

Assessment of phytoremediation potential of range vegetation for Lead (Pb) and Chromium (Cr) contamination in the Cholistan Desert, Pakistan



Muhammad Abid^{1*} and Muhammad Rafay¹

¹Institute of Forest Sciences, The Islamia University of Bahawalpur, Pakistan.
Corresponding author: m.abid@iub.edu.pk

Article Info

Abstract

Keywords:

Climate change, Chromium, Lead, Heavy metals, phytoremediation, Semi-arid

Date:

Received: 13 January 2025
Revised: 29 January 2025
Accepted: 17 April 2025
Online: 30 April 2025
Published: 30 May 2025
Volume: 04 (01), 2025



Copyright: © 2025 by the authors. This article is distributed under the Creative Commons Attribution (CC BY) license, allowing for open access and sharing under specified terms and conditions.
(<https://creativecommons.org/licenses/by/4.0/>)

The study was conducted to evaluate the phytoremediation potential of heavy metals by using xerophytes species; *Aristida hystrix* L. and *Aristida adscensionis* L. of Cholistan desert. The Phytoremediation, morphology and physiology of the grasses were studied. The experiment was conducted hydroponically in the experimental area of Forestry, range and Wildlife Management, The Islamia University of Bahawalpur. Results showed that under Chromium (Cr) and lead (Pb) stresses *A. hystrix* and *A. adscensionis*, exhibited significant differences in their morpho-physiological traits as compared to control treatments. Under 50 to 400 uM Pb treatments *A. adscensionis* exhibited reduction in root/shoot fresh weight (0.92 B) and (0.61 C) respectively and similar results were obtained in *A. hystrix*. Increasing Cr concentrations also significantly reduced the membrane stability index (MSI) of both grass species. Under 50 to 400 uM Pb concentrations *A. adscensionis* exhibit reduction in MSI from (87 A) to (42 C). Cr and Pb concentrations in *A. adscensionis* were increased with elevated treatments. At higher Cr concentration (80 uM) its values were (41 A) while under 20 uM it was (15 C). Pb levels were also increased with increasing Pb level in *A. hystrix* at 400 uM its concentration was (198 A) and it was (29 C) at 50 uM. Conclusively, both selected grass species have capability to absorb large amount of heavy metals from the soil at the cost of reduction in growth, however, considered as good options for the phytoremediation of Cr and Pb.

1 Introduction

Due to recent developments in industrial sector, heavy metals cause serious problems in the agro-ecosystem. From these industries, toxic compounds including heavy metal are discharged into surroundings which cause serious disruption in biogeochemical cycles (Ghosh *et al.*, 2015). Heavy metal contamination in the soil mainly depend on geological location and anthropogenic activities such as sludge application, automobile exhaust, waste water irrigation, waste disposal, smelting processes (Ban *et al.*, 2017; Rezaia *et al.*, 2015). In agriculture soil, generally heavy metal was considered as toxic if it is present above 0.2 mg/Kg for As, Cd, Cr, and 150 mg/Kg for Ni, Cu, Pb concentrations (Desai *et al.*, 2019; Klink, 2014; Yao *et al.*, 2012; Zhang, *et al.*, 2014). Non-degradable nature of heavy metals poses hazards through water and soil to human and animal body tissues and passes to different trophic levels. Bio magnification occurs during this passage in the ecosystem may resulted in mortality and morbidity (Desjardins *et al.*, 2018). Accumulation of different heavy metals affects plant growth differently. In plants accumulation of heavy metals affects many enzymatic reactions, chlorophyll biosynthesis, root length, plant biomass, respiration rate and seed germination (Beltrán, Gilberto *et al.*, 2012; Khalid *et al.*, 2017). Lead (Pb) poisoning in animals is a tissue poison and gets stored in different parts of the body especially in bones,

*Email: m.abid@iub.edu.pk

liver, kidney and brain. Lead ingestion is source of blood lead burden (Swarup *et al.*, 2005). Arsenic toxicity mostly causes in weight loss, capricious appetite, conjunctively and mucosal erythematic lesion including mouth ulceration and reduce milk yield in livestock. (Franzblau and Lilis, 1989). Lead toxicity causes abortions in goat and significant reduction is observed in production of egg in poultry (Thomas., 1996).

To mitigate heavy metal toxicity, phytoremediation is referred to exploitation of plants for detoxifying contaminants in the soil of environment (Khandare and Govindwar, 2015). In last few decades, phytoremediation was extensively used for decreasing soil contamination; first time used in 1983 (Wiangkham and Prapagdee, 2018; Zhu *et al.*, 2018). Plants are divided in to three categories based on ability to accumulate have metals i.e. excluders, accumulator and hyper accumulator. Literature shows that heavy metals is accumulated in plants from soil, water or air, nevertheless, soil is the major source (Khandare and Govindwar, 2015; Wiangkham and Prapagdee, 2018; Zhu *et al.*, 2018). Grasses can accumulate high amount of Pb, Cr, Cd, Ca, Cu and many other toxic heavy metals in it (Werle *et al.*, 2017). Therefore, it was common practice to grow grasses before growing major crop in India, Bangladesh, Pakistan (Madri *et al.*, 2004).

