

[Research]

## Evaluation of six cold-season turfgrasses responses to lead phytotoxicity for screening tolerant species

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### ABSTRACT

Lead (Pb) is one of the most hazardous heavy metal that caused serious problems for ecosystem at recent years. In this study, the effect of Pb on seed germination, incipient growth and antioxidant enzymes of six common used turfgrasses was investigated. The results showed that after implementation of different concentrations (0, 1000, 2000 and 3000  $\mu\text{M}$ ) of Pb, *Agropyron elongatum* exhibited the highest germination percentage (90%) under 3000  $\mu\text{M}$  Pb, followed by *Lolium perenne* L. (85.56%). The lowest germination parameters including germination vigor, rate, index and germination percentage was found in *Festuca ovina*, which means it is the most sensitive species to Pb than the other studied turfgrasses. In contrast, *F. rubra*, *F. ovina*, *A. elongatum* and *L. perenne* had the most significant aerial growth and the lowest chlorophyll degradation even at high lead concentration. An increase in *L. perenne* and *A. elongatum* antioxidant enzymes activities as well as a decrease in *F. ovina* observed after exposure to 3000  $\mu\text{M}$  Pb which correlated to their tolerance and sensitivity to Pb respectively. However, such increase in antioxidant activity was also found in *F. Rubra* and *Poa pratensis* but could not inhibit chlorophyll degradation and seedling growth retardation. It might support that there is suggesting the probable existence of a lead-injury mechanism rather than the oxidative stress. Overall, *A. elongatum* is introduced as the most tolerant turfgrass among studied ones, which could be cultivated in Pb-polluted sites.

**Key words:** *Agropyron*, Antioxidant enzymes, *Festuca*, Lead pollution, *Lolium*, *Poa*

### INTRODUCTION

Heavy metal-contaminated soil and water are serious worldwide problems at recent years that progressively increase. Lead (Pb) is the most common heavy metal contaminant which has been caused a great concern because of its wide spread occurrence, toxic nature, and long life in biological system. It originates from both natural sources and anthropogenic activities and causes noticeably annual reduction of crops productivity (Duruibe *et al.* 2007). Lead toxicity results in several damages from interception of germination to

retardation of plant growth in herbages (Lamhamdi *et al.* 2011) and tree species (Lin *et al.* 2005). Perturbations in cellular metabolism (Pandey & Sharma 2002), alteration in membrane permeability (Lukatkin *et al.* 2013), decrease in electron transport activity (Heyno *et al.* 2008), enzymes activities enhancement or inhibition (Shi & Zhu 2008), and particularly the elevated production of reactive oxygen species (ROS) (Steffens 2014) have been reported as consequences of plant exposure to excessive heavy metals. Moreover, seed germination, the initial event in the plant life,

has been considerably influenced by the heavy metal stresses (Moosavi *et al.* 2012). Turf, a plant with high irrigation requirement, is commonly used in landscapes at widespread scales throughout the world (Romero & Dukes 2014). Several factors such as water deficiency, water pollution, and heavy metal-contaminated soils have strongly restricted the plant germination and even subsequent growth and development. Bidar *et al.* (2007) reported that *L. perenne* was sensitive to heavy metal toxicity than *Trifolium repens*. The tolerance of *Poa annua* to high concentrations of Cu in soil was shown by Brun *et al.* (2003). Vamerali *et al.* (2010) considered *Festuca* spp. and *Lolium* spp. for phytoremediation of heavy metals-polluted soils. Moreover, Gąbka & Wolski (2011) showed that *F. arundinacea* Schreb. cv. Spirit increased its biomass yield under elevated Pb levels. However, they did not biochemically examine on them, nor introduced the most tolerant cultivar to the heavy metals.

The cessation of seed germination and the growth retardation have been illustrated as a response to elevated concentrations of lead in *L. perenne* (Bidar *et al.* 2007), *Elsholtzia argyi* (Islam *et al.* 2007), *Leucaena leucocephala* (Shafiq *et al.* 2008), *Triticum aestivum* L. (Lamhamdi *et al.* 2013), *Cicer arietinum* (Goswami *et al.* 2013) and *Sesamum indicum* (Rao & Lakshmi 2014). However, the studies about the effects of lead on the seed germination of turfgrasses species are little.

