American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

The Effects of Lead and Cadmium Individually and in Combinations on Germination and Seedling Growth of *Leucaena leucocephala* (Lam.) de Wit

Muhammad Kabir[a], Muhammad Zafar Iqbal[b], Muhammad Shafiq[c*], Zia-ur-Rehman Farooqi[d]

[a]Department of Biological Sciences, University of Sargodha, Sub Campus Bhakkar, Pakistan
[b,c,d]Department of Botany, University of Karachi, Karachi, 75270, Pakistan
[a]Email: kabir_botany82@yahoo.com
[b]Email: mziqbalbotuokpk@yahoo.com
[c]Email: shafiqeco@yahoo.com
[d]Email: farooqi_bot@yahoo.com

Abstract

Pollution by heavy metals in the environment is a worldwide problem due to anthropogenic, industrial and automobile activities. In present studies, the effects of selected heavy metals (Pb and Cd) individually and in combination on seed germination and seedling growth of *Leucaena leucocephala* (Lam.) de Wit. were investigated. Germination rate of *L. leucocephala* showed that increase in concentration of metal treatment from 25 to 100 ppm, significantly ($p<0.05$) reduced germination percentage which was more prominent for Pb treatments as compared to control. Seedling growth variables i.e. root and shoot length, seedling size, root/shoot ratio, seedling fresh and dry weights also declined significantly ($p<0.05$) as compared to control treatment. Seedlings growth of *L. leucocephala* gradually reduced with increased in concentrations of metals especially Pb and Cd as compared to control. The inhibitory effects of metals showed the order as Pb>Cd of effects at different concentrations. The combined metal treatments of Pb+Cd were also found more toxic as compared to individual's treatment. The seedlins of *L. leucocephala* were tested for tolerance to lead and cadmium. Tolerance indices and seedling vigor index of *L. leucocephala* for individuals and combined metal treatment also decreased with increase in concentration of Pb and Cd treatment as compared to control. The more reduction in seedling tolerance indices of *L. leucocephala* was recorded for combined metal treatment. This reduction was prominent for Pb+Cd at 100 ppm as compared to control treatments.

Key words: Heavy metals; phytoxicity; seed germination; seedling growth; tolerance index.

----

* Corresponding author.

1. Introduction

Heavy metals are a group of elements having a density greater than $5g/cm^3$ [1]. Heavy metal contamination of soil, water and air has caused serious environmental hazard in the biosphere [2] and the effects of combined heavy metals may be quite different from those of individual pollutants due to interactions between heavy metals [3]. The addition of the heavy metals in the environment has brought significant changes in the nature, structure and composition of the plant communities and involved in the extinction of some plant species to some extent from the universe. Plants absorb a number of elements from soil and some are known to be toxic even at low concentrations. Plants experience oxidative stress upon exposure to heavy metals that leads to cellular damage. To minimize the detrimental effects of heavy metal exposure and their accumulation, plants have evolved detoxification mechanisms [4]. Global industrial growth has contaminated the soil and water with many hazardous compounds, including heavy metals. These heavy metals are not only toxic to plants but also cause severe human health hazards when leach out into food chain [5]. Unlike organic contaminants, metal(loid)s do not undergo microbial or chemical degradation and persist for a long time after their introduction [6]. Furthermore, the addition of heavy metals in combination produced additive, antagonistic or synergistic effects on plant growth.

Among the heavy metals, lead and cadmium can be considered important heavy metals for ecotoxicology concern. Lead (Pb) is a potentially toxic heavy metal and has attracted considerable attention for its widespread distribution and potential risk to the environment. Lead is a widely distributed metal in the environment [7]. Pb contamination in soils resulted in soil fertility deterioration but also resulted in yield decline [8]. Cadmium (Cd)mainly is toxic element for both plants and animals when its concentration is exceeded a limit. Cadmium is strongly phytotoxic causing growth inhibition and even plant death [9] and its accumulation in the upper layers of forest soils affects plants, microorganisms and their interactions. Adequate strategies for the reforestation of metal contaminated sites are of vital importance was suggested [10]. Karachi is the largest industrial city of Pakistan and suffering by a series of environment related problems. The flora of the region is particularly has been affected due to constant increase of environmental pollutant such as heavy metals at alarming scale. Different researchers have carried out few studies on the level of heavy metals in city environment of Karachi. High levels of heavy metals were investigated in soil samples from various polluted areas of Karachi city [11,12]. Iqbal and his colleagues [13] carried out a survey of vegetation and trace metals in soils along the super highways near Karachi city recorded.

