# Heavy Metal Uptake, Translocation, and Bioaccumulation Studies of *Triticum aestivum* Cultivated in Contaminated Dredged Materials

**Ketia L. Shumaker[1]* and Gregorio Begonia[2]**

[1]College of Natural Sciences and Mathematics, University of West Alabama, UWA Station 7, Livingston, AL 35470-2099, USA
[2]Department of Biology, Jackson State University, 1400 Lynch St., P.O. Box 18540, Jackson, Mississippi 39217, USA
*Correspondence to Dr. Ketia Shumaker. Email: kshumaker@uwa.edu

**Abstract:** Phytoremediation is a technology that uses vegetation to remediate contaminants from water, soil, and sediments. Unlike traditional remediation techniques such as soil washing or vitrification, phytoremediation offers a technology that is solar-driven, aesthetically pleasing, and cost effective. Recent studies indicate that winter wheat (*Triticum aestivum* L.) is a potential accumulator for heavy metals such as lead (Pb) and cadmium (Cd) in hydroponic systems. Based on these findings, a laboratory study was conducted with the primary objective of determining the phytoaccumulation capability of this plant species for heavy metals from contaminated dredged materials (DMs) originating from two confined disposal facilities (CDF). The United States Army Corps of Engineers (USACE) manages several hundred million cubic meters of DMs each year, and 5 to 10 % of these DMs require special handling because they are contaminated with hazardous substances that can move from the substrates into food webs causing unacceptable risk outside CDFs. Phytoremediation may offer an alternative to decrease this risk. Chemical analyses by USACE personnel identified 17 metals in various DMs, but in this present study, only zinc (Zn) and Cd were investigated. Pre-germinated seeds of the test plants were planted under laboratory conditions in pots containing the various DMs and reference soil. Four weeks after planting, plants were harvested and separated into roots and shoots for biomass production and tissue metal concentrations analyses. Results showed that *T. aestivum* plants have the capacity to tolerate and grow in multiple-metal contaminated DMs with the potential of accumulating various amounts of Zn and Cd. Root and shoot biomass of *T. aestivum* were not significantly affected by the DMs on which the plants were grown suggesting that this plant species can grow just as well on DMs contaminated by various metals as in the reference soil. No significant differences in the Zn tissue concentrations were observed, differences in Cd tissue concentrations were noted. A maximum concentration of 26 mg Cd kg$^{-1}$ DW was detected in *T. aestivum* shoots. Although Cd tissue concentrations of *T. aestivum* plants in this study were below the Cd plant hyperaccumulation criterion of >100 mg kg$^{-1}$ Cd found in other studies, this plant species however may still have beneficial uses for phytoremediation studies. *T. aestivum* plants may serve as an indicator plant for environmental assessment and management, in which the concentration of heavy metals (e.g. Cd) mirrors the concentration in the substrate without dying due to phytotoxicity at low metal concentrations.

**Key words:** Phytoremediation, *Triticum aestivum*, dredged material, cadmium, zinc

## Introduction

Sediments from waterways, i.e., dredged material (DM), are often contaminated with several pollutants that enter the waterway via point (e.g., spills and industrial discharges) and non-point (e.g., surfaces runoff) sources. Due to these past and present pollutions, an increasing amount of DM is not available for beneficial use such as beach nourishment, habitat creation and restoration, landfill cover, and land site remediation. The United States Army Corps of Engineers (USACE) manages several hundred million cubic meters of DM each year. Five to ten percent of these DMs require special handling because they are contaminated with hazardous substances that can move from the substrates into food webs causing unacceptable risk outside confined disposal facilities (CDFs) [1]. An emerging technology known as phytoremediation uses vegetation to remove pollutants from water, soil, and sediments. Current remediation techniques involve

decontaminating the DM by means of mechanical, chemical, thermal, or biological processes, or any combination of them. Phytoremediation holds a better promise than current remediation practices for effective cleanup of hazardous waste sites because it is more cost-efficient and aesthetically pleasing. Phytoremediation may offer an alternative to decrease the environmental risk of contaminated DM.