The experiment was conducted to measure the reclamation potential of selected range grasses and their degree of tolerance towards heavy metal toxicity and their effect on the morpho-physiological traits.

2 Materials and Methods

2.1 Experimental conditions

Selected grass species *A. hystrix* and *A. adscensionis* were grown in Hoagland's solution containing essential and non-essential nutrients (see Table 1) for plant growth in 4-L pots for enhancing rate of water and nutrient uptake by grass species. pH of the Hoagland's solution was maintained below 6.0-6.5. Electric air-pump was used to continue aeration in the solution. The hydroponic solution was replaced with fresh solutions on weekly basis. These grass species were treated with Chromium (Cr) and lead (Pb), at the concentrations 20,40, and 80 $\mu\text{M/L}$ potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) and 50,200,400 μM Lead Nitrate ($\text{Pb}(\text{NO}_3)_2$) respectively. Both grass species were grown separately (in triplicates) in hydroponic solution containing different concentrations of heavy metals for six weeks. Plant growth was regularly monitored during this period. After six weeks of treatments, both grass species were harvested and analyzed for following morphological and physiological changes.

2.2 Parameters

Samples from each species were taken and washed through tape water and then rinsed with deionized water. Weight was recorded by using electrical balance. After this the amples were air dried after washing with tape water and deionization. Then these were oven dries at 70°C for three days.

Shoot/Root length

Root and Shoot were separated from whole plant. Lengths were measured by using measuring tape..

Determination of Chlorophyll contents

Chlorophyll contents were measured by using chlorophyll measuring spade.

Fresh and dry Weight

Weight was recorded by using electrical balance.

Relative Leaf Water Contents

LRWC was calculated by using the formula as giving by Lazcano-Ferrat and Lovatt (1999).

$$\text{LRWC \%} = (\text{FW}-\text{DW}) / (\text{TW}-\text{DW}) \times 100$$

Membrane Stability Index (MSI)

MSI was calculated as

$$\text{MSI} = [1-(C_1/C_2)] \times 100$$

Determination of Chromium and lead content

For the determination of chromium in root and shoot selected grass species, method of Wolf (1982) was used. Lead concentration in the roots and shoots were determined by already established protocol (Al *et al.*, 2014). Briefly, 100 mg of root and shoot samples were grinded in motor and pestle; dissolved in 10 ml of extraction buffer ($\text{HNO}_3:\text{HClO}_4$;3:1 ratio) in a pre-autoclaved flask and incubate for 12 hours at room temperature. After incubation, flasks were heated at 55°C for 20 min or till colorless point and allow them to cool at room temperature. All material was shifted to volumetric flasks and total volume was adjusted to 50 ml with distilled water. Flask were vigorously shaken for 2 min and then filtered. Absorbance was measured on Spectrophotometer and compared with standards.

2.3 Statistical analysis

The experiment was planned in completely randomized design (CRD). The data were subjected to statistical analysis using Microsoft® Excel software and SPSS (version 21.0) for the determination of analysis of variance (ANOVA) and LSD for comparison of mean values.

3 Results

The current research was aimed to evaluate the hidden potential of range grasses for phytoremediation of heavy metals and their effect on morpho-physiological traits.

3.1 Morphological parameters

Results showed that under heavy metal stress of Cr and Pb in both selected plants *A. adscensionis* and *A. hystris* exhibit significant difference in root length, shoot length, shoot fresh weight, root dry weight, shoot fresh weight and shoot dry weight as compared to control. For example, it was observed that from 50 to 200 uM Pb concentration, *A. adscensionis* exhibit more reduction in RFW as compared to *A. hystris*. Similar trend was observed at 20 uM Cr concentration, however at 40 uM Cr treatment, more reduction in root fresh weight was found in *A. hystris*. Similar trend was observed with other morphological parameters.

Table 1: Mean square values of morphological parameters of two range grass species under influence of different Chromium (Cr) treatments

Source	RL	SL	RFW	RDW	SFW	SDW
Grass ssp.	41.59*	91.66**	0.185**	0.06*	0.27**	10.51*
Cr Conc.	23.61*	268.41**	0.155**	0.03**	0.14*	8.59*
Grass Ssp. x Cr Conc.	8.22 ^{NS}	8.52 ^{NS}	0.31**	0.11*	0.13 ^{NS}	4.99*
Error	5.49	4.39	0.001	0.005	0.035	1.03

Table 2: Mean square values of morphological parameters of two range grass species under influence of different Lead (Pb) treatments

Source	RL	SL	RFW	RDW	SFW	SDW
Grass ssp.	38.51**	81.98*	0.129*	.215**	0.41**	9.95**
Pb Conc.	21.44**	94.15*	0.183*	.004*	0.28*	5.75*
Grass Ssp. x Pb Conc.	4.81 ^{NS}	17.65 ^{NS}	0.35**	0.19**	0.05 ^{NS}	7.22*
Error	4.77	13.75	0.013	0.01	0.04	1.39

