International Research Journal of Natural and Applied Sciences
Vol. 3, Issue 8, August 2016 IF- 3.827 ISSN: (2349-4077)
© Associated Asia Research Foundation (AARF)
Website: www.aarf.asia Email : editor@aarf.asia , editoraarf@gmail.com

APPLICATION OF *BRASSICA JUNCEA* (INDIAN MUSTARD) FOR PHYTOREMEDIATION OF LEAD AND ZINC IN POLLUTED YAMUNA SOIL

Rajni Gupta[1], Gaurav Chugh[1], Rakesh Kumar[2], Reena Saxena[2]

[1]Department of Botany, Kirori Mal College, University of Delhi, Delhi-110007, India.

[2]Department of Chemistry, Kirori Mal College, University of Delhi, Delhi-110007, India.

ABSTRACT

Yamuna is one of the most polluted rivers in the world due to high density population growth, rapid industrialization, especially around New Delhi, where 15 drains and numerous industries discharge waste water into the river which is held responsible for these toxic elements being discharged into the environment posing a great risk to ecological health and human wellbeing. Phytoremediation provides a sustainable alternative method to existing expensive, environmentally destructive conventional remediation techniques. It is a cost-effective, preserves soil microflora rendering long term applicability and considered to be a "Green Revolution" in the field of innovative clean up technologies. Brassica juncea, being a metal hyper-accumulator was investigated for its potential to extract heavy metals, particularly Lead (Pb) and Zinc (Zn) present in contaminated/Polluted Yamuna soil using phytoextraction. Yamuna soil was collected and studied for distribution of lead and zinc in soils and different parts of plant. Results indicate that Lead and zinc concentrations in plant of polluted Yamuna soil sample was higher than the garden soil sample. The plant study was done considering and measuring their root length and shoot length and analyzing them for their dry and fresh weight. The microwave digestion of soil and plant using EPA Digestion method-3051a was done and were analysed for Heavy Metal content by Flame Atomic Absorption Spectrometer Analyst 400 (Perkin Elmer, Shelton, CT, USA.).

KEYWORDS- *Brassica juncea*, Flame atomic absorption spectrometer, Phytoremediation, Yamuna soil

1. INTRODUCTION

The mankind is facing an unpleasant biological emergency brought by natural contamination. The quick pace of industrialization, ever increasing demand for energy and foolhardy abuse of natural resources during the most recent century have been fundamentally in charge of the natural holocaust, which is currently set to represent a greater risk to biodiversity and biological systems. Soil and water pollution by heavy metals is a standout amongst the most serious eco-biological issues everywhere throughout the world. Modernization, urbanization and industrialization have led to the formation of extensive number of desolate locales impregnated with lethal heavy metals [1]. In spite of the fact that trace elements are vital in micro quantities to support the metabolic exercises in the organisms, these turn out to be lethal past specific threshold limits. High amount of these metals in nature results in their incorporation, and ensuing biomagnification in higher trophic levels of nutritional pyramid, which unfavourably influence the behavioural, functional and structural exercises of living beings [2]. The sources of metal contamination incorporate smelting, gas fumes, electroplating and fuel generation, utilization of manures and mechanical assembling [3].Soils in agricultural fields are moderately rich in heavy metals by virtue of broad utilization of different agrochemicals containing such metals as constituents or contamination, for example, pesticides, fungicides or herbicides [4]. This has led to sharp increment in metal tainting of the biosphere and postures significant issues of environment and human wellbeing around the world [5].

1.1 Methods of Remediation

The recovery of polluted soils at a substantial rate is not as simple as it has all the earmarks of being, and needs the execution of a few regulatory strides. Different methodologies have been utilized or created to mitigate the contaminated soils and waters including the landfill/damping locales. These might be comprehensively named physicochemical and biological methodologies. The physicochemical method incorporates excavation of soil at dangerous waste site, inactivation, draining by utilizing acids, trailed by the arrival of clean residues to the site [6], precipitation, flocculation trailed by sedimentation, reverse osmosis and microfiltration. These ordinary physicochemical procedures are for the most part exorbitant and are not eco-friendly [7]. Also, these systems are in fact constrained to small zones and can definitely change the soil structure and fertility [8].Biological method of remediation incorporates: (i) the utilization of microorganisms to detoxify the metals by valence change, extra-cellular compound precipitation or volatilization, and (ii) utilization of particular plants to clean soil or water, by inactivating metals in rhizosphere or translocating them into above parts. The second approach is called phytoremediation, which is considered as another and profoundly encouraging system for the recovery of polluted locales, and is less expensive than physicochemical methods [9].