This laboratory study focused on the phytoextraction capability of a potential phytoaccumulator cultivated on DMs from two CDFs located in Bayport, Wisconsin and Monroe, Michigan (United States). Recent studies indicate that winter wheat (*Triticum aestivum* L.) [2], plants are potential accumulators for toxic heavy metals such as lead (Pb) and cadmium (Cd) in hydroponic systems. The current study was designed such that seed germination, fertilization, and watering required minimal care and maintenance. For phytoextraction to be a viable technology for industry, technology vendors and/or stakeholders, the contaminant must be available for uptake by the plant roots. Also, the root uptake and subsequent translocation of the heavy metal to the shoot is important, in that it eases harvesting and export of the aboveground plant material from the site [3]. Moreover, the success of phytoremediation of metals depends upon a plant's ability to tolerate and accumulate high concentrations of the metals at stake, while producing a large plant biomass [4].

The specific objectives of this study were to determine the following: 1) the total and bioavailable concentrations of the heavy metals of interest in the five DMs and in the reference soil, 2) the dry biomass yield of the test plant grown in the various substrates and, 3) the test plant's effectiveness for accumulating high levels of heavy metals in various parts of the plant's biomass (roots and shoots).

**Materials and Methods**

*Experimental Design*

This study was conducted using a randomized complete block design with six blocks (Bayport 1, Bayport 2, Bayport 3, Monroe 4, Monroe 5, and the reference soil) with four replicates per block of the test species. The study included a total of 24 experimental units.

*Substrates*

Substrates in this study are defined as the plant growth media. The test substrates were the DMs from two CDFs located in the Great Lakes Area. Baccto® Lite (obtained from Huttos Garden Center, Jackson, MS) was used as the reference soil to validate the performance of the test plant (Table 1). In this experiment, the reference soil was used as a plant control substrate to validate the performance of the test plant. The DMs were collected and shipped by USACE personnel. Best, Tatem, and Winfield [1] reported on the chemical and physical analyses of these DMs (Tables 2 and 3). Dredged materials were shipped to Jackson State University's Botanical Laboratory and stored in sealed

air-tight plastic containers. The DMs were dried at room temperature to reach a 50% moisture level. Moisture level was measured using a soil moisture meter (LI-COR Inc., Lincoln, NE). The DMs were mixed with vermiculite (obtained from Huttos Garden Center, Jackson, MS) to increase soil aeration [6]. The mixture was a 9:1 DM/vermiculite ratio.

**Table 1:** Reference soil profile [7].

| | Characteristics | Reference Soil |
|---|---|---|
| Total Metals (mg kg$^{-1}$ DW) | Cadmium | 1.24 |
| | Lead | 6.90 |
| | Nickel | 5.00 |
| | Vanadium | 5.70 |
| | Zinc | 18.20 |
| Other | Bulk density (g DWml$^{-1}$) | 1.27 |
| | Dry weight (% fresh weight) | 41.80 |
| | Organic matter (% dry weight) | 76.29 |
| | pH (water) | 5.79 |

**Table 2:** Bayport dredged material profile [1].

| | Characteristics | Bayport CDF-Wet Site |
|---|---|---|
| Organics (mg kg$^{-1}$ DW) | Pentacholorophenol | 0.003 |
| | Total PAH | 0.01 |
| | Total PCB | 1.29 |
| Total Metals (mg kg$^{-1}$ DW) | Aluminum | 17,658.00 |
| | Antimony | <3.24 |
| | Arsenic | 5.35 |
| | Beryllium | 1.07 |
| | Cadmium | 2.11 |
| | Chromium (IV) | <0.65 |
| | Copper | 86.00 |
| | Iron | 29,484.00 |
| | Lead | 87.80 |
| | Manganese | 771.00 |
| | Mercury (II) | 1.46 |
| | Nickel | 30.00 |
| | Selenium | <0.63 |
| | Silver | <0.41 |
| | Thallium | <1.62 |
| | Vanadium | 41.79 |
| | Zinc | 218.70 |
| Nutrients (mg kg$^{-1}$ DW) | Infinite-sink P* | 1.80 |
| | Nitrate-N | 0.01 |
| | Total-K | NA |
| Other | Bulk density (g DW ml$^{-1}$) | 0.88 |
| | Dry weight (% fresh weight) | 37.69 |
| | Organic matter (% dry weight) | 15.36 |
| | pH (water) | 7.29 |

