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Differential expression of Lead accumulation during two growing seasons by desert shrub *Acacia victoriae* L.

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Abstract

In desert reclamation strainer plants can be used for improvement and decrease pollution of soil and water. This technology can be used to remove both inorganic and organic contaminants in soil. In this study, one year old *Acacia victoriae* seedlings were exposed to Pb $(NO_3)_2$ in 5 different concentrations; 0, 50, 250, 500 and 1000 mg Pb L^{-1} for 45 days in two growing seasons. Subsequently, the heavy metal concentrations were measured in different plant tissues by Atomic Absorption Spectroscopy (AAS) for two growing periods. In addition, some physiological and morphological parameters (root and plant length, root diameter, leave area, dry weight, chlorophyll a, b and total) were measured. Based on the results, the visible toxicity symptoms (chlorosis and necrosis) appeared only to the highest concentration (1000 mg Pb L^{-1}) in both growing seasons. The results also showed that application highest concentration of Pb reduced the physiological and morphological parameters as compared to the control seedlings. The accumulation of Pb was influenced by the Pb concentration in the growth medium and the growing seasons as well. With respect to the more accumulation of Pb in the roots tissues than aboveground tissues, indicating *A.victoriae* as a good option for phytostabilization of Pb contaminated soils. Furthermore if *A.victoriae* is planted for Pb phytoextraction, therefore the harvest of aboveground should be done at the fall season. Meanwhile concentrations of Pb in the aboveground parts were more than roots at the fall season. In conclusion *A.victoriae* a native to the arid zone appeared to be hyper tolerate, accumulate high concentrations of Pb and it could be regarded as a potential accumulator. In addition *A.victoriae* have high application value in repairing Pb contaminated soils and is suitable and effective choice to be used as a tool of phytoremediation in industrial sites of the arid zones. Our findings suggest that *A.victoriae* has the advantages of high capacity for adaptation to poor, easy cultivation, deep root system, high tolerance to the drought, saline soils and Pb and could use as candidate plant for environmetals monitoring.

Keywords**:** Lead; Phytoextraction; Phytostabilization; Soil pollution; Toxicity symptom

1. Introduction

Heavy metal contamination of soils is an important environmental issue because of its impact on soil preservation and human health. Phytoremediation is an environmentally friendly and safe technique that employs the use of plants to recover/ clean polluted soils, particularly those polluted with heavy metals. The phytoremediation process can be divided into different classes on the removal of contaminants from the soil such as, phytostabilization, phytostimulation/phytovolatilisation, phytodegradation and phytoextraction (Souza *et al*., 2013). Pb is a non essential element that causes many problems within plants. This heavy metal is of particular concern to surface water and soil pollution. Pb afffected soils contain Pb in the range of 400-800 mg kg^{-1} soil whereas in industrialized areas the level may reach up to 1000 mg kg^{-1} Pb soil. The normal range of Pb that a plant can accumulate is between 0.2 to 20 mg kg^{-1} of dry tissues and 30 to 300 mg kg^{-1} Pb concentrations are considered toxic to plants (Mukhopadhyay and Maiti, 2010). Plants have developed different tolerance strategies to grow on soils rich in heavy metals. “Excluders” have

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a low uptake of trace elements, by active exclusion in the root system, even at high external concentrations in the soil solution. “Accumulators” are able to tolerate high concentrations of trace elements in their tissues and this accumulation can be produced even at low external concentrations in the soil solution. “Indicators” have a relatively constant root uptake over a wide gradient of trace elements in the soil (Khermandar, 2013). Hyperaccumulator term was then extended by others to describe plants that can accumulate heavy metals in their above ground parts 10–100 times greater than those of normal plants (Tang *et al.,* 2009). Hyperaccumulator must accumulate at least 100 mg g^{-1} (0.01% dry wt) of Cd, As and some other trace metals, 1000 mg g^{-1} (0.1% dry wt) of Pb, Co, Cu, Cr and Ni and 10000 mg g^{-1} (1% dry wt) of Mn and Ni (Mukhopadhyay and Maiti, 2010). Sarma (2011) reported the latest numbers of metal hyperaccumulators according to his report, more than 500 plant species consisting of 101 families are classified as metal hyperaccumulators. Besides hyperaccumulator herbaceous plants, several woody species are now considered of interest to this aim. Many woody plants are fast growing, have deep roots, produce abundant biomass are easy to harvest and several species revealed some capacity to tolerate and accumulate heavy metals (Khermandar, 2013).

-*Review of Literature* (done studies about species that belongs to Arid &Semiarid regions)

-Amira and Abdul Qados (2015) studied the remediation potentials of three tree species *Acacia saligna*, *Eucalyptus rostrata* and *Conocarpus erectus* to remove Pb from the contaminated soils in order to find out the most effective species that can be used to clean up the soil from Pb pollution. Obtained results showed that the highest level of pollution with Pb caused a significant reduction in vegitative growth parameters and photosynthetic pigments. Also results indicated that *A.saligna* was the most tolerant species to Pb pollution followed by *E.rostrara* while *C.erectus* showed low level of tolerance to Pb pollutants.

-Saikachout *et al*., (2015) studied the toxicity Pb to annual *Atriplex hortensis* and *Atriplex rosea*. The plant growth expressed as shoot and root dry weight of *Atriplex* plant was adversely inhibited when exposed to high concentrations of polluted soil. Significant increases in chlorophyll content were observed in leaves for *Atriplex* varieties after the plants were exposed to stress treatments. These results demonstrate that Pb contamination of soil has adversely affected the photosynthetic parameters of annual *Atriplex*. Finally, it was concluded that annual *Atriplex* has a high ability to tolerate Pb, so it might be a promising plant to be used for phytoremediation of Pb contaminated soil.