NS = non-significant; * = significant; ** = highly significant ($p < 0.05$)

RL = root length; SL = shoot length; RFW = root fresh weight; RDW = root dry weight; SFW = shoot fresh weight; SDW = shoot dry weight

3.2 Physiological parameters

In physiological parameters, relative leaf water content (RLWC), chlorophyll content (CC) showed a comparable reduction under Pb and Cr stress compares to control treatment. It was observed that RWC of *A. hystris* was more 34 than *A. adscensionis* in normal growth conditions. Cr concentration have significant effect on RWC of both grass species. from 50 to 200 uM Pb concentration, *A. adscensionis* exhibit more reduction in Chlorophyll content as compared to *A. hystris*. Similar trend was observed with Chlorophyll Content. However, in contrast, results showed that there was no significant change in MSI of both grass species under heavy metal treatments. Root of both grass species showed highest amount of Cr and Pb intake as compared to shoot. It was observed that under heavy metal stress of Cr and Pb, both selected plants *A. adscensionis* and *A. hystris* revealed comparable increment in metal content as compares to control.

Table 3: Mean square values of physiological parameters of two range grass species under influence of different Chromium (Cr) treatments

Source	RWC	MSI	Chl	CrR	CrS
Grass ssp.	18.41*	45.71*	125.8**	385.66**	198.60**
Cr. Conc.	446.88**	175.22**	151.4**	735.11**	168.21**
Grass Ssp. x Cr Conc.	10.51 ^{NS}	57.91*	17.6 ^{NS}	69.45*	35.17*
Error	3.15	4.55	5.49	3.55	1.85

Table 4: Mean square values of physiological parameters of two range grass species under influence of different Lead (Pb) treatments

Source	RWC	MSI	Chl	PbR	PbS
Grass ssp.	22.56*	22.50*	169.4**	1750.5**	1950.6**
Pb Conc.	495.88**	149.82**	124.2**	2510.1**	1802.9**
Grass Ssp. x Pb Conc.	18.12*	13.61*	7.91 ^{NS}	1011.5*	1235.5**
Error	5.55	3.72	4.99	171.6	241.9

NS = non-significant; * = significant; ** = highly significant ($p < 0.05$)

RWC = relative water content; MSI = membrane stability index; Chl = Chlorophyll; CrR = Chromium content in roots; CrS = Chromium content in shoots

