass was incinerated in an electric furnace at 550 °C for four. The ash obtained was mixed with 10 mL of two molar HCl. Subsequently, the concentration of the extracts was measured by atomic absorption AAS Vario 6. Finally, the experimental data were analyzed employing analysis of variance and the Duncan test using SAS software (SAS Institute, 2002).

## RESULTS AND DISCUSSION

According to the analysis of variance, lead had a significant main effect on all measured traits (Table 1). The highest plant height was measured on plants not exposed to lead (Table 2 and Fig. 6). The lowest plant height was found at a lead concentration of 200 mg per kg soil. We also found that increased levels of lead decreased dry weight per plant (Fig. 2). Therefore, Pb contamination reduced both, growth and plant biomass. It was also observed that the highest biomass was reached when both superabsorbent plant and mycorrhizal were present (Table 2). SAP (Super absorbent polymer) leads to an increase of water and nutrient uptake, through increased cell division and growth (Neumann & George, 2005). Mycorrhiza can develop at the dorsal root tip, the original position of cell elongation and nutrient uptake. Ultimately, this will also increase the availability of water and nutrients and may enhance cell division and plant height (Neumann & George, 2005). Our results are also in accordance with results of other researchers who linked the decrease of plant performance by heavy metals to lower amounts of chlorophyll in leaves, which slows down photosynthesis (Gajewska &

**Table 1.** Analysis of variance on wheat attributes affected by lead stress condition, mycorrhiza fungi and super polymer absorbent

S.O.V	d.f	Plant height	Shoot dry weight	Total chlorophyll	Superoxide dismutase	Shoot Pb concentration	Root Pb concentration
Lead	2	171.18**	307.13**	0.944**	8425**	0.035**	10.10**
SAP and AMF	3	6.22**	35.65*	0.015**	3658.88**	0.003**	0.527**
Lead * SAP and AMF	6	2.19*	8.93**	0.003**	826.06**	0.0006ns	0.062**
Error	24	1.20	7.94	0.001	94.52	0.0002	0.030
C.V (%)		1.74	10.19	1.95	2.01	6.38	13.68

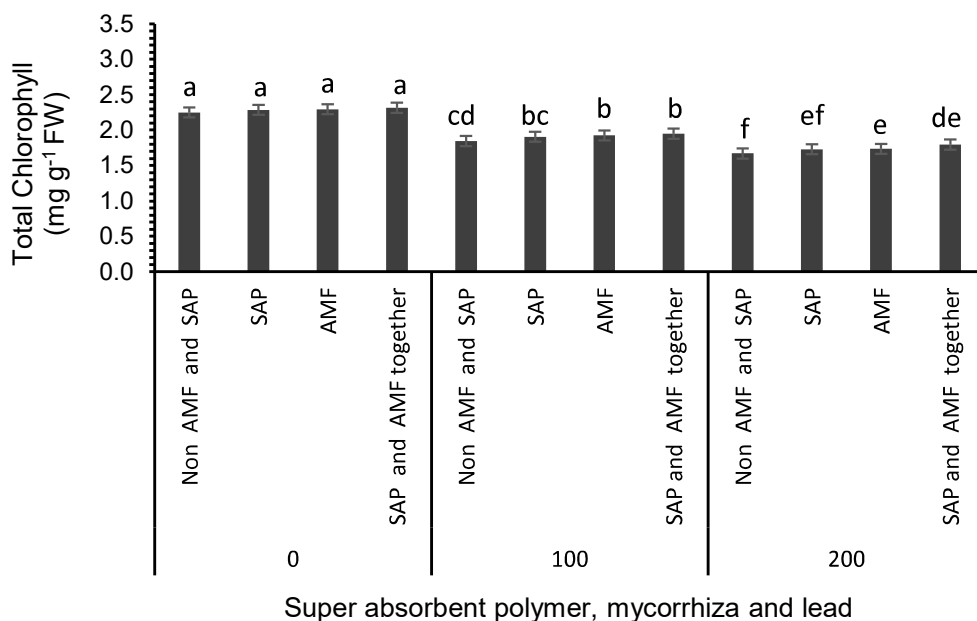
\*, \*\* and ns – significant at 0.05, 0.01 percentage and not significant; SAP = Super absorbent polymer; AMF = Arbuscular Mycorrhizal Fungi.

