

EFFECTS OF LEAD POLLUTION ON GERMINATION AND SEEDLING GROWTH OF TURFGRASS, *CYNODON DACTYLON*

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Abstract

Lead (Pb) pollution in soils often triggers a catastrophic impact on crop growth and creates concerns related to food safety while seriously affecting agricultural production. Turfgrass, which produces a large amount of biomass, has been widely grown as a heavy metal sink. The present study evaluates the germination and seedling growth responses of turfgrass, *Cynodon dactylon*, to different levels of Pb(NO₃)₂. The results indicated that 50–300 mg/kg of Pb(NO₃)₂ did not significantly influence germination. The germination percentage and index gradually decreased with an increase of Pb(NO₃)₂ from 300–1000 mg/kg. The growth of root, shoot, as well as contents of chlorophyll a and b decreased with increasing Pb concentrations. The contents of soluble sugar, proline, MDA, SOD, and POD activity increased with an increase of Pb(NO₃)₂. When the concentration of Pb was below 500 mg/kg, the activity of CAT would first increase and then decrease. The high tolerance and accumulation of Pb by *C. dactylon* make it a useful accumulator of Pb. Therefore, this species will be useful in the phytoremediation and revegetation of Pb contaminated soil.

Key words: *Cynodon dactylon*; Germination; Heavy metal; Lead; Phytoremediation; Turfgrass.

Introduction

Human activity has resulted in heavy metal pollution released from old mines, metal-related industries, and some rural areas where automotive exhaust pollutes the soil along roads and highways, as well as in land contaminated by fertilizers containing heavy metal (Wierzbicka & Obidzińska, 1998; Chen *et al.*, 2015). Lead is often an abundant toxic element in soil that has no biological function (Kushwaha *et al.*, 2018). Numerous products contain Pb, such as rubber toys, pottery, pesticides, paints, newsprint, glass, hair dyes, and gasoline (Shafiq *et al.*, 2008). The Agency for Toxic Substances and Disease Registry lists Pb as the element posing the second greatest potential threat to human health. Lead accumulates in human bodies, which can lead to fungal infection, chronic fatigue, allergies, gastrointestinal discomfort, joint and muscle pain, short-term memory loss, and headache (Jarvis & Leung, 2001; Kushwaha *et al.*, 2018). Early exposure to lead poisoning can cause potential multisystem disease susceptibility, low IQ, and lifelong behavioral disorders (Rodriguez-Barranco *et al.*, 2013; Sears *et al.*, 2012).

Heavy metals enter biological cycles and food chains through plants (Wierzbicka & Obidzińska, 1998). The exposure of plants to Pb can result in cellular damage and homeostasis disturbance of cell ions (Yoon *et al.*, 2006), which can damage lamellar tissues in chloroplasts and negatively affect chlorophyll production, transpiration, seedling development, and plant growth (Sharma & Dubey, 2005; Sengar *et al.*, 2009; Maestri *et al.*, 2010; Gupta *et al.*, 2010). Moreover, after accumulation in various parts of plants, Pb will enter food chain. Lead pollution in soil generally results in a reduction in biodiversity and damage to vegetation structure, poor soil patches occur, and trees grow sparsely or are even absent (Kushwaha *et al.*, 2018).

Conventional methods (excavation and landfill) or chemical treatment used to mitigate and repair Pb pollution are not only resource- and time-consuming. These methods are also likely to destroy the original habitat of plants and

induce secondary pollution. In recent years, phytoremediation has provided a new strategy with great potential for the remediation of pollutants by green plants. Blaylock *et al.*, (1997) used bioremediation to treat 1 acre of Pb polluted soil, saving 50–65% of remediation costs when compared with traditional methods. In phytoremediation, metal in the soil will be absorbed and transported to aboveground, toxic metals will be absorbed and concentrated in roots from polluted soils, and plants will decrease the mobility of heavy metal in the soil (phytostabilization) (Blaylock *et al.*, 1997; Maestri *et al.*, 2010; Kushwaha *et al.*, 2018). Studies of phytoremediation of soil contaminated by Pb demonstrate that Pb can accumulate in some plants at high levels (Huang *et al.*, 1997; Xiong, 1998; Sears *et al.*, 2012). Moreover, the extraction rate of Pb can be greatly improved by using synthetic chelates to treat contaminated soil (Huang *et al.*, 1997).

Turfgrass provides an important component of urban landscaping, which is characterized by having the advantages of strong adaptability, strong stress resistance, potent fertility, and a wide range of distribution (Bell, 2011). With a large planting area and large biomass, turfgrass has a good potential for use in urban environmental restoration. Soil heavy metal content can be gradually reduced through repeated clipping of turfgrass; this method can be used to achieve the goal of heavy metal removal by plants (Qu *et al.*, 2003).

Cynodon dactylon, also known as Bermudagrass, is a primary warm-season turfgrass. Its potential range extends to the south and center of the transition zone (Taliaferro, 1995; Bell, 2011). This species is widely used for golf courses, sports fields, school grounds, parks, roadsides, and lawns (Bell, 2011). Our study aimed to determine the ability of *C. dactylon* to tolerate soil toxicity and its ability to accumulate Pb. Specifically, the following three questions were answered: (1) Is *C. dactylon* tolerant to Pb during germination? (2) At what point will an increasing level of Pb in soil result in a decrease in the growth of *C. dactylon*? (3) Does elevated soil Pb influence the contents of Pb in different organs of *C. dactylon*?