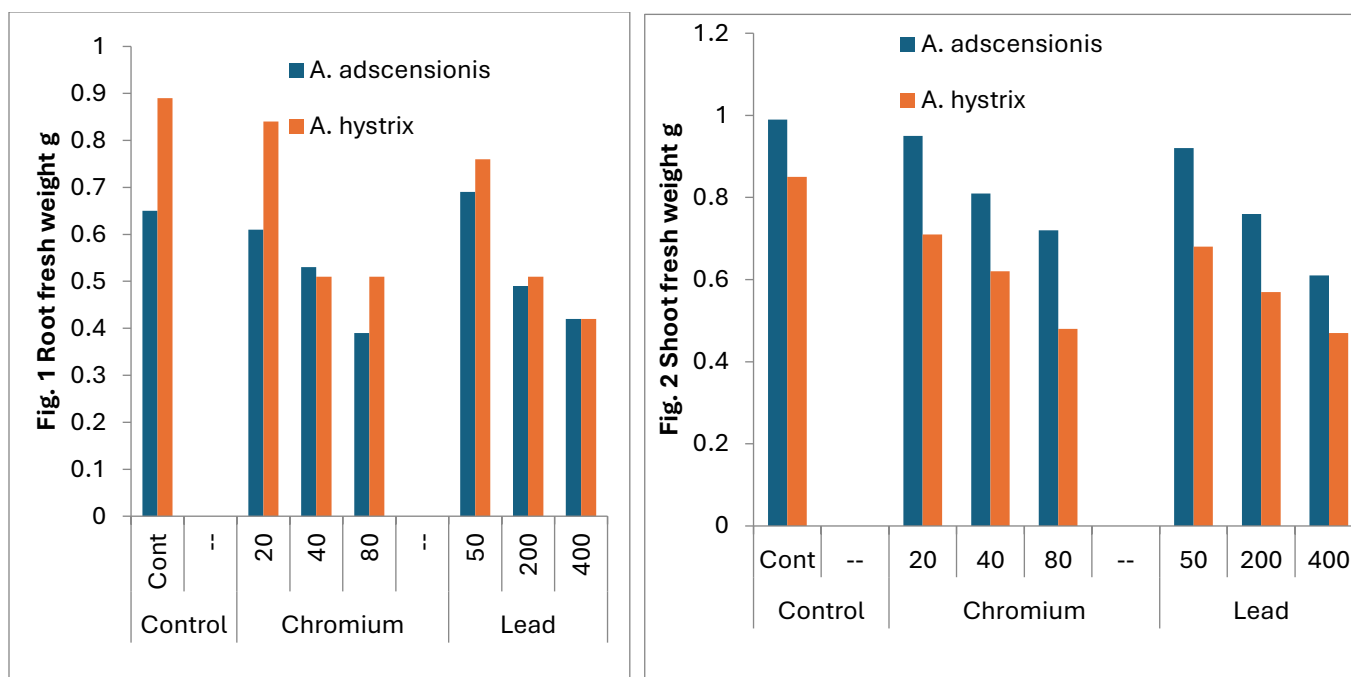


Figure 1,2. Root/shoot fresh weight

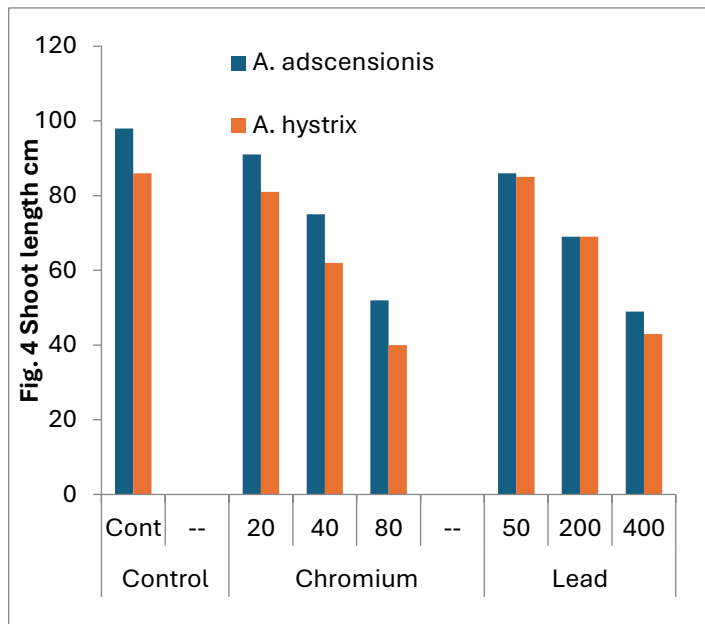
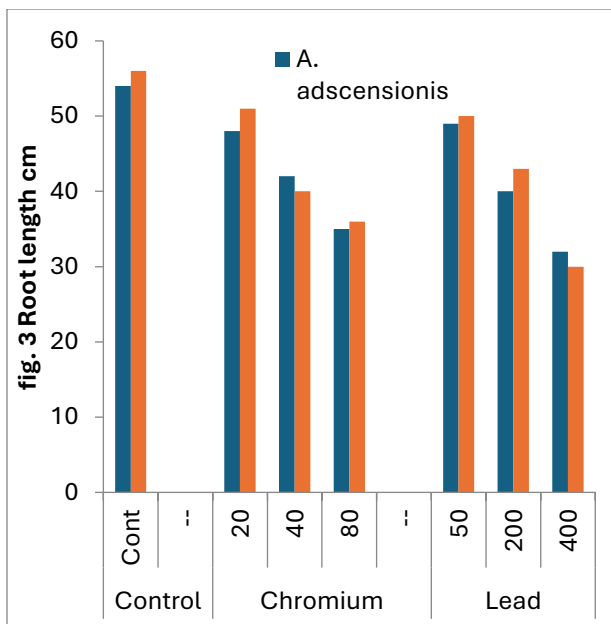