1.2 Phytoremediation as green clean technology

Phytoremediation, likewise termed as the "plant bioremediation", is turning into a territory of advanced research, which has gigantic prospects towards clean-up of contaminated soils and water [10]. The term phytoremediation refers to differing consortia of plant based innovations that uses native or GM plants, for cleaning polluted environment [11]. A living plant can be considered as a "sun oriented driven pump" which can accumulate specific components from the sullied soil and translocate these to shoots which can be in this way evacuated by harvesting. The gathered plant tissue, rich in contaminant can be effortlessly and securely handled by microbial, physical or chemical means. Plant based remediation is desirable over microbial remediation, despite the fact that the last is more flexible in degradative potential. Plants can expel contaminants from the soil through harvesting, which is eccentric with organisms and it is simpler to build up a successful plant populace than a microbial populace. The roots of plants likewise balance out the soil, and anticipate disintegration and spread of contaminants. Roots may likewise discharge compounds to upgrade microbial development, and enhance air circulation and microbial movement.

1.3 Effects of heavy metals on growth and metabolism of Plant

Pb is a non-essential toxic element to plant and animals, extensively studied due to its probable adverse effect on nervous system and other parts of our body [12]. In soil, Pb exists mainly in +2 oxidation state. It is the least mobile heavy metal in soil, especially under reducing or non-acid condition. It generally enters the soil through the automobile exhaust and mining activities. The major biochemical effect of Pb is its interference with heme synthesis, leading to hematological damage. It inhibits several of the key enzymes involved in the overall process of heme synthesis.Zn is considered essential for cell physiological processes, and in most living organisms, it is the second most abundant transition metal after Fe. Several enzymes contain Zn, like carbonic anhydrase, alcohol dehydrogenase, SOD and RNA polymerse [13]. When present at high concentrations, Zn can be phytotoxic, and plants affected show symptoms similar to those produced by Cd or Pb [14]. General symptoms of

Zn toxicity are observed to be turgorless cells, necrosis on older leaves and reduced growth [15]. Peralta et al. [16] reported alterations in vital growth processes such as membrane integrity, photosynthesis and chlorophyll biosynthesis, under excess Zn accumulation in plants.

1.4.*B. juncea*a- a promising candidate for Phytoextraction

Family Brassicaceae has a potential and promising number of plants to be utilized for phytoremediation. It contains countless species and their capacity to uptake heavy metals. They are additionally appropriate for in vitro procedures, and are alluring contender for the insertion of genes of interest for phytoremediation. Among the *Brassica* species, *B. juncea* has been the most broadly contemplated plant that displays unrivaled heavy metal aggregating capability [17]. As indicated by Renault [18], *B. juncea* can uptake metals upto 0.5% to 1% of its dry weight. The outcomes propose that a more prominent biomass can more than make up for a lower shoot metal concentration. Hence, *B. juncea* is considered as a promising option for phytoremediation furthermore a model framework to explore the physiology and natural chemistry of metal aggregation in plants.

1.5. Site characterization and selection

According to the Central Pollution Control Board (CPCB), 70% of the pollution in river is from untreated sewage and the remaining 30% is from industrial sources, agricultural run-off, garbage etc. The water quality of Yamuna River falls under the category "E" which makes it fit only for recreation and industrial cooling, completely ruling out the possibility for underwater life. Biological Oxygen Demand (BOD) load increased by 2.5 times between 1980 and 2005: from 117 tonnes per day in 1980 to 276 in 2005. Thus, the aim of the present work is to develop an effective and eco-friendly approach to control soil pollution near Yamuna River using phytoremediation.