Abbreviations: NA, not analyzed
*Plant available phosphorus-fraction

**Table 3:** Monroe dredged material profile [1].

| | *Characteristics* | *Monroe CDF-Wet Site* |
|---|---|---|
| Organics (mg kg⁻¹ DW) | Pentacholorophenol | <0.001 |
| | Total PAH | 0.02 |
| | Total PCB | 1.67 |
| Total Metals (mg kg⁻¹ DW) | Aluminum | 12,515.00 |
| | Antimony | <2.68 |
| | Arsenic | 8.57 |
| | Beryllium | 0.81 |
| | Cadmium | 1.21 |
| | Chromium (IV) | <0.54 |
| | Copper | 60.00 |
| | Iron | 24,522.00 |
| | Lead | 62.00 |
| | Manganese | 628.00 |
| | Mercury (II) | 0.31 |
| | Nickel | 36.18 |
| | Selenium | <0.54 |
| | Silver | <0.67 |
| | Thallium | <0.43 |
| | Vanadium | 28.81 |
| | Zinc | 201.00 |
| Nutrients (mg kg⁻¹ DW) | Infinite-sink P* | 2.80 |
| | Nitrate-N | 0.01 |
| | Total-K | NA |
| Other | Bulk density (g DWml⁻¹) | 1.26 |
| | Dry weight (% fresh weight) | 65.36 |
| | Organic matter (% dry weight) | 7.83 |
| | pH (water) | 7.58 |

Abbreviations: NA, not analyzed
* Plant available phosphorus-fraction

*Laboratory Growth Conditions and Procedure*

Seeds of *T. aestivum* were purchased from the Rainbow Whole Food Store in Jackson, MS. To obtain a sufficient amount of biomass from *T. aestivum*, ten plants were grown and harvested in the various substrates for four weeks in the laboratory. The laboratory conditions were monitored. The mean temperature was $24.7 \pm 0.56°C$ (mean $\pm$ SE), the mean light was level $111.96 \pm 2.46$ mol photons $m^{-2} s^{-1}$ (mean $\pm$ SE) for the entire study period, with a photoperiod of 16 hours. Light was measured from the top of the plants' canopy several times throughout the experiment using a light energy meter (Biospherical Instruments Inc. Model QSL-100). The light source was composed of four Ott-Lite® F96 80 watt fluorescent bulbs.

The plants were grown in 400 ml porous bottom pots (7.5 mm x 8 mm) each with its own 10.16 cm planting reservoir trays containing the needed solutions. The prepared DM/vermiculite mixtures were placed in the pots (350 g per pot). *T. aestivum* seeds were soaked in deionized water for 24 hours at room temperature before planting. After soaking, twenty seeds were initially planted in each pot and covered by a thin soil layer. After germination, *T. aestivum* was thinned to ten plants per pot on day 17. Thinning was done to ensure that a sufficient amount of dried biomass would be attainable for the acid digestion procedure and to avoid crowding.

The plants were watered every other day or as needed with distilled water and once a week with 100 ml of Hoagland's solution [8]. The plants were harvested after four weeks of cultivation. They were cleaned, washed successively with deionized water and a weak aqueous solution of sulfuric acid (1 ml of concentrated sulfuric acid per liter of deionized water) to remove any external heavy metal residue. The plants were then separated into roots and shoots, and placed in marked brown bags. The bags and contents were dried in a Blue M Electric Company (Blue Island, Illinois) convection laboratory oven at 100ºC for 24 hours. Plant parts were removed from bags and weighed using a balance (XE-100, Arvada, CO) to obtain root and shoot dry weights.