Figure 3,4. Root/shoot length

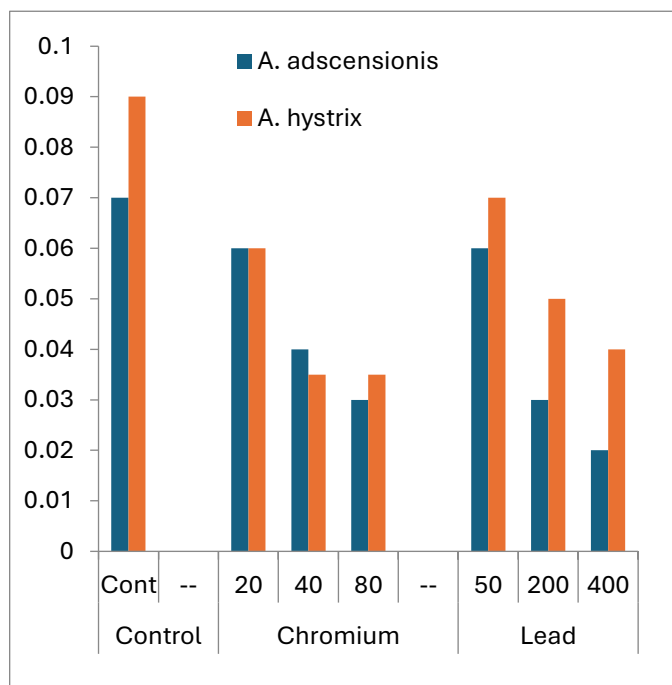
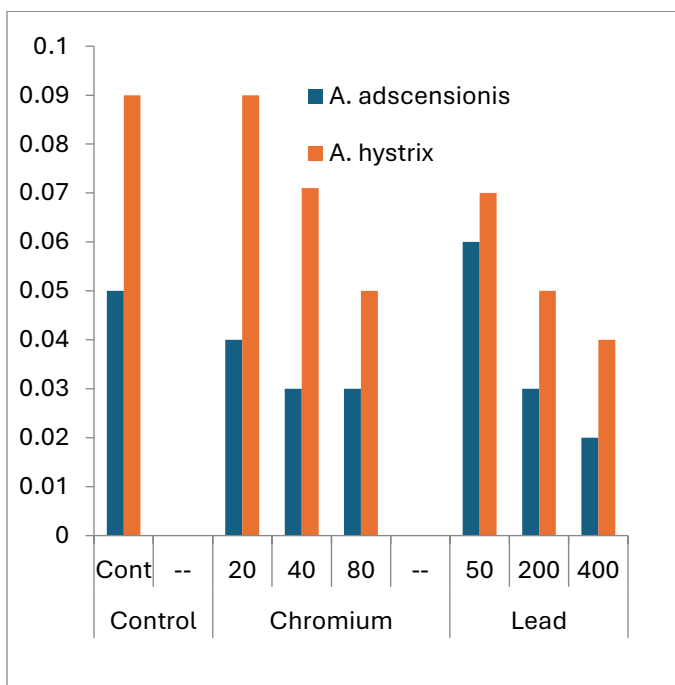