Leucaena is a deep-rooted species, which can extend its roots 5 meter to exploit underground water [14]. The tree has gained much impetus for its multifarious uses to mankind [15]. *Leucaena* is a tropical species requiring warm temperatures (25-30° C day temperatures) for optimum growth [16]. Young seedlings have survived extended periods of dry weather and soil and plant studies have confirmed that *Leucaena* exhibits better drought characteristics than a number of other tree legumes [17]. The genus *Leucaena* belongs to family Leguminosae (Fabaceae) and subfamily Mimosaceae. *Leucaena leucocephala* (Lam) de Wit. is the most wide spread member of the genus.

L. leucocephala is a fast growing woody tree which is well known for its adoptability in arid and semi-arid lands of the world including Pakistan. It is used for a variety of purposes, such as firewood, fiber and livestock feed. *L. leucocephala* growing at main busy roads of the Karachi, city and is under pressure of environmental pollution problem due to discharge of different types of automobile pollutants. The Roadside trees in the city are under pressure and are lost due to vehicular traffic infrastructure and other community needs [18]. Shafiq and Iqbal [19-20] had found some adverse effects of pollutants on plants growing at M.A. Jinnah road Karachi.

Little is known about the individual and combined effect of heavy metals such lead and cadmium on germination and seedling growth performance of an important arid plant species, *L. leucocephala* cultivated along the busy roads of the city of Karachi. The aim and goal of the present study was to evaluate the effects of selected heavy metals viz. lead, cadmium individually and in combination on seed germination and seedling growth performance of *L. leucocephala*.

2. Materials and Methods

The healthy seeds of *Leucaena leucephala* (Lam.) de Wit. were collected from the Karachi University Campus. The top ends of seeds were slightly cut with a clean scissor to remove any possible seed coat dormancy. The seeds were surface sterilized with 30% dilute solution of Sodium-hypo chlorite to prevent any fungal contamination. Ten seeds were placed in Petri dishes (90 mm diameter) on filter paper (Whatman No. 42). Solutions of lead nitrate and cadmium nitrate were prepared having 25, 50, 75 and 100 ppm concentrations for individual treatment and for combined treatments (Pb+Cd). At the start of experiment, 3ml of distilled water was added to each set of control and 3ml of each metal solution of 25, 50, 75 and 100 ppm concentrations to each set of respective treatment and every 2^{nd} day 1ml of 25, 50, 75 and 100ppm solutions of lead nitrate and cadmium nitrate were added to respective treatment. The control received only 1ml of distilled water on alternate days. The experiments were designed on the basis of three replicates and the Petri dishes were kept at room temperature (32±2°C) with 240 Lux light intensity and the experiment lasted for 12 days. The experiment was completely randomized. Seed germination, root, shoot, seedling lengths, seedling fresh weight were recorded and dry biomass was determined by placing the seedling in an oven at 80°C for 24 hours. Seedling fresh and dry biomass was measured with electrical balance. Seedling vigor index (S.V.I) was determined as per the formula given by Bewly and Black [21]. The seed germination and seedling growth data were statistically analyzed by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to determine the level of significance at $p < 0.05$ on personnel computer using COSTAT version 3. Tolerance indices of seedlings were determined with the help of following formula. Tolerance indices (T.I.) = Mean root length of treated seedlings / Mean root length of control seedlings x 100