Moreover, it has not been well understood to reveal the antioxidant enzymes profiles as determining factors in response of cold-season turfgrasses to various concentrations of lead. Bidar *et al.* (2007) evaluated only the superoxide dismutase (SOD) activity in *L. perenne* as affected by Cd, Zn, and Pb. Unfortunately, it has not been reported, to date, the analogy of tolerance/sensitivity of six commonly used cold-season turfgrasses, perennial ryegrass, tall fescue, blue sheep fescue, red fescue, kentucky bluegrass and agropyron, to lead-induced stress. Therefore, the present study was accomplished to

identify the most sustainable vegetation cover under toxic levels of lead for the employing of not only heavy metal-contaminated soils, but also wastewater in cultivation and irrigation purposes of the grasses. So, the germination and incipient growth parameters, as well as the biochemical analyses of the six types of turfgrass were evaluated and monitored under lead-induced stress.

The results of this study will be very helpful in phytomanagement of metal-polluted soils, and provide a tendency to the employing of lead-contained wastewater for irrigation of the grass in low water regions.

## MATERIALS AND METHODS

### Plant material

In the present study, the seeds of six turfgrasses; perennial ryegrass (*Lolium perenne*), tall fescue (*Festuca aurandinaceae*), blue sheep fescue (*Festuca ovina*), red fescue (*Festuca rubra*), kentucky bluegrass (*Poa pratensis*) and agropyron (*Agropyron elongatum*) were purchased from Pakan Bazr Company, Esfahan, Iran.

### Germination experiments

The first seeds were surface-sterilized with 5% (v/v) sodium hypochlorite for 10 minutes to prevent any fungal contamination, thereafter seeds were thoroughly rinsed with deionized water to ensure that no residual Na adhered to the seed surface and allowed to imbibe for 3 h. After water imbibition, the seeds were placed into Petri dishes (90 mm diameter) containing sterile filter sheets (Whatman No. 42) moistened with either 6 ml of distilled water as a control or Pb solutions (1000, 2000, 3000 µM). The lead solutions were freshly prepared by dissolving Pb (NO<sub>3</sub>)<sub>2</sub> in deionized water and adjusting their pH to 5.5 with HNO<sub>3</sub>. There were four replicates per each test concentrations and control. Petri dishes were kept in an incubator at 25 ± 2.5°C and 70% RH under a light irradiance of 300 mmol m<sup>-2</sup> s<sup>-1</sup> (12:12 h light: dark conditions), and the various parameters of germination and growth indices were recorded.

**Germination and seedling growth indices**  
The number of seeds germinated was determined in each treatment every 24 h intervals for 14 days using radicle protrusion (i.e., appearance of a radicle  $\geq 2$  mm in length) as a criterion (Gill *et al.* 2003), and the seed germination percent (GP) was calculated as follows: (number of seeds germinated/total number of seeds)  $\times 100$ . Day 14<sup>th</sup> of experiment was considered as the final day, because no more germination was observed and root and shoot length was measurable for all dishes. Germination rate (GR) was determined daily until the seedling stage (Ellis & Roberts 1980). Germination vigor (GV) and germination index (GI) were measured using following formulae: GV = seedling length (cm)  $\times$  GP, and GI = (GP/DT), where GP is the germination percentage on the T day, and DT is the day of germination. The length of shoots and roots were also recorded after two weeks from culturing.

### Biochemical analysis

#### Chlorophyll content

Weighed 0.5 g of leaf samples from controls and the different Pb treatments were homogenized in 20 ml 80% acetone. The absorbance of the supernatant measured at 663 and 645 nm using a Spectrophotometer (Ltd T80 + UV/VIS; PG Instruments, Leicestershire, UK). It was finally calculated according to Wellburn & Lichtenthaler (1984) and expressed as mg.g<sup>-1</sup> fresh weight (FW).

#### Preparations and assays of antioxidant enzymes

The leaves of the turfgrasses (200 mg) were homogenized in 1 ml ice-cold extraction buffer containing 50 mM phosphate buffer (pH 7), 1 mM EDTA and 1.5% (w/v) of PVP. The homogenate was centrifuged at 9000 g for 15 min. The supernatant was used as the crude extract for determination of enzyme activities (Zhang & Nan 2007).

The SOD activity was measured spectrophotometrically as described by Beyer & Fridovich (1987). The reaction solution (1 ml) contained 50 mM phosphate buffer (pH

7), 12 mM riboflavin, 13 mM methionine, 0.1 mM EDTA, 7 mM nitro blue tetrazolium (NBT) and 10  $\mu$ L of extracted enzyme solution. A solution with no enzyme was used as the control. Test tubes were irradiated under fluorescent lights at 100 mM m<sup>-2</sup> s<sup>-1</sup> for 20 min. The absorbance of each solution was measured at 560 nm using a spectrophotometer. One unit of enzyme activity was defined as the amount of enzyme that would inhibit 50% of NBT photo reduction.

