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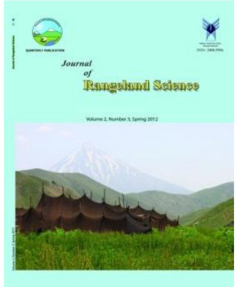

Full Length Article:

Germination, Growth and Uptake of Heavy Metals in Contaminated Soils (*Hordeum bulbosum* L.)

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Abstract. The use of metal-accumulating plants to clean the contaminated soil with toxic metals is the most rapidly developing component of phytoremediation as an environmentally friendly technology. Overcoming harsh soil conditions and accelerating the recovery of degraded soils remain a worldwide restoration challenge. This study evaluated the effect of different concentrations of Pb, Zn, Cu and Fe on the germination, growth and heavy metal uptake of *Hordeum bulbosum* L. Results obtained from the current research indicated that the plant gives dose-dependent responses to the contaminated soils. Reduction in germination, root and shoot height and biomass were significantly (P<0.05) different as compared to the control. Exposures of heavy metals in the treatment reduced the size of roots and shoots about 8.68% and 5.05% respectively as compared to the control. However, the concentration of heavy metals was increased at treatment four, the sizes of root and shoot are the same at two and three treatmens. Heavy metal concentrations were significantly higher in the root tissue than aboveground components and plant organs demonstrated a different affinity to take up heavy metals. In all the plant organs, the concentrations of heavy metals increased as the metal contents in the soil increased.

Key words: *Hordeum bulbosum*, Heavy metals, Germination, Phytoremediation.

Introduction

Heavy metal pollution of soil is one of the most important environmental problems throughout the world. In fact, heavy metals have a significant toxicity for humans, animals, microorganisms and plants (Bodar *et al*., 2006). Although a small portion of heavy metals in soils is derived from natural processes (e.g. bedrock weathering), a much higher amount originates from the anthropogenic sources such as mining and smelting industry, use of mineral fertilizers and pesticides and sewage sludge application (Komárek *et al*., 2008). Therefore, remediation of old industrial sites is a necessary step in order to meet the demand for urban housing, office and leisure spaces. Decreasing the concentration of these potentially toxic compounds is also beneficial to the environment. Many methods including removal, incineration and removal followed by thermal desorption have been used for cleaning up the contaminated soils although all these methods are cost-prohibitive (Joner and Leyval, 2001). To find more environmentally and economically acceptable options, scientists and engineers have recently started to generate cost-effective technologies that include the use of microorganisms, biomass and live plants in the cleaning process of polluted areas (Wasay *et al*., 1998; Gardea-Torresdey *et al*., 1996). In this way, phytoremediation that is to use green plants to decontaminate the metals out of the soil is an emerging technique with advantages of being in situ, cost effective and environmentally sustainable (Lasat, 2002).

Phytotoxicity varies according to the metal and plant species and it can occur beyond a certain threshold (Shamsi *et al*., 2008; Markovska *et al*., 2009; Mihucza *et al*., 2012), Therefore, for achieving maximum HMs reduction and successfully establishing stable vegetation cover, various criteria must be considered. The plants should be chosen carefully so that they provide a maximum root surface area (Aprill and Sims, 1990). Researchers have observed that some plant species are endemic to metalliferous soils and can tolerate greater rates than usual amounts of heavy metals or other toxic compounds (Peralta *et al.*, 2000; Blaylock and Huang, 2000). Considering the cost of more extensive testing, germination tests have frequently been used in selecting the species to be grown on the contaminated sites and most of these studies have been conducted using seedlings or adult plants (Gratton *et al*., 2000; Pichtel *et al*., 2000). In a few studies, the seeds have been exposed to the contaminants (Claire *et al*., 1991; Xiong, 1998). In order to explore an acceptable option for the biological control of small contaminated sites polluted by heavy metals, phytoremediation potential of *Hordeum bulbosum* has been considered as a local plant species in reducing the extent of contaminated soils with heavy metals in Takestan industrial city. Therefore, the germination, growth and heavy metal uptake of *H. bulbosum* were investigated in the contaminated soils. Furthermore, using germination rate as an indicator of subsequent establishment and growth of plant in the contaminated soils was investigated.

