Advances in Science and Technology, Vol. 5, No. 1, March / April, 2011, pp 70-75

# Phytoextraction of Cadmium and Responses of Indian Mustard Plants To Cadmium Contaminated Soil

Ketia L. Shumaker[1], Susmita Ghosh[2] and M. S. Zaman[3*]
[1]Department of Biological and Environmental Sciences, The University of West Alabama, Livingston, Alabama, 35470, USA; [2]Shiksha Center, Monroeville, Pennsylvania, 15146, USA; [3]Department of Biological Sciences, Alcorn State University, Alcorn State, Mississippi, 39096, USA.
(Submitted: December 15, 2009; Accepted: April 12, 2011)

## Abstract

Studies indicate that some plants are capable of removing various soil contaminants. Such observations have led to the establishment of an emerging technology, phytoremediation. In this study, *Brassica juncea* (Indian mustard) plants were grown in cadmium (Cd) contaminated soils. Data were analyzed for growth inhibition, chlorophyll synthesis, and tissue metal accumulation. Results indicated that growth was inhibited in most Cd treated groups, chlorophyll synthesis was inhibited in some Cd treated plants, and plant tissues accumulated high concentrations of Cd. Data from this study suggest that since *Brassica juncea* can tolerate high concentrations of Cd in soil and is able to hyperaccumulate Cd in the plant tissues, it could be a promising plant in phytoremediation studies.

**Keywords:** phytoremediation, phytoextraction, bioaccumulation, cadmium, heavy metals.

## 1.0 Introduction

Heavy metal pollution of soil and water is a crucial environmental concern as since metals get into the food chain and pose a serious threat to our health. Contaminants such as aluminum (Al), cadmium (Cd), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni), zinc (Zn), and other heavy metals have been proven to cause hazardous effects in animals and humans (Adriano, 1992; ATSDR, 2009; de Vries, 2007). Since soil cannot annihilate or decompose these metals, various conventional remediation techniques such as soil venting, washing, chemical treatment, and removal and burial of contaminated soil are used to remediate such contaminated soils. These techniques are exceedingly expensive and intrusive to the environment (Evanko & Dzombak, 1997). Studies indicate that the use of plants as soil remediators is relatively cost effective, reliable, and has little or no impact on the environment as compared to conventional methods (Hoova, 2007; López-Chuken, 2010). Phytoremediation, an emerging technology, is considered to be a "green revolution" in the field of innovative technologies to remediate metal contaminated soils. The idea and the use of metal accumulating plants to remove soil pollutants have existed for over 300 years (Chaney *et al.*, 1997). Chaney, an American scientist, reinstituted the phytoremediation concept (Chaney, 1983) which, within a short time generated excitement within the scientific community. So far, over 400 plant species have been reported to accumulate heavy metals from contaminated soil (Purakayastha and Chhonkar, 2010).

Cadmium is a tasteless and odorless natural element in the earth's crust. Due to anthropogenic activities over the past few decades, Cd soil concentrations have significantly increased, and, therefore, poses a serious health risk to humans. According to the United States Environmental Protection Agency (USEPA), soils containing above 100 ppm of Cd should be considered toxic and must be remediated before the land can be used for public use. While cadmium is naturally present in low concentrations in soil, <10 ppb, its concentration can reach the 100 ppm range in areas immediately adjacent to mines, smelters, and Ni-Cd battery plants (ATSDR, 1999). The purpose of this study was to investigate the responses of *Brassica juncea*, a potential metal ion hyperaccumulator (Salt *et al.*, 1995), to soil Cd pollution and its Cd phytoextraction potential.

*** Author's e-mail: zaman@alcorn.edu**

## 2.0 Methods

### 2.1 Experimental Plant and Growing Medium

Brassica juncea plants were grown in Memphis silt loam soil. This soil covers approximately 3.2-million acres of land in Mississippi, Louisiana, Alabama, and Tennessee. This is a well characterized soil containing about 70% silt, 20% clay, 9% sand, and 1% organic matter with a pH of 6.9 (Panicker, 1992). Surface soil (about 4" from the top) was collected from an undisturbed forest area of southwest Mississippi. Collected soil was air-dried in the laboratory for a week and then ground to pass through a 2mm sieve. Cadmium was added to this sieved soil at different concentrations as described in the following treatment protocol.