Materials and Methods

Seed collection: Freshly matured caryopsis (seeds) of *C. dactylon* “Bermuda” (hereafter *C. dactylon*) were collected from the campus of Yangzhou University (32°23'23"N, 119°25'10"E; 10 m a. s. l) in early September 2018. Before the experiment, seeds were dried in room conditions (20–25°C). Seed germination experiments were carried out within two weeks after seed collection.

Measurement of the effect of Pb(NO₃)₂ on germination of *C. dactylon* seeds: Germination experiments were started on September 18, 2018. The seeds were placed in 5 cm Petri dishes. Next, 2.5 mL of 50, 100, 300, 500 and 1000 mg/kg Pb(NO₃)₂ and distilled water was used to moisten the filter paper. Four replicates of 25 seeds were used in each Pb(NO₃)₂ concentration. Each dish was sealed with parafilm and then the dishes were incubated under a 12/12 h light/dark at 15/25°C for 30 days. A radicle length of ≥1 mm was the standard used to indicate germination. The velocity of germination was calculated following the method of Jiang *et al.*, (2018). Seed variability were determined based on Baskin *et al.*, (2014) at the end of each experiment.

Effect of Pb(NO₃)₂ on *C. dactylon* seedling growth and pigment contents: After 30 days of germination in Petri dishes, seedlings *C. dactylon* were collected and separated into roots and shoots. Root and shoot length of each seedling were measured with a Vernier caliper, and the root/shoot length ratio was calculated. The chlorophyll (Chl) a and b content were measured by spectrophotometry with wavelengths of 663, 645, and 470 nm according to the methods of Lichtenthaler (1987).

Effect of Pb(NO₃)₂ on the activities of SOD, CAT, and POD of *C. dactylon* seedlings: After 30 days of germination in Petri dishes, activities of SOD, CAT and POD were assayed [For more detail see García-Limones *et al.*, (2002)]. The activity of SOD was assayed according to its ability to inhibit the photochemical reduction of nitro blue tetrazolium. Ten mM permanganate titration was used to measure the residual H₂O₂ in the reaction solution and estimate the activity of CAT. Peroxidase activity was determined by the absorbance change rate of the reaction solution at 470 nm.

Effect of Pb(NO₃)₂ on the soluble sugar, proline, and MDA content of *C. dactylon* seedlings: After 30 days of germination in Petri dishes, soluble sugar and proline content were measured spectrophotometrically at 630 nm and 520 nm, respectively, according to Li (2000). MDA content was assayed according to Duan *et al.*, (2005) and Jiang *et al.*, (2018).

Pb accumulation of *C. dactylon* plants: After germination fresh seedlings were washed with tap water, divided into roots and shoots, and dried in an oven for 48 h at 75°C. Each dried sample was ground, digested in concentrated HNO₃ for 12 hours, then Pb content was determined according to Xiong (1998).

Statistical analysis: Percentage data were arcsin transformed prior to further analysis. The effects of different Pb concentrations on germination, root and shoot length, chlorophyll content, soluble sugar, proline, and MDA, activities of SOD, CAT, and POD, and the concentration of Pb in plant tissues were tested through one-way analysis of variance. If analysis of variance indicated significant effects ($p < 0.05$), the differences between treatments were determined by carrying out Duncan's test.

Results

Effects of Pb(NO₃)₂ on germination of *C. dactylon* seeds: The germination percentage and index of *C. dactylon* seeds were significantly affected by the Pb(NO₃)₂ concentration. Germination percentages and indices decreased as Pb(NO₃)₂ concentrations increased (Fig. 1). As the concentration of Pb(NO₃)₂ increased from 0 to 1000 mg/kg, the germination percentage of *C. dactylon* seeds decreased from 91% to 27%. At low or moderate concentrations of Pb(NO₃)₂ (50–300 mg/kg), the germination percentage of *C. dactylon* seeds remained high (>80%). The regression between the germination of *C. dactylon* seeds and the concentrations of Pb(NO₃)₂ was: $y = 94.47 - 0.06x$ ($R^2 = 0.97$). The critical (at ≥50% germination) and limit (0% germination) values in the simulation were 741 mg/kg and 1574 mg/kg.