2. EXPERIMENTATION

2.1. Instrumentation

A PerkinElmer AAnalyst 400 flame atomic absorption spectrometer equipped with deuterium lamp background correction and an air–acetylene burner for lead and zinc determination was used throughout the study. Microwave unit, Anton Paar, Model Multiwave 3000 was used to digest soil samples. To adjust the pH of solutions, ELICO pH meter model LI-614 was used.

For making all solutions and washing, double distilled water was used from Double Distillation unit.

2.2. Reagents and materials

All chemicals used were of analytical reagent grade. All working solutions were prepared using double distilled water. A stock solution of 1000 μg mL^{-1} of Pb (II) and Zn (II) were prepared Pb $(NO_3)_2$ and $Zn(NO_3)_2$ in a 100 mL standard volumetric flask and was used to prepare fresh Pb(II) solutions daily by dilution. To adjust the pH of metal ion solutions, 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} NaOH solution were used. All glass apparatus were cleaned with 10% (v/v) nitric acid and rinsed with double distilled water before use. Seeds of Indian mustard (*Brassica juncea*) were used for the experiment purpose. This plant belongs to family Brassicaceae, known for its ornamental flower, grows during the winter season. It is also important for its oil production property. Surface sterilization of the seeds was done by using 0.1% mercuric chloride. Then they were soaked for two days in sterile distilled water before sowing.

2.3 Site Characterization and Sampling

Soil was collected from the bank of Yamuna River as shown in Fig.1 at the depth of 0-20 cm. The controlled soil was collected from the garden and pH of the soils was studied using pH meter. 5 g of soil sample and 25 mL of water was taken in a 50 mL beaker. The beaker was kept for agitation for one hour. After one hour pH of an aliquot of the supernatant solution was taken. The pH meter was standardized by pH 4 and pH 7 buffer solution. Ten seeds of *Brassica juncea* per pot were used for sowing.

2.4. Green House Experiment

Green house pot culture experiment were conducted to study the effect of heavy metal i.e., lead and zinc on seed germination, root growth, shoot growth and phytoremediation by *Brassica juncea*. Earthen pots are used for this purpose. 350 gm of soil is taken in each pot and seeds were sown in each pot.

2.5. Digestion of Soil and Plant

2.5.1 Hot plate method

0.5g sample is dissolved in 4.5mL HCl and 1.5 mL HNO_3 and placed in round bottom flask. It is sealed with condenser and placed on magnetic stirrer for 3-4 h. After cooling the solution, it is filtered and placed in 100 ml volumetric flask and makeup till the mark.

2.5.2 Microwave digestion of soil and plant

EPA Digestion method-3051a (microwave, HNO_3-HCl, total-recoverable). This technique provides rapid, safe and efficient digestion and is not susceptible to losses of volatile metals. This technique is time saving and energy efficient and no acid fumes are generated. For all these reasons the above technique can be considered as a green technique. 0.5g of soil sample was dissolved in 4.5 mL conc. nitric acid and 1.5mL conc. hydrochloric acid and placed in quartz microwave vessel. The vessel was sealed and heated in the microwave unit, Anton Paar, Model Multiwave 3000. After cooling the solution was placed in 100 mL standard flask and makeup till the mark.

3. FIGURES AND TABLE

Fig. 1 - Site of Soil Collection

Fig. 2 - Green House Experiment

Table 1: Heavy Metal content in soil before and after Phytoremediation							
Sample	pH	Heavy Metal content (mg/kg)					
		Lead			Zinc		
		Before	After	%Remediation	Before	After	%Remediation
Yamuna	6.6	384	247	35.67	210	85	59.52
Garden	6.9	236	154	34.74	124	66	46.77