### 2.2 Planting and Treatment

*Brassica juncea* seeds were grown in 6.5 ounce-porous bottom planters with 150 grams of soil (dry weight) per planter. A one-centimeter depression was made in the center of the soil and one pre-germinated seed (radicle length of about 1 mm) was placed into each depression and then covered with soil. The planters were placed in reservoir trays. Each treatment group had its own separate reservoir tray. The plants were placed for 16 hour-light and 8 hour-dark cycles under color corrected lights with a light energy of 1.4 quanta/ sec/$cm^2$. Watering was done every alternate day or as needed with distilled water and once a week with modified Hoagland solution (Hoagland and Arnon, 1950). The plants were maintained under laboratory conditions for 30 days at 22.9 ± 0.45°C and a relative humidity of 58.0 ± 3.0%.

For the grade of growth inhibition (GGI) and tissue Cd accumulation evaluations, plants were divided into a control group and four Cd treated groups (grown in soils containing 0, 50, 100, 250, and 500 ppm Cd, respectively). For the chlorophyll evaluation the plants were divided into a control group and three Cd treated groups (soils containing 0, 100, 250, and 500 ppm Cd). For all the parameters there were 12 plants per group. The Cd was mixed with soil in the form of Cd $(NO_3)_2$ (Fisher Scientific, New Jersey, USA).

### 2.3 Growth Inhibition Analysis

For the GGI and tissue Cd accumulation evaluation the plants were harvested on day 30 of the experiment. After harvesting, the plants were washed with distilled water, separated into roots and shoots, and then completely dried at 75ºC for 96 hours in a laboratory oven. Based on the phytotoxicity protocol of Leita *et al*. (1993) the GGI was evaluated as such:

$$GGI = [(C - T / C] \times 100$$

where *C* and *T* represents the dry weight of tissues of control (*C*) and metal-treated (T) plants. Control group GGI = 0%, representing 100% growth.

### 2.4 Chlorophyll Analysis

For the chlorophyll evaluation plants were grown for 21 days. After harvesting, leaves from plants were randomly selected from the control and treated groups. The leaves from individual plants were immersed in 30 ml of 95% ethanol for 48 hours. The supernatant was collected and the process was repeated. The ethanol-chlorophyll solutions were pooled from each extraction. The leaves were dried at 70°C for 96 hours to determine the dry weight. Ethanol chlorophyll solutions were stored in darkness at room temperature. The absorbency (A) of the extract was determined at 665 and 649 nm on a spectrophotometer (Genesys 5, Spectronic Instruments, USA) to determine the chlorophyll concentration (µg chlorophyll/mg dry weight of leaf) and the total chlorophyll content of each plant. The following equations were used (Einhellig and Rasmussen, 1979):

$$\mu g\ chlorophyll\ a / ml\ \ solution = (13.70)(A665nm) - (5.76)(A649nm)$$

$$\mu g\ chlorophyll\ b / ml\ \ solution = (25.80)(A649nm) - (7.60)(A665nm)$$

### 2.5 Tissue Metal Analysis

For the Cd accumulation analysis, the USEPA Method 3050A (USEPA, 1986) was used to extract the Cd in the plant sample. Reagent blanks were used to determine if any contamination was detectable from the glassware, reagents, and/or other sources. To perform this procedure, 0.25 g of oven dried plant samples were transferred to 125 ml Erlenmeyer flasks. To each flask, 15 ml of nitric acid ($HNO_3$) and 10 ml of deionized water were then added. The samples were then heated on a hot plate for 45 minutes at medium heat. The samples were