The catalase (CAT) activity was assayed as reported by (Brouwer & Brouwer 1998). The reaction solution (0.5 ml) contained 25 mM phosphate buffer (pH = 7), 10 mM H<sub>2</sub>O<sub>2</sub> and 10  $\mu$ L of extracted enzyme solution. The reaction was initiated by adding the enzyme solution. Changes in absorbance at 240 nm were read every 10s for 60s using a spectrophotometer. One unit of CAT activity was defined as the absorbance change of 0.01 units per minute. The peroxidase (POD) activity was determined by the method of Chance & Maehly (1955). POD activity in leaves was assayed by the oxidation of guaiacol in the presence of H<sub>2</sub>O<sub>2</sub>. The increase in absorbance was recorded at 470 nm.

The reaction mixture contained 100  $\mu$ L of crude enzyme extract, 500  $\mu$ L of 5 mM H<sub>2</sub>O<sub>2</sub>, 500  $\mu$ L of 28 mM guaiacol, and 1900  $\mu$ L of 50 mM potassium phosphate buffer (pH 7.0). POD activity of the extract was expressed as activity U/g FW min.

#### Statistical analysis

Statistical analysis was performed using SAS software (Version 9.0). The significance of the differences among treatments was tested by One-Way ANOVA at  $p < 0.01$ . Values reported here are means of four replicates.

## RESULTS

#### Seeds germination characteristics

The effect of Pb concentrations on seed germination characteristics of different turfgrasses are summarized in Table 1. Germination index (GI) of studied turfgrasses was significantly affected by Pb concentrations. GI was significantly decreased

when Pb concentration increased in the solution except for *A. elongatum*. Among the lead concentrations, No significant difference was found for GI in *A. elongatum*. The lowest GI was observed in *F. ovina* when exposed to 3000 μM Pb (Table 1). Increasing Pb concentration in the solution caused a significant decreasing in germination percentage (GP) in the all turfgrass species. However no significant decreasing was found among Pb concentrations in *A. elongatum* and *L. perenne* (Table 1). The GP of the seeds of *F. ovina* was considerably affected by Pb treatments, so as it reduced from 93.6% in control to 8.9% in plants treated by 3000 μM Pb concentration. *A. elongatum* and *L. perenne* had the maximum GP about 90 and 85.56% respectively, whereas *F. ovina* showed only 8.9% GP. As shown in Table 1, by increasing Pb concentration in the solution, germination vigor (GV) also decreased in all studied turfgrass species, however, no significant difference was found between Pb concentrations in *A. elongatum*. The maximum and minimum GV were observed in *A. elongatum* and *F. ovina* as compared to their controls, with 57% and 97% reduction, respectively. The germination rate (GR) of *F. ovina* was more sensitive than that of other studied turfgrasses in response to the high Pb concentration in the solution, as shown by the 97% reduction at 3000 μM Pb solution.

The maximum GR was observed in *A. elongatum*, therefore this species could be less sensitive to Pb (Table 1).

### **Seedling shoot and root growth**

Pb significantly affected different turfgrasses seedling shoot and root length (Fig. 1a).

The root growth of all studied turfgrasses was significantly inhibited by Pb solutions. The highest suppressing effect was found in the *F. ovina* roots with 2 mm in length, while the lowest one was found in those of *A. elongatum* with 28mm in length (Fig 1a).

So that, it was suppressed in all turfgrasses as 50% as control at 1000 μM, except for *F. ovina*, which remained nearly unchanged.

This inhibition reached over 90% for the treatment with 3000 μM Pb. Unlike roots, the seedling shoots was less affected by Pb concentrations (Fig 1 b). No significant difference was found between 1000 μM Pb treated plants in comparison with control. But, the *F. rubra* and *F. ovina* shoots length significantly reduced after treatment with 2000 μM Pb solution. All turfgrass species (except for *L. perenne*) showed a significant reduction in their shoot length when exposure to 3000 μM Pb. The highest shoot growth was observed in *A. elongatum* with 96 mm in length comprising about 85% of control, whereas the minimum was in *F. rubra* with only 26 mm about 50% of control.

