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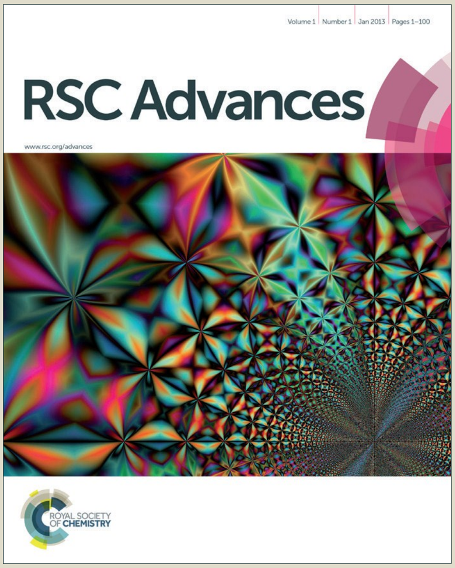

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Growth Responses and Accumulation of Cadmium in Switchgrass (*Panicumvirgatum* L.) and Prairie Cordgrass (*Spartinapectinata* Link)

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Abstract

Phytoextraction could be an efficient technique to remediate heavy metals from contaminated soils. Identifying bioenergy crops that can be produced successfully on marginal lands, such as those polluted by heavy metals, also reduces the pressure to produce energy crops on land that would otherwise be used to produce food crops. The objective of this study was to determine the phytoremediation capability of two warm season perennials, prairie cordgrass (*Spartinapectinata* Link, 'Savoy') and switchgrass (*Panicumvirgatum* L., 'Cave-In-Rock'(CIR)) through their growth response to cadmium (Cd). Growth rate, Cd tolerance, accumulation and translocation were measured at concentrations of 0 (control), 5, 10, 30, and 50 mg L^{-1} of Cd mixed with Hoagland's solution in an aerated hydroponic system. Although a reduction of plant growth was observed when Cd concentration was higher than 10 mg L^{-1} for both species, there were significant differences in Cdtolerance, translocation and accumulation between species. The tolerance index (T_i) was between 72.5 and 107.1in Savoy and 48.7 and 75.7 in CIR under Cd concentration of 50 mg L^{-1} and 5 mg L^{-1}, respectively. The translocation factor (T_f) for both species was increased with increasing Cd concentration in solution, but the T_f of Savoy was higher than CIR. The highest bio-concentration factor (BCF) of the roots reached 325.7 for Savoy and 144.5 for CIR when the Cd concentration was5mg $L^{-1}$and the BCF of the shoots in both species was consistently low (13.7 to 16.8 and 4.1 to 6.0for Savoy and CIR, respectively) indicating higher Cd retention in the roots than shoots. It was concluded that both species could be utilized in phytoremediation when the Cd concentration is less than 10 mg L^{-1}, however Savoy has the higher tolerance, translocation and accumulation capabilities which makes it a better candidate for phytoremediation and biomass production on Cd polluted soils.

Keywords: Energy crops, Cadmium, Tolerance, Phytoremediation, Bio-concentration factor, Translocation index

Introduction

Cadmium is a heavy metal that is toxic to humans, animals and plants and is a widespread, highly toxic environmental pollutant with a long biological half-life (10-30 years in animals).[1] Taken up in excess by plants, Cd directly or indirectly inhibits physiological processes, such as respiration, photosynthesis, cell elongation, plant and water relationships, nitrogen metabolism and mineral nutrition, resulting in poor growth and low biomass.[2] Response of plants to Cd stress is only partially understood. Cadmium may affect the uptake, transport and use of different macronutrients and micronutrients, especially Fe and Zn.[3,4] Cadmium is not essential to plant growth, and Cd accumulation in plant tissue can cause various phytotoxic symptoms including leaf chlorosis, root putrescence, and growth inhibition.The tolerable range of Cd concentration in leaf tissue (dry weight) of various agronomic crops is 0.05-0.2 mg kg^{-1}, and it is considered to reach excessive or toxic level within a range of 5-30 mg kg^{-1}.[5] The accumulation of Cd within the aquatic environment, sediments, and soils is a concern because the Cd taken up by plants can be passed on to humans through the food chain. Therefore, it is important to develop methods to remediate Cd-polluted soils.