allowed to cool and after adding 5 ml of $HNO_3$, the samples were then refluxed again for 30 minutes. The last step was repeated to ensure complete oxidation. The sample was then heated (without boiling) to evaporate to 5 ml. After this, the samples were allowed to cool again, and 2 ml of deionized water was added along with 3 ml of 30% hydrogen peroxide ($H_2O_2$) to each sample. The samples were then heated to start the peroxide reaction. 30% $H_2O_2$ was continually added in 1 ml aliquots until the effervescence became minimal. The acid-peroxide digestate was heated for a final time to reduce the digestate down to 5 ml. After cooling, the samples were diluted to a total volume of 100 ml with deionized water. The digestate was then filtered using a Whatman Number 1 filter paper (Fisher Scientific, New Jersey, USA) to remove any particulates that may have been present in the sample. The filtrate was then ready for heavy metal analysis using an Atomic Absorption Scan 4 Spectrometer (Jarell Ash, Franklin, MA). The following equation was used for the calibration curve: $x = (y + 0.0029)/0.0063$; where y was the absorbency from the spectrometer and x was the corrected absorbency.

### 2.6 Statistical Analysis

Data obtained in this study were analyzed by one-way Analysis of Variance (ANOVA) and the Tukey test.

## 3.0 Results

The results of the GGI study are shown in Table 1. The root GGI was significantly different in all Cd treated groups. The shoot GGI was significant in the 100, 250 and 500 *ppm* Cd treated groups. The total plant GGI was significant in all Cd treated groups. This data suggest that there is a positive correlation between the concentration of metals in the soil and plant growth inhibition. Similar observations were also reported by Zaman & Zereen (1998) using radish (*Raphanus sativus*) plants.

Table 1: The Grade of Growth Inhibition (GGI) of *Brassica juncea* grown in soil containing varying concentrations of Cadmium for 30 days.

| Treatment Groups | Root (Mean ± SEM) | Shoot (Mean ± SEM) | Total Plant (Mean ± SEM) |
|---|---|---|---|
| 50 ppm Cd | 36.9 ± 6.4* | 21.7 ± 5.0 | 25.8 ± 4.9* |
| 100 ppm Cd | 55.7 ± 10.5* | 49.9 ± 9.1* | 52.0 ± 9.1* |
| 250 ppm Cd | 82.7 ± 6.9* | 80.7 ± 6.1* | 81.1 ± 6.1* |
| 500 ppm Cd | 98.2 ± 0.2* | 93.0 ± 2.8* | 97.8 ± 0.4* |

GGI = [Control mean - Treated mean /Control mean] x 100 % (control group GGI = 0%, representing 100% growth).
*Significantly different at the $p < 0.05$ level: Tukey Test.

Results from the analysis of chlorophyll content (mg of total extractable chlorophyll) showed that there was a statistically significant reduction in chlorophyll content (chlorophyll a, b, and a + b) in 500 ppm Cd treated plants (Table 2). Data from the chlorophyll concentration (µg chlorophyll/mg leaf dry wt) analysis showed that there was a significant reduction in chlorophyll b and total chlorophyll concentration in 500 Cd treated group (See Table 3). Overall, data indicated a dose related inhibition in chlorophyll synthesis in all metal treated plants although these differences were not statistically significant at lower Cd levels. Hagg-Kerwer (1999) reported that exposure to Cd caused a decline in transpiration rate and leaf expansion without affecting photosynthesis in *B. juncea*.

Table 2: Chlorophyll content (mg of total extractable chlorophyll) of *Brassica juncea* grown in soil containing various concentrations of Cadmium for 21 days.

| Treatment Groups | Chl.a (Mean ± SEM) | Chl.b (Mean ± SEM) | Total Chl.(a+b) (Mean ± SEM) |
|---|---|---|---|
| Control | 15.2 ± 0.6 | 16.8 ± 1.1 | 32.0 ± 2.5 |
| 100 ppm Cd | 14.8 ± 0.4 | 10.2 ± 1.4 | 25.2 ± 1.7 |
| 250 ppm Cd | 12.6 ± 1.9 | 7.3 ± 1.2 | 19.9 ± 3.1 |
| 500 ppm Cd | 3.4 ± 1.0* | 1.3 ± 0.4* | 4.7 ± 1.4* |