Materials and Methods

Soil preparation

The soil for the greenhouse experiments was obtained from Takestan industrial city, an important industrial area of Qazvin (Northern Iran). The area is influenced by the industrial waste water; then, the soil is highly polluted with heavy metals. Soil samples were collected at four different points distributed along the wastewater channel from its mouth to 1 km landward. Soil samples of treatments one, two and three were taken outside of the channel in different distances and soil sample of treatment four was taken at the mouth of channel. Soil samples were divided into 4 groups considering the metal concentration in each group with 5 pots (replicates) (Table 1).

Soil sampling was obtained from the depth of 0–40 cm and mixed (Besalatpour *et al.*, 2008). All soil samples were sieved to 4 mm and moisture contents were adjusted to 70% water-holding capacity. Uncontaminated soil was used as the control throughout the study. Selected physical and chemical properties of contaminated and uncontaminated soil samples are listed in (Table 2). The soil texture (clay-loam) was determined by hydrometer method (Day, 1982), CEC (Cation Exchange Capacity) was measured by the method of Bower and Hatcher (1966), pH was determined in a 1:5 soil to distilled water slurry after 1 hour of agitation using a digital pH-meter (Thomas, 1996), Electrical Conductivity (EC) was measured using an EC-meter (Rhoades, 1996), total soil N was analyzed colorimetrically with a continuous flow ion analyzer following wet digestion in sulfuric acid (Bremner, 1996), organic carbon was measured by the Walkley-Black method (Nelson and Sommers, 1996). The $CaCO_3$ equivalent was determined by neutralizing with HCl and back titration with NaOH (Black *et al.*, 1965). Heavy metal (Pb, Zn, Cu and Fe) concentrations were determined as follows: dried soil samples were passed through a 2 mm diameter sieve. About 100 mg dry sediment was digested with HNO_3 and HCl (3:1) in a microwave oven. After mineralization, the samples were diluted, filtered and then analyzed for heavy metals using ICP/OES (Model GBC, 2004).

Table 1. Geographic coordinates and distance of four treatments from the wastewater channel mouth (m)

Treatment	Distance (m)	Coordinates
1	1000	35° 41′ 13"N 48° 38′28"E
2	500	35° 34′ 27"N 48° 27′16"E
3	200	35° 33′ 61"N 48° 42′21"E
4	0	35° 25′ 33"N 48° 42′17"E

Pot experiment

Seeds were obtained commercially and about 1 kg of soil samples was added to plastic pots (diameter 20×diameter 15×height 35 cm). A paper filter was placed at the bottom of each pot to prevent the soil escaping from the drainage holes. 25 seeds of the plant were placed evenly throughout each pot at least 1 to 2 cm from the edge. The pots were watered from the top during the germination period so that the soil moisture was kept near 70% of field capacity. For each treatment, germination was monitored closely over the first two weeks of the trials. Germinated seeds were counted daily according to the method proposed by Maguire (1962) and two germination parameters were determined: (1) final germination percentage (number of germinated seeds in each pot) and (2) Germination Rate (GR, a measure of germination speed with lower values indicating faster germination). Germination rates were calculated as follows:

$RG= \sum N_i D_i$

where, N_i is the number of germinant daily, D_i is the number of days from the initial sowing. In each trial, the plants were grown for 6 weeks after the germination period in the greenhouse. The minimum and maximum temperature was 21 and 28C°, respectively. The seedlings were harvested at the end of growing trial. The roots were carefully washed with deionized water to get rid of the sand. The plants were separated into root and shoot and dried in an oven at 70°C for 48 h. Then, the biomass (dry weight) was determined. Dried samples were ground, digested in the concentrated HNO_3 for 14 h, 12 h at room temperature first, then 2 h at 60°C, and further digested with concentrated HNO_3-$HClO_4$ (3:2, v/v) for 3 h at 140-160°C. After cooling, the extract was diluted with NHCl and made up to 25 ml.