### **Chlorophyll content**

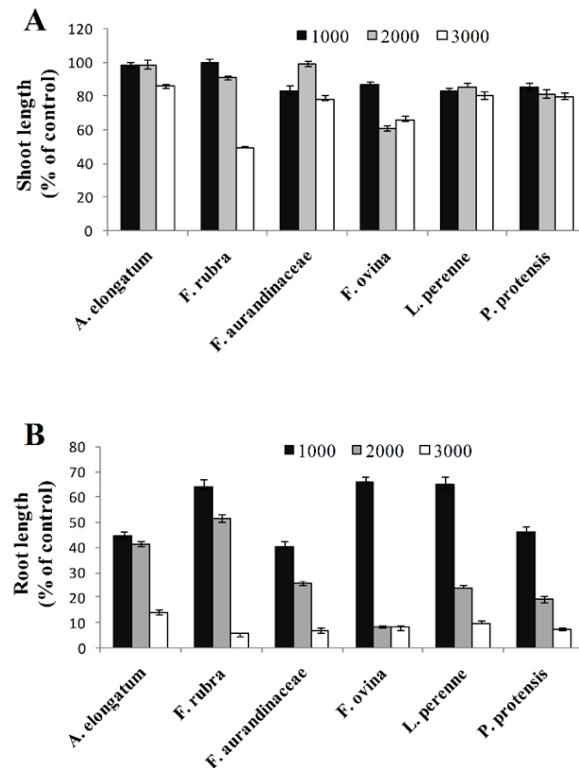
The chlorophyll content of all turfgrass species was significantly decreased with increasing Pb concentration in the solution, except for *A. elongatum* (Table 2). However, no significant difference was found between control and 1000 μM Pb from point view of chlorophyll content. The lowest chlorophyll content was found in *F. ovina*, when treated with 3000 μM Pb. It was declined as 45.8% as control in *F. ovina*. Therefore, this species could be the most sensitive turfgrass to the Pb contamination with respect to chlorophyll content.

### **Antioxidant enzymes activities**

Pb - induced oxidative stress was further confirmed by the dose-dependent increase in the antioxidant enzyme activities, as shown in Fig. 2A about the SOD activity in all Pb-treated seedlings, but *F. ovina*, tended to ascend when the lead concentration increased from 1000 to 3000 μM, in comparison with control. The SOD activity in *F. ovina* was significantly inhibited by the increasing of Pb concentration. The highest SOD activity was observed in *L. Perenne* with 263 U.g<sup>-1</sup> FW. As shown in Fig. 2B, except for *F. ovina*, the CAT activity continued to increase with the elevation of Pb concentration in all turfgrass species and peaked at the exposure of 3000 μM. So that, the maximum CAT activity was observed in *L. perenne* and *P. pratensis* (109 and 101 U.g<sup>-1</sup> FW,

respectively), whereas the minimum enzyme activity was shown in *F. ovina* (20.2 U. g<sup>-1</sup> FW). Moreover, when Pb concentration reached 3000 μM, CAT activity in *F. Rubra* and *A.*

*elongatum* became approximately 5.2 and 3.2-fold higher than in their controls respectively. Effects of Pb on POD activity are shown in Fig. 2C.