*Significantly different from the control at the 0.05 level: Tukey Test

Table 3: Chlorophyll concentration (µg chlorophyll/mg leaf dry wt) of *Brassica juncea* grown in soil containing various concentrations of Cadmium for 21 days.

| Treatment Groups | Chl.a (Mean ± SEM) | Chl.b (Mean ± SEM) | Total Chl.(a+b) (Mean ± SEM) |
|---|---|---|---|
| Control | 14.3 ± 1.4 | 14.8 ± 1.1 | 29.1 ± 0.8 |
| 100 ppm Cd | 16.0 ± 0.9 | 10.7 ± 0.9 | 26.7 ± 0.8 |
| 250 ppm Cd | 16.1 ± 1.5 | 9.1 ± 1.1 | 25.2 ± 2.6 |
| 500 ppm Cd | 13.3 ± 2.0 | 5.0 ± 0.8* | 18.3 ± 2.8* |

*Significantly different from the control at the 0.05 level: Tukey Test

Data from tissue Cd analysis revealed significant Cd accumulations in all Cd treated groups when compared to the control plants. Cadmium accumulations in the root and shoot were not significantly dif-

ferent among treated groups. Root Cd uptake was higher than the shoot uptake in the 100 ppm group, and shoot Cd uptake was found to be higher than the root uptake in the 250 ppm group (Table 4). Tissue Cd evaluation could not be performed in 500 ppm Cd treated groups due to lack of enough tissue samples to allow Cd analysis (as this group of plants had a very high GGI- see Table 1). Root and total plant Cd accumulations were higher in the 100 ppm Cd treated group as compared to 250 ppm Cd treated group (Table 4). This was probably contributed by cytotoxic effects of Cd that affected plant's metabolic process. Metal transport mechanisms may have been affected in this high Cd treated group.

Addae *et.al.* (2010) reported Cd and Pb uptake by cabbage plants. They observed that cabbage plants could tolerate high levels of Cd and Pb in soil and the metal uptakes were dose related. Such dose related accumulations of Cd in collard and Indian mustard plants were also recently reported by Zaman and Lockett (2010).

Table 4: Cadmium accumulations (Mean ± SEM) in the root, shoot, and total of the *Brassica juncea* after 30 days of growth in cadmium-contaminated soil.

| Treatment Groups | Chl.a (Mean ± SEM) | Chl.b (Mean ± SEM) | Total Chl.(a+b) (Mean ± SEM) |
|---|---|---|---|
| Control | 0.0 ± 0.00 | 0.0 ± 0.00 | 0.0 ± 0.00 |
| 50 ppm Cd | 210.0 ± 1.64* | 205.0 ± 3.14* | 415.0 ± 3.90* |
| 100 ppm Cd | 362.0 ± 3.30* | 223.0 ± 9.76* | 585.0 ± 12.00* |
| 250 ppm Cd | 232.4 ± 1.98* | 281.0 ± 18.40* | 514.0 ± 19.20* |

*Significantly different at the $p < 0.05$ level: Tukey Test

## 4.0 Discussions

Phytoremediation occurs through a series of complex interactions between plants and soil. The success of a plant species as a hyperaccumulator depends on its metal tolerance, and metal uptake and accumulation efficiency, bioavailability of the metal, and the physicochemical properties of the contaminant and the media (such as soil pH, soil texture, soil organic matter content, etc.; Adriano, 1992; Morel, 1997; Gérard *et.al.*, 2000; Saygideđer, 2000). Studies have been conducted using hydroponic systems where the plants were exposed to various contaminants without the use of soil, but with inert media, such as gravel, peat or sand (Resh, 1991; Dushenkov *et.al.*, 1995; Salt *et.al.*, 1995; Ghosh and Rhyne, 1998). Since hydroponic systems prevent the loss of metal bioavailability, such studies provide important preliminary data towards the interaction between the plant and the metal. But unlike hydroponic system, metals in a typical soil system form complexes that are made up of cations and exchange materials. Since natural soil is the ultimate medium of interest, studies of interactions between the soil metal pollutants and the plants will provide more meaningful data to fully understand and explore phytoremediation processes.