3. Results

The treatment of different concentration (0, 25, 50, 75 and 100 ppm) of selected heavy metals (Pb and Cd) on seed germination and seedling growth of *Leucaena leucocephala* (Lam.) de Wit. was observed. The low percentage of seed germination of *L. leucocephala* was recorded when treated with lead (Pb) as compared to cadmium (Cd). Similarly, the result indicated that root is strongly affected by these metal treatments as compared to shoot. With increased concentration of Pb more profound affects on root and shoot of *L.*

leucocephala were recorded as compared to the other metal treatments. The percentage of seed germination was reduced to 60% at 100 ppm Pb treatment. For other treatment of Cd this percentage was reduced to 63 (Table 1). Heavy metals treatment showed that seedling growth of *L. leucocephala* was decreased with increase in concentration up to 100 ppm of lead and cadmium. Germination rate in control was 93 % which lowered with increased concentration of lead and cadmium at 100 ppm treatments this rate was reduced to 63 % both for lead and cadmium. Results showed that seedling growth parameters were also declined with increase in concentration from 25 to 100 ppm of Pb and Cd. Seedling size of *L. leucocephala,* which includes the length of root and shoot was recorded as 13.46 cm for control which decreased to 7.46 cm for lead and 8.30 cm for cadmium when treated with 100 ppm solution of these metals. Combined treatment of Pb and Cd showed more adverse affects on germination and seedling growth of *L. leucocephala* as compared to individually treatments of these two metals. Different concentration of Pb+Cd varying from 25 to 100 ppm illustrated that germination rate was reduced from 83% to 53% for these various treatments as compared to control in which germination of *L. leucocephala* is recorded as 86%. Root and shoot length was also reduced by combined treatments of Pb+Cd but this reduction was more for the root as compared to shoot resulting in overall, inhibition of seedling size.

Table 1: Effects of lead (Pb) and cadmium (Cd) individually and in combination on different growth parameters of *Leucaena leucocephala*

Metals	**Treatments (ppm)**	**Germination (%)**	**Root length (cm)**	**Shoot length (cm)**	**Seedling size (cm)**
Pb	00	93.33±3.33a	5.63±0.20a	7.83±0.37a	13.46±0.52a
	25	90.00±5.77ab	4.50±0.76ab	7.06±0.18ab	11.53±0.63b
	50	76.66±3.33bc	4.06±0.71abc	6.73±0.29b	10.80±0.81bc
	75	73.33±3.33c	3.46±0.52bc	5.86±0.23c	9.66±0.39c
	100	63.33±6.67c	2.46±0.14c	5.00±0.21d	7.46±0.20d
Cd	00	93.33±3.33a	5.80±0.06a	8.10±0.15a	13.90±0.10a
	25	86.67±3.33ab	5.47±0.09b	7.77±0.18a	13.24±0.26a
	50	80.00±5.77bc	4.63±0.12c	6.73±0.34b	11.36±0.44b
	75	73.33±3.33cd	4.00±0.06d	5.87±028c	9.87±0.52c
	100	63.33±3.33d	3.13±0.09e	5.17±0.18c	8.30±0.21c
Pb+Cd	00	86.00±3.33a	3.6±0.14a	7.7±0.08a	11.4±0.10a
	25+25	83.00±3.33a	3.2±0.14b	7.2±0.10ab	10.43±0.24b
	50+50	76.00±3.33a	2.7±0.14c	6.8±0.21b	9.56±0.34b
	75+75	60.00±5.77b	2.1±0.10d	5.5±0.40c	7.60±0.45c
	100+100	53.00±3.33b	1.6±0.11e	4.9±0.20c	6.53±0.08d

Number followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at p<0.05 level. ± Standard Error

Results also showed that seedling fresh and dry weights of *L. leucocephala* were also declined with increased concentration of combine treatment of Pb+Cd. Overall, reduction in mass was calculated when seedling were treated with combine treatments of Pb+Cd and this reduction was more prominent with increasing concentration of these combined metals (Table 2).