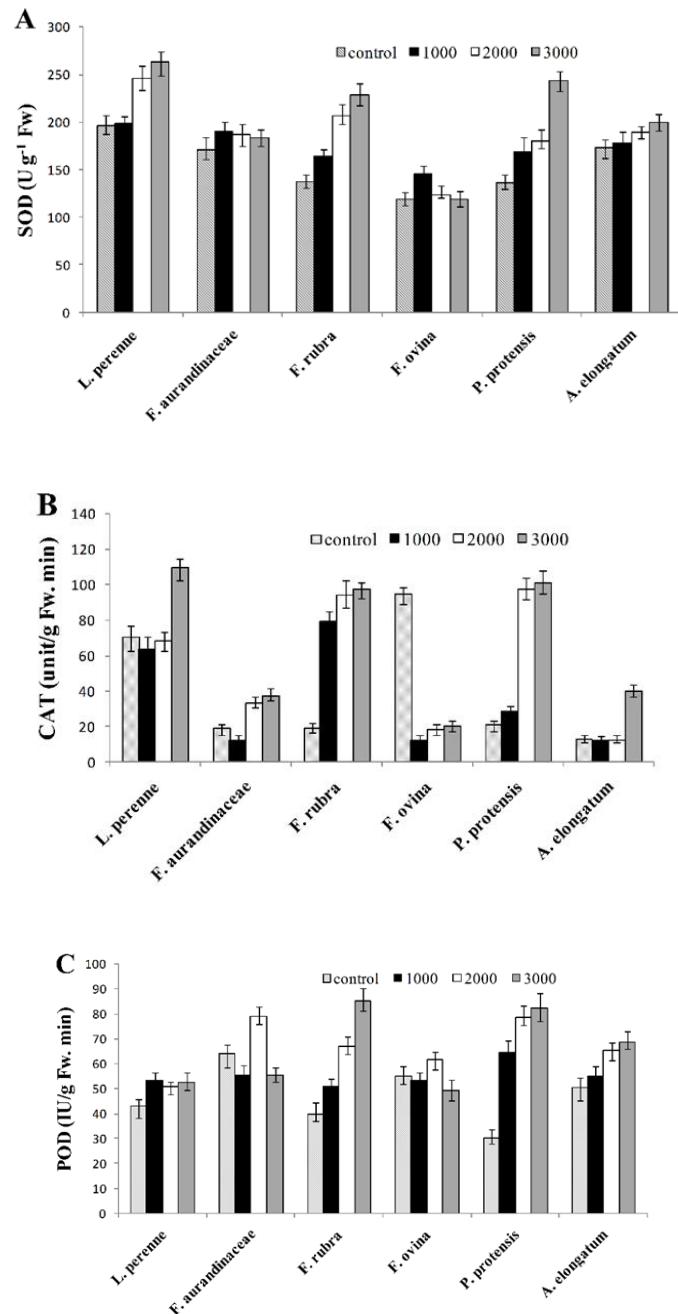


**Fig. 1.** Percentage decrease in shoot length (A) and root length (B) of turfgrass seedlings at different concentrations of Pb (NO<sub>3</sub>)<sub>2</sub> (1000, 2000 and 3000 μM). Bars indicated mean ± SE (n = 3).

**Table 1.** Germination index, percentage, vigor and rate of six turfgrass species in response to different concentrations of Pb.

Indices	Pb (NO <sub>3</sub> ) <sub>2</sub> concentration (μM)	<i>A. elongatum</i>	<i>F. rubra</i>	<i>F. aurandinaceae</i>	<i>F. ovina</i>	<i>L. perenne</i>	<i>P. pratensis</i>
Germination Index	0	35.1 <sup>aA</sup>	24.76 <sup>cA</sup>	22.7 <sup>cA</sup>	27.26 <sup>bA</sup>	32.86 <sup>abA</sup>	28.06 <sup>cA</sup>
	1000	36.4 <sup>aA</sup>	23.1 <sup>cA</sup>	22.8 <sup>cA</sup>	25.9 <sup>cA</sup>	30.8 <sup>bA</sup>	22.03 <sup>cB</sup>
	2000	33.1 <sup>aA</sup>	21.9 <sup>bA</sup>	20.33 <sup>bA</sup>	6.3 <sup>b</sup>	29.46 <sup>aA</sup>	15.26 <sup>bC</sup>
	3000	34.4 <sup>aA</sup>	17.7 <sup>cB</sup>	16.16 <sup>cB</sup>	2.7 <sup>b</sup>	25.5 <sup>bB</sup>	11.03 <sup>dC</sup>
Germination percentage	0	88.33 <sup>aA</sup>	84.11 <sup>aA</sup>	87.13 <sup>aA</sup>	93.36 <sup>aA</sup>	91.1 <sup>aA</sup>	88.23 <sup>aA</sup>
	1000	88.86 <sup>aA</sup>	81.86 <sup>aA</sup>	69.46 <sup>bb</sup>	89.96 <sup>aA</sup>	89.9 <sup>aA</sup>	64.43 <sup>bB</sup>
	2000	86.46 <sup>aA</sup>	59.03 <sup>cB</sup>	65.56 <sup>bcBC</sup>	38.86 <sup>dB</sup>	85.33 <sup>aB</sup>	58.9 <sup>cC</sup>
	3000	88 <sup>aA</sup>	49.13 <sup>bC</sup>	60 <sup>bC</sup>	8.9 <sup>cC</sup>	83.56 <sup>aB</sup>	47.8 <sup>bD</sup>
Germination vigor	0	26111 <sup>aA</sup>	10176 <sup>cA</sup>	13084 <sup>bA</sup>	10753 <sup>cA</sup>	14069 <sup>bA</sup>	10473 <sup>cA</sup>
	1000	17823 <sup>aB</sup>	7162.2 <sup>cB</sup>	7088.86 <sup>cB</sup>	7843.3 <sup>cB</sup>	10227.2 <sup>bB</sup>	6771.1 <sup>cB</sup>
	2000	14421 <sup>aC</sup>	6766.6 <sup>BC</sup>	7102.2 <sup>cB</sup>	1410 <sup>eC</sup>	8858.6 <sup>bBC</sup>	3420 <sup>dC</sup>
	3000	12262.8 <sup>aC</sup>	4344.4 <sup>cC</sup>	3935.5 <sup>cC</sup>	360 <sup>dC</sup>	6090.9 <sup>bC</sup>	2957.7 <sup>cC</sup>
Germination rate	0	9.2 <sup>aA</sup>	4.93 <sup>cA</sup>	4.46 <sup>cA</sup>	5.26 <sup>bcA</sup>	6.86 <sup>bA</sup>	4.06 <sup>cA</sup>
	1000	11 <sup>aA</sup>	4.56 <sup>cA</sup>	4.2 <sup>cA</sup>	5 <sup>cA</sup>	6.76 <sup>bA</sup>	4.63 <sup>cA</sup>
	2000	8.7 <sup>aA</sup>	3.6 <sup>cAB</sup>	3.83 <sup>cAB</sup>	1.46 <sup>dB</sup>	6.1 <sup>bAB</sup>	3.3 <sup>cB</sup>
	3000	10 <sup>aA</sup>	2.6 <sup>cB</sup>	2.5 <sup>cB</sup>	0.66 <sup>dB</sup>	5.03 <sup>bB</sup>	2.6 <sup>cB</sup>