Figure 5,6. Root/shoot dry weight

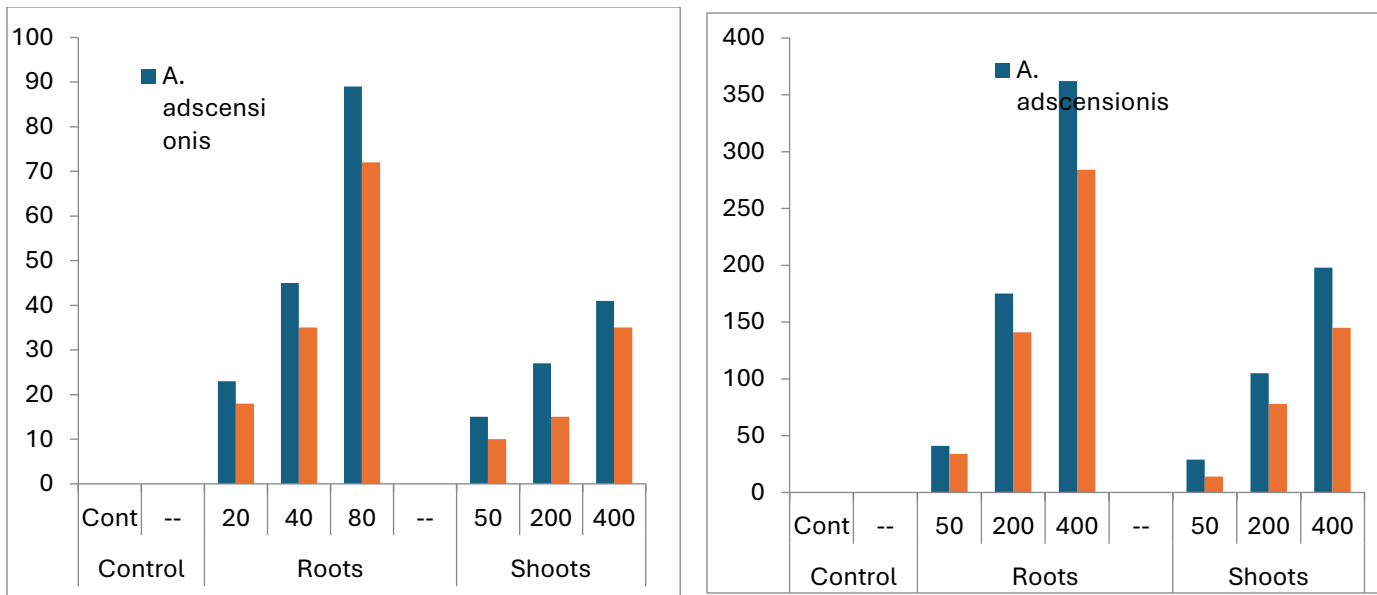


Figure 7,8. Chromium and lead concentrations ppm

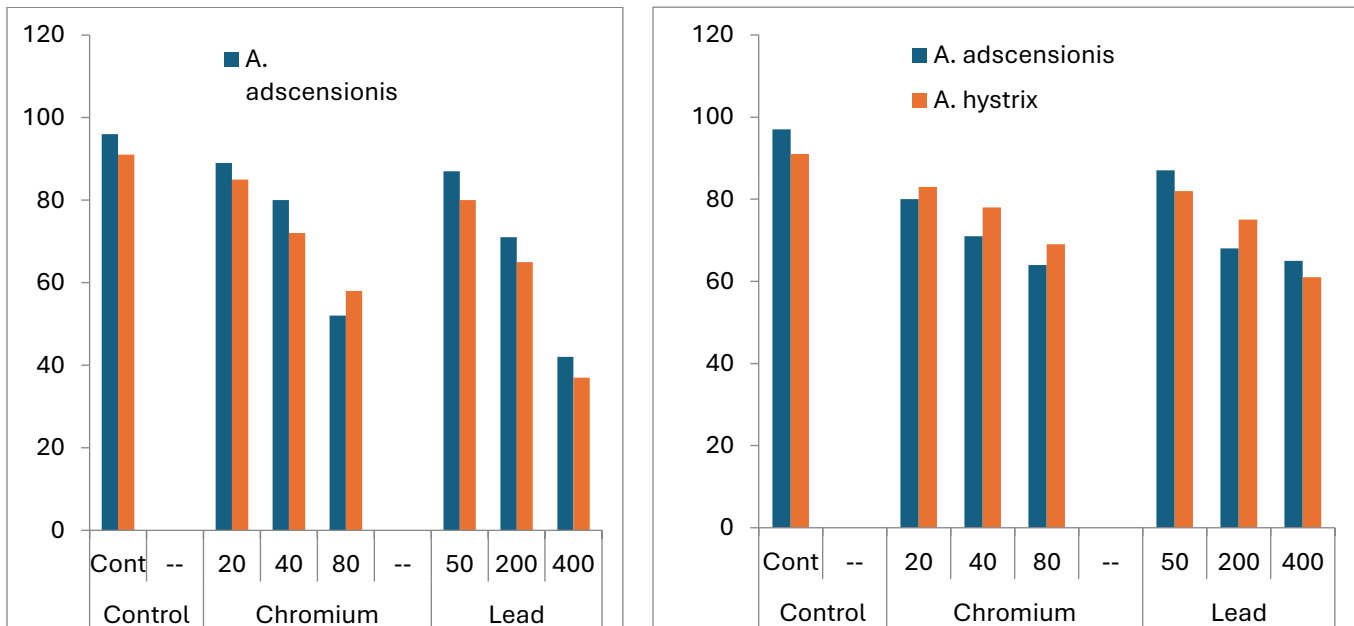
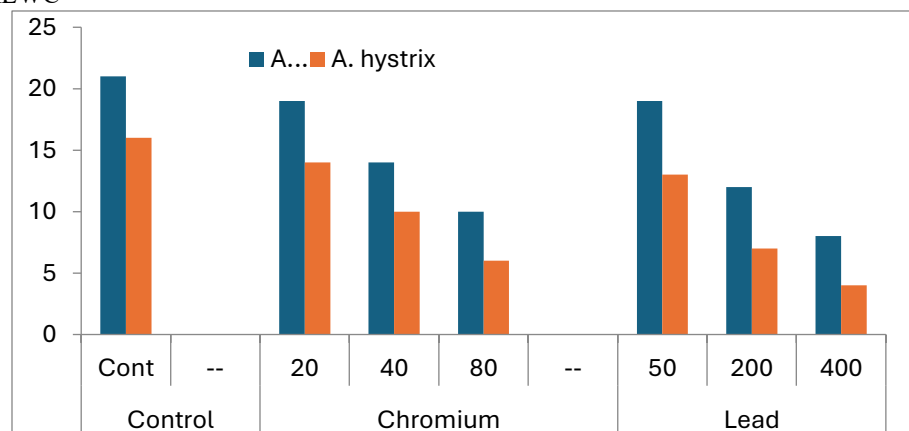


Figure 9,10. MSI and RLWC



4 Discussion

Under access heavy metal stress, *Lycopersicon esculentum* showed no effect in root and shoot length, however, overall fruit production reduced (Ho *et al.*, 2013). *Sesbania drummondii* also exhibit reduction in shoot length under Cr and Pb stress (Richard and Weidhaas, 2014). (X. Zhang *et al.*, 2014) study different parameters under heavy metal (Zn, Cr, Pb, Ni) stress in king grass (*Pennisetum purpureum*). In his research he concluded that root fresh weight is affected by increasing concentration of Cr and Pb. Similar results were observed by (Yao *et al.*, 2012) in which Pb exhibit significant effect on RFW reduction (25 %). Lipid peroxidation and chlorophyll breakdown is induced in plant resulting in an enhanced production of reactive oxygen species (ROS) (Hegedüs *et al.* 2004). Cr toxicity has a direct impact on the cellular metabolism which cause stunted growth in plants (Shanker *et al.* 2005).