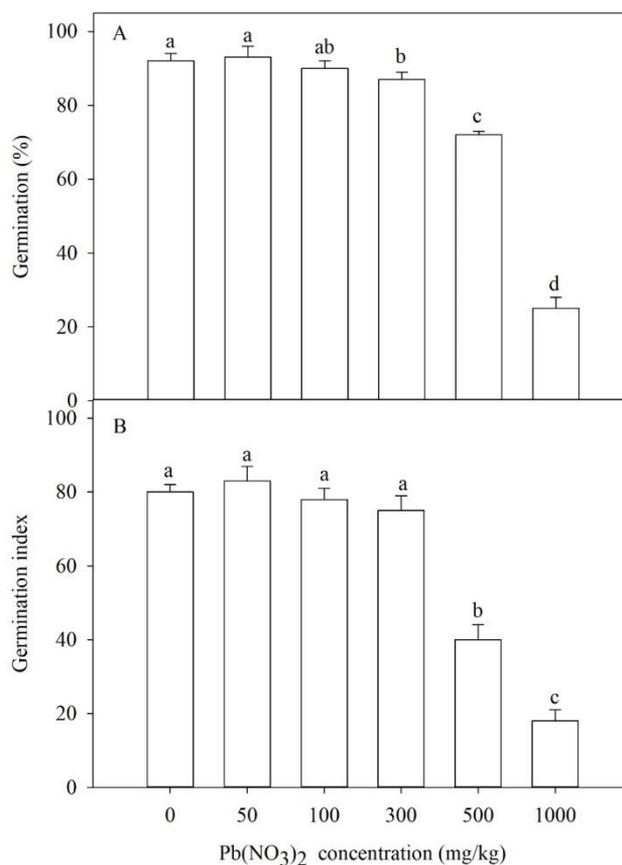


Fig. 1. Effect of Pb(NO₃)₂ on germination percentages (A) and germination index (B) of *Cynodon dactylon* seeds. Different letters indicate significant differences in germination percentages or germination index among different Pb(NO₃)₂ concentrations with Duncan's test ($p < 0.05$).

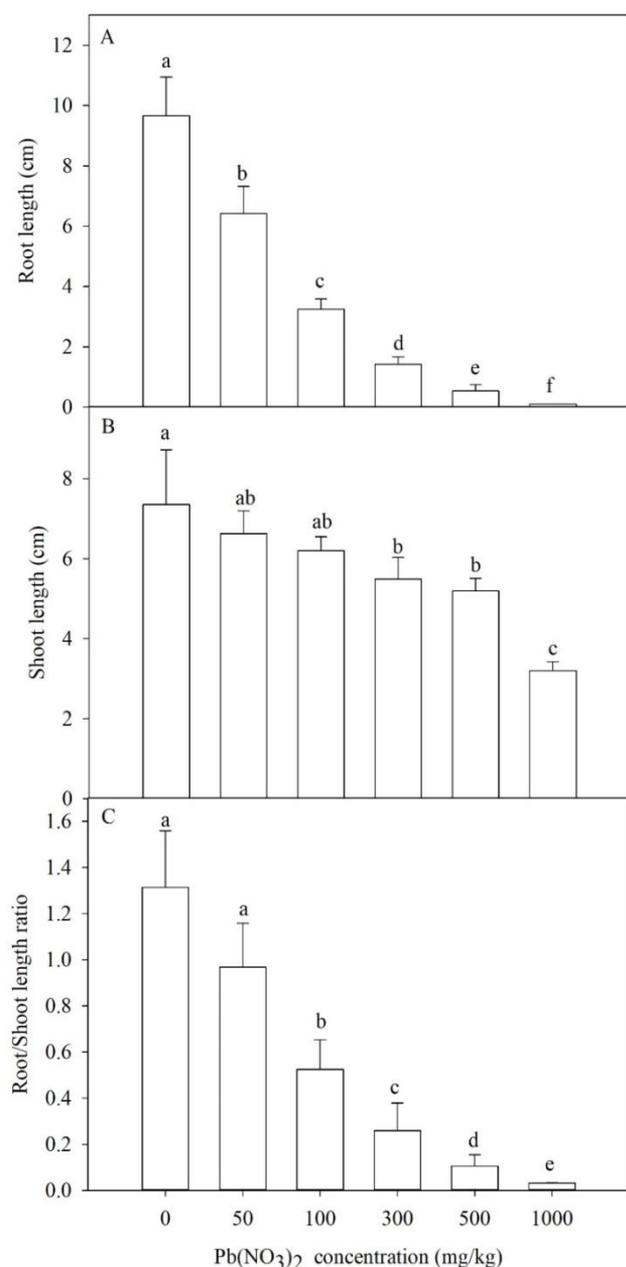


Fig. 2. Effect of Pb(NO₃)₂ on root length (A), shoot length (B) and root/shoot length ratio (C) of *Cynodon dactylon* seeds. Different letters indicate significant differences in each measure among different Pb(NO₃)₂ concentrations with Duncan's test ($p < 0.05$).

Effects of Pb(NO₃)₂ on *C. dactylon* seedling growth and pigment content:

Root and shoot length and their ratio in *C. dactylon* were significantly affected by Pb(NO₃)₂ concentration. With an increase of Pb(NO₃)₂ concentration, length of root (Fig. 2A), and root/shoot length ratio (Fig. 2C) decreased significantly. However, length of shoot did not differ with 0–100 mg/kg Pb(NO₃)₂, but only decreased at 300, 500, and 1000 mg/kg Pb(NO₃)₂ (Fig. 2B). When compared with 0 mg/kg Pb(NO₃)₂, the reductions of length of root, shoot, and root/shoot length ratio were 97.03%, 50.06% and 96.15% under 1000 mg/kg Pb(NO₃)₂, respectively.

Chlorophyll a was not affected by 50, 100, 300 mg/kg concentrations of Pb(NO₃)₂ (Fig. 3A). However, for seedlings grown at 500 and 1000 mg/kg Pb(NO₃)₂,

Chl a decreased significantly. Chlorophyll b was significantly affected by Pb(NO₃)₂ concentration. With the increase of Pb(NO₃)₂ concentration, the contents of Chl b decreased significantly (Fig. 3B). When compared with 0 mg/kg Pb(NO₃)₂, the reductions of Chl a and Chl b were 74.20% and 49.18% under 1000 mg/kg Pb(NO₃)₂, respectively.