Phytoremediation is a process where plants are used to degrade, extract, contain or immobilize contaminants from soil and water.[6,7] It is a cost-effective, environmentally friendly, and technically applicable technology used to restore polluted sites in situ.[8-10] A plant species with an exceptional capacity to accumulate metals is the key for phytoremediation.[9-11] Potential candidates must be high biomass producers and have strong developed root systems, excellent transpiration, and the ability to remove heavy metals from contaminated soils.[12,13] Although over

450 plant species have been identified as hyperaccumulators of heavy metals, only a few are known to hyperaccumulate Cd.[6,13-18] Grasses such as *Festucaovina* L., *F. rubra* L., *Agrostiscapillaris* L., *A. delicatula* Pourr.ex Lapeyr and *A.stolonifer a* L. have high degrees of metals tolerance but they do not hyperaccumulate metals.[19]

Both prairie cordgrass and switchgrass are C_4 perennial rhizomatous grasses and have been recognized as excellent energy crops for marginal lands. [20-22] Several studies have demonstrated the tolerance of prairie cordgrass and certain switchgrass cultivars for high soil salinity.[23-25] Prairie cordgrass is well adapted to soils that are wet throughout the growing season. Even though there is limited information available on stress tolerances of prairie cordgrass including abiotic stress and heavy metal toxicity, *Spartina* spp is known as highly stress tolerant. Two other species in the genus, austral cordgrass (*S. densiflora* Brongon) has proven to be a vigorous plant, which can grow well in salt marshes[26] and is tolerant of very high levels or continues exposure to Zn.[27] Prairie cordgrass has been reported to havea high tolerance for conditions of water logging, high salinity, and low pH.[28,29] Certain switchgrass cultivars have been reported to be tolerant of Cd when grown in sand.[30] Switchgrass can be grown on Cd-contaminated sites to produce acceptable levels of biomass; however, the plant material produced should not be used as animal feed without testing and verification of the Cd concentration.[30]

However, no direct comparison has been made between switchgrass and prairie cordgrass for Cd tolerance or performance on marginal land. Since prairie cordgrass and switchgrass play an important role for feedstock production on marginal land, direct comparison between two species will provide useful information for developing the future energy crops for sustainable bioenergy feedstock on marginal land. The objective of this study was to determine the effects of Cd on plant growth, biomass distribution, and Cd accumulation in ‘Cave-in-Rock’ switchgrass (*Panicum virgatum* L.) and ‘Savoy’ prairie cordgrass (*Spartina pectinata* Link), there by assessing Cd toxicity tolerance and ability to accumulate Cd in biomass.

Materials and Methods

The seeds of Savoy prairie cordgrass (Savoy) originated from central Illinois, USA and were obtained from our breeding program, and Cave-in-Rock switchgrass (CIR) seeds were purchased froma commercial seed source (Millborn Seeds Inc., Brookings, SD, USA).

A hydroponicexperiment was conducted in a controlled greenhouse setting at the University of Illinois, Urbana, IL, USA. A 20-day-old seedling was transplanted into a 3 L pot containing 20% Hoagland’s nutrient solution [31] without Cd. After 5 days of seedling culture, the solution was replaced by a 100% Hoagland’s nutrient solution without Cd for another 5 days of seedling culture, and then exposed to a 100% Hoagland’s nutrient solution with 0, 5, 10, 30, 50 or 100 Cd^{2+} mg L^{-1}. The solutions with different Cd^{2+} concentrations were refreshed every three days. The six treatments for each species were performed in a randomized complete block design and each treatment was replicated four times and the experiment was repeated twice. The pH of solution was maintained at 6.0±0.5 modification with 1 mol L^{-1} HCl or 1 mol L^{-1} NaOH every day. Air was pumped with EcoPlus Air Pumps for 30min every 3 hr each day in order to keep the dissolved oxygen in solution. The plants were harvested 20 d after exposure to the Hoagland’s nutrient solutions containing Cd. Biomass was measured as the fresh weight (g F.W) of plant from both before and after treatment.