According to Guo *et al.* (2014), chlorophyll content (CC) was decreased in maize plants under Cr contamination. Similar results were observed in many other studies (Al *et al.*, 2014; V. Chandra *et al.*, 2016; Ghosh *et al.*, 2015; Gupta *et al.*, 2014; Ohlbaum *et al.*, 2018; Papazoglou and Luisa, 2017; Roongtanakiat and Akharawutchayanon, 2017; Todd *et al.*, 2016; X. Zhang *et al.*, 2014). However, in contrast, our results showed that there was no significant change in the membrane stability index (MSI) of both grass species under heavy metal treatments. Enzymatic activity associated with the photosynthetic carbon reduction cycle and all of three phases of the Calvin cycle is delayed by the heavy metal stress in plants. (Krupa and Baszynski 1995; Prasad 1995; 1997). The heavy metal toxicity can affect plants essential processes causing stress in plants for example decreased water loss, (i.e., enhanced water conservation), decrease in number and size of leaves, stomatal size, number and diameter of xylem vessels, increased stomatal resistance, enhancement of leaf rolling and leaf abscission, higher degree of root suberization (Barcelo and Poschenrieder 1990). Cr toxicity decreased the water potential relative water content and transpiration rates, and in cauliflower leaves (Chatterjee and Chatterjee 2000).

Conclusion

Knowledge of the history, resources and hazards associated with the heavy metal toxicity leads to an emphasis on the selection of an optimum remedial techniques. Remediation of soil contaminated by heavy metals is of great importance due to risks associated with it and management of the land resources to make them available for Agriculture, Forestry and Food security. Phytoremediation is listed among the best available cost, effective and environment friendly technique for cleaning up heavy metal contamination in soil. Range grasses are highly recommended as remediation tool for field applicability in the developing countries which are under extreme pressure of land use change, urbanization, and industrialization which contributes towards environmental degradation.

Acknowledgment

We would like to gratefully acknowledge the staff of the Institute of Forest Sciences, The Islamia University of Bahawalpur, Pakistan, for providing support in the field work.

References

- Al, Z., N. Amer, K. Smets, J. Yperman, R. Carleer, S. Dumontet, and J. Vangronsveld. 2014. ScienceDirect Evaluation of flash and slow pyrolysis applied on heavy metal contaminated Sorghum bicolor shoots resulting from phytoremediation. *Biomass and Bioenergy*, 63:268–279.
- Ban, Y., Y. Jiang, M. Li, X. Zhang, S. Zhang, Y. Wu, and Z. Xu. 2017. Chemosphere Homogenous stands of a wetland grass living in heavy metal polluted wetlands harbor diverse consortia of arbuscular mycorrhizal fungi. *Chemosphere*, 181:699–709.
- Barcelo, J., CH. Poschenrieder. (1990) Plant water relations as affected by heavy metal stress: a review. *J Plant Nutr*, 13:1–37
- Beltrán, M., L. Gilberto, T. Bustillos, and A. González. 2012. Assessment of in situ and ex situ phytoremediation with grass mixtures in soils polluted with nickel, copper, and arsenic. *Physics and Chemistry of the Earth*, 39(2):52–57.
- Chatterjee, J., C. Chatterjee. (2000) Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environ Pollut*, 109:69–74
- Chandra, R. and H. Kang. 2016. Mixed heavy metal stress on photosynthesis, transpiration rate, and chlorophyll content in poplar hybrids. *Forest Science and Technology*, 12(2):55–61.
- Desai, M., M. Haigh, and H. Walkington. 2019. Science of the Total Environment Phytoremediation: Metal decontamination of soils after the sequential forestation of former opencast coal land. *Science of the Total Environment*, 656:670–680.
- Desjardins, D., N. J. B. Brereton, L. Marchand, J. Brisson, F. E. Pitre, and M. Labrecque. 2018. Complementarity of three distinctive phytoremediation crops for multiple-trace element contaminated soil. *Science of the Total Environment*, 611(2):1428–1438.
- Franzblau, A. and R. Lilis. (1989): *Archives of Environmental Health*. 44: 385–390.
- Gupta, S. K., T. Herren, K. Wenger, R. Krebs, and T. Hari, “In situ gentle remediation measures for heavy metal-polluted soils,” in *Phytoremediation of Contaminated Soil and Water*, N. Terry and G. Bañuelos, Eds., pp. 303–322, *Lewis Publishers*,
- Ghosh, M., J. Paul, A. Jana, A. De, and A. Mukherjee. 2015. Use of the grass, *Vetiveria zizanioides* (L.) Nash for detoxification and phytoremediation of soils contaminated with fly ash from thermal power plants. *Ecological Engineering*, 74:258–265.
- Gupta, D. K., S. Chatterjee, S. Datta, V. Veer, and C. Walther. 2014. Chemosphere Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals. *Chemosphere*, 108:134–144.
- Hegedüs, A., Erdei S, Janda T, Toth E, Horvath G, Dubits D (2004) Transgenic tobacco plants over producing alfa aldose/aldehyde reductase show higher tolerance to low temperature and cadmium stress. *Plant Sci* 166:1329–1333
- Ho, Y., J. Hsieh, and C. Huang. 2013. Construction of a plant – microbe phytoremediation system: Combination of vetiver grass with a functional endophytic bacterium, *Achromobacter xylosoxidans* F3B, for aromatic pollutants removal. *Bioresource Technology*, 145:43–47
- Khandare, R. V and S. P. Govindwar. 2015. Phytoremediation of textile dyes and effluents: Current scenario and future prospects.