-Patil and Umadevi (2014) studied Pb effect on four *Eucalyptus* species. The results show that Pb exhibited toxic effect at higher concentrations on *Eucalyptus* species viz., *E.tereticornis, E.globulus, E.camaldulensis* and *E.citriodora*. The growth parameters of seedling like shoot and root length, dry biomass were inhibited more severely in *E.tereticornis* and *E. camaldulensis* while *E.citriodora* was least affected by Pb. *E.citriodora* showed tolerance to Pb and recorded maximum seedling growth attributes as compare to other *Eucalyptus* species.

-Pereira *et al*., (2013) studied Pb tolerance of *Schinus molle* plants and the resulting anatomical and physiological modifications during growth. The seedling growth were unaffected and the accumulation of Pb was higher in the roots than in the shoots. Thus, *S.molle* accumulated Pb to concentrations above toxic levels and showed only favorable modifications to the anatomical structure and growth, suggesting this species is Pb tolerant and has the potential for use in phytoremediation systems.

-Farooqi *et al*., (2011) studied tolerance of *Albizia lebbeck* benth to different levels of Pb in natural field conditions. The results show that Pb produced significant effects on different growth parameters of *A.lebbeck*, such as, root, shoot and seedling length, leave area, number of leaves, plant circumference and seedling dry biomass in natural field conditions. Seedling growth performance of *A.lebbeck* showed low level of tolerance with increasing concentrations of Pb.

Acacia victoriae as a woody plant belongs to *leguminous* family that has been assessed in polluted areas because of their low implementation cost and high capacity for adaptation to poor and degraded contaminated soils in dry lands. The *leguminous* family can a heavy metal hyperaccumulator, because of symbiotic association with nitrogen fixers and all species in this family nearly are associated with mycorrihzal fungi (Ricardo *et al.,* 2012) and can obviously absorb more heavy metals from the landfill site leachate than non *leguminous* species. Also they have a deep root system for remediating deep soil/water depths. In general, *Acacias* are relatively short lived, lasting from 20–30 years and recover well from pruning, which allows for the ready removal of polluted plant material (Khermandar, 2013). This study is focused on the phytoremediation potential of Pb heavy metal contaminated soils by *A.victoriae*. Our assumptions in this study is that the accumulator plants uptake the heavy metals according to the seasonal growth patterns and matching the phytoextraction practices to those patterns can maximize the remediation efficiency of contaminated soils and increase metal uptake and accumulation. However there is not much knowledge about the pattern of heavy metal uptake and accumulation into the tissues during the growing seasons. This understanding is important to schedule phytoremediation practices to coincide with periods of high metal accumulation in aboveground tissues thereby maximizing the phytoextraction efficiency. Also to our knowledge, this is the first study to show Pb phytoremediation potential of *A.victoriae* for two growing periods and will open new ways in this field for the young scientists of the area. It is important to understand seasonal heavy metal accumulation in different parts of plants in order to develop the best phytoremediation practices for contaminated soils. In addition, the objective of the study is to investigate the effects of high concentration of Pb in the soil medium on growth and photosynthetic characteristics of *A.victoriae* in two growing seasons. The results of this study show the potential use of *A.victoriae* trees for phytoremediation purposes in Pb contaminated soils.

2. Materials and Methods

2.1. Study area

In this study, one year old *A.victoriae* seedlings were procured from Mehran (a city in Ilam province, west of Iran) nursery which is located in an arid zone and were transported to the research and educational nursery in Ilam University. This pot experiment was conducted in the plant nursery of department of natural resources at University of Ilam, Iran. Considered region is located in 46^o 22' 15" to 46^o 25' 27" eastern longitude and 33^o 41' 01" to 33^o 43' 13" northern latitude and altitude of above sea level, average annual temperature and precipitation were 1174 m, 16.9^{o}C and 525 mm, respectively.

2.2. Methodology

30 seedlings (15 seedlings for each period) of one year old *A.victoriae* were replanted into 30 cm blackened plastic pots, filled with 2.5 Kg (dry weight) of loamy-silt soils (the soil used in the experiment was collected from the surface layer (0-30 cm) of Botanical Garden–Ilam University, which had no pollution of heavy metals. The soil was dried and passed through a 2 mm sieve for analysis. Particles size distribution determined by hydrometer method), by proportions of 1:1:2 for manure, sand and soil, respectively (Table 1). The seedlings were exposed to Pb $(NO_3)_2$ solution in 5 different concentrations; 0, 50, 250, 500 and 1000 mg Pb L^{-1} by supplementing them into irrigation water (Irrigation was based on 60% of Field Capacity) each time (Seedling were irrigated 20 times) during 45 days in both seasons. For each Pb concentration three replicates were evaluated.

Table 1. Physico-chemical characterizations of control soil

Soil investigation	pH	EC (ds m^{-1})	Total N (%)	OM (%)	OC (%)	Available K (mg kg^{-1})	Available Na (mg kg^{-1})	Soil Texture (%)			Soil Texture
								Sand	Silt	Clay	
Control soil	7.2	3.59	0.164	2.48	1.44	42	12	41	54	5	loamy-silt

2.3. Physiological and morphological measurements

Any symptoms of metal toxicity (e.g. discoloration, pigmentation, yellowing, stunting, chlorosis, necrosis) exhibited by plants were visually noted during the experimental period. Before harvest, for each replicate, plant growth was evaluated by measuring plant length from the soil level to the top (root and plant length), root diameter and leaves area were measured. No yellow leave parts were found in the control seedlings. *A.victoriae* plants were harvested after 45 days exposure of seedlings to Pb treatments in both growing seasons. *A.victoriae* seedlings were carefully removed from the pots, separated into leave, shoot and root portions, rinsed thoroughly twice with deionized water to remove any soil, adhering debris and surface dust. The collected plant samples were placed in plastic bags, labeled carefully and brought to the laboratory. All samples were weighed and they were placed in paper bags, leave, shoot and roots were dried in an oven 70◦C for 48 h, the dry weights of them were recorded. Growth measurements were used to estimate the Pb Tolerance Index (TI). TI was calculated by dividing the root length of the plant exposed to stress Pb by that measured during growth in the control. The following equation was used (Sedzik *et al*., 2015).