Effects of Pb(NO₃)₂ on the activities of SOD, CAT, and POD of *C. dactylon* seedlings:

Activities of SOD, CAT, and POD of *C. dactylon* seedlings were significantly affected by Pb(NO₃)₂ concentration. The SOD activities of *C. dactylon* seedlings increased with increasing concentrations of Pb(NO₃)₂ (Fig. 4A). Compared with seedlings grown in distilled water, SOD activities increased by 90%, 64%, 129%, 101%, and 104% at 50, 100, 300, 500, and 1000 mg/kg Pb(NO₃)₂, respectively. Catalase activity displayed an increasing trend at 0–500 mg/kg Pb(NO₃)₂, and decreased at 1000 mg/kg Pb(NO₃)₂. When the concentration of Pb(NO₃)₂ was 500 mg/kg, CAT activity increased 87.11%. However, it reduced by 12.54% at 1000 mg/kg (Fig. 4B). The POD activity increased with an increase of Pb(NO₃)₂ concentration (Fig. 4C), increasing by 169.57% at 500 mg/kg Pb(NO₃)₂ (Fig. 4C).

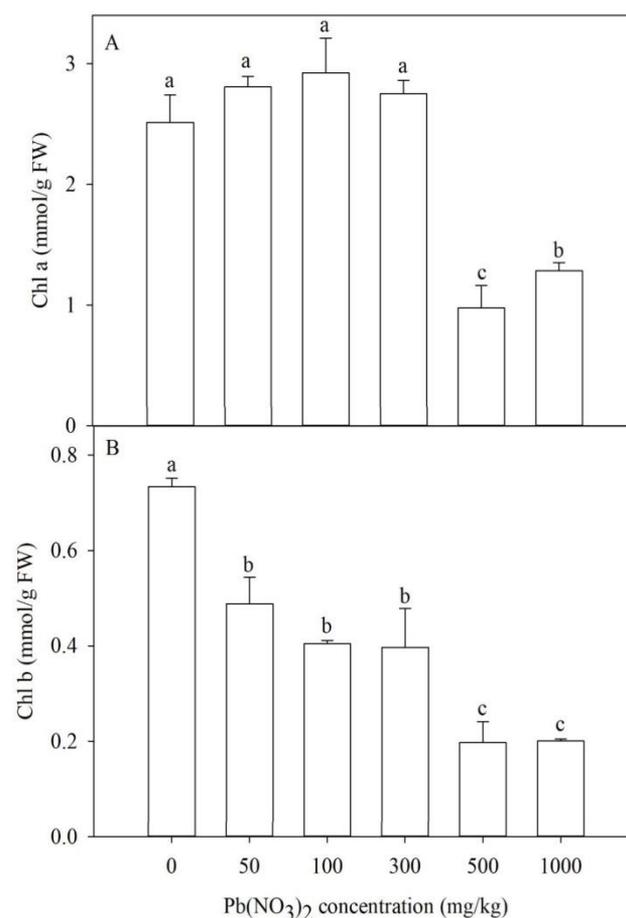


Fig. 3. Effect of Pb(NO₃)₂ on chlorophyll a content (A) and chlorophyll b content (B) of *Cynodon dactylon* seedlings. Different letters indicate significant differences in each measure among different Pb(NO₃)₂ concentrations with Duncan's test ($p < 0.05$).

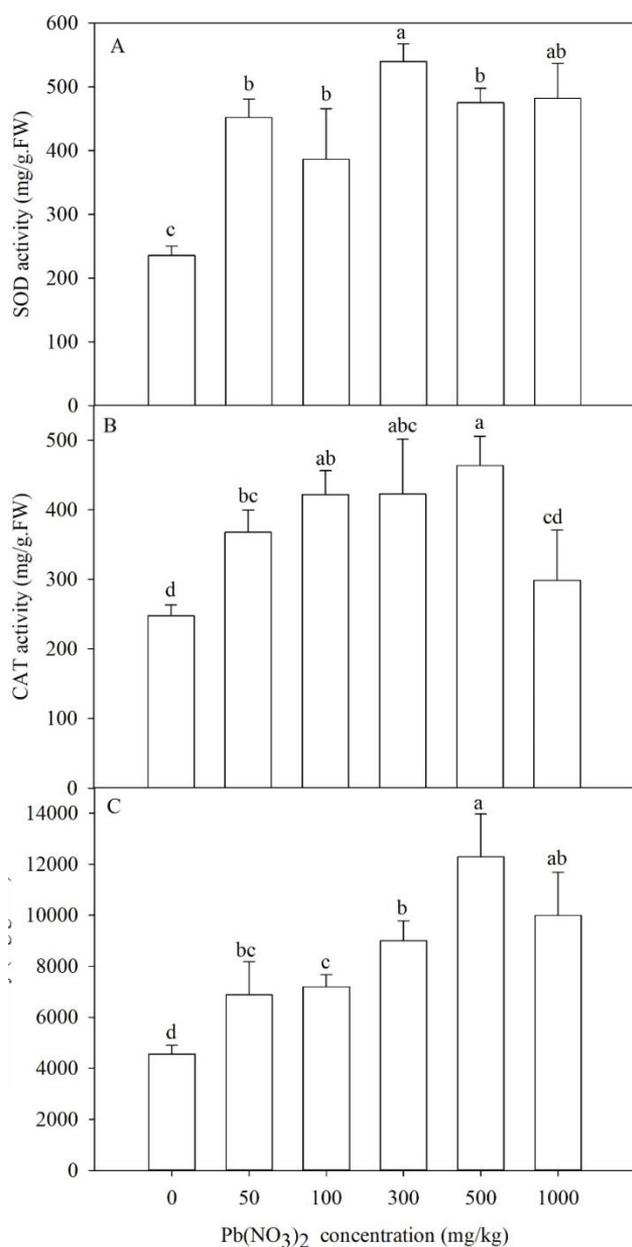


Fig. 4. Effect of Pb(NO₃)₂ on the activities of SOD (A), CAT (B), and POD (C) of *Cynodon dactylon* seedlings. Different letters indicate significant differences in each measures among different Pb(NO₃)₂ concentrations with Duncan's test ($p < 0.05$).