Phytoaccumulation is the plant's ability to take up and store significant amounts of heavy metals in the plant tissue. And hyperaccumulation is the plant's ability to accumulate metals at levels 100-fold greater in the shoot tissue as compared to the common nonaccumulator plants (Lasat, 2002).

A typical Cd hyperaccumulator should bioaccumulate at least 100 ppm Cd (Baker *et.al.* 2000) in its shoot part. *Brassica juncea* is a relatively small plant with a root system that can be extended 6 to 9 inches deep into the soil. Since data from this study indicate that *Brassica juncea* can easily tolerate over 100 ppm Cd toxicity in soil and can phytoaccumulate significant amounts of Cd from the soil (See Table 4), it may be a significant plant for further understanding of phytoremediation mechanisms. It may also be a suitable plant to phytoremediate soils with shallow Cd contamination.

**Acknowledgements:** The authors would like to acknowledge Dr Charles Rhyne, former professor at Jackson State University, Jackson, MS, USA, and Dr Alice Powell, former professor at Alcorn State University, Alcorn State, MS, USA, for their assistance in this project.

## References

Addae, C.L., M. Piva, A.J. Bednar, and M.S. Zaman, 2010, "Cadmium and Lead Bioaccumulation in Cabbage Plants Grown in Metal Contaminated Soils", Adv. Sci. Tech. **4**(1), 79-82.

Adriano, D.C. 1992, "Biogeochemistry of trace metals," Lewis Publication, Boca Raton, Florida, USA, 109-158.

Agency for Toxic Substances and Disease Registry (ATSDR), 2009, "Agency for Toxic Substances and Disease Registry: Toxicological Profile for Cad mium," Update, (Final Report) United States De partment of Health and Human Services, Atlanta, GA.

Baker, A.J.M., Reeves, R.D. and Smith, J.A.C. 2000, "Metal accumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils," In Phytoremediation of Contaminated Soil and Wa ter,. Lewis Publication, Boca Raton, FL., pp 85-107.

Chaney, R.L. 1983, Plant uptake of inorganic waste constituents. In F.J. Parr, P.B. Marsh and J.M. Kla. [eds], "Land Treatment of Hazardous Wastes," Noyes Data Corp., Park Ridge, New Jersey USA, pp 50-76.

Chaney, R.L., Malik, M., Li Y.M., Brown, S., Brewer, E.P., Angle, J.S. and Baker, A.L.M. 1997, Phytoremediation of soil metals, [online], Website http://www.soils.wisc.edu/~barak/temp/opin_fin. html [accessed 12 November, 2010].

Chaney R.L., Angle, J.S., Broadhurst, C.L., Peters, C.A., Tappero, R.V., and Sparks, D.L. 2007, "Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies," Journal of Environmental Quality. **36** (5), 1429-43.

De Vries, W., Römkens, P.F., and Schütze, G. 2007, "Critical soil concentrations of cadmium, lead, and mercury in view of health effects on humans and animals." Reviews of Environmental Contamination & Toxicology **191**, 91-130.

Dushenkov, V.N., Kumar, H.M. and Raskin, I. 1995, "Rhizofiltration: The use of plants to remove heavy metals from aqueous streams," Environmental Science and Technology, **29**, 1239 - 1245.

Einhellig, F.A. and Rasmussen, J.A. 1979, "Effects of three phenolic acids on chlorophyll content and growth of soybean and grain surghum seedlings," Journal of Chemical Ecology, **5**, 815 - 823.

