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Research Article

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Response of Six Turf Species to Cadmium Stress: Displaying the Most Tolerant Cultivar

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Abstract

This study was accomplished to monitor the interspecific differences in response to cadmium (Cd) stress among six turfgrass species through investigating the seed germination, seedling growth and some biochemical characteristics. After an environmentally controlled experiment under different Cd treatments (0, 100, 200 and 300 μM), *Agropyron elongatum* showed the highest germination percentage (80.33%) under 300 μM Cd, followed by *Lollium perenne* with 72.59%. The lowest germination parameters including germination vigor, index and percentage were found in *Festuca ovina,* which referred as the more sensitive species to Cd than other studied turfgrasses. The highest shoot growth followed by the lowest chlorophyll degradation was observed in *Agropyron elongatum*, *Festuca rubra* and *Lolium perenne* respectively. Furthermore, the low chlorophyll degradation and the high seedling growth in turf species were correlated with increasing in some antioxidant enzymes activities. It might display the crucial indirect effect of Cd, that is, oxidative stress. Overall, *Agropyron elongatum* is introduced as the most tolerant cool-season turfgrass among studied species, which could be considered for engaging in advanced studies of heavy metal stresses.

Keywords

Agropyron; Antioxidant enzymes; *Festuca*, *Lollium*; *Poa*

Introduction

Contamination of soil and water with toxic metals is severe environmental problem [1]. Heavy metals are mainly considered as both natural and anthropogenic-induced stress, caused by weathering of rocks, industrial effluents and agricultural runoffs [2]. Cadmium (Cd) is one of the most phytotoxic heavy metals to plants because it is highly soluble in water and promptly taken up by plants, and can interfere with numerous biochemical and physiological processes including photosynthesis, respiration, nitrogen and protein metabolism, and nutrient uptake [3]. Since it is active at concentrations much lower than those of other toxic elements, Cd could greatly influence the plants germination and growth, such as decreasing the amount of chlorophyll, depressing photosynthesis, disturbing the uptake and translocation of mineral nutrients, dwarfing plants, inhibiting the growth of roots, decreasing biomass, and even leading to death in some severe cases [4,5].

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It has been reported that seed germination and early seedling growth are crucial in plant life and are extremely responsive to the environmental alterations [6]. Delay in germination has been often observed after exposure to heavy metals [7]. This can be associated with disorders in germinating metabolism, which is a highly complex multistage process regulated at the physiological, biochemical and molecular programs [8]. Moreover, Cd as a non-redox metal is unable to participate in Fenton-type reactions, but it causes oxidative stress by generating reactive oxygen species [9]. It has also been reported that great variation exists in heavy metal accumulation and tolerance among plant species [10]. Therefore, selection or development of Cd tolerant genotypes through screening in germination as well as biochemical and physiological variations in plants under different concentrations of Cd would be promising approaches to decrease the flow of Cd from contaminated soils and water into the food chain and to ameliorate Cd-induced stress.

Turf, a plant with high irrigation requirement, is commonly used in landscapes at widespread scales throughout the world [11]. 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. [12] reported that *L. perenne* was more sensitive than *Trifolium repens* to heavy metal toxicity. González [13] showed that some grass species such as *Festuca arundinacea*, *F. rubra genuina*, *F. rubra fallax* and *Lolium perenne* were able to hyper-accumulate Cu, Ni, Zn, Pb and Cd in their tissues, but the more tolerant species among them were remained unknown.

The cessation of seed germination and the growth retardation have been illustrated as a response to elevated concentrations of Cd in *Triticum aestivum* [14], *Poa pratensis L.* [15], *Oryza sativa L.* [16], *Sorghum bicolor L.* [17], *Peganum harmala* and *Halogeton glomeratus* [18], soybean [19], *Elymus dahuricus* [20], *F. arundinacea* and *P. pratensis* [21]. However, most studies about the effects of Cd on seed germination and growth of turfgrass species have been relied only on morphological parameters not biochemical ones.

Moreover, the role of antioxidant enzymes as determining factors in response of cool- season turfgrasses to various concentrations of Cd has not been well understood. Bidar et al. [12] evaluated only superoxide dismutase (SOD) activity in *L. perenne* as affected by Cd, Zn, and Pb. Xu and Wang [21] also reported only growth parameters in *P. pratensis* and *F. arundinacea* under Cd exposure.