**Table 2.** Mean comparison of main effects of lead stress, mycorrhiza fungi and super polymer absorbent on some attributes of wheat

Treatment	Plant height, (cm)	Shoot dry weight, (g)	Total chlorophyll, (mg g <sup>-1</sup> FW)	Superoxide Dismutase, ( $\Delta A$ mg pro.min <sup>-1</sup> )	Shoot Pb concentration, (mg.g <sup>-1</sup> DW)	Root Pb concentration, (mg.g <sup>-1</sup> DW)
<b>Lead concentration</b>						
0	66.30a	33.33a	2.287a	194.46c	0.043c	0.391c
100	62.90b	25.89b	1.907b	533.87b	0.124b	1.233b
200	58.75c	23.67b	1.740c	716.62a	0.146a	2.225a
<b>Mycorrhiza and Super absorbent</b>						
Non-AMF and SAP	61.66c	25.22b	1.922c	507.10a	0.127a	1.288b
SAP	62.44bc	26.94ab	1.974b	484.66b	0.112ab	0.966c
AMF	62.83ab	28.57a	1.995ab	476.32b	0.097bc	1.555a
SAP and AM together	63.66a	29.80a	2.021a	458.53c	0.081c	1.322b

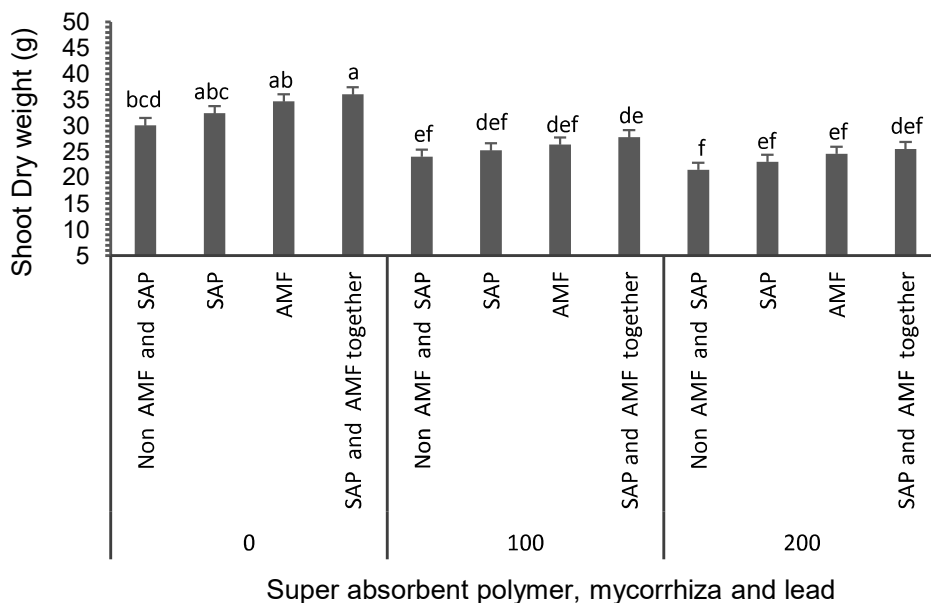
Treatment means followed by the same letter within each column are not significantly different ( $P < 0.05$ ) according to Duncan's Multiple Range test; SAP = Super absorbent polymer; AMF = Arbuscular Mycorrhizal Fungi.

Skodowska, 2007; Ghasemi et al., 2009; Pakdaman et al., 2013). Accordingly, we found that the synthesis of chlorophyll of plants growing in soils contaminated with lead was reduced (Fig. 1). Heavy metals also increase decomposition of the chlorophyll pigment, thereby reducing the amount of chlorophyll in the tissue (Gajewska et al., 2006).