Table 2: Plant and Soil Biomass Analysis									
Sample		Shoot length (cm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)	Plant % moisture content	Soil fresh weight (g)	Soil dry weight (g)	Soil % moisture content
Yamuna	Nov.	7.5	1.3	2.965	2.156	37.52	50	43.0	16.27
	Dec.	15.2	2.1	3.676	2.545	39.31	50	42.3	18.20
	Jan.	21.0	2.5	4.877	3.376	41.78	50	41.7	19.90
	Feb.	26.8	2.7	5.963	4.395	35.67	50	41.1	21.64
Garden	Nov.	7.4	1.5	3.065	2.353	31.24	50	46.5	7.53
	Dec.	16.1	2.5	3.878	2.891	35.46	50	46.1	8.45
	Jan.	20.5	3.0	5.232	4.064	40.17	50	45.6	9.64
	Feb.	25.6	3.4	6.147	5.008	30.06	50	44.9	11.35

Table 3: Concentration of lead and Zinc in soil and Plants					
Sample		Heavy Metal			
		Lead (mg/kg)		Zinc (mg/kg)	
		Soil	Plant	Soil	Plant
Yamuna	November	365	18	198	11
	December	334	51	157	49
	January	281	99	109	97
	February	247	131	85	122
Garden	November	217	13	113	10
	December	199	32	99	23
	January	178	59	78	44
	February	154	78	66	57