So far, the analogy of tolerance/sensitivity of six commonly used cool-season turfgrasses, perennial ryegrass, tall fescue, blue sheep fescue, red fescue, kentucky bluegrass and agropyron, to Cd-induced stress, has not been reported. Therefore, the present study was accomplished to identify the most sustainable ground cover under toxic levels of Cd for use not only in heavy metal-contaminated soils, but also in cultivations which are irrigated using waste water.

Materials and Methods

Plant material

In the present study, the seeds of six turf species; perennial ryegrass (*Lollium 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 and took to the Horticultural laboratory of University of Guilan, Iran.

Germination experiments

The first seeds were surface-sterilized using 5% (v/v) sodium hypochlorite for 10 min., followed by thoroughly rinsing with sterile deionized water and then 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 Cd solutions (100, 200, 300 µM). The cadmium solutions were freshly prepared by dissolving $CdCl_2$ in deionized water and their pH was adjusted to 7.0–7.2. There were four replicates per each treatment. Petri dishes were kept in an incubator at 25 ± 2.5 ˚C and 70% RH under a light irradiance of 30 mmol m^{-2} s^{-1} (12/12 h light/dark conditions), and the various parameters of germination and growth indices were recorded after two weeks from culturing.

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 ≥ 2 mm in length) as a criterion [22], and the seed germination percent (GP) was calculated as follows: (number of seeds geminated/total number of seeds) × 100. Day 14^{th} of the experiment was considered as the final day, because no more germination was observed and roots and shoots length were measurable for all dishes. Germination rate (GR) was determined daily until the seedling stage [23]. Germination vigor (GV) and germination index (GI) were measured using following formulae: GV=seedling length (cm) × GP, and GI=(GP/DT), where GP is the germination percentage on the T days, 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 Cd treated seedlings were homogenized in 20 mL 80% acetone. The absorbance of the supernatant measured at 663 and 645 nm using a Spectrophotometer (Itd T80 + UV/VIS; PG Instruments, Leicestershire, UK). It was finally calculated according to Wellburn and Lichtenthaler [24] and expressed as mg g^{-1} 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 [25]. The SOD activity was measured spectrophotometrically as described by Beyer and Fridovich [26]. The reaction solution (1 mL) contained 50 mM phosphate buffer (pH=7), 12 mM riboflavin, 13 mM methionine, 0.1mM EDTA , 7 mM nitro blue tetrazolium (NBT) and 10 µL of extracted enzyme solution. A solution with no enzyme was used as the control. Test tubes were irradiated under fluorescent lights at 100 mmol m^{-2} s^{-1} 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 percent of NBT photo reduction.

The catalase (CAT) activity was assayed as reported by Brouwer and Brouwer [27], The reaction solution (0.5 mL) contained 25 mM phosphate buffer (pH=7), 10 mM H_2O_2 and 10 µL of extracted enzyme solution. The reaction was initiated by adding the enzyme solution. Changes in absorbance at 240 nm were read every 10 s for 60 s 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 and Maehly [28]. POD activity in leaves was assayed by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance was recorded at 470 nm. The reaction mixture contained 100 µL of crude enzyme extract, 500 µL of 5 mM H_2O_2, 500 µL of 28 mM guaiacol, and 1,900 µL 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 germination indices (germination index, percentage, vigor and rate) of different turfgrass seeds treated with $CdCl_2$ are summarized in Table 1. Germination index (GI) of all studied turfgrasses was significantly affected by Cd concentrations. With increasing Cd levels in the solution GI decreased in the all studied species, except for *A. elongatum*. The maximum and minimum GI was observed in *A. elongatum* and *F. ovina*, as compared to their controls, with 6.01% and 47.24% reduction, respectively.

As the Table 1 shows, the germination percentage (GP) of seeds declined, when Cd level in the solution increased from 100 to 300 µM. However no significant difference was found for GP in *L. perenne* and *F. aurandinaceae* when exposed to Cd from 0 to 200 µM. Furthermore, no significant difference was found between Cd levels in *A. elongatum*. The lowest GP was observed in *F. ovina*, when exposed to 300 µM Cd (Table 1).

The germination vigor (GV) of all studied turf species was significantly decreased when Cd concentration increased in the solution (Table 1). The GV of the seeds of *F. aurandinaceae* was considerably affected by Cd treatments, so as it reduced from 13084 in control to 5280 in plants treated by 300 µM Cd. In other words, declined 59.64% compared to control. But *L. perenne* had the maximum GV about 35% compared to control when exposed to 300 µM Cd.