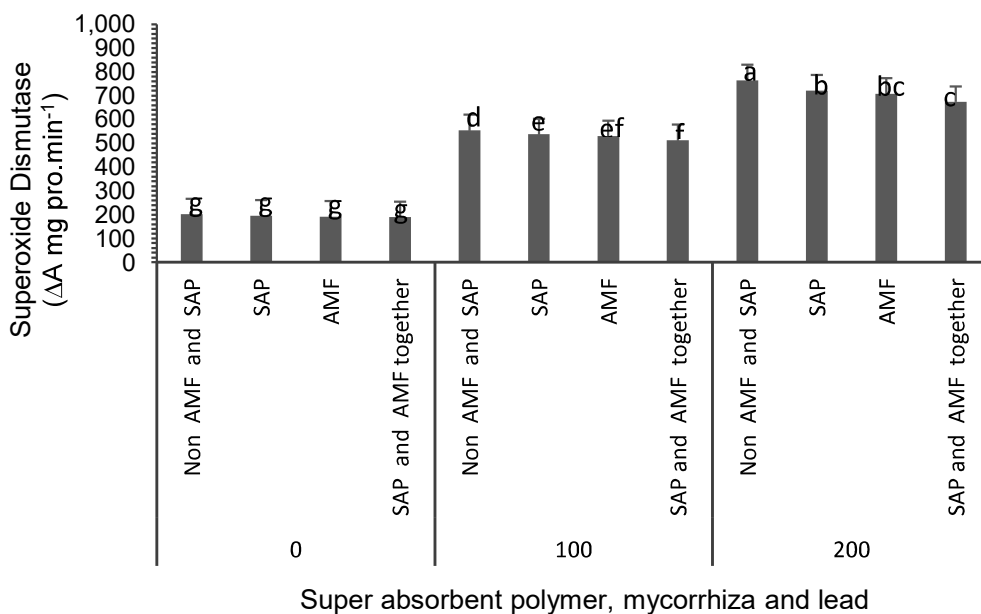


**Figure 1.** Interaction between lead stress (0, 100 and 200 mg kg<sup>-1</sup>), AMF and SAP on Total Chlorophyll (mg g<sup>-1</sup>FW). Treatment means followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan's Multiple Range test; SAP = Super absorbent polymer; AMF = Arbuscular Mycorrhizal Fungi.

Other researchers have shown that the use of superabsorbent polymers can increase the chlorophyll content of sunflower plants under stress (Nazarli et al., 2010). In addition, mycorrhiza coexisting in plants can increase the speed and rate of photosynthesis and consequently biomass production. This is probably due to improved nutrient and water uptake through the host plant (Demir, 2004). Accordingly, Goussous & Mohammad (2009) report that the fungus *Glomus intraradices* affects the absorption of nutrients and increases growth of onions. In case of superoxide dismutase enzyme activity, our results showed a significant difference between treatments, i.e. the highest activity of superoxide dismutase was observed in the application of lead at a concentration of 200 mg per kg soil. And also the results indicated that the combined use of superabsorbent and mycorrhiza reduced the amount of superoxide dismutase enzyme from 764.88 to 672.93 ( $\Delta A \text{ mg pro. min}^{-1}$ ) (Table 2 and Fig. 3). Superoxide dismutase is a key enzyme to protect cells against oxidative stress, since it accelerates the conversion of  $O_2^-$  to  $H_2O_2$  and  $O_2$  (Fukai & Ushio-Fukai, 2011). The highest activity of superoxide dismutase was found at a lead concentration of 200 milligrams per kilogram of soil. Plants exposed to heavy

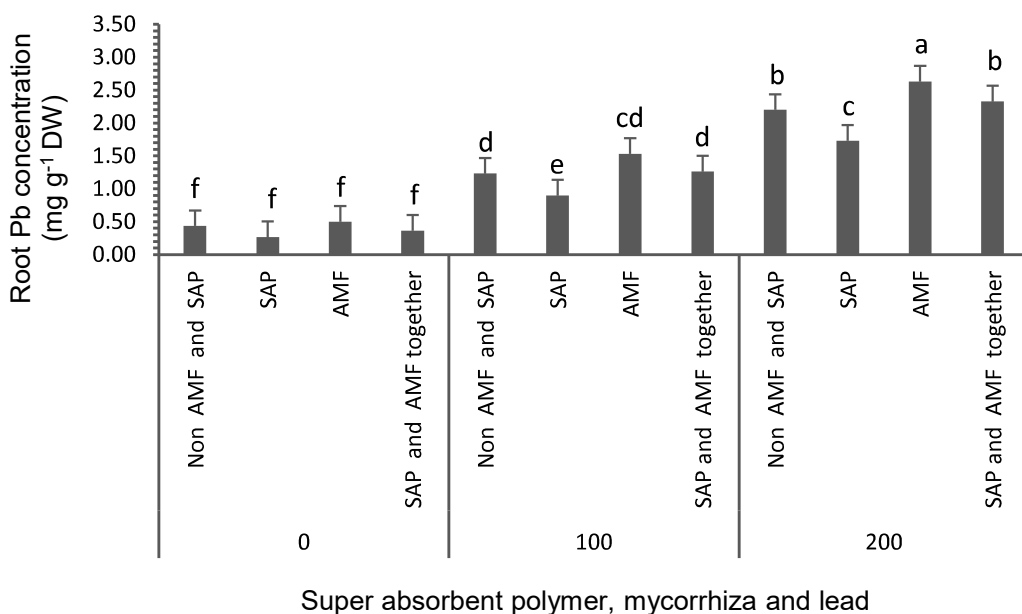


**Figure 2.** Interaction between lead stress (0, 100 and 200 mg kg<sup>-1</sup>), AMF and SAP on Shoot Dry weight (g). Treatment means followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan's Multiple Range test; SAP = Super absorbent polymer; AMF = Arbuscular Mycorrhizal Fungi.