Table 2: Effects of lead (Pb) and cadmium (Cd) individually and in combination on seedling fresh weight, seedling dry weight and root/shoot ratio of *Leucaena leucocephala*

Metals	**Treatments (ppm)**	**Seedling fresh weight (mg)**	**Seedling dry weight (mg)**	**Root/shoot Ratio**
Pb	00	149±4.48a	84±1.20a	0.76±0.04a
	25	127±5.69b	77±1.76b	0.64±0.12a
	50	111±0.88c	71±0.88c	0.61±0.10a
	75	98±2.18d	69±0.58c	0.59±0.10a
	100	88±3.78d	52±0.33d	0.49±0.04a
Cd	00	168±3.76a	94±2.96a	0.71±0.02a
	25	156±3.21b	88±0.88a	0.70±0.06a
	50	143±3.18c	81±0.88b	0.68±0.02a
	75	128±3.28d	74±2.18c	0.68±0.02a
	100	112±2.96e	63±2.73d	0.60±0.02b
Pb+Cd	00	168±5.13a	101±1.53a	0.48±0.02a
	25+25	147±3.28b	87±1.15b	0.45±0.01ab
	50+50	133±6.80b	85±2.18b	0.41±0.01abc
	75+75	106±5.36c	75±2.96c	0.38±0.02bc
	100+100	95±1.20c	63±0.88d	0.33±0.04c
Number followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at $p<0.05$ level. ± Standard Error				

The results of this study showed that the tolerance in seedling growth of *L. leucocephala* differed in their sensitivity to lead, cadmium individually and in combination treatments. The seedlings of *L. leucocephala* were found resistant to lead, cadmium individually and in combination toxicity at low concentration to some extent while increase in concentration of selected heavy metals treatment increased the toxicity and lower the tolerance indices in seedling growth performance of *L. leucocephala* as compared to control treatment. Low percentage of tolerance indices for seedlings of *L. leucocephala* were recorded with 100 ppm at combined treatment of Pb +Cd as compared to individual treatment of Pb and Cd (Fig. 1). Tolerance indices of *L. leucocephala* for individuals and combined metal treatment also decreased with increase in concentration of Pb and Cd treatment as compared to control. The more reduction in seedling tolerance indices of *L. leucocephala* was recorded for combined metal treatment. This reduction was prominent for Pb+Cd at 100 ppm as compared to control treatments.

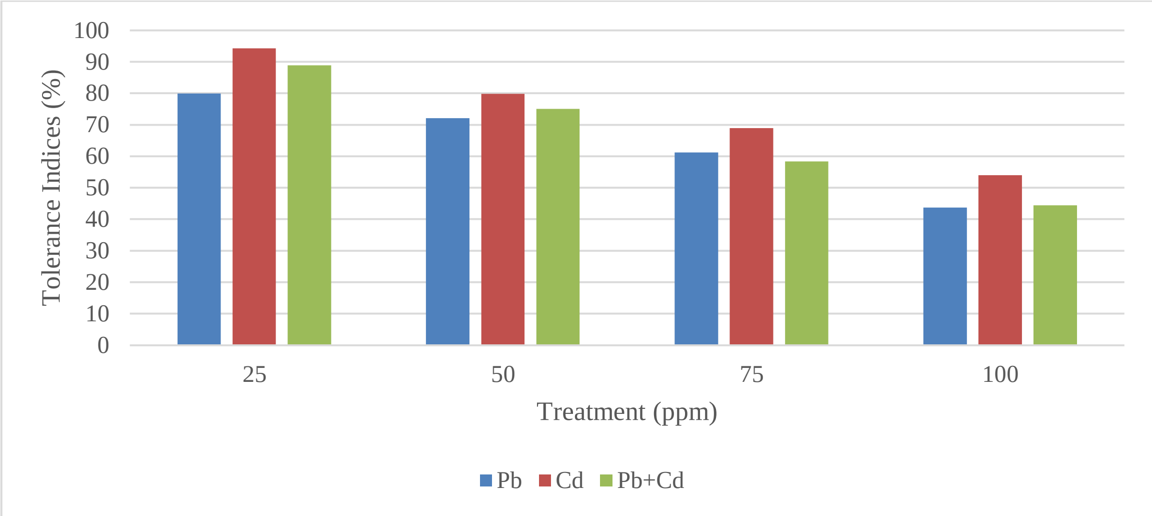

Figure 1: Percentage of tolerance in Leucaena leucocephala (Lam.) de Wit. using different concentration (25, 50, 75, 100 ppm) of lead (Pb), cadmium (Cd) individually and in combination (Pb+Cd) as compared to control

Seedling Vigor Index (S.V.I.) for *L. leucocephala* was highest in control seedling and gradually declined with the increase in concentration of Pb and Cd treatments from 25 to 100 ppm (Fig. 2). Seedling vigor index of *L. leucocephala* for individuals and combined metal treatment decreased with increase in concentration of Pb and Cd treatment as compared to control. The more reduction in seedling tolerance indices of *L. leucocephala* was recorded for combined metal treatment. This reduction was prominent for Pb+Cd at 100 ppm as compared to control treatments.