Means in each row and column followed by different letters were significantly different (P< 0.01 level). Capital and small letters show statistical differences for data in columns and rows, respectively.



**Fig. 2.** The activities of SOD (A), CAT (B) and POD (C) of six turfgrass seedlings treated with 0, 1000, 2000 and 3000  $\mu\text{M}$  Pb ( $\text{NO}_3$ )<sub>2</sub>. Data are expressed as mean  $\pm$  SE ( $n = 4$ ).

**Table 2.** Shoot chlorophyll content of six turfgrass species ( $\text{mg g}^{-1}$  fresh weight) when exposed to different Pb concentrations.

Pb ( $\text{NO}_3$ ) <sub>2</sub> concentration ( $\mu\text{M}$ )	<i>A. elongatum</i>	<i>F. rubra</i>	<i>F. aurandinaeae</i>	<i>F. ovina</i>	<i>L. perenne</i>	<i>P. pratensis</i>
0	4.25 <sup>a</sup>	4.38 <sup>a</sup>	5.49 <sup>a</sup>	3.72 <sup>a</sup>	3.91 <sup>a</sup>	3.35 <sup>a</sup>
1000	4.21 <sup>a</sup>	4.34 <sup>a</sup>	5.67 <sup>a</sup>	3.69 <sup>a</sup>	3.92 <sup>a</sup>	3.31 <sup>a</sup>
2000	4.12 <sup>a</sup>	3.75 <sup>b</sup>	4.97 <sup>b</sup>	2.67 <sup>b</sup>	3.66 <sup>ab</sup>	2.73 <sup>b</sup>
3000	4.01 <sup>a</sup>	3.19 <sup>c</sup>	4.28 <sup>c</sup>	2.02 <sup>c</sup>	3.39 <sup>b</sup>	2.16 <sup>c</sup>

Values within the same column with different letters are significantly different at  $P < 0.01$ .

As found in the results, POD activity increased significantly in the most of turfgrass species when exposure to the higher Pb concentrations. For *P. pratensis* and *F. rubra* seedlings, POD activity significantly increased in 3000 µM (276.65 and 214.27% of controls, respectively) in comparison with that in the other Pb concentrations. But, by increasing the Pb concentration from 2000 to 3000 µM, POD activity was significantly declined in *F. ovina* and *F. aurandinaceae*.

## DISCUSSION

The plant seed germination and seedling growth can be regarded as general responses associated with heavy metal toxicity (Kopyra & Gwóźdż 2003; Radić *et al.* 2010). In the present study, seed germination-related parameters of all tested turfgrasses, except for *A. elongatum* and *L. perenne* were significantly affected by Pb-induced stress. Sengar *et al.* (2009) have reported that inhibition of seed germination may result from the interference of Pb with germination-involved enzymes. In contrast, in *A. elongatum* and *L. perenne*, both seed GP and GV as well as the incipient growth of roots and shoots were not considerably affected by Pb toxicity. The *F. ovina* seed germination was notably inhibited more than that of other turfgrasses at higher Pb concentrations; its root growth was not as reduced as that of *P. pratensis*, *F. aurandinaceae* and *F. rubra*. Gąbka & Wolski (2011) Have showed that *F. ovina* "Noni" was able to accumulate heavy metals in their tissues, but its general aspect (aesthetic value) became worse than that of non-heavy metal - accumulated plants.

According to finding of the present study, the terrestrial parts of all Pb-treated turfgrasses were more strictly affected by Pb than the aerial ones. It has been well established that the roots growth is more sensitive to metal toxicity than seed germination, because they are the primary targets of metal ions. Hence, root testing is widely used for evaluating the toxicity levels of heavy metals (Márquez-García *et al.* 2013). This is confirmed by the

results of the present study, so the roots of all turfgrasses were affected particularly under the highest Pb concentration.