Evanko, C.R. and Dzombak, D.A. 1997, "Remediation of metals-contaminated Soils and ground water," Ground-Water Remediation Technologies Analysis Center Series, Pittsburgh, PA. pp 1 - 24.

Gérard, E., Echevarria, G., Sterckeman, T. and Morel, J.L. 2000, "Cadmium availability to three plant species varying in cadmium accumulation pattern", Journal of Environmental Quality **29**, 1117 - -1123.

Ghosh, S. and Rhyne, C. 1998, "A search for hyper-accumulating plants in the laboratory," Journal of the Mississippi Academy of Sciences, pp 43, 11.

Glass, D.J. 2000, Economic potential of phytoremediation. In B. Ensley and I. Raskin [eds.], "Phytoremediation of Toxic Metals: Using Plants to Clean-Up the Environment," John Wiley & Sons, New York, USA, pp 15-31.

Hagg-Kerwer, A., Schafer, H.J., Heiss, S., Water, C. and Ransch, T. 1999, "Cadmium exposure in *Brassica juncea* causes a decline in transpiration rate and leaf expansion without effect on photosynthesis", Journal of Experimental Botany **50**, 1827-1835.

Hoagland, D.R. and Arnon, D.I. 1950, "The water culture method for growing plants without soil," California Agriculture Experiment Station Circular 347, Berkeley, California, USA.

Hooda, V. 2007, "Phytoremediation of toxic met als from soil and waste water," Journal of Environ mental Biology, **28**(2 Suppl), 367-76.

Lasat, M.M. 2002, "Phytoextraction of Toxic Metals: A Review of Biological Mechanisms," Journal of Environmental Quality, **31**, 109-120.

Leita L, Nobili, M.D., Mondini, C., and Garcia, M. T.B. 1993, "Response of Leguminosae to cadmium exposure," Journal of Plant Nutrition, **16**(10) 2001-2012.

López-Chuken, U.J., Young, S.D, and Sánchez-Gonzlez, M. 2010, "The Use of Chloro-Complexation to Enhance Cadmium Uptake by *Zea mays* and *Brassica juncea*: Testing a "Free Ion Activity Model and Implications For Phytoremediation," International Journal of Phytoremediation, **12**,pp7.

Morel, J.L. 1997, "Bioavailability of trace elements to terrestrial plants," In CRC Press Inc, Soil Ecotoxicology, Boca Raton, Florida USA, 141-175.

Panicker, G.K. 1992, "The effects of pine needles, gypsum and polymers on soil crusting, seedling emergence, and yield of snap beans," M.S. thesis, Alcorn State University, Alcorn State, Mississippi, USA.

Purakayastha, T.J. and Chhonkar, P.K. 2010, "Phytoremediation of Heavy Metal Contaminated Soils," Soil Biology **19**, 389-429.

Resh, H.M. 1991, "Hydroponic food production," Woodbridge Press Publishing Company California, USA.

Salt, D.E., Prince, R.C., Pickering, I.J., and Raskin I. 1995, "Mechanisms of cadmium mobility and accumulation in Indian mustard," Plant Physiology, **109** (4), 1427-1433.

Saygideđer, S., 2000, "Sorption of cadmium and their effects on growth, protein contents, and photosynthetic pigment composition of *Veronica anagallis-aquatica* L. and *Ranunculus aquatilis* L", Bulletin of Environmental Contamination and Toxicology ,pp 459 – 464.

United States Environmental Protection Agency (US EPA), 1986, "Test methods for evaluating solid waste, In Environmental Protection Agency, Office of Solid Waste and Emergency Response Laboratory Manual Physical and Chemical Methods".

Zaman, M.S., and Zereen F. 1998, "Growth responses of radish plants to soil cadmium and lead contamination." Bulletin of Environmental Contamination and Toxicology **61**, 44 – 50.

Zaman, M.S. and Lockett, C., 2010, "Cadmium Uptake by Collard and Indian Mustard Plants Grown in Cadmium Contaminated Soil", J. Miss. Acad. Sci. **55**(2), 149-153.