Effects of Cd on germination rate (GR) are shown in Table 1. Except for *F. rubra*, no significant difference was found among control, 100 and 200 µM Cd treated turf seeds in GR. But, with increasing Cd level from 200 to 300 µM, GR was significantly declined in all turfgrass species except for *A. elongatum*. Therefore, this species could be the less sensitive to Cd heavy metal.

Seedlings shoot and root growth

Shoot and root length of all studied species were decreased when exposed to Cd treatment as compared to control (Figure 1A and 1B). The highest dose of Cd (300 µM) markedly hindered root length. The highest suppressing effect of Cd contamination on root elongation was found in *F. aurandinaceae* (about 91% of control) and the lowest

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

		Turfgrass species					
Indices	**$CdCl_2$ concentration (µM)**	***Agropyron elongatum***	***Festuca rubra***	***Festuca aurandiance***	***Festuca ovina***	***Lolium perenne***	***Poa pratensis***
	0	35.1aA	24.76cA	22.7cA	27.26bA	32.86abA	28.06cA
Germination	**100**	34.51aA	23bcAB	20.04cA	23.58bcA	31.94aA	25.49bAB
Index	**200**	33.7aA	20.6bcBC	19.13bcA	18.75cB	30.61aAB	22.92bB
	300	32.99aA	18.7cC	15.68dB	14.38dC	27.93bB	19.89cB
	0	88.33aA	84.11aA	87.13aA	93.36aA	91.1aA	88.23aA
Germination	**100**	86.71aA	76.18bB	75.19bB	77.16bB	87.61aAB	77.93bB
percentage	**200**	83.56aAB	66.98bC	71.43bB	56.49cC	81.51aB	66.81bC
	300	80.33aB	58.4cD	56.09cC	37.18dD	72.59bC	53.36cD
	0	26111aA	10176cA	13084bA	10753cA	14069bA	10473cA
Germination	**100**	18969aB	8362.1bcAB	10966bA	7684.2cA	10871bB	8053bcAB
vigor	**200**	16487aB	7168bBC	5528.1cB	6395cB	9362bB	6925cB
	300	14297aB	5841.6cC	5280cB	4911.6cB	9078.3bB	5221.7cB
	0	9.2aA	4.93cA	4.46cA	5.26bcA	6.86bA	4.06cA
Germination	**100**	9.51aA	4.38cAB	4.31cA	5.18bcA	6.5bA	4cA
rate	**200**	9.66aA	3.7cB	4.06cA	4.59cAB	6.1bA	3.85cAB
	300	9.73aA	3.54cB	3.48cB	4.07bcB	5.3bB	3.01cB

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.

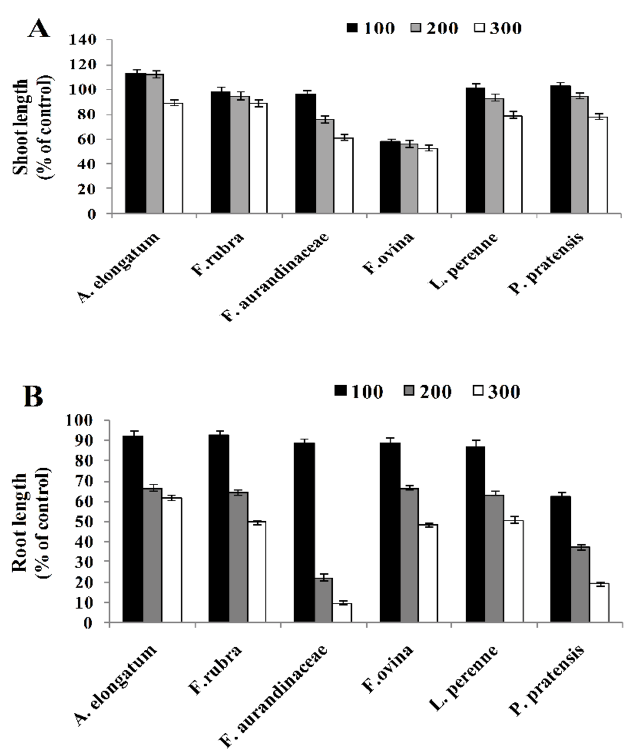

Figure 1: Percentage decrease in shoot length (**A**) and root length (**B**) in *Agropyron elongatum, Festuca rubra, Festuca aurandiance, Festuca ovina, Lolium perenne and Poa pratensis* seedlings seedlings at different concentrations of $CdCl_2$ (100, 200 and 300 µM). Bars indicated mean ± SE (n=3).

one was in *A. elongatum* roots with 70.87 mm about 38% of control in length (Figure 1A).