*Substrate and Tissue Analysis*

*Diethyltriamine-pentaacetic acid (DTPA) Extraction*

This method was used to quantify the biologically available fractions of heavy metals in the DMs and reference soil using a mild extractant, DTPA [9]. The DTPA mixture was composed of 0.005 M of DTPA, 0.01 M $CaCl_2$, and 0.1 M triethanolamine (TEA) all buffered at a pH of 7.3 using 0.1 N and 1.0 N HCl. The moist weights of the DM and reference soil were determined before oven drying. Twenty-grams of each oven-dried substrate were placed in 500 ml centrifuge bottles. Then, 50 ml of DTPA mixture was added to the centrifuge bottle and amended with deionized water to bring the volume up to 100 ml. Reagent blanks were prepared to determine if any contamination was detected from the glassware, reagents, or other sources. The mixture was shaken for 24 hours at room temperature and then centrifuged at 9,000 rpm (13,702 g) for 30 minutes, with 5-minute acceleration and 30-minute deceleration. The supernatant was filtered through a Whatman No. 42 filter paper in a Buchner funnel, and then analyzed for heavy metals lead (Pb), zinc (Zn), and cadmium (Cd) using the Atomic Absorption Flame Emission Spectrophotometer (AA Model 6701F, Shimadzu, Japan).

*Acid digestion*

After determining the shoot and root dry biomass, shoot and root tissues were pooled for each experimental group and used for tissue metal extraction and analysis. The United States Environmental Protection Agency (USEPA) Method 3050A [10] was used to extract the metals in the plant materials and the substrates. Substrate concentrations obtained from the acid digestion procedure were used for the total substrate metal concentrations. Acid digestion procedures for the plant materials were carried out separately from the substrate digestion procedure. Reagent blanks were used to determine the contamination, if any, from glassware, reagents, or other sources. To perform the plant metal extraction, 0.1 - 0.5 g of each plant subsample was transferred to a 125 ml Erlenmeyer flask. For the substrate metal extraction, 1.0 – 2.0 g of the substrate was transferred to a 125 ml Erlenmeyer flask. The flasks were then amended with 15 ml of 100 % nitric acid ($HNO_3$) and 10 ml of deionized water. 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 100% HNO₃, the sample was refluxed again for 30 minutes. The last step was repeated to ensure complete oxidation. The sample was then heated, without boiling and evaporated to 5 ml. After this, the samples were allowed to cool again, and 2 ml of deionized water were 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. The 30% $H_2O_2$ were continually added in 1ml aliquots until the effervescence became minimal. The acid-peroxide digestate was heated for a final time to reduce the volume to 5 ml. After cooling, the samples were diluted to 100 ml with deionized water. The digestate was filtered using a Whatman No. 1 filter to remove any particulates that may have been present in the sample. A different filter and sulfuric acid-cleansed funnel was used for each sample to avoid cross-contamination of the samples. The filtrate was then ready for metal analysis.

*Metal Analysis*

Metal concentrations were determined using an atomic absorption flame emission spectrophotometer (AA Model 6701F, Shimadzu, Japan). Fresh standards of 1, 5, and 10 parts per million (ppm) Pb; 0.1, 0.2, and 0.4 ppm Zn and 0.2, 0.4, and 0.6 ppm Cd concentrations were prepared for the atomic absorption analysis. The solutions were aspirated and their concentrations were calculated and recorded. The Zn and Cd concentrations in the DM, reference soil, and plant materials were measured.

*Statistical Analysis*

The data were analyzed using analysis of variance [11]. The metal concentrations of the five DMs were compared with the metal concentrations of the reference substrate using Dunnet's multiple comparison tests. The Duncan's Multiple Range Test was used as a mean separating procedure for the total and bioavailable metal concentrations. All total and bioavailable metal concentrations were based on the measurements derived from the substrate analysis protocol in this study. The block effects were examined to determine if there were significant variations in responses due to situation of the planted pots within the blocks. The criteria for statistical differences were determined at both the 5% (*p-value* $\leq$ 0.05) and the 10 % significance level (*p-value* $\leq$ 0.1).