The longest roots and shoots were recorded at the initial treatment with Cd and at harvest. The elongation of the longest roots and height of the shoots were calculated using the final length at harvest minus the initial length. The number of tillers was observed before treated with Cd and at harvest. The increase in the number of tillers was calculated as the tillers at treatment with Cd subtracted from the total tillers at harvest .The fresh biomass of energy crops was recorded at harvest and the plant samples were washed with tap water and rinsed with distilled water three times. The washed samples were separated into roots and shoots, then the roots were immersed into a 500 ml solution of Na_2-EDTA (10 mmol L^{-1}) for 10 min to remove the Cd attached to the root surface. The samples were washed again with distilled water before further processing. The roots and shoots were dried at 105℃ for 30 min and then at 65℃ for 2 d, and the dried root weight and shoot weight were measured. Then, the dry plant tissues were ground to pass a 1mm screen to determine the concentration of Cd.

The plant tissue samples were digested with microwave assisted acid digestion.[31] 0.2500 g representative plant samples, 9 mL of concentrated nitric acid and 3 mL hydrofluoric acid were placed in a 50mL inert polymeric microwave vessels, and then the vessels were sealed and heated in the microwave system for 15 minutes at 180±5 ℃ temperature. After cooling, the vessel contents were filtered and then decanted, diluted to 100mL, and analyzed by inductively coupled plasma-atomic emission spectrometry (Optima8000, PE Company, USA).[32]

The tolerance index (T_i) was calculated to measure the ability of the plant to grow in the presence of a given concentration of Cd:[33,34]

$$T_i(\%) = \frac{W_{Cd}}{W_{CK}} \times 100$$

where W_{Cd} is the total dry biomass of plants grown in Hoagland's nutrient solution with different Cd^{2+} concentrations (mg) and W_{CK} is the total dry biomass of plants grown in the control solution without Cd (mg).

The translocation factor (T_f) was calculated to evaluate the capability of the plant to accumulate Cd in the roots and shoots: [34]

$$T_f(\%) = \frac{C_{Cd,shoots}}{C_{Cd,roots}} \times 100$$

where $C_{Cd,shoots}$ is the Cd concentration in the shoots (mg kg^{-1}) and $C_{Cd,roots}$ is the Cd concentration in the roots (mg kg^{-1}).

The Cd bio-concentration factor (BCF) was calculated as follows:[35]

$$BCF = \frac{C_{Cd,plant}}{C_{Cd,solution}}$$

where $C_{Cd,plant}$ is the Cd concentration in the plant (mg kg^{-1}) and $C_{Cd,solution}$ is the Cd concentration in the solution (mg L^{-1}).

Analysis of variance (ANOVA) for Cd concentration, species, and their interaction was performed with a significance level of $P < 0.05$ using the GLM procedure of SAS software (SAS Institute, Cary, NC).The effect of experiments was considered random and was not significant. The data presented are the least squared mean values and is compared as the mean separations using Tukey's Studentized Range (HSD) test (P-value< 0.05).

Results

Cadmium tolerance and growth response

The tolerance of plants to toxic metals is frequently measured by comparing the rates of root and shoot growth in culture solutions with and without the addition of a target heavy metal. [33] Plant growth responses including root and shoot growth, number of tillers, and biomass under Cd stress for 20 days are shown in Figures 1, 2, 3, and 4, respectively, which indicate that growth was diminished by Cd stress. Negative effects of Cd on plant growth responses of both grasses were increased by increasing Cd concentration in the solution. Root growth of Savoy was not significantly different from the control when the concentration of Cd in Hoagland's solution was less than 10 mg L^{-1} (Fig. 1). However, root growth of Savoy was significantly inhibited when the concentration of Cd was more than 30 mg L^{-1}. Roots of Savoy stopped growing and began to decay when the concentration of Cd was more than 50mg L^{-1}, but Savoy continued to survive at100 mg L^{-1}. Cave-in-Rock was more sensitive to Cd and its growth was inhibited when the concentration of Cd was 5 mg L^{-1} or higher (Fig. 1; Fig. 5). Root elongation of CIR stopped growing and began to decay when the concentration of Cd was more than 10 mg L^{-1}. Cave-in-Rock plants died when the concentration of Cd was higher than 50 mg L^{-1} and therefore no data was shown at this concentration.