$$\mathrm{TI}\ (\%) = 100 \times \frac{\text{Root length under Pb treatment}}{\text{Root length in the control}} \qquad (1)$$

Chlorophyll content in *A.victoriae* leaves sample was determined on fresh weight basis. For this 1 g of freshly collected leaves were taken and homogenized properly in a mortar with 10 ml of 80% acetone. This was then centrifuged at 3000 rotations min^{-1} for 15 minutes at room temperature. Then, 1 ml of the surface solution was taken and adjusted with 4 ml 80% acetone. Finally the optical density of the solution was measured by PD-303UV spectrophotometer at wavelengths 645 and 663 nm and chlorophyll content (mg g^{-1}) was calculated as follows (Khermandar, 2013).

$$\text{Col a} = 12.7\,(\text{A } 663) - 2.69\,(\text{A } 645) \qquad (2)$$

$$\text{Col b} = 22.9\,(\text{A } 645) - 4.68\,(\text{A } 663) \qquad (3)$$

$$\text{Col Total} = 20.2\,(\text{A } 645) - 8.02\,(\text{A } 663) \qquad (4)$$

To test the *A.victoriae* resistance of Pb, the Plant Resistance Index (PRI) was calculated as follows. (Khermandar, 2013).

$$\text{PRI} = \frac{\text{dry weight of tissues of metal treated plants}}{\text{dry weight of tissues of control}} \times 100 \qquad (5)$$

2.4. Determination of Pb accumulation

To assess the Pb concentration in both treated and control seedlings (for both growing seasons). Dried plant samples of leaves, shoots and roots were digested with using of HNO_3, of H_2SO_4 and of $HClO_4$ (Moreira *et al.*, 2011) and Pb concentrations were measured by the Flame Atomic Absorption Spectrophotometry (CTA-2000 AAS). The chemical analyses for each organ and treatment were carried out on three replicates and the concentration of Pb was represented in mg per Kg dry weight.

2.5. Evaluation of phytoremediation parameters

After analyzing the results of Pb accumulation by *A.victoriae* different parts, two important factors were studied to understand the possible role of *A.victoriae* in phytoremediation. The two investigated factors are the TF and BCF. Both can be used to estimate a plant's potential for phytoremediation purpose. BCF and the TF are considered when investigating whether a plant is a hyperaccumulator of a metal (Hegazy *et al.*, 2011).

Bioconcentration factor (BCF) is bioaccumulation factor was calculated as the ratio of a given Pb concentration in the plant tissues at harvest to the concentration of the Pb in the external environment. When BCF>1 indicate that the plant is an Accumulator, BCF<1 indicate the plant is an Excluder (Hegazy *et al.*, 2011).

$$\text{BCF} = \frac{[\text{Pb }]\text{plant}}{[\text{Pb }]\text{sediment}} \qquad (6)$$

Translocation factor (TF) is the capability of plants to take up heavy metals in their roots and to translocate them from the roots to their aboveground parts. Therefore, it is the ratio of heavy metal concentration in aerial parts of the plant to that in its roots. This specific criterion for hyperaccumulators should reach >1 (Liu *et al.*, 2010). TF > 1 indicate the plant is effective in the translocation of a metal from root to shoot tissue. (Hegazy *et al.*, 2011).

$$\text{TF} = \frac{[\text{Pb }]\text{shoot}}{[\text{Pb }]\text{root}} \qquad (7)$$

2.6. Statistical Analysis

Combined analysis of variance was used to estimate the average response of given treatments and to test the consistency of the responses from summer season to fall season i.e. interaction of the treatment effects with seasons. The combined analysis of variance provided an overview of the magnitude of variance between the experimental seasons, the variation between treatments (Pb concentrations) and especially the treatments * seasons interaction. The used design was randomized block design and the least significant difference test (LSD) for comparing means. The level of statistical significance was set at P<0.01 and <0.05. All the results are expressed as means and letters that indicate the statistical differences between the means. The software used for the combined analysis of variance was the SAS for windows program (SAS-9.1-portable).

3. Results and Discussion

3.1. Analysis of variance

In the combined analysis of variance over seasons, the effects of Pb concentrations were compared to the Pb accumulations in different plant tissues and grwoth characteristics. The results showed that some traits such as Pb contents in different tissues, TF and BCF had different responses related to treatments in two seasons (Table 2). In addition, significant mean squares of traits for treatments at 1% and 5% probability level were detected for all traits (Table 2).

Table 2. Combined analysis of variance from the evaluation of *A.victoriae* traits in two seasons for 5 Pb concentrations

Source of Variation	df	Mean Square								
		Root length	Diameter	Plant length	Leave area	Leave dry matter	Shoot dry matter	Root dry matter	TI	PRI
Seasons	1	832.13**	21.35**	2650.80**	0.03**	189.55**	112.55**	43.03**	177.63**	175.69**
Error 1	4	8.60	0.21	15.10	0.16	0.28	0.17	0.12	106.26	20.29
Treatments	4	159.75**	3.30**	490.63**	0.39**	1.98**	4.11**	7.53**	1921.33**	2323.42**
Treatment vs. Season	4	2.38^{ns}	0.03^{ns}	1.80^{ns}	0.08^{ns}	0.52*	0.03^{ns}	0.42^{ns}	162.80^{ns}	117.00^{ns}
Error 2	16	8.91	0.44	11.76	0.03	0.18	0.15	0.30	120.39	40.87
C.V		9.04	16.20	5.32	19.03	8.38	7.51	14.73	10.11	6.09