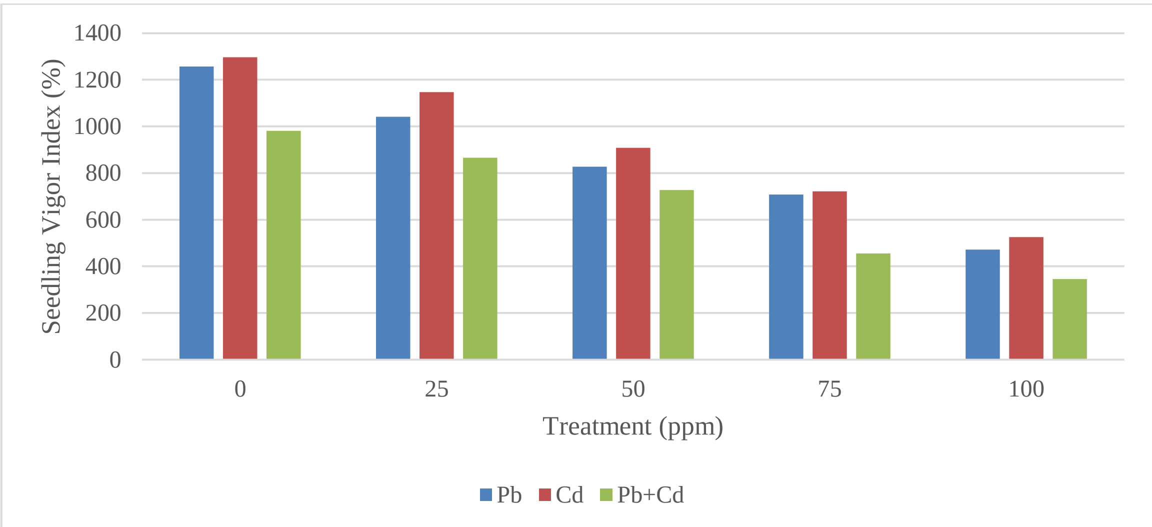

Figure 2: Seedling vigor index for Leucaena leucocephala (Lam.) de Wit. using different concentration (0, 25, 50, 75, 100 ppm) of lead (Pb), cadmium (Cd) individually and in combination (Pb+Cd) as compared to control

4. Discussion

We aimed to find the toxicological impacts of Pb and Cd in single dozes and in combinations on *L. leucocephala* seedling. The results indicated that increasing concentrations of the Pb and Cd significantly ($p < 0.05$) reduced the seedlings growth. Germination and seedling establishment are critical stage in the plant life cycle. The plant under stress conditions adversely affected by heavy metals treatments. In present study, the toxicity and tolerance to selected heavy metals viz. lead and cadmium individually and in combination on seed germination and seedling growth performances of *Leucaena leucocephala* were significantly ($p<0.05$) affected. The percentage of seed germination of *L. leucocephala* was recorded highly reduced to 60% at 100 ppm Pb treatment. While for other treatments of Cd, this percentage was less reduced to 63 as compared to control. Excessive amount of toxic element usually caused reduction in plant growth [22] and the damage may occur at any stage of plant growth and on any parts of the plant. In lab conditions, when *L. leucocephala* was subjected to different metal treatments, Pb was found more toxic as compared to Cd. Similarly, in another study, the physiological responses and tolerance mechanisms to different concentration of Pb stress viz. 0, 50, 150, 300, 600, 800, 1000 mg/L were reported for the xerophils, *Salsola passerina* Bunge and *Chenopodium album* L. in terms of the seed germination rate [23]. The effect of the Pb and Cd at 25 ppm concentration on root growth of *L. leucocephala* was also observed and agreed with the findings of Ismail and his colleagues [24]. An increase in the concentrations of lead at 1000 mg/kg reported significantly inhibitory effect on seed germination percentage, root length, shoot length, tolerance index, fresh weight and dry weight of soybean (*Glycine max* (L.) Merr in seven days old seedlings as compared to control treatment [25]. Exposure to 25ppm concentration of Cd reduced the root length of *L. leucocephala* as compared with the root length of control. Similarly, the increasing concentrations of Cd in half Murashige and Skoog nutrient solution culture were found significantly inhibited the growth of young sweet potato plants (*Ipomoea batatas*) in a dose dependent manner for each metal reported earlier [26]. Results also showed that seedling fresh and dry weights of *L. leucocephala* were also declined with increased concentration of combine treatment of Pb+Cd and this reduction was more prominent with increasing concentration of these combined metals.