- Biotechnology Advances*, 33(8):1697–1714.
- Khalid, S., M. Shahid, N. Khan, B. Murtaza, I. Bibi, and C. Dumat. 2017. A comparison of technologies for remediation of heavy metal contaminated soils. *Journal of Geochemical Exploration*, 182:247–268
- Klink, A. 2014. Trace metal bioindication and phytoremediation potentialities of *Phalaris arundinacea* L. (reed canary grass). *Journal of Geochemical Exploration Journal*, 146:27–33.
- Richard, T. and J. Weidhaas. 2014. Dissolution, sorption, and phytoremediation of IMX-101 explosive formulation constituents: 2, 4-dinitroanisole (DNAN). *Journal of Hazardous Materials*, 280:561–569.
- Roongtanakiat, N. and T. Akharawutchayanon. 2017. Evaluation of vetiver grass for radiocesium absorption ability. *Agriculture and Natural Resources*, 51(3):173–180.
- Rezania, S., M. Ponraj, A. Talaiekhosani, F. Sabbagh, and F. Sairan. 2015. Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. *Journal of Environmental Management*, 163:125–133.
- Swarup D, Patra RC, Naresh R, Kumar P, Shekhar P. [http://www.ncbi.nlm.nih.gov/entrez/\(2005\) Science of the Total Environment. 15\(1-3\): 67-71](http://www.ncbi.nlm.nih.gov/entrez/(2005) Science of the Total Environment. 15(1-3): 67-71)
- Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. *Environ Int* 31:739–751
- Krupa Z, Baszynski T (1995) Some aspects of heavy metals toxicity towards photosynthetic apparatus – Direct and indirect effects on light and dark reactions. *Acta Physiol Plant* 17:177–190
- Madrid, F., M. S. Liphadzi, and M. B. Kirkham. 2004. Heavy metal displacement in chelate-irrigated soil during phytoremediation. *Journal of Hydrology*, 272(3):107–119.
- Ohlbaum, M., S. L. Wadgaonkar, J. J. A. Van Bruggen, Y. V Nancharaiiah, and P. N. L. Lens. 2018. Phytoremediation of seleniferous soil leachate using the aquatic plants *Lemna minor* and *Egeria densa*. *Ecological Engineering*, 120(5):321–328.
- Papazoglou, E. G. and A. Luisa. 2017. Industrial Crops & Products Preliminary studies on the growth, tolerance and phytoremediation ability of sugarbeet (*Beta vulgaris* L.) grown on heavy metal contaminated soil. *Industrial Crops & Products*, 107(6):463–471.
- Prasad MNV (1995) Cadmium toxicity and tolerance in vascular plants. *Environ Exp Bot* 35: 525–540
- Prasad MNV (1997) Trace metals. In: Prasad MNV (ed) *Plant ecophysiology*. Willey, New York, pp 207–249
- Todd, L. F., K. Landman, and S. Kelly. 2016. Urban Forestry & Urban Greening Phytoremediation: An interim landscape architecture strategy to improve accessibility of contaminated vacant lands in Canadian municipalities. *Urban Forestry & Urban Greening*, 182:42–256.
- Thomas, A.C. 1996. Drinking water quality for poultry. North Carolina Cooperative Extension Service. Publication number: *PS and T* = 4
- Werle, S., D. Bisorca, A. Katelbach-wo, M. Pogrzeba, I. Ratman-k, and J. Krzy. 2017. Phytoremediation as an effective method to remove heavy metals from contaminated area e TG / FT-IR analysis results of the gasification of heavy metal contaminated energy crops. *Journal of the Energy Institute*, 90:408–417.
- Wiangkham, N. and B. Prapagdee. 2018. Chemosphere Potential of Napier grass with cadmium-resistant bacterial inoculation on cadmium phytoremediation and its possibility to use as biomass fuel. *Chemosphere*, 201:511–518.
- Yao, Z., J. Li, H. Xie, and C. Yu. 2012. Review on remediation technologies of soil contaminated by heavy metals. *Procedia Environmental Sciences*, 16:722–729.
- Zhang, X., X. Zhang, B. Gao, Z. Li, H. Xia, H. Li, and J. Li. 2014. Effect of cadmium on growth, photosynthesis, mineral nutrition and metal accumulation of an energy crop, king grass (*Pennisetum*). *Biomass and Bioenergy*, 67:179–187.
- Zhu, G., H. Xiao, Q. Guo, B. Song, G. Zheng, Z. Zhang, ... C. Peter. 2018. Ecotoxicology and Environmental Safety Heavy metal contents and enrichment characteristics of dominant plants in wasteland of the downstream of a lead-zinc mining area in Guangxi, Southwest China. *Ecotoxicology and Environmental Safety*, 151(1):266–271.