Shoot growth was also decreased with increasing Cd concentrations in Petri dish solution. Unlike roots, the seedling shoots were less sensitive to Cd (Figure 1B). No significant difference was found between 100 µM Cd treated seedlings and control for shoot growth except for *F. ovina*. Thereafter, with increasing Cd level from 100 to 200 µM, shoot length was significantly declined in *F. aurandinaceae*, *F. ovina* and *L. perenne*. Except for *F. rubra*, all turfgrass species showed a significant reduction in their shoot length when exposed to 300 µM Cd. The highest shoot growth was observed in *F. rubra and A. elongatum* with 89.09% and 87.58% of control, respectively, and the minimum was found in *F. ovina* with 53.22% of control.

Chlorophyll content

Leaf chlorophyll contents of the six turfgrass species was generally declined with increasing Cd concentrations (Table 2). However, no significant difference was found between control and 100 µM Cd in some turfgrass species. The lowest chlorophyll content was found in *F. ovina*, when treated with 300 µM Cd. It was declined as 45.96% as control in *F. ovina*, therefore, this species could be the most sensitive turf to Cd contamination for chlorophyll content.

Antioxidant enzymes activities

Effects of Cd stress on SOD activity are shown in Figure 2A. As the results showed, SOD activity gradually increased when Cd concentration increase from 100 to 300 µM and the maximum activity was assayed at 300 µM Cd, although there were no statistical difference. The maximum SOD activity was observed in *L. perenne* and *A. elongatum* (234 and 217 U g^{-1} Fw, respectively) after exposure to 300 µM Cd, whereas the minimum activity was shown in *F. ovina* (125 U g^{-1} Fw).

CAT activity was also increased significantly in the most of turfgrass species, when exposed to 100 and 200 µM Cd (Figure 2B), but it significantly decreased at 300 µM. CAT activity was continuously suppressed in *F. ovina*, and this inhibition was strengthened as Cd concentrations were increased. Also in *F. aurandinaceae* seedlings, CAT activity was significantly decreased at 300 µM. The highest increase in CAT activity was observed in *A. elongatum*, as compared to its control.

Table 2: Shoot chlorophyll content of six turfgrass species (mg/g fresh weight) when exposed to different Cd concentrations.

			Turfgrass species			
$CdCl_2$ concentration (μM)	***Agropyron elongatum***	***Festuca rubra***	***Festuca aurandiance***	***Festuca ovina***	***Lolium perenne***	***Poa pratensis***
0	4.25abA	4.38abA	4.92aA	3.72bA	3.91bA	3.95bA
100	4.18abAB	4.2abAB	4.79aAB	3.49bA	3.82bA	3.85bA
200	3.85abAB	3.69bB	4.25aB	2.69cB	3.51bAB	2.81cB
300	3.51aB	3.46bC	3.48aC	2.01cC	3.09bB	2.076cC

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.

POD activity tended to increase when Cd concentration increased from 100 to 200 μM, but suppressed at 300 μM Cd in the all turf species except for *F. aurandinaceae* (Figure 2C). The highest and lowest increase in POD activity at 300 μM Cd were observed in *A. elongatum* and *F. ovina*, about 33% and 13%, respectively with respect to their controls. POD activity in *F. aurandinaceae* significantly increased and reached its peak value at 300 μM.

Discussion

Seed germination and early growth performance of plants are usually considered in evaluating the toxicity of heavy metals [29]. In the present study, seed germination response of different turf species to Cd treatment was completely species dependent. Only *A. elongatum* and *L. perenne* have been shown to germinate even under the highest level (300 μM) of Cd. All seed germination parameters, except for GR, were significantly affected by high concentrations of Cd. Previous study also showed the toxic effects of the heavy metal on the seed germination and seedling growth Sengar et al. [30] have reported that inhibition of seed germination may result from the interference of Cd with germination-involved enzymes. Decrease in seed germination of plant can be attributed to the accelerated breakdown of stored nutrients in seed and alterations of selection permeability properties of cell membrane, due to negative effects of heavy metals [31]. The seed germination of *F. ovina* was inhibited more than that of other studied species at higher Cd levels; however, its root growth was not as reduced as that of *P. pratensis* and *F. aurandinaceae*. Gąbka and Wolski [32] showed that *F. ovina* "Noni" was able to accumulate heavy metals in its tissues, but its general aspect (aesthetic value) became worse than that of non-heavy metal accumulated plants.