Heavy metals are known to pose a potential threat to terrestrial and aquatic biota [27]. The seedling growth of *L. leucocephala* was decreased with increase in concentration up to 100 ppm of cadmium and reduced to 63 % both for lead and for cadmium. Seedling size, which includes the length of root and shoot, was highly decreased when treated with 100 ppm solution of these metals. Cd showed a greater inhibitory effect on root than shoot growth, predominantly in *Sinaps alba* L. In addition to growth inhibition, Cd reduced biomass production (Fresh Mass, Dry Mass), mainly in the shoots. The adverse effect of Cd was also confirmed by a significant reduction in protein sulfhydryl groups [28]. Combined treatment of Pb and Cd showed more adverse effects on germination and seedling growth of *L. leucocephala* as compared to individually treatments of these two metals. The treatment of lead produced poor root development of *L. leucocephala*. Excessive amount of toxic element usually caused reduction in plant growth [29]. In an earlier studies on tobacco the results reported that the amounts of exchangeable Cd^{2+} and Pb^{2+} and carbonate bound Cd^{2+} and Pb^{2+} in soil increased with the amounts of Cd^{2+} and Pb^{2+} added to soil, and the contents of both Cd^{2+} and Pb^{2+} in roots were significantly increased along with stress time and the amounts of Cd^{2+} and Pb^{2+} added to soil. The growing of tobacco in Cd^{2+} and Cd^{2+}+Pb^{2+} polluted soil for 50, 100, and 150 d resulted in some abnormal external morphological and anatomical changes in ripe region of lateral roots [30]. The physiological effects of Cd and Cu have been highlighted in several studies over the last years. At the cellular level, oxidative stress has been reported as a common mechanism in both stress situations [31]. *Sesbania drummondii* seedling growth was found significantly inhibited with metal treatments lead (Pb), copper (Cu), nickel (Ni) and zinc (Zn). The uptake of metals followed the order Pb > Cu > Zn > Ni in roots and Pb > Zn > Cu > Ni in shoots of lead (Pb), copper (Cu), nickel (Ni) and zinc (Zn). In addition, uptake of a single metal by *S. drummondii* was affected by the presence of a second metal, suggesting an antagonistic effect or competition between metals at the plant uptake site [32]. Low tolerance indices for *L. leucocephala* seedlings were recorded for 100 ppm at combined treatment of Pb +Cd as compared to individual treatment of Pb and Cd. The mechanism of action of toxicity depends on the availability of chemical compounds [33] in the substrate. The purslane seedlings were reported more sensitive to the heavy metals in comparison with shoot. The uptake patterns showed antagonistic impacts on each other and were reflected in response to growth parameters. The combine toxicities of Cd, Pb and Zn (Cd/Pb, Cd/Zn and Pb/Zn) were found more than the toxicity due to single dose of each element but less than their additive sums [34].

5. Conclusion

The toxicities of lead using seed germination and seedling growth performance of *L. leucocephala* was determined. It was concluded that lead and cadmium treatment produced toxic effects on seed germination and seedling growth of *L. leucocephala* as compared to control. The tolerance indices and seedling vigor index was found less at higher concentration of both metal treatment. It is suggested that the value of difference in tolerance indices and seedling vigor index should be consider while planting *L. leucephala* in metal contaminated areas.

Acknowledgement

We are highly grateful to Higher Education Commission (HEC) of Pakistan for funding this study which is a part of project No. 20-1073/07. We are also thankful to the chairperson, Department of Botany and the University authorities for providing us space and facilities for conducting the research.