The plant height was also affected by Cd concentration in solution. Shoot growth of Savoy and CIR was significantly decreased under all Cd concentrations (Fig. 2; Fig. 5). Shoot growth in both grasses maintained 50% level of the control when the concentration of Cd was 5mg L^{-1}, and growth was negligible when the concentration of Cd reached higher than 10mg L^{-1}, as compared with the control (Fig. 2).The number of tillers of Savoy and CIR responded differently to Cd concentrations in solution (Fig. 3). The number of tillers of CIR continuously decreased as Cd concentration increased. However, the number of tillers of Savoy under any given Cd concentrations was not different, though they were lower than the control. The biomass yield of both grasses decreased under Cd stress, but biomass accumulation patterns between grasses were different (Fig.4).For Savoy, biomass with the control and 5 mg L^{-1} was not different, decreased from 5 mg L^{-1} to 10 mg L^{-1}, and were not different under Cd concentrations between 10 and 50 mg L^{-1}. However, CIR biomass accumulation continuously decreased as Cd concentration increased and no biomass accumulated above 30 mg L^{-1}.

From the growth response of both grass species to Cd stress, including the elongation of longest roots, height of shoot, the number of tillers and total biomass, it was generally observed that the tolerance to Cd of Savoy was higher than that of CIR. The difference between the two species with regards to Cd tolerance was further confirmed by the analysis of the tolerance index, T_i (Fig.6). On the basis of the total dry biomass of each species, the T_i revealed that on average Savoy had a greater tolerance to Cd than CIR. The T_i% of Savoy and CIR was 107.13% and 75.74%, respectively, when the Cd concentration was 5 mg L^{-1} which indicated that the growth was not affected. But the growth of both Savoy and CIR was affected when Cd concentration was 10mg L^{-1} or above.

Accumulation of Cd

The concentration of Cd detected in the roots and shoots of Savoy and CIR after exposure for 20 days to various concentrations of Cd is shown in Fig. 7. In the control, the Cd concentration in the shoots of both grasses was below the threshold of detection. High concentrations of Cd were detected in the roots and shoots of both grasses and Cd concentrations increased as the Cd concentration increased in the solution. The roots and shoots of Savoy accumulated Cd ranging from 1628.7 to 2912.0 mg kg^{-1} and 74.8 to 416.2 mg kg^{-1}, respectively for the various Cd treatments. The roots and shoots of CIR accumulated Cd ranging from 722.7 to 1695.7 mg kg^{-1} and 21.1 to 145.6 mg kg^{-1}, respectively. Root accumulation of Cd represented approximately 87% to 96% and 92% to 97% of total accumulation in biomass of Savoy and CIR, respectively. The Cd concentration in Savoy was 2.2 to 4.0 times higher in the shoots and 1.7 to 2.8 times higher in the roots than in CIR at the same Cd treatment.

The Cd bio-concentration factor (BCF) and translocation factor (T_f)

To evaluate the ability of Savoy and CIR to extract and accumulate Cd in the plant, the bio-concentration factor (BCF) was calculated. The root BCF was higher than the shoot BCF in both Savoy and CIR, being 7 to 22 times and 12 to 34 times higher, respectively and Savoy BCF was higher than CIR (Fig. 8).In the roots of both species, the BCF decreased as the Cd concentration increased. However, in the shoots of both species, the BCFs were not different among Cd treatments.

The capability of Savoy and CIR to accumulate Cd in the above ground tissues was further confirmed by calculating the translocation factor (T_f), which indicated the percentage of the amount of absorbed metal that reached the shoots with respect to the amount present in the roots (Fig. 9).The T_f in both energy crops showed different translocation capabilities. The higher T_f was observed in Savoy, and the T_f of both Savoy and CIR increased as the concentration of Cd increased in the solution.