The inhibition of root growth by Pb has also been shown to affect nitrogen assimilation through the enzymes nitrate reductase and glutamine synthetase in *Cucumis sativus* and *Glycine max*, respectively (Sharma & Dubey 2005).

*A. elongatum* and *L. perenne* had most appropriate growth even under high lead concentrations which may be resulted from less chlorophyll degradation. In contrast, the other turfgrass such as *F. ovina* and *F. rubra* showed enhanced chlorophyll decomposition at elevated level of Pb. So, their shoots were eventually dried. Reduction in chlorophyll contents by excess Pb has been reported in *Oryza sativa* L. (Lou *et al.* 2007) and *Zea mays* L. (Ekmekeci *et al.* 2009). It has been revealed that Pb can displace metal cofactors from metalloenzymes, which includes Mg in the chlorophyll porphyrin ring, resulting in interference with photosynthesis by reducing chlorophyll levels (Patra *et al.* 2004). Sharma & Dubey (2005) have also found that the key chlorophyll biosynthesis enzymes were also strongly inhibited by Pb, as well as many enzymes in the Calvin cycle followed by reducing the rate and efficiency of CO<sub>2</sub> fixation.

It has been established that Pb toxicity results in enhanced ROS generation (Verma & Dubey 2003). ROS are dangerous primarily due to reaction with lipids, proteins, and nucleic acids (Halliwell & Gutteridge 2011). In the present study, the POD, CAT and SOD activities in *A. elongatum* were less than those in *F. rubra*, which had the lowest growth. It could indicate that the antioxidant enzymes activity manner was not in accordance with *F. rubra* responses to exogenous Pb toxicity. Contrary to *F. rubra* and *F. ovina*, *A. elongatum* and *L. perenne* had either the most aerial growth or the less chlorophyll degradation even at high Pb concentration. An increase in the *L. perenne* and *A. elongatum* antioxidant enzymes as well as a decrease in those of *F.*

*ovina* observed after exposure to 3000 µM Pb, were in accordance with their tolerance and sensitive, respectively, indicating a strong relationship between Pb tolerance and antioxidant systems in the grasses. Inversely, such increase in *F. rubra* and *P. pratensis* could not prevent from chlorophyll degradation and growth reduction. It may be due to the probable existence of a Pb-injury mechanism rather than the oxidative stress. *A. elongatum* and *L. perenne* tolerance mechanism to high Pb concentrations could be widely attributed to high efficient of antioxidant enzymes, particularly CAT and SOD. According to the finding of Malecka *et al.* (2001), although Pb is not a redox metal and cannot generate ROS directly, it cause oxidative stress indirectly as shown by the increased lipid peroxidation in rice and pea plants exposed to the metal. According to our finding, SOD and CAT activities are more strongly related to the Pb tolerance capabilities than to the POD activity. The increased antioxidant activities particularly in *L. perenne*, *A. elongatum* after exposure to the high concentration of Pb may indicate that the turfgrasses cells presented endogenous protective effects and the antioxidant enzymes were induced to prevent against Pb-induced ROS. However, our results showed that the efficiency of antioxidant enzymes activities is species-dependent in counteracting the adverse effects of Pb phytotoxicity. It was not in parallel to that of wheat seedlings reported by Yang *et al.* (2010). They have reported that appropriate growth of wheat seedlings exposed to lead toxicity was only due to the ROS scavenging by highly activated antioxidant enzymes.

According to its tolerance under Pb stress condition, *A. elongatum* seems to have a strong defense system during both seed germination and subsequent seedling growth. It may be due to the perturbation in Pb ion entrance into the tissues resulted from more thickness of the turfgrass seed coat in comparison with that of any other studied turfgrasses. Krammer & Colville (2011) have found that the influences of metals on seed germination in different

plants depend mainly on interspecies differences in seed structure, particularly the seed coat anatomy. Jian-Ling & Li-Qiang (2014) have also reported that seed coat plays a crucial role in protecting seed to access the unique ability for obstruction and retention of Pb. In addition, it has been reviewed that Pb inhibited germination widely in species with Pb-permeable seed coats (Krammer & Colville 2011). It is in consistent with our seed germination results which indicated that the seeds of *A. elongatum*, apart from thick coat, may have a substantially higher threshold for toxicity than other turfgrasses species. Overall, *A. elongatum* and *L. perenne* not only had much seed germination under high Pb concentrations, but also showed better growth during subsequent stages. It may be hypothesized that there was a relationship between seed germination sensitivity/tolerance and that of subsequent plant growth to lead stress.