Source of Variation	df	Mean Square										
		Col a	Col b	Col T	Leave	Shoot	Root	S/R	L/R	L/S	TF	BCF
Seasons	1	0.81**	0.01**	0.60**	1456403**	1820403**	2054083**	2.01**	1.52**	0.05^{ns}	6.99**	122.21**
Error 1	4	0.01	0.02	0.05	7363.33	4773.33	11506.67	0.01	0.02	0.01	0.06	0.58
Treatments	4	0.87**	0.17**	1.81**	995941**	1835711**	6124420**	0.62**	0.42**	0.74**	2.11**	342.54**
Treatment vs. Season	4	0.09*	0.01^{ns}	0.05^{ns}	125645**	200495**	880316.67**	0.16**	0.11**	0.01^{ns}	0.50**	27.79**
Error 2	16	0.02	0.02	0.06	4538.33	11510.83	11735.83	0.01	0.01	0.01	0.02	1.09
C.V		15.73	15.47	17.06	10.83	13.30	8.31	18.30	17.08	18.09	15.69	16.00

** Significant at the 0.01 probability level, * Significant at the 0.05 probability level, ns non significant respectively

3.2. Physiological and morphological responses of seedlings

Many studies have shown that when plants absorb Pb at high levels, several physiological systems are damaged and visible symptoms of Pb toxicity include chlorosis and necrosis also reduction of length, leave area, fresh and dry weight (Kumar and Jayaraman, 2014), reduction in leaves number and root diameter (Naz *et al*., 2013), reduction in chlorophyll a, b and total (Sing *et al*., 2012). In this study there was a significant different between treatments but the trend of Pb concentrations effect on TI, root length, dry matter of different tissues (leaves, shoots and roots) for both seasons were the same (Fig. 1, 2).

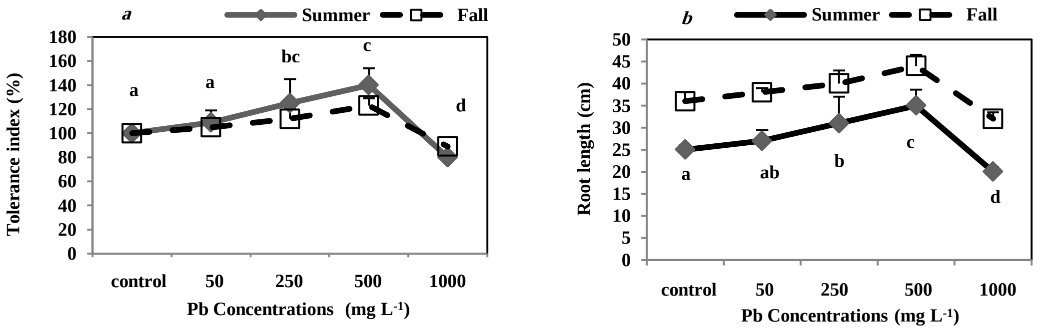

Fig. 1. Effect of Pb concentrations on the Tolerance Index (TI) (a) and root length (b) of *A.victoriae* seedlings in two seasons.

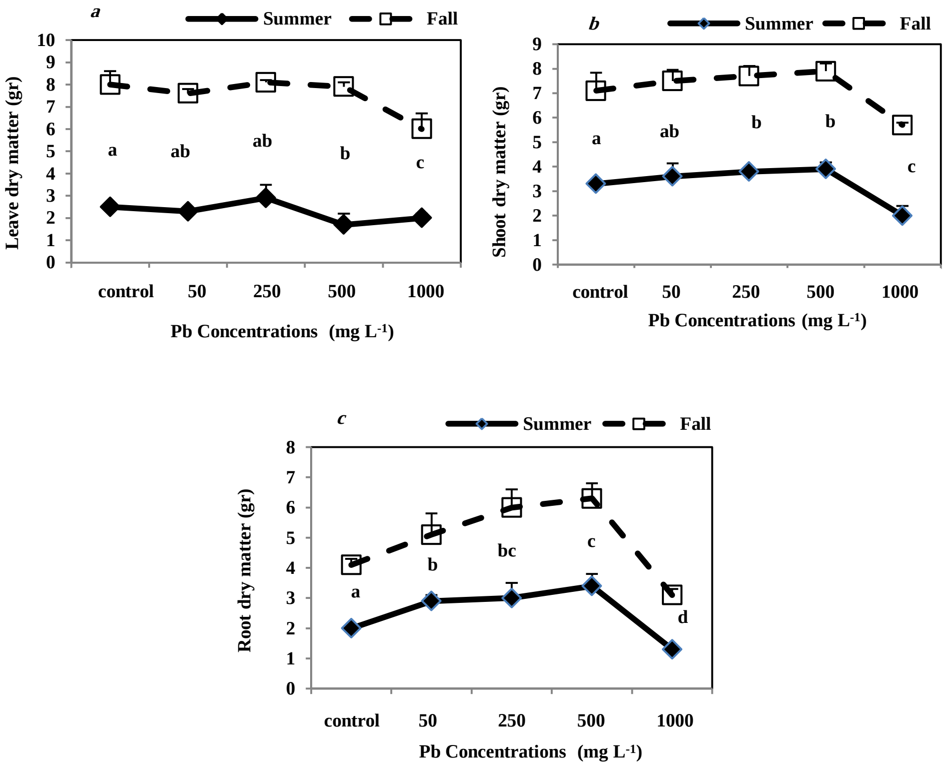

Fig. 2. Effect of Pb concentrations on the leave (a) shoot (b) and root dry matter (c) of *A.victoriae* seedlings in two seasons