In general, the terrestrial parts of all Cd-treated turfgrasses were more strictly affected than the aerial parts. These results have been certified by other researchers. It has been well established that root growth is more sensitive than seed germination to metal toxicity, because they are the primary targets of metal ions, and hence root testing is widely used for evaluating the toxicity levels of heavy metals [33]. In this study, the roots of six turfgrass species were affected when high levels of Cd were applied. Shafiq et al. [31] have been explained that the reduction in root length of *Leucaena leucocephala* under Cd treatments could be due to the reduced mitotic cells in meristematic zone of root. Lerda (1992) had similar observations in roots of onion (*Allium cepa*). The exact reason for different responses of root growth to heavy metals is not known but might be due to either the rapid accumulation of heavy metals in root than shoot or the faster rate of detoxification in shoot than root [31].

F. rubra and *A. elongatum* had an appropriate growth even under high Cd concentrations which may be resulted from less chlorophyll degradation. In contrast, the other species such as *F. ovina* and *P. pratensis* showed enhanced chlorophyll decomposition at elevated levels of Cd, so as their shoots were eventually died. Reduction in chlorophyll contents by excess Cd has been reported in *Bacopa monnieri* and hybrid Bermudagrass, which might be due to the interaction of Cd to –SH group of enzymes of chlorophyll biosynthesis.

It has been well established that Cd toxicity results in enhanced reactive oxygen species (ROS) generation [3]. ROS are dangerous primarily due to reaction with lipids, proteins, and nucleic acids [34]. In this study, POD, CAT and SOD activities were further in more tolerant species such as *A. elongatum* and *F. rubra* than sensitive ones such as *F. ovina* and *F. aurandinaceae*. It could indicate that the antioxidant enzymes activities manner was in accordance with the growth of turfgrasses exposed to exogenous Cd toxicity. In addition, an increase in antioxidant enzymes of *A. elongatum* and *F. rubra* as well as a decrease in those of *F. ovina* observed after exposure to 300 μM Cd were in accordance with their tolerance and sensitivity, respectively, might indicate a strong relationship between Cd tolerance and antioxidant system in these grasses. Zhang et al. [3] also reported that *Vicia sativa* was more tolerant to Cd than *Phaseolus aureus* because of higher leaf symplastic SOD and APX activities. Therefore, the more tolerance to Cd levels in *A. elongatum* and *F. rubra* could be related to the higher efficiency of antioxidant enzymes. Zhang et al. [35] have reported that Cd stress induces intracellular oxidizing conditions leading to the production of ROS, as shown by the increased lipid peroxidation in *Achnatherum inebrians* exposed to the heavy metal. According to our findings, CAT and POD activities were more strongly related to the Cd tolerance capabilities than SOD. The increased antioxidant activities particularly in *A. elongatum* and *F. rubra* after high concentration Cd exposure may indicated that the turfgrasses cells presented endogenous protective effects and the antioxidant enzymes were induced to prevent against Cd-induced ROS. However, our results showed that the tolerance induction to Cd stress through antioxidant enzymes activities was species-dependent. Moreover, It was in parallel to that of kenaf (*Hibiscus cannabinus*) seedlings reported by Li et al. [36]. They have reported that appropriate growth of kenaf seedlings when exposed to Cd toxicity can be due to the ROS scavenging by highly activated antioxidant enzymes.