Discussions

Metal tolerance, and consequently the protection of the integrity and functionality of the primary physiological and metabolic processes, is an essential pre-requisite for a plant to be utilized in phytoremediation.[36] In this study, the responses of Savoy and CIR to Cd stress, which were analyzed by growth parameters (elongation of longest roots, height of the shoot, the number of tillers, total biomass) and Cd uptake, indicated there were significant differences in tolerance, accumulation and translocation of Cd. These fundamental aspects should form the criteria used to screen plants for selection in phytoremediation. However, phytoremediation needs to be a combination of heavy metal accumulation and reduction of damaging effects on biomass production, and not merely due to metal extraction.[34] Metal tolerance could be estimated based on root elongation and growth since tolerance at the root level represents the first step in metal absorption and loading into the xylem vessels.[37,38] Previous research has shown that 40 μM (4.49 mg L^{-1}) and 1 μM (0.11 mg L^{-1}) the solution with Cd^{2+} can reduce growth of barley and soybean, respectively, by 50%.[39,40] Indian mustard (*Brassica juncea* L. Czern.) showed a decline in growth vigor under 25 μM (2.81 mg L^{-1}) the solution with Cd^{2+} concentration even though it is considered a Cd hyperaccumulator.[41,42] In a similar study, *P. austral is* showed a 50% depression in root elongation when grown in Cd concentrations ranging from 2.85 to 3.20 μM (0.32 mg L^{-1} to 0.36 mg L^{-1}), and the T_i was reduced to less than 80% when the Cd concentration was above 8.8μM (0.99 mg L^{-1}).[35] Reed[30] indicated that different switchgrass cultivars showed different levels of tolerance to Cd in solution, and approximately one third of the control biomass yield was lost when grown at 16 mg L^{-1} Cd concentration. In this study, Savoy and CIR were tolerant to the Cd concentration of 5 mg L^{-1} Cd, with a T_i of 92.2% and 87.9%, while Cd concentrations above 10 mg L^{-1} reduced the T_i to 60.8% and 57.7%, respectively. These results suggested that both energy crops were tolerant to Cd stress.

Based on the total dry biomass of each energy crop, the T*i* revealed that on average Savoy absorbed much more Cd than CIR. Most vascular herbaceous species accumulate greater concentrations of heavy metals in the roots than that in shoots.[43,44] Under our experimental conditions, the roots and shoots of Savoy accumulated Cd to the highest concentrations of 2912.0 mg kg^{-1} and 416.2 mg kg^{-1}, respectively, with a BCF ranging 325.7 and 16.8 respectively, for the different Cd treatments. The roots and shoots of CIR accumulated Cd to the highest concentrations of 1695.7mg kg^{-1} and 145.6mg kg^{-1} with a BCF ranging from 56.5 to 144.5 and 4.2 to 6.0, respectively for the different Cd treatments. Our results demonstrated that, even though the majority of Cd was found in the roots, substantial amounts were also in the shoots. The Cd concentration in both grasses was higher than 100 mg kg^{-1} dry mass. This level reached the threshold concentration defined by Van der Ent for a Cd hyperaccumulator.[18] In this study, the bioconcentration factor (BCF) values under lower concentration of Cd treatment indicated that Savoy had double or triple the capacity to remove metal from the solution compared to CIR. In a study comparing two wooden species (poplar and willow) seedlings performance under a similar condition, Zacchini reported a range of BCF values from 52 to 290 in roots and 2.5 to 14.5 in shoots, respectively.[45] Other studies on herbaceous plants showing higher BCF values compared to the current experiment were largely conducted under lower cadmium concentrations or trace element supply.[35,36] The ability to accumulate heavy metal in the shoots and roots can be better illustrated by calculating the translocation fact or (T_f). In this study, the range of the T_f of Savoy and CIR was 4.6 to14.3 and 2.9 to 8.6, respectively. Due to a different experimental setting, the T_f measured in this study is lower than that indicated in other plants cultivated in soil.[46,47] In general, Savoy showed a higher Cd accumulation capability in its harvestable parts and accumulated more Cd per plant base since it produced more biomass than CIR under each soluble Cd concentration. Wetland plants are generally not hyperaccumulators according to the definition by Baker & Brooks[48] and Wei & Zhou[49]. However, perennial wetland plants can store metals in below ground parts other than roots. In a study comparing four emergent rhizome type wetland plants, Zhang found that a significant amount of Cd was accumulated by rhizome under soluble Cd concentration 20 mg L^{-1}.[50] In engineering a remediation system on marginal land, especially wetlands, the perennial nature of these two species can provide both roots and rhizomes as preferable alternatives to the destructive mechanical harvesting of aboveground tissue. Moreover, both species in the current study were able to survive under Cd concentration in solution at 10 mg L^{-1} which already causes net weight loss for other hyperaccumulators such as water hyacinth (*Eichhorniacrassipes*) and duckweed (*Lemnaminor L.*).[51, 52]