The plants and roots growth were influenced variably in Pb contaminated treatments. The increasing of Pb concentrations from 50 to 500 mg L^{-1} had significantly increased the plant length of seedlings (between 2% to 21% higher than the control seedlings). Same trend was observed in plant length as well as root length (Table 3). Root length of seedlings exposed 50 to 500 mg PbL^{-1} were between 7% to 28% higher than the control seedlings (same results by Nagaraju *et al*., 2015; Ano *et al*., 2013; Wang *et al*., 2011). However, nitrate had few inhibitory on the growth. The primary effect of Pb toxicity on plants is the rapid inhibition of root growth, probably due to the inhibition of cell division in root tips. Root system is the main part that absorbs and excludes heavy metal. The root length of sensitive plant is obviously inhibited by heavy metal, while that of tolerant plant is only slightly affected (Wang *et al*., 2011). Root growths of seedlings treated with 1000 mg Pb L^{-1} were reduced (almost 14% lower than the control) as compared to the untreated seedlings (Fig. 1). High concentration of heavy metals leads root hair browning is the result of suberin deposits. Reduction in mitotic cells in meristematic zone of root is the result of decreased root length due to metal stress (Nagaraju *et al*., 2015). Reduction the plant length of seedlings was recorded (almost 18% lower than the control) in response to 1000 mg Pb L^{-1} as compared to untreated control. Reduced seedling length in metal Pb (NO$_3$)$_2$ treatments could be the reduction in meristematic cells present in this region and some enzymes contained in the cotyledon and endosperms. Cells become active and begin to digest and store food which is converted into soluble form and transported to the shoot and root tips for enzyme amylase which converts starch into sugar and protease act on protein. So when activities of hydrolytic enzyme are affected, the food does not reach to the root and shoot affecting the seedling length (Kumar and Jayaraman, 2014). Also the reduction in plant growth during stress is due to low water potential, hampered nutrient uptake and secondary stress such as oxidative stress. Further Pb can also disturb microtubule organization in meristematic cells (Minakshi *et al.*, 2012). Thus the reduction in growth of seedlings by Pb was attributed to the suppression of the cells elongation which was affected due to an irreversible inhibition exerted by Pb on the proton pump responsible for the process (Amira and Qados, 2015). Similarly maximum reduction in root diameter was observed in response to 1000 mg Pb L^{-1} (almost 41% lower than the control) (Table 3) (same results by Naz *et al*., 2013; Pereira *et al*., 2013). Because, plants try to avoid growing roots in the contaminated zones. The inhibition of root after exposure to Pb may be due to a decrease in mineral elements in the root, leading to a decrease in cell division or cell elongation. Also accumulation of metals with in roots reduces the mitotic rate in meristematic zone particularly by blocking metaphase in meristematic cells and thereby reduces their length (Imtiyaz *et al.*, 2014). However, roots of plants treated with the lowest concentration (50 to 500 mg Pb L^{-1}) were not negative affect by the Pb. Pb toxicity in roots of seedlings exposed to 1000 mg Pb L^{-1} is apparent as a reduction in the growth of main root, fewer and shorter lateral roots. TI of seedlings exposed 50 to 500 mg Pb L^{-1} were between 7% to 31% higher than the control seedlings (same results by Wang *et al*., 2011; Amalia *et al*., 2011) to 84% the lowest value (in seedlings exposed to 1000 mg Pb L^{-1}). Therefore, when Pb concentration in the soil medium was increased up to 1000 mg L^{-1}, the TI showed a significant decrease in the value (Fig. 1). The reduction in the growth of *A.victoriae* could be also due to the suppression of the elongation growth rate of cells. The reduction in growth expressed as reduced leave area and decreased plant matter (Table 3). Dry matter of different parts of seedlings increased significantly with the increasing Pb concentrations from 50 to 500 mg L^{-1} in the medium as compared to the controls (Table 3). The maximum leave, shoot and root dry matter (almost 11%, 15% and 62% respectively, higher than the control) were found in seedlings exposed with 500 mg Pb L^{-1} (same results by Nagaraju *et al*., 2015; Tang *et al.,* 2009). Reduction the leave, shoot and root dry matter seedlings (almost 18%, 25% and 25% respectively, lower than the control) were recorded in response to 1000 mg Pb L^{-1} as compared to untreated control (Table 3 and Fig. 2). The reason for reduction in shoot and roots dry matter due to heavy metals could be ascribed to the reduction in meristematic cells (Amira and Qados, 2015). The reduced matter indicates the plants sensitivity to various stress viz heavy metal stress that damage physiological functions (Nagaraju *et al.,* 2015). The results showed that the leave area was reduced (in plants treated with 1000 mg Pb L^{-1} almost 42% lower than the control (Table 3) (same results by Kumar and Jayaraman, 2014; Imtiyaz *et al.,* 2014; Farooqi *et al*., 2011; Bhardwaj *et al*., 2009). The reduction of the leave area ratio is a natural response and may be related to the higher leave area ratio during the early plant developmental stages (Pereira *et al*., 2013). Also reduction in leave area and seedling dry matter might be due to accumulation of Pb in soil which physically blocks water uptakes from root to shoot and is related with the rate of photosynthesis, mainly associated with the water content and CO_2 absorption (Farooqi *et al*., 2011). In addition, PRI in all treatments ranged from 122% the highest value (in seedlings exposed to 500 mg Pb L^{-1}) to 73% the lowest value (in seedlings exposed to 1000 mg Pb L^{-1}) (Table 3). Tolerance to heavy metals in plants may be defined as the ability to survive in a soil that is manifested by an interaction between a genotype and its environment (Farooqi *et al*., 2011). Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity (Amira and Qados, 2015). Chlorophyll b content was found lesser than chlorophyll a in *A.victoriae* seedlings leaves. Heavy metal has deleterious effects on the content and functionality of the photosynthetic pigments. This can be caused by the inhibition of pigment synthesis or direct oxidative damage to the pigments. They comprise impairments of chlorophyll synthesis resulting in chlorotic leaves, changed ratios of chlorophyll a, b and photosynthetic activity, dwarfism of plants or effects on root ultrastructure (Saikachout *et al*., 2015). In all treatments exposed to Pb, the chlorophyll contents were lower than the control seedlings (same results by Amira and Qados, 2015; Sedzik *et al*., 2015; Minakshi *et al.,* 2012; Sing *et al*., 2012; Debrito *et al*., 2011; Bhardwaj *et al*., 2009; John *et al*., 2009). Maximum decrease in chlorophyll (a, b & total) content in *A.victoriae* leaves was recorded in 1000 mg Pb $L^{−1}$ treatments lower than the control seedlings in both growing seasons (Fig. 3).