References

[1] Stobrawa, K., & G. Lorenc-Plucinska. 2008. Thresholds of heavy-metal toxicity in cuttings of European black poplar (Populus nigra L.) determined according to antioxidant status of fine roots and morphometrical disorders. Sci. of The Total Environ. 390(1): 86-96.

[2] Xiong, Z.T. 1998. Lead uptake and effects on seed germination and plant growth in a Pb hyperaccumula tor Brassica pekinensis Rupr. Bull. of Environ. Cont. and Toxiol, 60: 285–291.

[3] MacFarlane, G.R., & M.D. Burchett. 2002. Toxicity, growth and accumulation relationships of copper lead and zinc in the grey mangrove Avicennia marina (Forsk.) Vierh. Mar. Environ. Res., 54: 65–84.

[4] Yadev, S.K. 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and

phytochelatins in heavy metal stress tolerance of plants. Afr. J. Bot, 76(2): 167-179.

[5] Kumar, S., M.H Asif., D. Chakrabarty, R.D. Tripathi., R.S. Dubey & P.K. Trivedi. 2013. Expression of a rice Lambda class of glutathione S-transferase,OsGSTL2, in Arabidopsis provides tolerance to heavy metal and other abiotic stresses. J. of Hazardous Materials, 248-249, 228-237.

[6] Bolan, N., A. Kunhikrshnan., R. Thangarajan., J. Kumpiene., T. Makino., M.B. Krikham & K. Scheckel. 2014. Remediation of heavy metal (loid)s contaminated soils – To mobilize or to immobilize?. J. of Hazardous Materials, 266: 141-166.

[7] Toplan, S., D. Ozcelik., T. Gulyasar & M.C. Akyolcu. 2004. Changes in hemorheological parameters due to lead exposure in female rats. J. of Trace Elements in Medicine and Bio, 18 (2): 179 - 182.

[8] Majer, B.J., D. Tscherko & A. Paschke. 2002. Effects of heavy metal contamination of soils on micronucleus induction in Tradescantia and on microbial enzyme activities: a comparative investigation. Mutation Res., 515: 111-124.

[9] Sandalio, L.M., H.C. Dalurzo., M. Gomez, M.C. Romero-Puertas & L.A. Rio. 2001. Cadmium induced changes in the growth and oxidative metabolism of pea plants. J. of Expt. Bot., 52: 2115–2126.

[10] Sousa, N.R., M.A. Ramos., A.P.G.C. Marquest & P.M. L. Castro. 2012. The effect of ectomycorrhizal fungi forming symbiosis with Pinus pinaster seedlings exposed to cadmium. Sci. of The Total Environ. 414: 63-67.

[11] Ara, F, M.Z. Iqbal. & M.S. Qureshi. 1996. Determination of heavy metals contamination of trees and soils due to vehicular emission in Karachi city. Kar. Uni. J. of Sci. 24: 80 - 84.

[12] Khalid, F., M.Z. Iqbal & M.Z. Qureshi. 1996. Concentration of heavy metals determined in leaves and soil from various areas of Karachi, city. Environ. Sci, 4: 213-219.

[13] Iqbal, M.Z., A.K. Sherwani & M. Shafiq. 1998. Vegetation characteristics and trace metals (Cu, Zn and Pb) in soils along the super highways near Karachi, Pakistan. Studia Bot. Hungarica, 29: 79-86.

[14] Brewbaker, J.L., D.I. Plucknett and V. Gonzales (1972). Varietal trials of Leucaena leucocephla ("Koahaole") in Hawaii. Honolulu: University of Hawaii, College of Agriculture, Hawaii Agricultural Experiment Station, 26-29.

[15] Annonymous, 1984. Leucaena: Promising forage and tree crop for the tropics, 2^{nd} ed. National Academy Press Washington D.C. 1-100.

[16] Brewbaker, J.L., N. Hegde., E.M. Hutton., R.J. Jones., J.B. Lowry., F. Moog. & R.V. Beldt. 1985. Leucaena - Forage Production and Use. NFTA, Hawaii. 39 pp.