Effects of Pb(NO₃)₂ on the soluble sugar, proline, and MDA content of *C. dactylon* seedlings: Soluble sugar, proline, and MDA content *C. dactylon* were significantly affected by Pb(NO₃)₂ concentration (Fig. 5). With an increase of Pb(NO₃)₂ concentration, content of soluble sugar, proline, and MDA increased. Compared with 0 mg/kg Pb(NO₃)₂, contents of soluble sugar, proline, and MDA increased 117.58%, 196.28% and 114.33%, respectively, under 1000 mg/kg Pb(NO₃)₂.

Pb accumulation of *C. dactylon* plants: The accumulation of Pb in *C. dactylon* seedlings root and shoot were significantly influenced by Pb(NO₃)₂ concentration. With the increase of Pb(NO₃)₂ concentration, the Pb concentration of both root and shoot increased (Fig. 6).

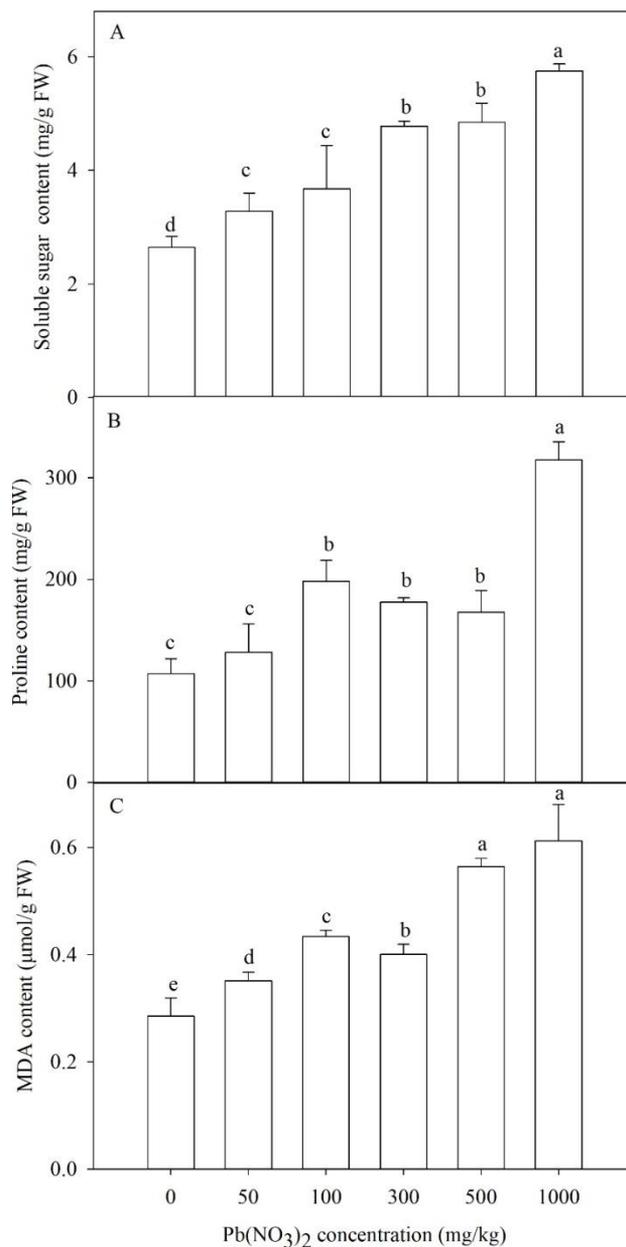


Fig. 5. Effect of Pb(NO₃)₂ on soluble sugar (A), proline (B) and MDA (C) content of *Cynodon dactylon* seedlings. Different letters indicate significant differences in each measures among different Pb(NO₃)₂ concentrations with Duncan's test ($p < 0.05$).

Moreover, the Pb concentration in *C. dactylon* shoots was higher than that in *C. dactylon* roots (Fig. 6).

Discussion

Turfgrass is prevalent in golf courses, athletic fields, parks, institutional, commercial and residential lawns, and urban landscape (Milesi *et al.*, 2005; Liu *et al.*, 2008; Bell, 2011). Recent studies have shown that turfgrasses can promote the degradation of organic pollutants (Fiorenza *et al.*, 2000; Qu *et al.*, 2003; Duo *et al.*, 2005; Cheng *et al.*, 2007). However, the tolerance of turfgrass to heavy metals and its metal absorption ability have seldom been evaluated. Our data demonstrate the high tolerance of *C. dactylon* seeds to Pb during germination. Therefore, this species can be planted in soil contaminated by Pb.