Fig.3- Heavy Metal content in soil before and after Phytoremediation

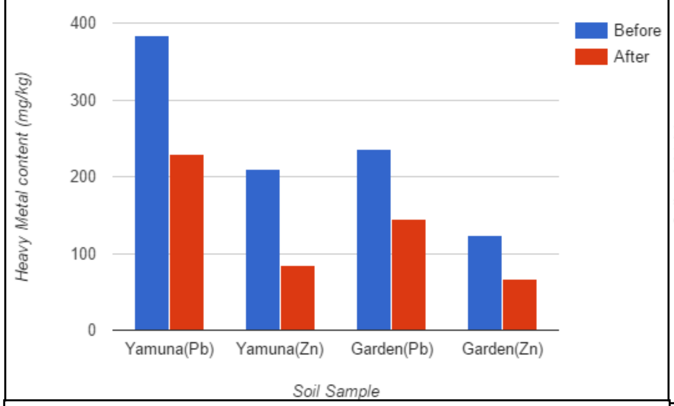


Fig.4- Biomass(fresh and dry weight) analysis of Soil samples

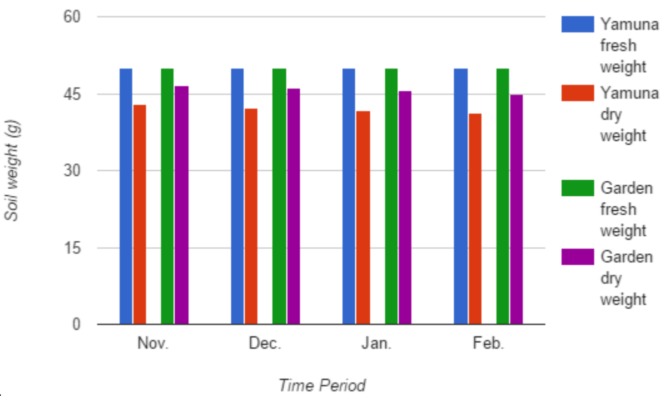


Fig.5- Root and Shoot length of Brassica species

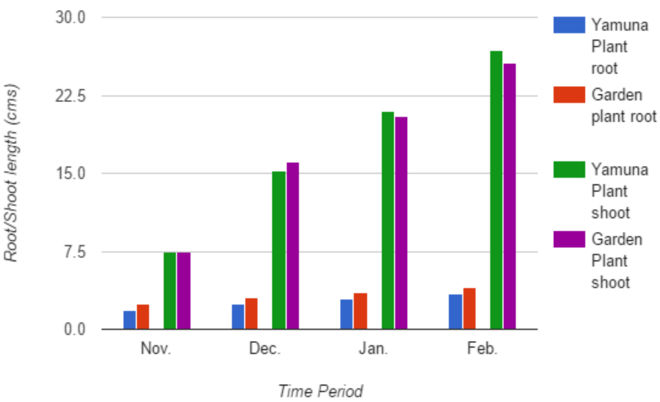


Fig.6- Biomass(fresh and dry weight) analysis of Brassica species

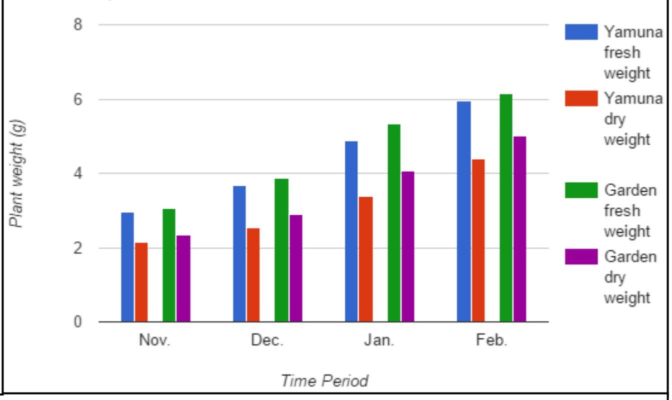


Fig.7- Amount of Lead in Yamuna soil and plant

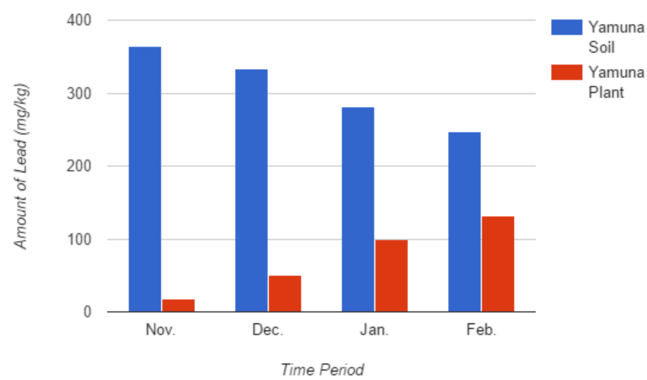


Fig.8- Amount of Lead in Garden soil and plant

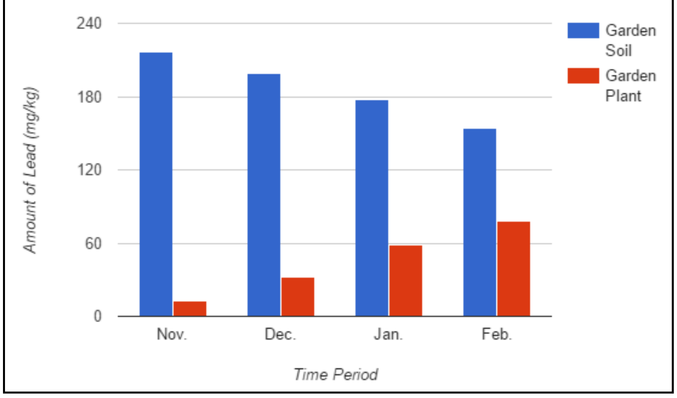
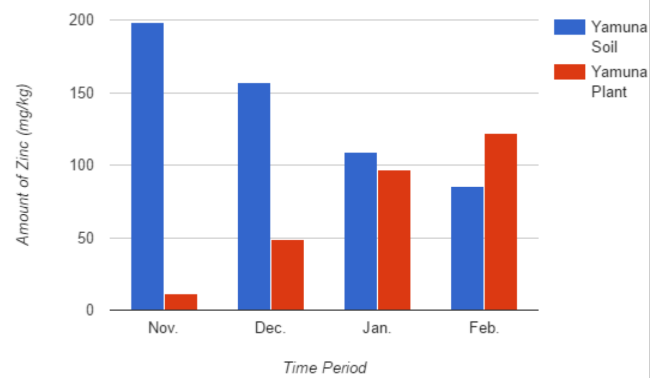