Table 3. Growth parameters of *A.victoriae* seedlings, grown in pots and exposed to various concentrations of Pb in two seasons

		Root length (cm)	**Diameter (mm)**	**Plant length (cm)**	**Leave area (cm^2)**	**Leave dry matter (gr)**	**Shoot dry matter (gr)**	**Root dry matter (gr)**	**TI (%)**	**PRI (%)**
Seasons	**Summer**	27.73^a	3.28^a	55.00^a	1.36^a	2.55^a	3.35^a	4.95^a	110.93^a	107.33^a
	Fall	38.26^b	4.97^b	73.80^b	1.29^a	7.57^b	7.23^b	2.56^b	106.06^a	102.49^a
Treatments Pb (mg L^{-1})	**Control**	30.50^a	5.05^a	62.50^a	1.50^a	5.00^a	5.19^a	3.05^a	100.00^a	100.00^a
	50 $^{(mg\ L-1)}$	32.66^{ab}	4.31^{ab}	63.50^a	1.46^a	5.31^{ab}	5.61^{ab}	4.05^b	107.33^a	109.96^b
	250 $^{(mg\ L-1)}$	36.00^b	4.18^b	69.33^b	1.43^a	5.37^{ab}	5.77^b	4.55^{bc}	119.00^{bc}	118.28^c
	500 $^{(mg\ L-1)}$	39.00^c	4.10^b	75.50^c	1.35^a	5.53^b	5.97^b	4.93^c	131.50^c	122.95^c
	1000 $^{(mg\ L-1)}$	26.16^d	2.98^c	51.16^c	0.87^b	4.09^c	3.90^c	2.19^d	84.66^d	73.36^d

* Means with the same letter are not significantly different

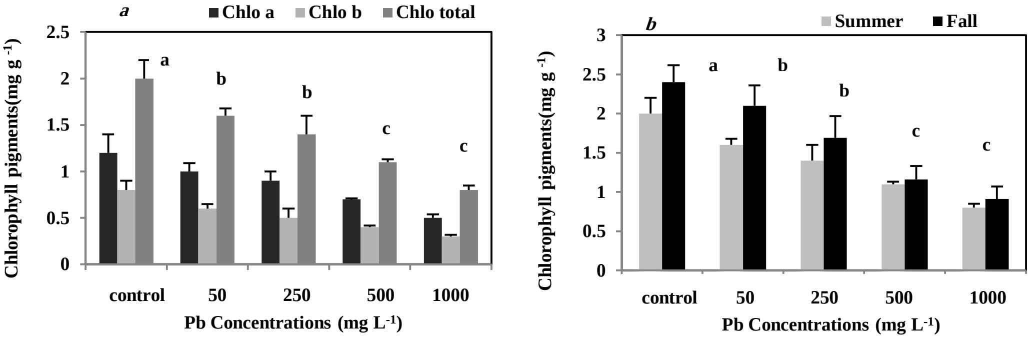

Fig. 3. Chlorophyll pigments (a, b & total) in *A.victoriae* leaves (a) and comparison of total chlorophyll between two seasons (b)

There were not significant differences in chlorophyll (b and total) contents between two seasons in all treatments (Table 2). Decreased chlorophyll content associated with heavy metal stress may be due to the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Heavy metals severely inhibit the plant growth and even cause plant death by disturbing the uptake of nutrients and thereby decreasing the photosynthesis via degradation of chlorophyll (Debritto *et al*., 2011). It can be assumed that Pb may inhibit chlorophyll biosynthesis by impairing the uptake of essential photosynthetic pigment elements, such as Ca, Mg, K and Fe (Amira and Qados, 2015). However, results showed physiological and morphological parameters treated with the highest concentration were significantly affected as decreasing in 1000 mg Pb L^{-1} treatments (Table 2 and Fig. 1, 2). Pb is toxic to the plant growth and only a small number of plant species were reported to accumulate this metal without signs of toxicity. The most common effect of metal toxicity in plants is stunted growth, leave chlorosis and alteration in the activity of many key enzymes of various metabolic pathways (Saikachout *et al*., 2015). The visible symptoms of root necrosis in cultures exposed to higher concentrations of Pb can be at least partially explained by the excess of Pb in the nutrient medium, which can lead to reduction in plant growth, inhibition of photosynthesis, cell injury and tissue necrosis, and might even be lethal (Cassanego *et al*., 2015). The seedlings exposed to a Pb concentration of 1000 mg Pb L^{-1} did visible symptoms of Pb toxicity such as chlorotic spots, necrotic lesions at the leave surface and the length decrease in roots and plant length (same results by Gupta and Chakrabart, 2013; Rappe *et al*., 2011). Chlorosis of leave is one of the physiological symptoms of Pb action on plants. Chlorosis is caused by inhibition of synthesis of photosynthetic pigments (Sedzik *et al*., 2015). Also reduction of total chlorophyll and were promoted by reduction in growth and dry matter (Table 3). The present study reveals that the *A.victoriae* had no growth and matter inhibition but even showed a higher amount of growth and matter in seedlings treated with 50 to 500 mg Pb L^{-1}. These results indicated of *A.victoriae* tolerance in high amounts of Pb. Plants that survive in Pb contaminated soil may present morphological, physiological and biochemical alterations and may develop mechanisms for detoxification and tolerance such as accumulating Pb in the vacuol and cell walls of roots and aerial parts (Cassanego *et al*., 2015).

3.3. Pb contents in A.victoriae tissues

Generally, the amount of accumulated Pb in the tissues increased with increasing concentration of applied Pb. The results of combined analysis of variance over seasons, showed that Pb accumulation contents in all the treatments was significantly different between two seasons (Table 2). Metal concentrations in different parts of the plants were different. The two growing seasons of *A.victoriae* seedlings accumulated large concentrations of Pb in their root tissues in all treatments. This shows *A.victoriae* has more potential for Pb accumulation in roots. Fig. 4 (a, b) shows that concentrations of Pb in leave and shoots of *A.victoriae* seedlings in fall growing season were significantly larger than summer. While in all treatments, Pb concentrations in root tissues of *A.victoriae* seedlings in summer growing season were greater than fall season (Fig. 4c).