Statistical analysis

Analyses were carried out using SPSS release 16.0. Data were tested for normality with the Kolmogorov–Smirnov test and for variance homogeneity with the Levene test (P<0.05) (Mansourfar, 2006). Variations in heavy metal concentrations in the soils and plant organs were compared by one-way analysis of variance. Tukey's t-test between means was calculated only if F-test was significant at the 0.05 level of probability. A probability of 0.05 or lower was considered as significant.

Results

Soil properties

Soils from the contaminated and uncontaminated sites had clay loam textures and electrical conductivity of soils were ranged between 2.30-2.66 dSm^{-1}. pH varied between 8.3-8.8 (Table 2). The results revealed that the soils are similar in terms of physical and chemical characteristics. Concentrations of heavy metals are listed in Table 3, the highest concentration was measured for treatment 4 and treatment 1 had the lowest heavy metal concentration.

Table 2. Physical and chemical characteristics of soil samples were collected for the greenhouse treatments

Site	Texture	CEC (meq)	N%	OC%	EC ($dS\ m^{-1}$)	pH	$CaCO_3$ (%)
1	Clay loam	36.30	0.13	0.14	2.55	8.4	12.21
2	Clay loam	35.40	0.12	0.14	2.50	8.3	12.00
3	Clay loam	36.20	0.14	0.15	2.66	8.4	12.50
4	Clay loam	35.00	0.15	0.16	2.30	8.6	13.60
Control	Clay loam	29.30	0.10	0.16	2.40	8.8	15.30

Soils were sampled from 0 to 40 cm depth with a 5 cm-diameter hand-driven corer.

Table 3. Total concentration of heavy metals ($mgkg^{-1}$, n=5) for contaminated treatments based on soil collected from the industrial area

Treatment	Pb	Zn	Cu	Fe
1	720.12±16.00 a	520.11±16.72 a	21.83±3.00 a	245.13±52.20 a
2	1095.00±96.11 b	1633.42±25.09 b	50.40±2.73 b	990.77±64.20 b
3	2600.42±94.70 c	2208.03±33.00 c	73.00±5.61 b	1563.9±130.1 c
4	5571.35±83.22 d	2823.17±46.11 c	115.62±77.4 c	2588.6±175.66 d

Results are mean±SE. Different letters indicate significant difference between treatments (Tukey's test).

Effects of heavy metals on seed germination

Table 4 shows the effects of various concentrations of Pb, Zn, Cu, and Fe on seed germination of *H. bulbosum* grown in the contaminated and uncontaminated soils. In general, there was a reduction in seed germination as metal concentrations in the soils increased. Significant decreases ($P<0.05$) of germination rate and percentage with the increase of heavy metal concentrations in the solution have been observed. These parameters at treatment one are the same with those of the control and at treatment four, the parameters are as high as 37.33% and 38.20% of the control, respectively. At treatment one, the root length is significantly different from the control decreasing to a half of the control. At treatment four (the highest content of metals), the root length is only as long as one tenth of the control.

Effects of heavy metals on root and shoot growth

Although the response to heavy metal concentration varied between root and shoot length, it demonstrated an overall dose dependent response to the contaminated soil and a decrease in the root and shoot growth was evident as compared to the control treatment. At treatment one (the lowest concentration), the reduction in root and shoot size was about 8.68% and 5.05% respectively as compared to the root and shoot size of the control. However, the concentration of heavy metals was increased at treatment four, root and shoot sizes are the same at treatments two and three.