[17] Swasdiphanich, S. 1992. Environmental influences on forage yields of shrub legumes. Ph.D. thesis, The University of Queensland.

[18] Jim, C.Y. 1998. Pressure on urban trees in Hong Kong: Pervasive problem and possible amelioration. Arbor. J., 22: 37-60.

[19] Shafiq, M. & M.Z. , Iqbal. 2005. The toxicity effects of heavy metals on germination and seedling growth of Cassia siamea Lamark. J. of New Seeds, 7: 95-105.

[20] Shafiq, M. & M.Z. Iqbal. 2012. "Impact of Automobile Pollutants on Plants". ISBN 978-3-8443-8504-5. LAP LAMBERT Academic Publishing GmbH & Co. KG Heinrich-Böcking-Str. 6-8, 66121, Saarbrücken, Germany. 132 pp.

[21] Bewly, J.D. & B.M. Black. 1982. Germination of seeds. In: Physiology and biochemistry of seed germination. Ed: A.A. Khan, Springer Verlag, NewYork, pp. 40-80.

[22] Prodgers, R.A. & W.P. Inskeep. 1981. Heavy metals tolerance of inland salt grass Distichlis spicata. Great Basin Naturalist, 51: 271-278.

[23] Hu, R., K. Sun., X. Pan., Y. Zhang & X. Wang. 2012. Physiological responses and tolerance mechanisms to Pb in two xerophils: Salsola passerina Bunge and Chenopodium album L. J of Hazardous Materials, 205-206: 131-138.

[24] Ismail, S., F. Khan & M.Z. Iqbal. 2013. Phytoremediation: Assessing tolerance of tree species against heavy metals (PB and CD) toxicity. Pak. J. Bot., 45 (6): 2181-2186.

[25] Gupta, S., Meena, M.K. & Datta, S. 2016. Effect of selected heavy metals (Lead and Zinc) on seedling growth of soybean Glycine max (L.) Merr. Int J Pharm Pharm Sci, 8(8): 302-305.

[26] Kim, Y., H.S. Lee & S.S. Kwak. 2010. Differential responses of sweet potato peroxidases to heavy metals. Chemosphere, 81(1): 79-85.

[27] Agoramoorthy, G, F., Chen & M.J.Hsu. 2008. Threat of heavy metal pollution in halophytic and mangrove plants of Tamil Nadu, India. Environ. Pollut., 155 (2): 320-326.

[28] Molnárová, M & A. Fargašová. 2012. Relationship between various physiological and biochemical parameters activated by cadmium &in Sinapis alba L. and Hordeum vulgare L. Ecological Eng., 49: 65-72.

[29] Prodgers, R.A. & W.P. Inskeep. 1991. Heavy metal tolerance of inland salt grass (Distichlis spicata). Great Basin Naturalist, 5(3): 271-278.

[30] Yuan, Z., S. Xiong., C. Li & X. Ma. 2011. Effects of chronic stress of cadmium and lead on

anatomical structure of tobacco roots. Agricultural Sci. in China, 10 (12): 1941-1948.

[31] Smeets, K., K. Opdenakker., T. Remans., S.V. Sanden., F.V. Belleghem., B. Semane., B. Horeman., Y. Guisez., J. Vangronsveld & A. Cuypers. 2009. Oxidative stress-related responses at transcriptional and enzymatic levels after exposure to Cd or Cu in a multipollution context. J of Plant Physio, 166(18): 1982-1992.

[32] Israr, M., A. Jewell., D. Kumar & S.V. Sahi. 2011. Interactive effects of lead, copper, nickel and zinc on growth, metal uptake and antioxidative metabolism of Sesbania drummondii. J. of Hazardous Materials, 186(2-3), 1520-1526.

[33 [Ren, S. 2003. Phenol mechanism of toxic action classification and prediction: a decision tree approach. Toxicol. Lett., 144 (3): 313 – 323.

[34] Naz, A. , Khan, S. , Qasim, M. , Khalid, S. , Muhammad, S. & Tariq, M. 2013. Metals toxicity and its bioaccumulation in purslane seedlings grown in controlled environment. Natural Science, **5:** 573-579.