In conclusion, the evaluation of the T_i, T_f and BCF for Savoy prairie cordgrass and CIR switchgrass in this study confirms that both grass species have a considerable potential to remove Cd from a contaminated environment with Savoy having a greater potential to remediate polluted soils because it has a higher tolerance, greater ability to translocate heavy metals to its shoots, and is a good Cd accumulator. The high accumulation in belowground tissues of both species could be used either as rhizofiltrations to remediate wastewater or phytostabilization to limit Cd flowing into water.

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Figure 1. Changes in root growth of ‘Savoy’ prairie cordgrass and ‘Cave-In-Rock’ (CIR) switchgrass affected by Cd concentrations.

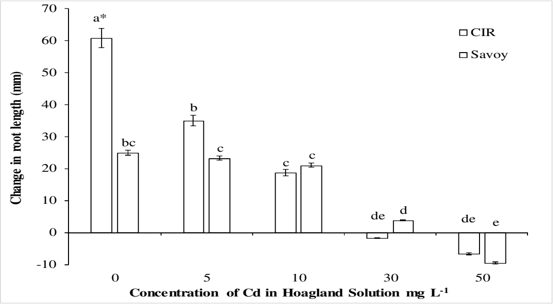

Figure 2.Changes in shoot growth of ‘Savoy’ prairie cordgrass and ‘Cave-In-Rock’ (CIR) switchgrass affected by Cd concentrations.

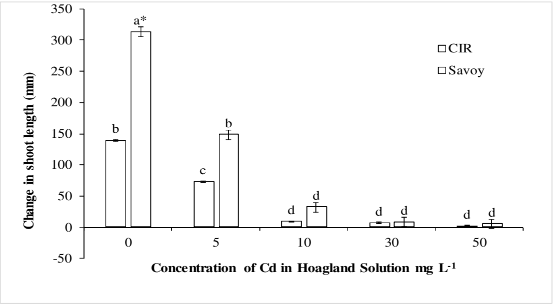

Figure 3. Changes in tiller number of ‘Savoy’ prairie cordgrass and ‘Cave-In-Rock’ (CIR) switchgrass affected by Cd concentrations.

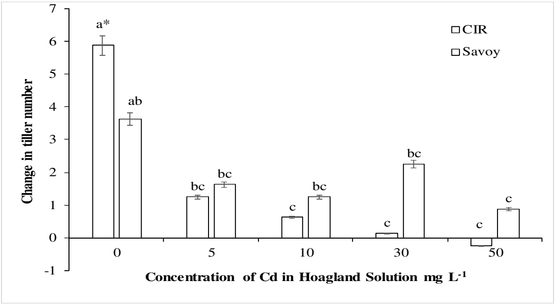

Figure 4. Changes in biomass yield of 'Savoy' prairie cordgrass and 'Cave-In-Rock' (CIR) switchgrass affected by Cd concentrations. Biomass was measured as the fresh weight (g F.W) of plant from both before and after treatment.