The relationship between plant responses (tissue metal concentration and biomass production) and substrate metal concentrations (total and bioavailable) were derived by linear regression. The p-value in the regression model was set at a 5 % significance level (p-value $\leq$ 0.05). The $R^2$ value of the regression model was used to indicate the explained variance in the model, whereby $R^2$ values of $\geq$ 0.50 (explaining at least 50 percent of the variance in the data set) was considered meaningful. Linear regression equations were used to predict the tissue metal concentrations resulting from the substrate metal concentrations. The biota to soil accumulation factor (BAF), which is the ratio between the shoot metal concentration in the various plant species and the substrate metal concentration, was also calculated as an estimate for the potential trophic transfer of the metal of interest from the DM into the plants [7].

## Results

No symptoms of phytotoxicity, such as chlorosis or necrosis, were noticeable from the plant species under investigation. The level of Pb in the plant biomass was below detection. This may be due to the binding of Pb by the substrate in the aged DM, since in the preliminary experiment the test plant species accumulated detectable levels of Pb in both root and shoot biomass (data not shown). However, the levels of Zn and Cd were measurable.

Results of *T. aestivum* biomass are shown in Figure 1. Root biomass ranged from 0.21 ± 0.02 to 0.27 ± 0.01 g DW plant⁻¹. No significant differences were observed in the root biomass of *T. aestivum* plants grown in the various DMs when compared to the plants grown in the reference soil. The shoot biomass of plants grown on Bayport 1 (0.62 ± 0.03 g DW plant⁻¹) and Bayport 3 (0.50 ± 0.02 g DW plant⁻¹) DMs were significantly lower than those plants grown on the reference soil (0.97 ± 0.06 g DW plant⁻¹) (p $\leq$ 0.05). The shoot biomass of plants cultivated on Monroe 4 DM (0.67 ± 0.10 g DW plant⁻¹) was significantly lower than that of the reference plants (p $\leq$ 0.1). Statistically, the total plant mass behaved identical to shoot mass at the 5 % significance level.
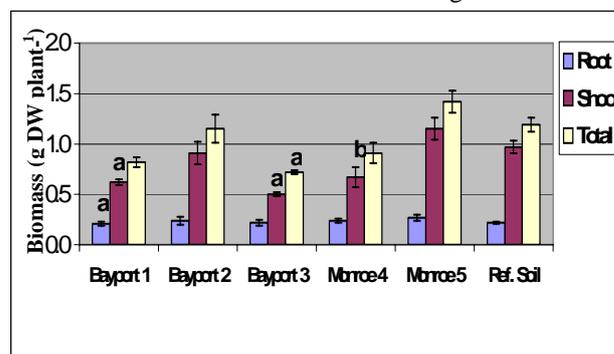


**Figure 1:** Biomass of *Triticum aestivum* (mean and standard error; N=4) grown in substrates for 4 weeks. ᵃStatistically significant from the reference soil at the 5 percent significance level; ᵇat the 10 percent significance level according to Dunnet test.
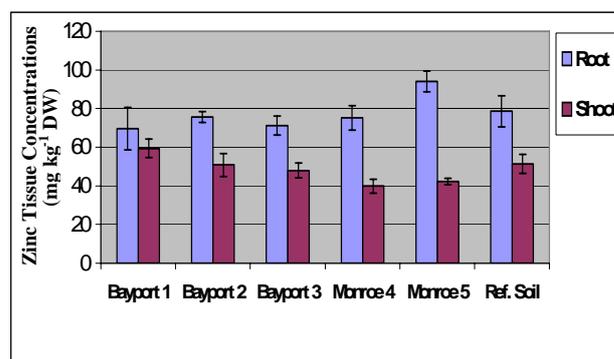


**Figure 2:** Mean concentrations of zinc in *Triticum aestivum* (mean and standard error; N=4). No significant differences observed when compared to plants grown in the reference soil according to Dunnet test.