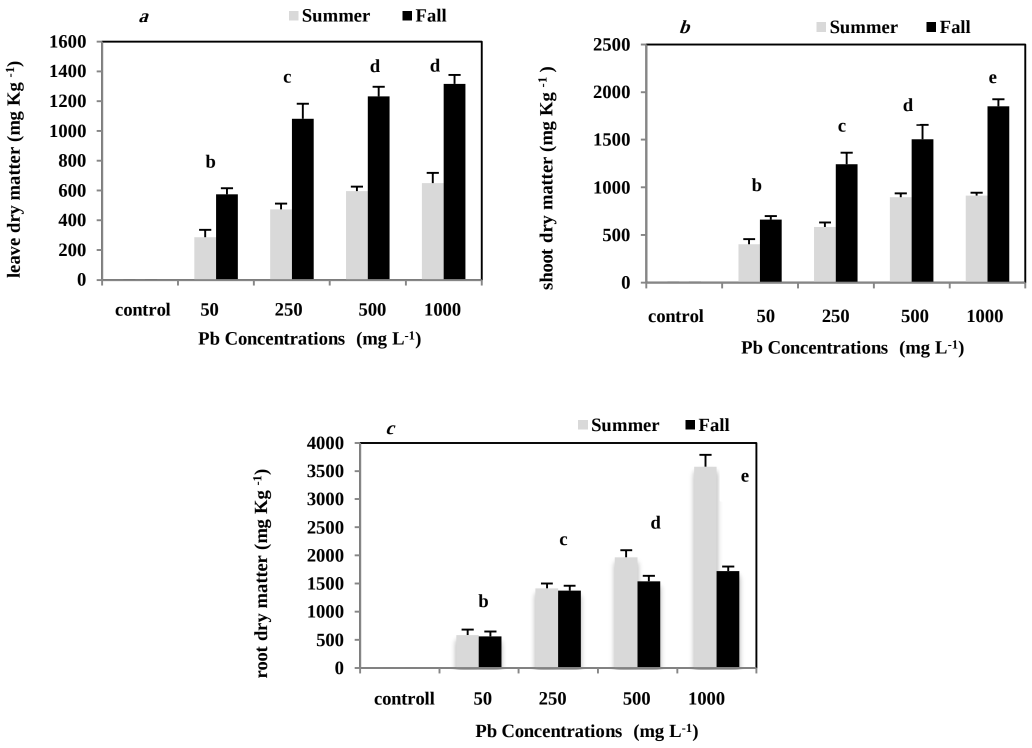

Fig. 4. Pb concentrations in leaves (a), shoots (b) and roots (c) of *A. victoriae* seedlings subject to different concentration of Pb treatments in two seasons.

A.victoriae accumulated substantial amounts of Pb in root tissues and it was almost five times more than in the shoots and six times more than in the leaves in summer (Table 4). In all treatments, most of transported Pb to aboveground tissues was located in the shoots. The concentration gradients of Pb in different parts of seedlings ranked as follows: roots> shoots> leaves (Fig. 4). The amounts of Pb in roots were increased from 710 to 2651.67 mg kg^{-1} of dry matter of 50 to 1000 mg Pb L^{-1}, respectively. There were also significant differences between the amounts of Pb in roots for treatments at 50 to 1000 mg Pb L^{-1} as compared to control seedlings. Similar to the roots, the amounts of Pb in leaves and shoots increased from 431.67 to 983.33 mg kg^{-1} and from 533.33 to 1381.67 mg kg^{-1} of dry matter for 50 to 1000 mg PbL^{-1} treatments, respectively (Table 4). There were significant differences between the amounts of Pb in shoot and roots for treatments at 50 to 1000 mg Pb L^{-1} as compared to the control, but there were no significant differences between the amounts of Pb in leaves of 500 and 1000 mg Pb L^{-1} (Table 4).

Table 4. Pb contents (mg Kg $^{-1}$ dry matter) in leave, shoot and roots, Pb translocation factors (TFs; shoot/root Pb ratios (S/R), leave/root Pb ratios (L/R), leave/shoot Pb ratios (L/S) and TF and bioconcentration factor (BCF) of *A.victoriae* seedlings grown in pots and exposed to various concentrations of Pb in two seasons.

		Leave (mg kg^{-1})	**Shoot (mg kg^{-1})**	**Root (mg kg^{-1})**	**S/R (mg kg^{-1})**	**L/R (mg kg^{-1})**	**L/S (mg kg^{-1})**	**TF (mg kg^{-1})**	**BCF**
Seasons	**Summer**	401.33^{a}	560.00^{a}	1564.00^{a}	0.31^{a}	0.22^{a}	0.58^{a}	0.54^{a}	4.51^{a}
	Fall	842.00^{b}	1052.67^{b}	1041.00^{b}	0.83^{b}	0.68^{b}	0.65^{a}	1.51^{b}	8.55^{b}
Treatments Pb (mg L^{-1})	**Control**	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
	50 $^{(mg\ L-1)}$	431.67^{b}	533.33^{b}	710.00^{b}	0.83^{b}	0.68^{b}	0.79^{bc}	1.52^{b}	19.30^{b}
	250 $^{(mg\ L-1)}$	778.33^{c}	915.00^{c}	1396.67^{c}	0.71^{bc}	0.56^{c}	0.85^{b}	1.26^{c}	6.77^{c}
	500 $^{(mg\ L-1)}$	915.00^{d}	1201.67^{d}	1756.67^{d}	0.67^{c}	0.55^{c}	0.74^{bc}	1.23^{c}	4.24^{d}
	1000 $^{(mg\ L-1)}$	983.33^{d}	1381.67^{e}	2651.67^{e}	0.66^{c}	0.47^{c}	0.71^{c}	1.13^{c}	2.36^{e}

* Means with the same letter are not significantly different

Different translocation dynamics and accumulation capacity of plant tissues for heavy metals could be explained by the insufficient selectivity of various ion transporting channels or by the different cell wall permeability for ions, which both could influence the effectivity of intracellular storage dynamics (Mala *et al*., 2010). In this study, the BCF values were found between 2.36 and 19.30 for treatments at 1000 and 50 mg Pb L^{-1}, respectively, were greater than 1 which indicated the phytoremediation potential of *A.victoriae* for Pb (Fig. 5).