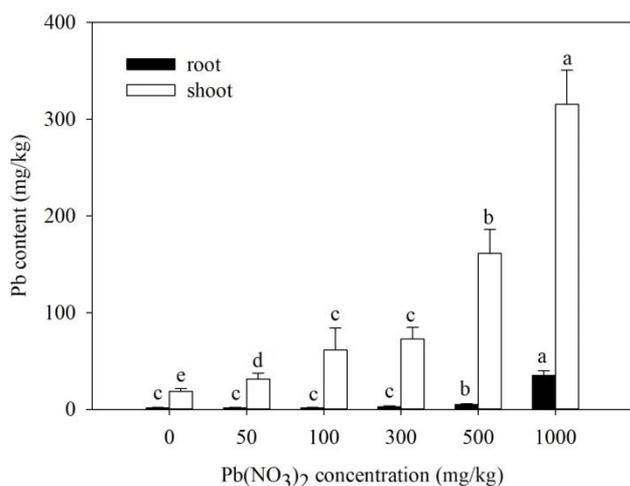


Fig. 6. Effect of $\text{Pb}(\text{NO}_3)_2$ on Pb content in root and shoot of *Cynodon dactylon* seedlings. Different letters indicate significant differences in Pb contents of root or shoot with Duncan's test ($p < 0.05$). A significant difference was observed between the Pb content of root and shoot for each $\text{Pb}(\text{NO}_3)_2$ concentration ($p < 0.05$).

Germination is a key stage in plant life history (Jiang *et al.*, 2018). Gaining a better understanding the effects of Pb solutions on seed germination is of great importance for accumulating knowledge about the revegetation of soil contaminated by Pb through the direct seeding method. The results of the present study demonstrate *C. dactylon* seeds have a germination rate of 50% for 731 mg/kg $\text{Pb}(\text{NO}_3)_2$ at 15/25°C (Fig. 1). Obviously, this species has a high tolerance level to Pb. Germination of *Lepidium sativum* and *Sinapis alba* seeds decreased when the concentration of Pb was above 0.01%, and was totally inhibited when the concentration reached 0.5% (Piotrowska *et al.*, 2009; Baskin & Baskin, 2014). Germination of *Eruca sativa* seeds in 1.5 and 2 mmol/L Pb was slightly higher than that in 0 mM Pb (control). However, in 2.5, 3.0, and 3.5 mmol/L Pb, its germination is significantly lower than that in the control (Faheed, 2005).

At 100 mg/kg $\text{Pb}(\text{NO}_3)_2$, root length decreased significantly (Fig. 2). The root and shoot were different in length between the control and 1000 mg/kg $\text{Pb}(\text{NO}_3)_2$. Therefore, it seems that root has higher sensitivity to Pb. Similar results have also been reported in *Brassica pekinensis* (Xiong, 1998), *Sesamum indicum* (Kumar *et al.*, 1993), *Sinapis alba* (Fargašová, 1994), and in *Lactuca sativa* and *Raphanus sativus* (Nwosu *et al.*, 1995). Roots are more sensitive to heavy metals because they are the absorptive organ which is affected earlier and subjected to heavy metal accumulation when compared with shoots. This also explains why the length of root is commonly used to evaluate the ability of plants to tolerate heavy metals (Xiong, 1998).

Lead stress causes the inhibition of photosynthesis in plants (Xiong *et al.*, 2006; Cenkci *et al.*, 2010). The chlorophyll level in several plant species decreased under the impact of heavy metals. The total chlorophyll content of the two wheat varieties decreased by 50% and 70% under the application of Cd and Pb, respectively (Oncel *et al.*, 2000). In *C. dactylon*, both Chl a and Chl b decreased with an increase of $\text{Pb}(\text{NO}_3)_2$ concentration

(Fig. 3). The inhibition of the enzymes activities responsible for chlorophyll biosynthesis is likely to decrease the content of chlorophyll related to heavy metal stress (Vodnik *et al.*, 1999).

The tolerance of *C. dactylon* to Pb during germination can also be reflected by different physiological parameters. Organic solutes determine the osmotic adjustment that occurs in plants (Martino *et al.*, 2003). Seeds of *C. dactylon* accumulated high levels of soluble sugar and proline during germination under different Pb treatments (Fig. 5). Obviously, *C. dactylon* can tolerate high concentrations of Pb by increasing the organic solute content of the plants. These organic solutes may include various enzymes (Rausser, 1995; Srivastava *et al.*, 2004).

Lead pollution will also result in enhanced ROS generation (Verma & Dubey, 2003). Our results indicate that SOD activity in actively growing *C. dactylon* seedlings increased with an increase in the Pb concentration. It is believed that SOD provides plants with the first line of defense against ROS because SOD acts on superoxide radicals, which are precursors of other ROS (Fatima & Ahmad, 2005). Substrate induction can also explain the increase of CAT (Reddy & Kumar, 2005). As a stress enzyme, POD has a broad specificity for phenolic substrates (Reddy & Kumar, 2005). The increase of POD is related to Pb stress, which indicates it is an intrinsic defense tool (Verma & Dubey, 2003).