Fig.9- Amount of Zinc in Yamuna soil and plant



4. RESULT AND DISCUSSION

B. juncea is known for its ability to accumulate heavy metals in substantial amounts, and is considered as a promising contender for the commercialization of phytoremediation. The present work was embraced to investigate its phytoremediation potential under heavy metal contamination with reference to the investigation of metal interactions and their consequences on development and metal uptake arrangement of the plants. The fresh weight and dry weight of the plants was studied to compare the biomass amount and was found to be high in garden soil plants as compared to Yamuna soil plants. Observation regarding healthy growth rate of garden soil plants, considering they are of same age, is much better than that of Yamuna soil plants due to the presence of various contaminants (heavy metals) in Yamuna soil. The root length of Yamuna soil plants is considerably less than that of garden soil plant (Table 2) which is attributed to root as being the primary zone for accumulation of lead and zinc thus preserving metabolically and functionally active parts of plant (leaves and shoot) from deleterious effect of heavy metal. As growth of plant increases, the amount of lead and zinc in soil decreased while it increased in plant due to phytoextraction potentiality of *Brassica* species. The heavy metal accumulation capacity for zinc was observed to be higher than that of lead in terms of heavy metal content in soil samples before and after remediation (Table 1)

The amount of Zinc taken up by plants in both soil samples is more than that of amount of Lead up taken because zinc being an essential micro-nutrient is required for proper growth and development in optimum concentration and hence is taken up by the plants more readily. Lead being toxic even at very low concentration affects the physiological and metabolic state of the plants thus limit uptake of lead. The amount of lead and zinc relatively present in soil and plant at different growth phases is summarized in Table 3. Hence the % remediation shows efficiency of *Brassica juncea* as hyper accumulator of Lead and zinc employing phyto-extraction. High concentration of metal accumulation is observed in underground parts which is a constitutive and adaptive mechanism of a plant saving the metabolically active part of the plant from deleterious effects of excess heavy metals.

4. CONCLUSION AND FURTHER RECOMMENDATIONS

The present study delineates the effects of heavy metal interactions on the growth and function of *B. juncea*, and highlights its potential in phytoremediation of heavy metal contaminated sites. Improved growth of *B. juncea* leads to enhanced yield in terms of greater biomass production, thus significantly increase in metal removal from the multi heavy metal contaminated sites. Phytoremediation has the potential to treat sites polluted with more than one type of pollutant and is a promising technology for ecological restoration and biodiversity conservation. It is still a relatively new technology and is mostly in its testing stages and as such has not been used in many places as a full scale application. However it has been tested successfully in many places around the world for many different contaminants. Since lot of biomass is generated during this process raising a need for proper disposal and management. After burning of such plants, they can either be disposed securely in specific dumps or if monetarily attainable, prepared for bio-recovery of coveted metals (a practice known as phytomining) [25]. 'Mining with plants', or phytomining, is being considered as a safe and viable method for waste disposal. Phytomining is the production of a `crop' of a metal by developing high-biomass plants that gather metal in high amounts. This biomass containing heavy metals can be combusted to get crucial energy and the remaining slag is considered as "bio-mineral". This bio-mineral can be arranged for the recovery or extraction of the heavy metals.

In conclusion, Phytoremediation is more economically viable using the same tools and supplies as agriculture and is less disruptive to the environment and does not involve waiting for new plant communities to decolonize the site. It avoids excavation and transport of polluted media thus reducing the risk of spreading the contamination. And hence, is more likely to be accepted by the public as it is more aesthetically pleasing than traditional methods.

REFERENCES

[1] Lone, M.I., He, Z., Stoffella, P.J. and Yang, X. Progress and perspectives of phytoremediation. *Journal of Zhejiang University. 9*, 2008, 210-220.

[2] Islam, E., Yang, X.E., He, Z.L. and Mahmood, Q. Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. *Journal of Zhejiang University Science B. 8*, 2007, 1-13.

[3] Blaylock, M.J. and Huang, J.W. Phytoextraction of metals. In: Raskin, I. and Ensley, B.D. (eds). Phytoremediation of Toxic Metals: Using Plants to CleanUp the Environment.2000 pp 53-70. John Wiley & Sons, Inc., New York.

[4] Clemens, S. Toxic metal accumulation, responses to exposure and mechanisms of tolerance in *plants.Biochimie. 88*, 2006, 1707-1717.

[5] Liu, X.M., Wu, Q.T. and Banks, M.K. Effect of simultaneous establishment of Sedum alfridii and Zea mays on heavy metal accumulation in plants. *International Journal of Phytoremrdiation. 7*, 2005, 43-53.