According to the length difference of root and shoot between the lowest heavy metal concentration and control, root seems to be more sensitive to heavy metals than the shoot. The data corresponding to the biomass with various treatments are presented in (Table 4). Similar to the seed germination and root and shoot length, the mean biomass is significantly different from the treatment four decreasing to the half of the control. It is interesting to note that the response of *H. bulbosum* to the heavy metals seems to be a combination of some stimulating and inhibiting effects and the biomass reached its minimum at treatment four.

Table 4. Germination rate, germination percentage (after 14 days of treatment), biomass (DW), roots and shoots length of *H. bulbosum* at five different treatments (at the end of growing trial)

Treatment	Germination Rate (%)	Germination Percentage	Root Length (mm)	Shoot Length (mm)	Biomass (mg)
1	93.67±0.00 a	94.00±1.64 a	40.32±2.20 a	35.71±2.10 a	50.72±0.92 a
2	66.55±1.70 b	62.40±1.47 b	22.37±2.50 b	25.09±2.00 b	33.41±1.36 b
3	50.13±1.55 c	44.81±1.50 c	20.40±1.30 b	20.23±1.70 b	30.50±1.54 b
4	37.33±1.40 c	38.20±1.74 c	18.61±1.20 b	15.12±1.22 b	30.80±1.00 b
Control	100.00±0.00 a	100.00±1.20 a	48.00±2.16 a	39.51±1.54 a	71.84±2.09 a

Results are mean±SE. Different letters indicate significant difference between treatments (Tukey's test).

The seedling heavy metal uptake

The heavy metal concentrations in the roots and shoot of *H. bulbosum* are listed in (Table 5). Heavy metal concentrations were significantly higher in the root tissue than aboveground components (leaf and stem) and plant organs demonstrated a different affinity to take up heavy metals. It is interesting to note that heavy metal concentration in the plant is significantly affected by the content supplied in the soil and the plant tissue as well as the interaction between two factors (Table 5). Mean values for organs showed the maximum accumulation at treatment 4 that showed a significant accumulation ($P<0.05$) comparing with other treatments. However, data demonstrated an increase in the concentration of heavy metals, there was no significant difference between content of heavy metals in the root and shoot between treatments 2 and 3.

Table 5. Heavy metal concentration (mg/kg) in seedling root and shoot tissues of *H. bulbosum* at the end of growing trial

Treatment	Pb		Zn		Cu		Fe	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
1	903.10 ±14.00 C-b	228.66 ±7.09 C-a	747.22 ±10.05 C-b	288.30 ±9.53 C-a	79.00 ±5.40 C-a	66.40 ±7.33 C-b	330.56 ±14.00 D-a	127.00 ± 4.76 C-b
2	4321.00 ±53.33 B-b	978.20 ±11.35 B-a	2208.46 ±18.00 B-b	823.07 ±7.77 B-a	244.39 ±8.90 B-a	103.57 ±8.00 B-b	1240.22 ±17.30 C-a	1005.00 ±14.23 B-b
3	4799.15 ±47.18 B-b	1054.06 ±13.40 B-a	2401.52 ±24.70 B-b	1444.82 ±16.24 B-a	253.28 ±10.72 B-a	124.04 ±6.42 B-b	2247.27 ±24.00 B-a	1340.46 ±23.08 B-b
4	6420.50 ±76.00 A-b	2300.92 ±25.00 A-a	4900.10 ±57.05 A-b	2805.38 ±29.50 A-a	724.06 ±11.50 A-a	446.50 ±12.37 A-b	4358.12 ±63.90 A-a	1970.10 ±33.50 A-b
Control	ND	ND	ND	ND	ND	ND	ND	ND

Results are mean±SE. Different capital letters in each column indicate significant differences between treatments. Different lower case letters in each row indicate significant differences between organs (Tukey’s test). ND= NOT Detected/ Below detectable range.