With the increase of $\text{Pb}(\text{NO}_3)_2$ concentration, Pb concentration of both root and shoot increased. Moreover, Pb concentration in *C. dactylon* shoots was higher than that in *C. dactylon* roots (Fig. 6). A higher shoot/root ratio of heavy metal accumulation content is of great significance to the phytoremediation of soils polluted by heavy metal. Only by harvesting the aboveground part of the plant can the heavy metal contaminated soil be remediated, thus simplifying this agricultural practice. The translocation of Pb in plants is an important factor determining the distribution of Pb in different plant tissues. The translocation of heavy metal in plants involves several physiological, biochemical, and anatomical mechanisms (Salt *et al.*, 1995), thereby complicating the accumulation and distribution of heavy metal in aboveground tissues. However, little is known about the absorption, compartmentalization, and translocation of heavy metals in turfgrasses and the relevant mechanisms, or the degradation mechanism of toxic organics by turfgrass. If a large amount of heavy metal can be translocated from the ground, the lawn grass species can be harvested (Kumar *et al.*, 1995). Heavy metals can be effectively removed from the polluted soil through the removal of turfgrass clippings.

Conclusion

Seeds of *C. dactylon* have high tolerance to Pb, while *C. dactylon* seedlings have high Pb tolerance and accumulation Pb rate. The contents of Pb in shoots and roots indicate that the species could be a Pb-accumulator. Seeds and plants of *C. dactylon* have a high tolerance to Pb, showing that this species directly can be sown in soil contaminated by Pb and can be used

for phytoremediation and revegetation. However, it is unknown whether soil properties can positively or negatively influence the level of Pb resistance and absorption from soil. Multivariate experiments will be carried out in the future to answer this question.

Acknowledgments

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References

- Baskin, C.C. and J.M. Baskin. 2014. *Seeds: ecology, biogeography, and evolution of dormancy and germination*. 2nd edn. Academic Press, San Diego.
- Bell, G.E. 2011. *Turfgrass physiology and ecology: Advanced management principles*. Cambridge University Press, London.
- Blaylock, M.J., D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley and I. Raskin. 1997. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technol.*, 31: 860-865.
- Cenkci, S., I.H. Cigerci, M. Yildiz, C. Özyay, A. Bozdağ and H. Terzi. 2010. Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. *Environ. Exp. Bot.*, 67: 467-473.
- Chen, H., Y. Teng, S. Lu, Y. Wang and J. Wang. 2015. Contamination features and health risk of soil heavy metals in China. *Sci. Total Environ.*, 512: 143-153.
- Cheng, H., W. Xu, J. Liu, Q. Zhao, Y. He and G. Chen. 2007. Application of composted sewage sludge CSS as a soil amendment for turfgrass growth. *Ecol. Eng.*, 29: 96-104.
- Duan, B., Y. Lu, C. Yin, O. Junntila and C. Li. 2005. Physiological responses to drought and shade in two contrasting *Picea asperata* populations. *Physiol. Plant.*, 124: 476-484.
- Duo, L.A., Y.B. Gao and S.L. Zhao. 2005. Heavy metal accumulation and ecological responses of turfgrass to rubbish compost with EDTA addition. *J. Integ. Plant Biol.*, 47: 1047-1054.
- Faheed, F.A. 2005. Effect of lead stress on the growth and metabolism of *Eruca sativa* M. seedlings. *Acta Agron. Hung.*, 53: 319-327.
- Fargašová, A. 1994. Effect of Pb, Cd, Hg, As, and Cr on germination and root growth of *Sinapis alba* seeds. *Bull Environ. Contam. Toxicol.*, 52: 452-456.
- Fatima, R.A. and M. Ahmad. 2005. Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in wastewater. *Sci. Total Environ.*, 346: 256-273.
- Fiorenza, S., C.L. Oubre and C.H. Ward. 2000. *Phytoremediation of hydrocarbon-contaminated soil*. CRC Press, Boca Raton.
- García-Limones, C., G. Dorado, J.A. Navas-Cortés, R.M. Jiménez-Díaz and M. Tena. 2002. Changes in the redox status of chickpea roots in response to infection by *Fusarium oxysporum* f. sp. *ciceris*: apoplastic antioxidant enzyme activities and expression of oxidative stress-related genes. *Plant Biol.*, 11: 194-203.
- Gupta, D.K., H.G. Huang, X.E. Yang, B.H.N. Razafindrabe and M. Inouhe. 2010. The detoxification of lead in *Sedum alfredii* H. is not related to phytochelatin but the glutathione. *J. Hazard Mater.*, 177: 437-444.
- Huang, J.W., J. Chen, W.R. Berti and S.D. Cunningham. 1997. Phytoremediation of lead-contaminated soils: Role of synthetic chelates in lead phytoextraction. *Environ. Sci. Technol.*, 31: 800-805.
- Jarvis, M.D. and D.W.M. Leung. 2001. Chelated lead transport in *Chamaecytisus proliferus* Lf. link ssp. *proliferus* var. *palmensis* H. Christ.: an ultrastructural study. *Plant Sci.*, 161: 433-441.
- Jiang, L., L. Wang and C.Y. Tian. 2018. High lithium tolerance of *Apocynum venetum* seeds during germination. *Environ. Sci. Pollut. Res.*, 25: 5040-5046.
- Kumar, G. and R.P. Singh. 1993. Nitrate assimilation and biomass production in *Sesamum indicum* L. Seedlings in a lead enriched environment. *Water Air Soil Poll.*, 66: 163-171.
- Kumar, P.N., V. Dushenkov, H. Motto and I. Raskin. 1995. Phytoextraction: the use of plants to remove heavy metals from soils. *Environ. Sci. Technol.*, 29: 1232-1238.
- Kushwaha, A., N. Hans, S. Kumar and R. Rani. 2018. A critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies. *Ecotox. Environ. Safe.*, 147: 1035-1045.
- Li, H.S. 2000. *Experimental principles and techniques of plant physiology and biochemis*. Higher Education Press, Beijing, China.
- Lichtenthaler, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Method Enzymol.*, 148: 350-382.
- Liu, D., T.Q. Li, X.F. Jin, X.E. Yang, E. Islam and Q. Mahmood. 2008. Lead induced changes in the growth and antioxidant metabolism of the lead accumulating and non-accumulating ecotypes of *Sedum alfredii*. *J. Integ. Plant Biol.*, 50: 129-140.
- Maestri, E., M. Marmiroli, G. Visioli and N. Marmiroli. 2010. Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. *Environ. Exp. Bot.*, 68: 1-13.
- Martino, C., S. Delfine, R. Pizzuto, F. Loreto and A. Fuggi. 2003. Free amino acids and glycine betaine in leaf osmoregulation of spinach responding to increasing salt stress. *New Phytol.*, 158: 455-463.
- Milesi, C., S.W. Running, C.D. Elvidge, J.B. Dietz, B.T. Tuttle and R.R. Nemani. 2005. Mapping and modeling the biogeochemical cycling of turf grasses in the United States. *Environ. Manag.*, 36: 426-438.
- Nwosu, J.U., A.K. Harding and G. Linder. 1995. Cadmium and lead uptake by edible crops grown in a silt loam soil. *Bull. Environ. Contam. Toxicol.*, 54: 570-578.
- Öncel, I., Y. Keleş and A.S. Üstün. 2000. Interactive effects of temperature and heavy metal stress on the growth and some biochemical compounds in wheat seedlings. *Environ. Pollut.*, 107: 315-320.
- Piotrowska, A., A. Bajguz, B. Godlewska-Żyłkiewicz, R. Czerpak and M. Kamińska. 2009. Jasmonic acid as modulator of lead toxicity in aquatic plant *Wolffia arrhiza* (Lemnaceae). *Environ. Exp. Bot.*, 66: 507-513.
- Qu, R.L., D. Li, R. Du and R. Qu. 2003. Lead uptake by roots of four turfgrass species in hydroponic cultures. *Hort. Sci.*, 38: 623-626.
- Rausser, W.E. 1995. Phytochelatin and related peptides. Structure, biosynthesis, and function. *Plant Physiol.*, 109: 1141-1149.
- Reddy, A.M., S.G. Kumar, G. Jyothsnakumari, S. Thimmanai and C. Sudhakar. 2005. Lead induced changes in antioxidant metabolism of horsegram *Macrotyloma uniflorum* Verdc. and bengalgram *Cicer arietinum* L. *Chemosphere*, 60: 97-104.
- Rodríguez-Barranco, M., M. Lacasaña, C. Aguilar-Garduño, J. Alguacil, F. Gil, B. González-Alzaga and A. Rojas-García. 2013. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. *Sci. Total Environ.*, 454: 562-577.