Zinc accumulation in the root ranged from 69.56 ± 11.01mg kg⁻¹ DW in *T. aestivum* plants grown on Bayport 1 DM to 94.01 ± 5.34 mg kg⁻¹ DW in plants grown on Monroe 5 DM (Figure 2). Zn accumulation in

the shoot ranged from 39.86 ± 3.59 mg kg$^{-1}$ DW in plants grown on Monroe 4 DM to 59.39 ± 4.87 mg kg$^{-1}$ DW in plants grown on Bayport 1 DM. However, no significant differences were observed in the root or shoot Zn accumulation by *T. aestivum* plants grown in the various DMs compared to reference plants. Cadmium accumulation in *T. aestivum* plants is summarized in Figure 3. Cadmium accumulation in the roots was significantly higher in plants grown in Bayports 1 and 2 DMs than in reference plants. Shoot Cd accumulations were significantly higher in plants grown on Bayport 1 DM, but significantly lower in plants grown on Bayport 2, Bayport 3, and Monroe 4 DMs than in reference plants. Shoot Cd concentrations ranged from 2.46 ± 0.30 mg kg$^{-1}$ DW in plants grown on Bayport 2 DM to 26.34 ± 0.45mg kg$^{-1}$ DW in plants grown in Bayport 1 DM. At a lower significance level, (i.e. 10%), significant differences in tissue Cd concentrations were found (Figure 3). No significant relationship between the shoot metal concentrations (Zn and Cd) and substrate metal concentrations were found using linear regression techniques (data not shown). Consequently, BAFs were not calculated. Statistical analysis of the relationship between the total plant biomass production and substrate metal concentrations showed no significant correlation.
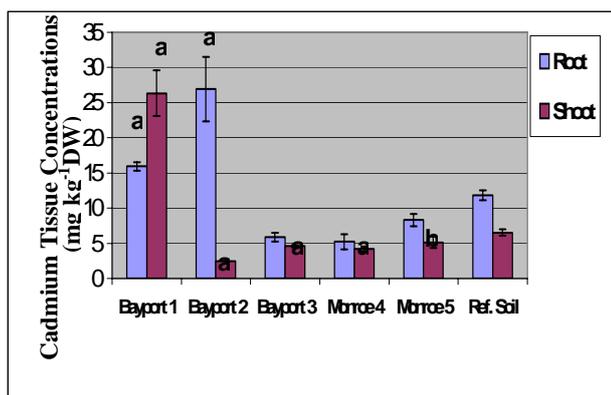


**Figure 3:** Mean concentrations of cadmium in *Triticum aestivum* (mean and standard error; N=4). [a]Statistically significant from the reference soil at the 5 percent significance level; [b]at the 10 percent significance level according to Dunnet test.

*Substrate Metal Concentrations*

*Zinc*

The total Zn concentrations were significantly higher in Bayport 1, Bayport 2, and Monroe 4 DMs than in the reference soil ($p \leq 0.05$) (Table 4). At the 10 % significance level, the total substrate Zn concentration was also significantly higher for the Bayport 3 DM (Table 4). Concentrations of the bioavailable Zn in the substrates in this study ranged from 2.53 ± 0.00 mg kg$^{-1}$ DW in the reference soil to 6.60 ± 0.05 mg kg$^{-1}$ DW in Monroe 4 DM. The bioavailable Zn concentrations in all DMs were significantly higher than in the reference soil.

**Table 4:** Total and bioavailable zinc concentrations (mean values and standard error, N=3).

| Substrates | Total Zn (mg kg$^{-1}$ DW) | Bioavailable Zn (mg kg$^{-1}$ DW) |
|---|---|---|
| Reference Soil | 39.67 ± 1.29 | 2.53 ± 0.00 |
| Bayport 1 | 115.62 ± 3.93[a] | 6.17 ± 0.39[a] |
| Bayport 2 | 126.78 ± 1.12[a] | 6.31 ± 0.08[a] |
| Bayport 3 | 105.61 ± 34.68[b] | 5.44 ± 0.05[a] |
| Monroe 4 | 127.37 ± 22.41[a] | 6.60 ± 0.05[a] |
| Monroe 5 | 92.47 ± 6.23 | 6.24 ± 0.01[a] |

[a]Significantly different from the reference soil at the 5 percent significance level; [b]significantly different from the reference soil at the 10 percent significance level, according to Dunnet test.