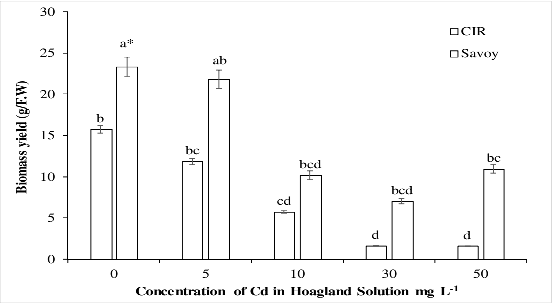

Figure. 5 Morphological aspects of Savoy prairie cordgrass (A) and CIR switchgrass (B) plants 20 d after exposed to cadmium solution with different concentrations

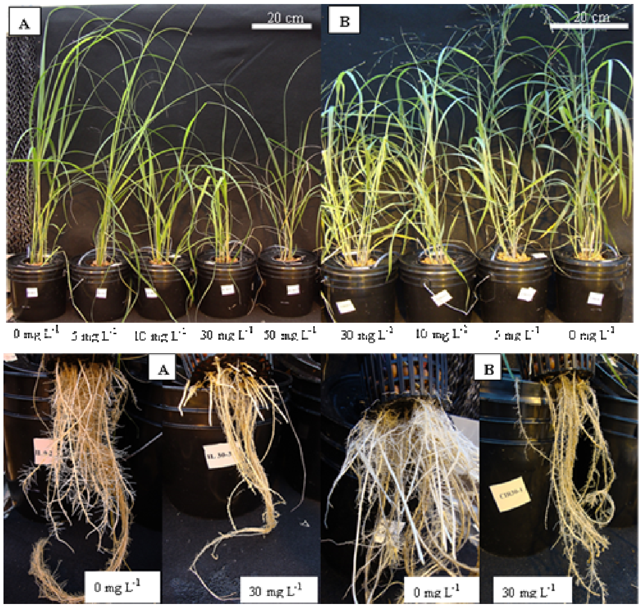

Figure 6 Tolerance index (T_{i}, %) of Savoy prairie cordgrass and Cave-In-Rock (CIR) switchgrass under different Cd concentrations.

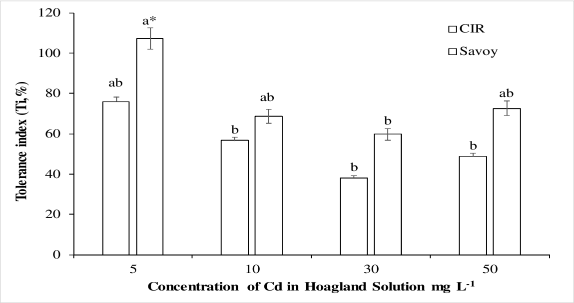

Figure 7 Concentration of Cd in root and shoot of ‘Savoy’ prairie cordgrass and ‘Cave-In-Rock’ (CIR) switchgrass under different Cd concentrations.

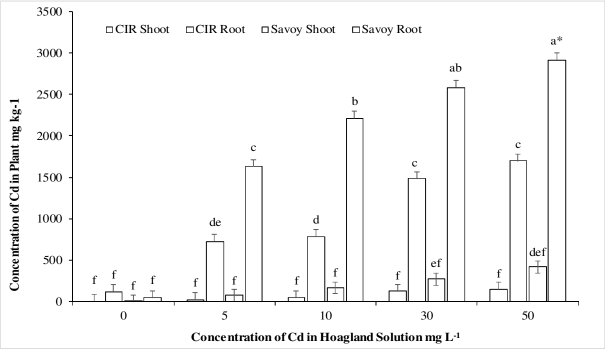

Figure 8.Bio-concentration factor (BCF) of ‘Savoy’ prairie cordgrass and ‘Cave-In-Rock’ (CIR) switchgrass under different Cd concentrations.

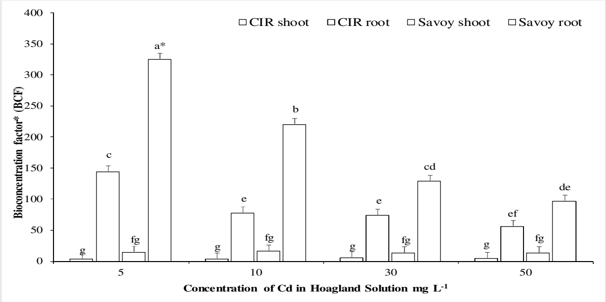

Figure 9. Translocation factor (T_f) of ‘Savoy’ prairie cordgrass and ‘Cave-In-Rock’ (CIR) switchgrass under different Cd concentrations.