- Salt, D.E., M. Blaylock, N.P. Kumar, V. Dushenkov, B.D. Ensley, I. Chet and I. Raskin. 1995. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. *Nat. Biotechnol.*, 13: 468.
- Sears, M.E., K.J. Kerr and R.I. Bray. 2012. Arsenic, cadmium, lead, and mercury in sweat: A systematic review. *J. Environ. Public Health*, 2012: 184745.
- Sengar, R.S., M. Gautam, R.S. Sengar, S.K. Garg, K. Sengar and R. Chaudhary. 2009. Lead stress effects on physiobiochemical activities of higher plants. *Rev. Environ. Contam. Toxicol.*, 196: 73-93.
- Shafiq, M., M.Z. Iqbal and A. Mohammad. 2008. Effect of lead and cadmium on germination and seedling growth of *Leucaena leucocephala*. *J. Appl. Sci. Environ. Manag.*, 12: 61-66.
- Sharma, P and R.S. Dubey. 2005. Lead toxicity in plants. *Braz. J. Plant Physiol.*, 17: 35-52.
- Srivastava, S., R.D. Tripathi and U.N. Dwivedi. 2004. Synthesis of phytochelatins and modulation of antioxidants in response to cadmium stress in *Cuscuta reflexa* — an angiospermic parasite. *J. Plant Physiol.*, 161: 665.
- Taliaferro, C.M. 1995. Diversity and vulnerability of Bermuda turfgrass species. *Crop Sci.*, 35: 327-332.
- Verma, S. and R.S. Dubey. 2003. Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Sci.*, 164: 645-655.
- Vodnik, D., G. Jentachke, E. Fritz, N. Gogala and D.L. Godbold. 1999. Root-applied cytokinin reduces lead uptake and affects its distribution in Norway spruce seedlings. *Physiol. Plant.*, 106: 75-81.
- Wierzbicka, M. and J. Obidzińska. 1998. The effect of lead on seed imbibition and germination in different plant species. *Plant Sci.*, 137: 155-171.
- Xiong, Z.T. 1998. Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumulator *Brassica pekinensis* Rupr. *Bull. Environ. Contam. Toxicol.*, 60: 285-291.
- Xiong, Z.T., F. Zhao and M.J. Li. 2006. Lead toxicity in *Brassica pekinensis* Rupr: effect on nitrate assimilation and growth. *Environ. Toxicol.*, 21: 147-153.
- Yoon, J., X. Cao, Q. Zhou and L.Q. Ma. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci. Total Environ.*, 368: 456-464.

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