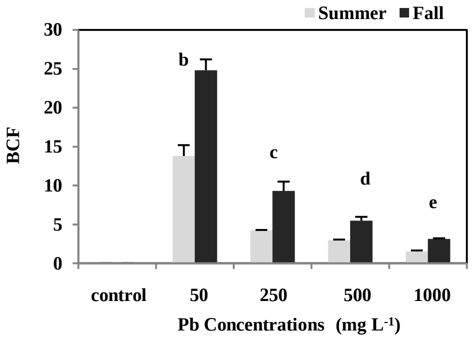

Fig. 5. BCF of *A. victoriae* seedlings for different concentration of Pb treatments in two seasons

BCF is a common important factor when considering the phytoremediation potential of a given species (Hegazy *et al.,* 2011). These high BCF shows the higher amount of metal accumulated in the roots than shoots and leaves. In all the treatments, an average of 43% to 53% from the Pb accumulated in the entire plant tissues was retained in the roots of 50 to 1000 mg Pb L^{-1}, respectively (Table 4 and Fig. 5). These results were associated with values ($0.47 \leq$ TFs ≤ 0.83) of the TFs from roots in all the treatments (Table 4). The movements of heavy metal from soil to roots and shoots, indicating the efficiency to uptake the bioavailable metals from the system. The TF (Translocation Factor) were averagely found between 1.13 and 1.52 for treatments at 1000 and 50 mg Pb L^{-1}, respectively. The TF higher than 1 in the fall season (1.83 and 2.3 for treatments at 1000 and 50 mg Pb L^{-1}) indicating that *A.victoriae* has effectively transferred Pb from roots to shoots. Therefore, *A.victoriae* could be considered as hyper accumulators, because TF higher than 1 and *A.victoriae* accumulated more than 1000 mg g^{-1} (0.1% dry wt) Pb in aerial parts. Also suitable for phytoextraction purposes (metal exclusion strategies of plant, without destroying soil structure and fertility) and aboveground should be harvested at the fall season, because the concentration of the metal in the aboveground tissues is maximal (same results by Naz *et al*., 2016; Sawidis *et al.,* 2011; Tang *et al.,* 2009). The present study indicated that concentrations of Pb differed between two seasons. The seedlings response to soil Pb varied both in nature and in the amount in two seasons (Fig. 4 a, b and c). In addition, *A.victoriae* was able to tolerate relatively high amounts of Pb, produce dry matter (up to 1000 mg L^{-1}) as much as uncontaminated treatments and bioaccumulate in roots (same results by Amira and Qados, 2015; Eissa *et al.,* 2014; Gupta and Chakrabart, 2013; Pereira *et al*., 2013; Hazrat *et al*., 2012; Minakshi *et al.*, 2012; Qiu *et al.*, 2011; Wang *et al.*, 2011; Mala *et al*., 2010; John *et al*., 2009). In summer, limited the metal translocation to above ground tissues and survives despite of high amount of Pb has the potential to be used for Pb phytostabilization. Plant roots physically stabilize the soil, thus preventing erosion and reduce water percolation through the soil. Sorption or precipitation of metals can occur in the rhizosphere. Moreover, Pb tends to be accumulated in roots more than in aerial parts. Because of actively growing roots provide a barrier which restricts the movement of Pb to the aboveground parts of plants. But at the treatment 1000 mg Pb L^{-1} the Pb concentrations in the leaves and shoots of *A.victoriae* seedlings exceeded 1000 mg kg^{-1} in summer growing season (Table 4).

4. Conclusion

The use of fast growing woody plants with high capability to uptake and accumulate toxic compounds forms an important objective of contemporary environmental politics of industrially developed countries. To the best of our knowledge, the present work is considered one of the first studies focusing on the use of *A.victoriae* species in during the growing seasons in phytoremediation and rehabilitation of Pb contaminated soils. The results showed that *A.victoriae* a native to the arid zone appeared to be hyper tolerate, accumulate high concentrations of Pb and it could be regarded as a potential accumulator. Certain levels of Pb in solution induced a stimulatory effect on some physiological and morphological parameters of *A.victoriae*. These indicated that this species may possess certain interesting mechanisms for Pb, detoxification. The discovery of this species thus provides a new material to study the underlying mechanisms of stimulatory effect and accumlation of Pb. In addition, in this study we also understand the pattern of Pb accumulation in *A.victoriae* in two growing seasons so that we can effectively manage Pb contaminated areas. The highest concentrations of Pb were found in roots. Therefore *A.victoriae* could be considered as a root bioaccumulation species. However, significant concentrations of Pb were found in both shoots and leaves, especially in the fall season. Therefore, *A.victoriae* will provide an important material for exploring the tolerance strategies of Pb accumulation in plant cells and have high application value in repairing Pb contaminated soils. There is need to consider variety responses to heavy metal polluted soils in selection schemes for *A.victoriae* improvement in areas where soil pollution is prevalent. Finally, from the results of study, it can be concluded that *A.victoriae* due to its ability to accumulate high amount of Pb in its tissues relatively large biomass and high tolerance to the drought and saline soils, is suitable and effective choice to be used as a tool of phytoremediation (phytostabilization or phytoextraction) in industrial sites of the arid zones. Our findings suggest that *A.victoriae* is highly tolerant to Pb stress and has the advantages of high capacity for adaptation to poor, easy cultivation and deep root system, which most heavy metal tolerant plants lack and will supply a gap of most Pb tolerant plant discovery. The study revealed the potential use of *A.victoriae* as candidate plants for environmetals monitoring. Also biotechnological and genetic engineering based approaches can be used to enhance the naturally occurring plants to detoxify hazardous compounds.

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