Discussion and Conclusion

In the current research study, the effect of heavy metals on the seed germination and growth of *H. bulbosum* was assessed. In general, there was a reduction in seed germination and growth as metal concentration in the soils increased. Claire *et al.*, (1991) obtained similar results in a study using nickel and other heavy metals on cabbage, lettuce, millet, radish, turnip and wheat. Similar results have also been observed in *Pisum sativum* L. (Sabrine *et al.*, 2010), *Sinapis alba* (Fargasova, 1994), *Brassica pekinensis* (Xiong, 1998), lettuce and radish (Nwosu *et al.*, 1995) and *Medicago sativa* (Peralta *et al.*, 2000).

Decrease in the root and shoot growth was evident as compared to the control treatment. Arduini *et al.*, (1994) found the similar result in a research on morphology of *Pinus pinea* and *Pinus pinaster* using cadmium and copper. Peralta *et al.*, (2004) reported that a reduction in chlorophyll could diminish the aboveground organ growth. In current study, the data revealed that the root seems to be more sensitive to heavy metals than the shoot because an increase in heavy metal concentrations of soil leads to a root elongation reduction more than the shoot. One of the explanations for the roots to be more responsive to the contaminants in environment might be that the roots were the specialized absorptive parts so that they were affected earlier and subjected to accumulation of more toxic elements than any other parts. This could also be the main reason for that the root length was usually used as a measure for determining heavy metal-tolerant ability of plants. Xiong (1998) found a similar effect using lead in *B. pekinensis* seed.

The highest total dry biomass (roots plus shoots) was observed in the control treatment followed by treatment one and the lowest total dry biomass was seen in treatment four (the highest heavy metal concentration). Decreasing the dry biomass might be due to a decrease in water absorption in plant tissues by toxic metals causing undesirable impacts on the plant growth (Fuentes *et al.*, 2006). Similar results have also been reported in the studies of Inckot *et al.*, (2011), Papazoglou *et al.*, (2005) and Peralta *et al.*, (2004).

Regarding the concentration of metals in the aerial tissues, root played a more important role and the absorption of metals in the root was higher. Therefore, the metals investigated in the root of plant showed a significant increase and a relationship between the plant root and metal accumulation was clearly visible. So, the roots of plant could be considered as an index for Pb, Zn, Cu and Fe absorption. The relatively low accumulation of heavy metals in the aboveground tissues was probably due to the need of plants to prevent toxicity to the photosynthetic apparatus as suggested by other authors (Stoltz and Greger, 2002; Bragato *et al.*, 2006). In all the tissues (root and shoot), the concentrations of heavy metals increased without exception as the metal contents in the soil was increased. Published data revealed that heavy metal content in the plant is a function of heavy metal concentration in the soil. Kabata-Pendias and Pendias (1984) reported that the plants would take up the metal via the root system when the soil is contaminated with heavy metals. In this study, root and shoot are directly subjected to heavy metals. Thus, the heavy metal concentration in the tissues is more closely related to that of the soil.

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References

Aprill, W., Sims, R. C. 1990. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. *Chemosphere*. **20:** 253–265.

Arduini, I., Godbold, D. L., Onnis, A. 1994. Cadmium and copper change root growth and morphology of *Pinus pinea* and *Pinus pinaster* seedlings. *Physiologia Plantarum*. **92:** 675-680.

Besalatpour, A., Khoshgoftarmanesh, A. H., Hajabbasi, M. A., Afyuni, M. 2008. Germination and growth of selected plants in a petroleum contaminated calcareous soil. Soil and sediment contamination. **17:** 665–676.

Black, C. A., Evans, D. D., White, J. L., Ensminger, L. E., Clark, F. E. 1965. Methods of Soil Analysis. Part 2. Agron. Monogr. 9. ASA, Madison, WI.

Blaylock, M. J., Huang, J. W. 2000. Phytoextraction of metals, In: I. Raskin and B. D. Ensley (Ed.) Phytoremediation of toxic metals: using plants to clean up the environment, John Wiley and Sons, Inc, Toronto, Canada. p, 303.