**Figure 3.** Interaction between lead stress (0, 100 and 200 mg kg<sup>-1</sup>), AMF and SAP on Superoxide Dismutase (ΔA mg pro.min<sup>-1</sup>). Treatment means followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan's Multiple Range test; SAP = Super absorbent polymer; AMF = Arbuscular Mycorrhizal Fungi.

metals often show specific enzyme activities and plant species exposed to heavy metals often respond differently (Dhir et al., 2009). Research by Parlak (2016) showed that increasing the concentration of nickel increased superoxide dismutase activity in wheat seedlings. Tohidi Moghadam (2017) also reported that SAP application could reduce harmful effects of arsenic by alleviating oxidative stress. Also in the present study, the use of super absorbent and mycorrhiza reduced the activity of superoxide dismutase in the leaves (Table 2 and Fig. 3). In addition, the results demonstrated that the highest lead content in shoots and roots was found at a lead concentration of 200 milligrams per kilogram of soil (Table 2, Figs 4 and 5). There the highest lead content was found in shoots of the control, i.e. no mycorrhiza and no super absorbent polymer (Fig. 5) and the highest lead content of roots is related to the level of mycorrhizal treatment (Table 2 and Fig. 4). Levels of lead in the shoots of wheat in the mycorrhiza treatment were lower. Comparing wheat without mycorrhiza addition with those plants in the mycorrhiza treatments prove a reduced transfer of lead from roots to shoots.

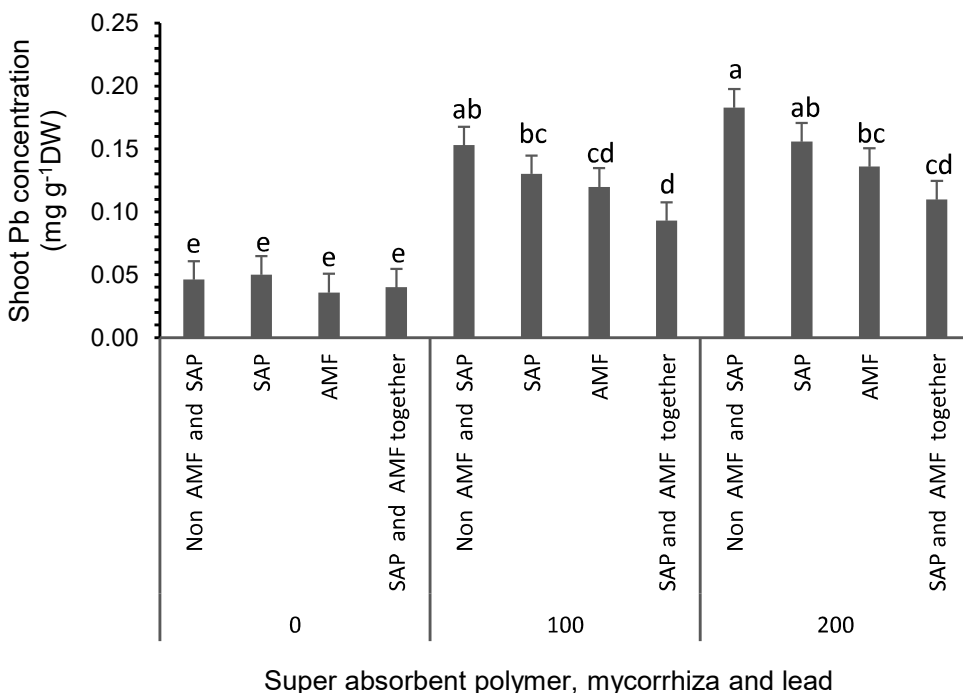


**Figure 4.** Interaction between lead stress (0, 100 and 200 mg kg<sup>-1</sup>), AMF and SAP on Root Pb concentration (mg g<sup>-1</sup>DW). Treatment means followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan's Multiple Range test; SAP = Super absorbent polymer; AMF = Arbuscular Mycorrhizal Fungi.