*Cadmium.*

Total Cd concentrations in the various substrates ranged from 0.48 ± 0.09 mg kg$^{-1}$ DW in the reference soil to 1.34 ± 0.04 mg kg$^{-1}$ DW in Bayport 1 DM (Table 5). No significant differences were found in the total Cd concentrations in the DMs, except for Bayport 1 DM, compared to the reference soil ($p \leq 0.05$). Bioavailable Cd concentrations in all the DMs were significantly higher than in the reference soil.

**Table 5:** Total and bioavailable cadmium concentrations (mean values and standard error, N=3).

| Substrates | Total Cd (mg kg$^{-1}$ DW) | Bioavailable Cd (mg kg$^{-1}$ DW) |
|---|---|---|
| Reference Soil | 0.48 ± 0.09 | 0.09 ± 0.01 |
| Bayport 1 | 1.34 ± 0.04[a] | 0.45 ± 0.01[a] |
| Bayport 2 | 1.09 ± 0.01[b] | 0.30 ± 0.02[a] |
| Bayport 3 | 1.08 ± 0.25[b] | 0.35 ± 0.02[a] |
| Monroe 4 | 0.93 ± 0.12 | 0.36 ± 0.03[a] |
| Monroe 5 | 0.59 ± 0.12 | 0.23 ± 0.01[a] |

[a]Significantly different from the reference soil at the 5 percent significance level; [b]significantly different from the reference soil at the 10 percent significance level, according to Dunnet test.

**Discussion**

All plants can take up metals from the substrate in which they are cultivated at varying degrees. Baker [12] stated that there are two ways in which higher plants can tolerate the presence of metals in their environment:
(1) Exclusion, which occurs when the transportation of metals is restricted and low, relatively constant, metal concentrations are maintained in the shoot over a wide range of soil concentrations.
(2) Accumulation, which occurs when metals are taken up in a nontoxic form in the shoot at both high and low soil concentrations. He suggested that accumulators can be characterized by a shoot:root metal concentration ratio of  >1 due to the tendency to

298

*Int. J. Environ. Res. Public Health* **2005**, *2(2)*

translocate metals from the root to the shoot, whereas excluders are characterized by a ratio of $< 1$. Baker [12] further stated that an intermediate response of an indicator plant is also likely, whereby the shoot metal concentrations reflect those in the substrate.

No significant differences in the Zn tissue concentrations were observed, differences in Cd tissue concentrations were noted in *T. aestivum* plants. A maximum concentration of 26 mg Cd kg$^{-1}$ DW was detected in *T. aestivum* shoots. Although Cd tissue concentrations of *T. aestivum* plants in this study were below the Cd plant hyperaccumulation criterion of $>100$ mg kg$^{-1}$ Cd found in other studies [13], this plant species however may still have beneficial uses for phytoremediation studies. Zaman and Zereen [14] and McGrath et al. [15] concluded that although not capable of hyperaccumulation, *T. aestivum* plants may pose as an indicator plant, in which the concentration of heavy metals (e.g. Cd) mirrors the concentration in the substrate without dying due to phytotoxicity at low metal concentrations.

Substrate-metal binding has been documented in numerous soil chemistry studies [16]. This may explain why the plant tissue Pb accumulation was below detection in the laboratory experiment, although it was demonstrated in the preliminary hydroponic experiment. Studies have shown that Pb can be strongly retained in many soils, thereby hindering Pb mobility into plant tissue [17, 18, 19]. McBride [20] stated that the preeminent way to evaluate soil-metal mobility was to understand the soil properties and conditions that affect the long- and short-term fate of the metals in soils. Ross [21] stated that the exclusion of the large number of influential factors (i.e. bulk density, temperature, aeration, redox potential, pH, and organic matter quantity and quality) and soil interaction was a main problem in McBride's [20] approach to understanding metal processes in soil. Results from the current study concur with those reported by Best et al. [7] who also failed to detect Pb, but did recover Zn and Ni in plants grown in Monroe CDF-DM.

**References**

1. Best, E. P. H., H. E. Tatem, and L. Winfield. Toxicological and bioaccumulation testing of dredged material placed into upland confined disposal facilities using plants and worms. *Dredging Operations & Environmental research Tech Note. DOER-C22. September* **2001**. http://www.wes.army.mil/el/dots/doer/pdf/doere22.pdf.