Bodar, C. W., Pronk, M. E., Sijm, D. T. 2006. The European Union risk assessment on zinc and zinc compounds: the process and the facts. *Integrated Environmental Assessment and Management*. **1:** 301–319.

Bower, C. A., Hatcher, J. T. 1966. Simultaneous determination of surface area and cation-exchange capacity: Soil Sci. Soc. Am. Proc. **30:** 525-527.

Bragato, C., Brix, H., Malagoli, M. 2006. Accumulation of nutrients and heavy metals in *Phragmites australis* (Cav.) Trin. ex Steudel and *Bolboschoenus maritimuss* (L.) Palla in a constructed wetland of the Venice lagoon watershed. *Environmental Pollution*. **144:** 967–975.

Bremner, J. M., 1996. Nitrogen total. Pages 1085–1122 in J. M. Bartels, editor. Methods of soil analysis. Part 3. Chemical methods—SSSA book series No. 5. Soil Science Society of America, Madison, Wisconsin.

Claire, L. C., Adriano, D. C., Sajwan, K. S., Abel, S. L., Thoma, D. P., Driver, J. T. 1991. Effects of selected trace metals on germinating seeds of six plant species. *Water, Air and Soil Pollution*. **59:** 231-240.

Day, P. R., 1982. In methods of soil analysis, part 2; Page, A. L., *et al.*, Eds.; Agronomy Monograph 91 American Society of Agronomy: Madison, WI. 935-951.

Fargasova, A., 1994. Effect of Pb, Cd, Hg, As and Cr on germination and root growth of *Sinapis alba* seeds. Bulletin of Environmental Contamination and Toxicology. **52:** 452-456.

Fuentes, D., Disante, K. B., Valdecantos, A., Cortina, J., Vallejo, V. R. 2006. Response of *Pinus halepensis* Mill. Seedling to biosolids enrich with Cu, Ni and Zn in three Mediterranean forest soils. *Environmental Pollution*. **20:** 1-8.

Gardea-Torresdey, J. L., Polette, L., Arteaga, S., Tiemann, K. J., Bibb, J., Gonzalez, J. H. 1996. Determination of the content of hazardous heavy metals on *Larrea tridentata* grown around a contaminated area. Proceedings of the Eleventh Annual EPA Conference On Hazardous Waste Research, Edited by L. R. Erickson, D. L. Tillison, S. C. Grant and J. P. McDonald, Albuquerque, NM. p. 660.

Gratton, W. S., Nkongolo, K. K., Spiers, G. A. 2000. Heavy metal accumulation in soil and Jack pine (*Pinus banksiana*) needles in Sudbury, Ontario, Canada, *Bulletin of Environmental Contamination and Toxicology*. **64:** 550-557.

Inckot, R. C., Santos, G. O., Souza, L. A., Bona, C. 2011. Germination and development of *Mimosa pilulifera* in petroleum-contaminated soil and bioremediated soil Flora. **206:** 261–266.

Joner, E. J., Leyval, C. 2001. Influence of Arbuscular mycorrhiza on clover and ryegrass grown together in a soil spiked with polycyclic aromatic hydrocarbons. *Jour. Mycorrhiza*. **10:** 155–159.

Kabata-Pendias, A., Pendias, H. 1984.Trace elements in the soils and plants. CRC Press, Florida.

Komárek, M., Tlustoˇs, P., Száková, J., Chrastny´, V. 2008. The use of poplar during a two-year induced phytoextraction of metals from contaminated agricultural soils. Environ Pollut. **151:** 27–38.

Lasat, M. M., 2002. Phyto-extraction of toxic metals: a review of biological mechanisms. *Jour. Environmental Quality*. **31:** 109–120.

Maguire, J. D., 1962. Speed of germination: Aid in selection and evaluation of seedling emergence and vigor. *Jour. Crop Science.* **2:** 176–177.