## CONCLUSION

In conclusion, Pb treatment reduces seed germination parameters and seedling growth, especially root growth of the six tested turfgrasses. The results also showed that those species having much seed germination under high Pb concentrations are more tolerant during subsequent growth. Root growth was more sensitive to Pb than seedlings shoot. An increase in *L. perenne* and *A. elongatum* antioxidant enzymes after exposure to higher Pb concentrations followed by both less chlorophyll degradation and less growth reduction could indicate that Pb tolerance was associated with antioxidant enzymes in the grasses. Vice versa, such increase in *F. rubra* and *P. pratensis* could not prevent chlorophyll degradation and growth reduction. In our opinion, either Pb toxicity threshold or its mechanism was species-dependent. Among the six turfgrasses, *A. elongatum* was the most tolerant, while *F. ovina* was the most sensitive to Pb as indicated by their seed germination, growth, chlorophyll content and antioxidant enzymes activities. Therefore, *A. elongatum*

could be considered as a promising species in Pb - contaminated sites for both aesthetic and phytoremediation purposes.

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## ارزیابی پاسخ‌های شش گونه چمن سردسیری به سمیت سرب جهت انتخاب گونه‌های با تحمل ک. غلامی‌پور فرد<sup>۱</sup>, م. قاسم‌نژاد<sup>۲\*</sup>, ه. زکی‌زاده<sup>۱</sup>, م. کافی<sup>۲</sup>, ف. رجالی<sup>۳</sup>

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### چکیده

سرب یکی از خط‌ناک‌ترین فلزات سنگین است که در سال‌های اخیر مشکلات جدی برای اکوسیستم به وجود آورده است. در این مطالعه تاثیرات سرب بر روی جوانه‌زنی بذر، رشد اولیه و آنزیم‌های آنتی‌اکسیدانی شش گونه چمن سردسیری که به طور رایج استفاده می‌شوند، مورد پژوهش قرار گرفت. پس از کاربرد غلظت‌های مختلف نیترات سرب (صفر، ۱۰۰۰، ۲۰۰۰ و ۳۰۰۰ میکرومولار) گونه‌ی علف‌گندمی بلند با ۹۰٪ و به دنبال آن گونه‌ی لولیوم با ۸۵/۵۶٪، بیشترین درصد جوانه‌زنی را با تیمار ۳۰۰۰ میکرومولار سرب نشان دادند. کمترین پارامترهای جوانه‌زنی شامل بنیه بذر، سرعت، شاخص و درصد جوانه‌زنی به ترتیب با ۳۶۰، ۰/۶۶، ۰/۰۷ و ۰/۸٪ در فستوکای آبی مشاهده شد که به این معنی است که این گونه حساس‌ترین گونه نسبت به دیگر چمن‌های مورد مطالعه است. در مقایسه با فستوکای قرمز و فستوکای آبی، گونه‌های علف‌گندمی بلند و لولیوم با ۹۶٪ و ۶۴ میلی‌متر به ترتیب با حدود ۸۵ و ۸۱٪ نسبت به شاهد، بیشترین میزان رشد اندام هوایی و کمترین میزان تجزیه کلروفیل را حتی در غلظت بالای سرب نشان دادند. افزایش فعالیت آنزیم‌های آنتی‌اکسیدانی در گونه‌های علف‌گندمی بلند و لولیوم و همچنین کاهش فعالیت این آنزیم‌ها در گونه‌ی فستوکای آبی پس از در معرض قرار گیری با غلظت ۳۰۰۰ میکرومولار سرب مشاهده شد. فعالیت آنزیم‌های آنتی‌اکسیدانی همراه با پارامترهای جوانه‌زنی ممکن است با میزان تحمل و حساسیت گونه‌ها به سرب مرتبط باشد. اگرچه این افزایش در فعالیت آنتی‌اکسیدانی در گونه‌های فستوکای قرمز و پوا نتوانست از تجزیه کلروفیل و تاخیر رشد گیاه‌چه‌ها جلوگیری کند. این موضوع ممکن است بیشتر وجود یک مکانیسم آسیب‌رسان سرب را تایید کند تا اینکه موید یک تنفس اکسیداتیو باشد. در مجموع بین ۶ گونه چمن مورد مطالعه، گونه‌ی علف‌گندمی بلند به عنوان پرتحمل‌ترین گونه معرفی شد و می‌تواند در مکان‌های آلوده به سرب کاشته شود.

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