2. Chaudri, A. M.; McGrath, S. P.; Zhao, F. J.; Crosland, A. R.: Cadmium in British wheat grain. Extended Abstracts 1$^{st}$ International Conference on Contaminants and the Soil Environment. *Adelaide, UK,,* **1996**, p. 77.

3. Best, E. P. H.; Geter, K. N.; Larson, S. L.: Exploration of the potential for phytoremediation of lead based paint contaminated soil at Fort Lewis, WA. *ERDC/EL TR-02, U. S. Army Engineer Research and Development Center, Vicksburg, MS,* **2002**.

4. Blaylock, M. J.; Salt, D. E.; Dushenkov, S.; Zakharova, O.; Gussman, C.; Kapulnik, Y.; Ensley, B. D.; Raskin, I.: Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Tech.,* **1997**, *31,* 860-865.

5. Ebbs, S. D.; Kochian, L. V.: Toxicity of zinc and copper to *Brassica* species: implications for phytoremediation. *J. Environ. Qual.,* **1997**, *26,* 776-781.

6. Best, E. P. H.: Personal communication, **2000**.

7. Best, E. P. H.; Geter, K. N.; Tatem, H. E.: Validation of pathway analysis of metals from aged dredged material using plants and worms. DOER Technical Notes Collection (ERDC TN-DOER-C32), U.S. *Army Engineer Research and Development Center, Vicksburg, MS,* **2003**.

8. Hoagland, D. R.; Arnon, D. I.: The water culture method of growing plants without soil. *California Agriculture Experiment Station.* **1938**, p. 347.

9. Lindsay, W. L.: Chemical equilibria in soils. USA, *John Wiley and Sons,* **1979**.

10. United States Environmental Protection Agency. Test methods for evaluating solid waste. Environmental Protection Agency, Office of Solid Waste and Emergency Response. Vol. IC: *Laboratory Manual Physical and Chemical Methods,* **1986**.

11. SAS Institute. Statistical analysis system. *SAS Institute. Cary, North Carolina,* **1999**.

12. Baker, A. J. M.: Accumulators and excluders – strategies in the response of plants to heavy metals. *J. Plant Nutr.,* **1981**, *3,* 643-654.

13. Erikkson, J.; Ledin, S.: Changes in phytoavailability and concentration of cadmium on soil following long term Salix cropping. *Water, Air, and Soil Pollution,* **1999**, *114,* 171-184.

14. Zaman, M. S.; Zereen, F.: Growth responses of radish plants to soil cadmium and lead contamination and lead contamination. *Bull. Environ. Contam. Toxicol,* **1998**, *61,* 44-50.

15. McGrath, S. P.; Dunham, S. J.; Correll, R. L.: Potential for phytoextraction of zinc and cadmium from soils using hyperaccumulator plants. In Phytoremediation of contaminated soil and water. *Florida: Lewis Publishers,* **2000**.

16. Sposito, G. S.; The chemistry of soils. New York: *Oxford University Press,* **1989**.

17. Nolan, A. L.; Lombi, E.; McLaughlin, M. J.: Metal bioaccumulation and toxicity in soils - why bother with speciation? *Australia J. Chem.,* **2003**, *56,* 77-91.

18. Shen, Z.; Li, X.; Wang, C.; Chen, H.; Chua. H.: Lead phytoextraction from contaminated soil with high-biomass plant species. *J. Environ. Qual.,* **2002**, *31,* 1893-1900.

19. Cooper, E. M.; Sims, J. T.; Cunningham, S. D.; Huang, J. W.; Berti, W. R.: Chelate-assisted phytoextraction of lead from contaminated soils. *J. Environ. Qual.,* **1999**, *28,* 1709-1719.

20. McBride, M. B.: Reactions controlling heavy metal solubility in soils. *Adv. Soil Sci.,* **1989**, *10,* 31-56.

21. Ross, S. M.: Toxic metals in soil-plant systems. *John Wiley and Sons, USA,* **1994**.