Mansourfar, K., 2006. Advanced statistical method using applied software. University of Tehran press. p, 459.

Markovska, Y., Gorinova, N., Nedkovska, M., Miteva, K. 2009. Cadmium-induced oxidative damage and antioxidant responses in *Brassica juncea* plants. Biol Plant. **53:** 151–4.

Mihucza, V. G., Csogd, A., Fodore, F., Tatára, E., Szoboszlaia, N., Silaghi-Dumitrescuf, L., Záraya, G. 2012. Impact of two iron (III) chelators on the iron, cadmium, lead and nickel accumulation in poplar grown under heavy metal stress in hydroponics. *Jour. Plant Physiology.* **169:** 561– 566.

Nelson, D. W., Sommers, L. E. 1996. Total carbon, organic carbon, and organic matter. Pages 961–1010 in J. M. Bartels, editor. Methods of soil analysis. Part 3. Chemical methods—SSSA book series no. 5. Soil Science Society of America, Madison, Wisconsin.

Nwosu, J. U., Harding, A. K., Linder, G. 1995. Cadmium and lead uptake by edible crops grown in a silt loam soil. *Bulletin of Environmental Contamination and Toxicology*. **54:** 570-578.

Papazoglou, E. G., Karantounias, G. A., Vemmos, S. N., Bouranis, D. L. 2005. Photosynthesis and growth responses of giant reed (*Arundo donax* L.) to the heavy metals Cd and Ni. *Environment International*. **31:** 243-249.

Peralta, J. R., De la Rosa, G., Gonzalez, J. H., Gardea-Torresdey, J. l. 2004. Effect of the growth stage on the heavy metal tolerance of alfalfa plants. *Advances in Environmental Researc.* **8:** 679-685.

Peralta, J. R., Gardea-Torresdey, J. L., Tiemann, K. J., Gómez, E., Arteaga, S., Rascon, E., Parsons, J. G. 2000. Study of the effects of heavy metals on seed germination and plant growth on alfalfa plant (*Medicago sativa*) grown in solid media. *Proceedings of the Conference on Hazardous Waste Research.* 135-140.

Pichtel, J., Kuroiwa, K., Sawyer, H. T. 2000. Distribution of Pb, Cd, and Ba in soils and plants of two contaminated sites, *Environmental Pollution.* **110:** 171-178.

Rhoades, J. D., 1996. Salinity: Electrical conductivity and total dissolved solids. In: Methods of Soil Analysis. Part 2. pp 417–435. (Page, A. L., ed.). American Society of Agronomy, Madison, WI.

Sabrinea, H., Afifb, H., M, Banni., Hamadic, B., Mariab, H. 2010. Effects of cadmium and copper on pollen germination and fruit set in pea (*Pisum sativum* L.). Scientia Horticulturae. **125:** 551–555.

Shamsi, I., Wei, K., Zhang, G., Jilani, G., Hassan, M. 2008. Interactive effects of cadmium and aluminum on growth and antioxidative enzymes in soybean. Biol Plant. **52:** 165–9.

Stoltz, E., Greger, M. 2002. Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing on submerged mine tailings. *Environmental and Experimental Botany.* **47:** 271-280.

Thomas, G. W., 1996. Soil pH and soil acidity. Pages 475–490 in J. M. Bartels, editor. Methods of soil analysis. Part 3. Chemical methods-SSSA book series no. 5. Soil Science Society of America, Madison, Wisconsin.

Wasay, S. A., Barrington, S. F., Tokunaga, S. F. 1998. Using *Aspergillus niger* to biorremediate soils contaminated by heavy metals, *Bioremediation Jour.* **2(3):** 183-190.

Xiong, Z. T., 1998. Lead uptake and effects on seed germination and plant growth in a Pb Hyperaccumulator *Brassica pekinensis* Rupr. *Bulletin of Environmental Contamination and Toxicology.* **60:** 285-291.440.