[6] Blaylock, M.J. and Huang, J.W. Phytoextraction of metals. In: Raskin, I. and Ensley, B.D. (eds). Phytoremediation of Toxic Metals: Using Plants to CleanUpthe Environment.2000 pp 53-70. John Wiley & Sons, Inc., New York.

[7] Salt, D.E., Blaylock, M., Kumar, P.B.A.N., Dushenkov, V., Ensley, B.D., Chet, L. and Raskin, L. Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plant. *Biotechnology. 13*, 1995, 468-474.

[8] Salt, D.E., Smith, R.D. and Raskin, I. Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology. 49*, 1998, 643-668.

[9] Ghosh, M. and Singh, S.P. A review on phytoremediation of heavy metals and utilization of its byproducts. *Applied Ecology Environmental Research. 3,* 2005, 1-18.

[10] Ghosh, M. and Singh, S.P. Comparative uptake and phytoextration study of soil induced chromium by accumulator and high biomass weed species. *Applied Ecology and Environmental Research. 3,* 2005, 67-79.

[11] Qiu, R., Fang, X., Tang, Y., Du, S., Zeng, X. and Brewer, E. Zinc hyperaccumulation and uptake of Potentillagriffithii Hook. *International Journal of Phytoremediation. 8,* 2006, 299-310.

[12] Garbisu, C. and Alkorta, I. Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. BioresourceTechnology. 77, 2001, 229-236.

[13] Lai, H. and Chen, Z. In-situ selection of suitable plants for the phytoremediation of multi-metal-contaminated sites in central Taiwan. *International Journal of Phytoremediation*. *11*, 2009, 235-250.

[14] Cherian, S. and Oliveria, M.M. Transgenic plants in phytoremediation: recent advances and new possibilities. *Environmental Science and Technology. 39,* 2005, 9377-9390.

[15] Dowling, D.N. and Doty, S.L. Improving phytoremediation through biotechnology. *Current Opinion in Biotechnology. 20,* 2009, 204-206.

[16] Nriagu, J.O., Pacyna, J.M., Quantitative assessment of worldwide contamination of air, water and soils by trace metals. *Nature, 333*, 1988, 134-139.

[17] Mengel, K. and Kirby, E.A. Principles of Plant Nutrition.pp 687, 1987 International Potash Institute, Berne.

[18] Foy, C.D., Chaney, R.L. and White, M.C. The physiology of metal toxicity in plants. Annual Review on Plant Physiology. 29, 1978, 511-566.

[19] Wallnofer, P.R. and Engelhardt, G. Schadstoffe, die ausdem Boden aufgenommenwerden. In: Hock, B. and Elstner, E.F. (eds). Pflanzentoxikologie.pp., 1984, 95-117.

[20] Peralta, J.R., Gardea, T. J.L., Tiemann, K.J., Gomez, E., Arteaga, S. and Rascon, E. Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicagosativa).*Bulletin of Environmental Contamination and Toxicology. 66,* 2001, 727-734.

[21] Blaylock, M.J., Salt, D.E., Dushenkow, S., Zakharova, O. and Gussman, C. Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents. *Environmental Science and Technology. 31,* 1997, 860-865.

[22] Ebbs, S.D. and Kochian, L.V. Toxicity of Zn and Cu to Brassica species: Implications for phytoremediation. *Journal of Environmental Quality. 26*, 1997, 776- 781.

[23] Ebbs, S.D. and Kochian, L.V.Phytoextraction of zinc by oat (Avena sativa), barley (Hordeumvulgare), and Indian mustard (Brassica juncea). *Environmental Science and Technology. 32,* 1998, 802-806.

[24] Ren, H., Chen, X., Sun, G. and Wang, Y. Response of wheat seedlings with different drought resistance to water deficiency and NaCI stresses. *Ying Yong Sheng Tai XueBao. 11,* 2000, 718-722.

[25] Salt, D.E., Smith, R.D., Raskin, I., Phytoremediation. *Annu.Rev. Plant Physiol. Plant Mol. Biol. 49*, 1998, 643–668.