In general, uptake of heavy metals by plants and the ability to accumulate these differs between species and will increase with increasing heavy metal pollution of the soils (Burken et al., 2011). This phenomenon is likely to be a physiological trait of the species. While some species can absorb high amounts of heavy metals from the environment without suffering serious injuries, others show a lower ability to absorb heavy metals and might experience poisoning and damage already at low heavy metal

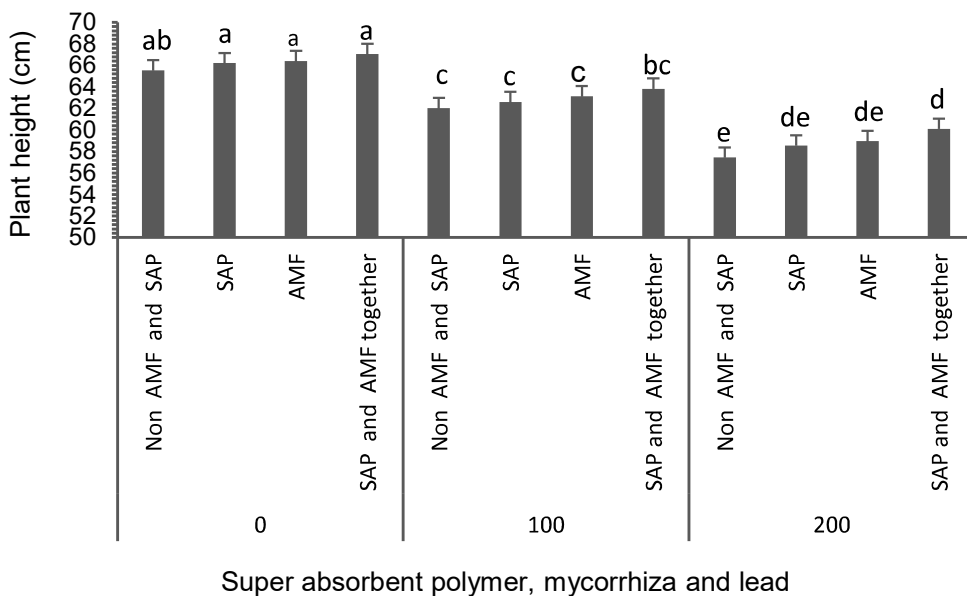
levels (Gosh & Singh, 2005; Baycu et al., 2006). In this situation, superabsorbent polymers can improve soil physical and chemical properties (Bai et al., 2010). Super absorbent polymers stabilise heavy metals in the soil and consequently the absorption and accumulation of heavy metals in plants is reduced.

Interestingly, mycorrhizal fungi actively increase absorption of heavy metals through roots-mycorrhiza complex but at the same time maintain the active uptake of nutrients such as nitrogen and phosphorus. Reduced lead contents in the shoots of plants in the mycorrhiza treatments might be due to the deposition of heavy metals in the parenchyma cells of fungi in non-toxic form (Vivas et al., 2006; Gonzalez-Guerrero et al., 2008). Mycorrhizal fungi produce a non-soluble glycoprotein called glomalin that binds to toxic elements such as heavy metals and prevents their transfer into plants (Gonzalez-Chavez et al., 2004; Pyrzynska, 2007). Still, increasing levels of uptake will lead to increased lead contents in shoots and roots (Figs 4, 5). Under these circumstances, the use of super absorbent and mycorrhiza together simultaneously reduced the lead content of the shoot. Thus, there are two different mechanism that reduce heavy metal content in the plant: while mycorrhiza increases the amount of lead in the root-mycorrhiza system but hampers their uptake into the plant, super absorbent polymers prevent the uptake of heavy metals by immobilizing heavy metals.



**Figure 5.** Interaction between lead stress (0, 100 and 200 mg kg<sup>-1</sup>), AMF and SAP on Shoot Pb concentration (mg g<sup>-1</sup>DW). Treatment means followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan's Multiple Range test; SAP = Super absorbent polymer; AMF = Arbuscular Mycorrhizal Fungi.





**Figure 6.** Interaction between lead stress (0, 100 and 200 mg kg<sup>-1</sup>), AMF and SAP on Plant height (cm). Treatment means followed by the same letter are not significantly different ( $P < 0.05$ ) according to Duncan's Multiple Range test; SAP = Super absorbent polymer; AMF = Arbuscular Mycorrhizal Fungi.

## CONCLUSION

Our study showed that levels of lead in the shoots of wheat in the mycorrhiza treatment were lower. The current results compare to wheat without mycorrhiza, prove the reduced transfer of lead from roots to shoots. In contrast, the content of lead in the mycorrhizal roots is increased. As well as the results illustrated that the transfer of lead from the soil into the roots is higher than the transfer of lead from roots to the shoots. In addition, lead is immobilized in the soil by super absorbent polymers or stored in the plant root-mycorrhiza system. Both processes prevent transfer of lead into shoots and are effective in the phytoremediation of heavy metals. Therefore, the combined application of super absorbent polymer and mycorrhizal fungi may be a feasible measurement to re-integrate polluted soils with heavy metals into agricultural management.

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