According to these results *A. elongatum* seems to have a strong defense system during both seed germination and subsequent seedling growth. It might be due to the perturbation in Cd ion entrance into the tissues resulted from its thicker seed coat in comparison with that of any other studied turfgrasses. Kranner and Colville [37] have found that the influences of metals on seed germination in different plants depend mainly on interspecies differences in seed structure, particularly seed coat anatomy. Jian-Ling and Li-Qiang [38] have also reported that seed coat plays a crucial role in protecting seed for having the unique ability to obstruction and retention of the heavy metal. It is consistent with our seed germination results indicating that seeds of *A. elongatum*, apart from thick coat, may have a substantially higher threshold for toxicity than other studied species. Overall, *A. elongatum* and *F. rubra* not only had the more seed germination under high Cd concentrations, but also showed

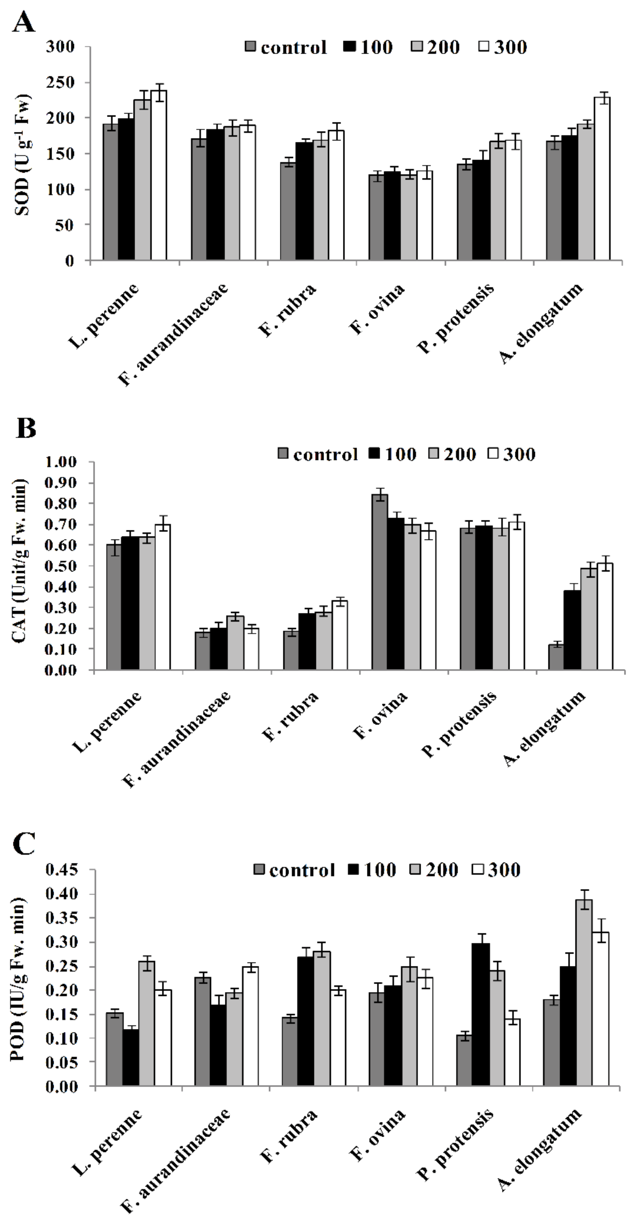

Figure 2: The activities of SOD (A), CAT (B) and POD (C) in *Agropyron elongatum, Festuca rubra, Festuca aurandiance, Festuca ovina, Lolium perenne and Poa pratensis* seedlings treated with 0, 100, 200 and 300 μM $CdCl_2$. Data are expressed as mean ± SE (n=4).

the better growth during subsequent stages. It may be hypothesized that there was a relationship between seed germination sensitivity/tolerance and subsequent plant growth to Cd stress.

Conclusion

In conclusion, the response of cool-season turfgrasses to Cd stress was species dependent. Cd treatment, particularly at high concentrations influenced seed germination and growth rate of the studied turfgrasses. Our results demonstrated that the higher seed germination was correlated with the more tolerance to the contamination during early seedling growth. In all studied species, shoot growth was not influenced as much as root growth to Cd treatment. The higher antioxidant enzymes (CAT, SOD, and POD) activities could relatively mitigate the adverse effects of Cd, and keep on their appropriate growth. It might disclose the important role of the side-effect of Cd, that is, oxidative stress. In general, based on the different assessments from seedling growth to chlorophyll content, the tolerance of the six studied turfgrasses was ordered as follows; *A. elongatum* > *F. rubra* > *L. perenne*> *P. pratensis* > *F. aurandinacea*> *F. ovina*. Overall, *A. elongatum*, from a morpho-physiologically and biochemically point of view, could be introduced as the most tolerant turfgrass, which could be used not only for cultivation in Cd-polluted sites, but also for more researches on the deciphering of the exact mechanisms of the heavy metal tolerance.

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