

# A Sediment Phytoattenuation Evaluation by Four Sessions of Vetiver Planting and Harvesting

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## Abstract

Phytoattenuation is a novel green remediation approach which can be employed in both a sediment and a soil decontamination. Pot experiments have been conducted to evaluate two pollution levels of a sediment consisting of high pollution from swine industries and low contamination from campus wetland. EDTA demonstrated satisfactory metal uptake and mobility enhancement has been achieved. Pb is less mobile which induced low vetiver translocation while Zn is the most mobile which possesses high bioavailability. Four sessions of planting and harvesting gradually decreasing Cu and Zn levels the constrictions decreasing to achieve local sediment criteria which can be used for agricultural soil conditioning. The results of this study are prominent with gradually mitigating sediment contamination is less detriment to sediment properties relative to commonly used soil washing.

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**Index terms**— phytoattenuation, Cu, Zn, vetiver, heavy metal.

## 1 Introduction

In Taiwan the brand new sediment reuse and management Act has been in progress. The lingering sediment illegal dumping expected to be solved after the enactment of a sediment management regulatory standard. The main contaminant management was risk based control while in situ capping phytoremediation and ex situ sediment pressured chelator pretreated followed by phytoextraction has been proposed. Phytoattenuation is a novel concept to denominated sediment metal contents without abrasively destroy sediment properties rendering for agricultural soil fertilizing.

Vetiver is known for its effectiveness in sediment erosion control due to its unique morphological and physiological characteristics. Vetiver is also a high biomass plant with remarkable photosynthetic efficiency which renders it tolerant against various harsh environmental conditions. Vetiver with deep-rooted and higher water-use can effectively stabilize soluble metals in sediments (Chen et al., 2004). These properties enable vetiver to be an ideal candidate for phytoextraction.

EDTA, a synthetic chelator, is poorly biodegraded in soils though its effectiveness at completing metals. Excess amounts of EDTA may leach to groundwater and cause subsurface water contamination. However, due to its high chelation ability, potential leaching to groundwater should be into serious concern.

A novel green remediation approach intends to convey in this paper by employing plant to gradually reduce soil metal contamination through several rounds of planting and harvesting. Unlike phytoextraction, phytoattenuation aims to reduce soil metal pollution in a gradually and less aggressive approach such as chelator assisted remediation. The initial pollution level generally is lower than most soil contamination sites. Therefore, plant is easier to propagate to increase biomass inducing reliable metal uptake. The conceptual model is shown in Fig. 1.

Attenuation is borrowing from the concept "natural attenuation" which has been commonly proposed as a remediation approach for organic pollutants such as DNAPL (dense non-aqueous liquid) solvent TCE (tri-chloro ethylene) and PCE (tetra-chloro ethylene) or LNAPL (light non-aqueous liquid) petroleum product BTEX

(benzene, toluene, ethyl benzene, and xylene. Natural attenuation mainly used natural pollution mitigation mechanism including microbial degradation, adsorption, volatilization, etc. This approach is targeted to pollutant which is not degraded in a reasonable time using conventional remediation techniques, technical imperfectability, or the cost beyond the affordable monetary amounts, economical imperfectability.

Cu and Zn are used as the fodder additives for preventing swine diarrhea and skin abrasion (Yeh and Wu, 2009). Cu has been reported the toxicity to phytoplankton and been employed as algicide for serious eutrophication mitigation. The careless management of Cu and Zn wastewater from swine industries could damage the water and a soil environment. Previous studies regarding (Bioconcentration factor) BCF and (Translocation factor) TF are summarized in Table ?? EDTA, DTPA, EDDS, citric acid, and

The objectives of this study were to research the phytoattenuation to gradually mitigate the a sediment Cu and Zn pollution via employing EDTA chelator enhancement. Possible a sediment metal fraction and vetiver uptake evaluation also be conducted. The recent reference is listed in Table ?. b) Total metal content, a sediment retained fractionation and plant metal uptake analysis Plant after last session of operation was harvested, careful washed, and air dried for metal analysis. Plant samples were dried at 103° in an oven until completely dried. Dried plant samples were divided into root and shoot for metal accumulation assessment. These pretreated plants were digested in a solution containing 11:1 HNO<sub>3</sub> : HCl solution via a microwave digestion apparatus (Mars 230/60, CEM Corporation) and diluted to 100 mL with deionized water. 0.2 g of dried a sediment adding aqua regia rendering for microwave digestion and 2.5 g of dried for sequential extraction experiments. Metals analyses were conducted via an atomic absorption spectrophotometry (AAS, Perkin Elmer). c) Harvested Plant tissue and final a sediment metal content analysis

Plant was harvested, careful washed, and air dried for metal analysis. Plant samples were dried at 103° in an oven until completely dried. Dried plant samples were divided into root and shoot for metal accumulation assessment. These pretreated plants were digested in a solution containing 11:1 HNO<sub>3</sub> : HCl solution via a microwave digestion apparatus and diluted to 100 mL with deionized water. 0.2 g of dried a sediment was added aqua regia rendering for microwave digestion. Metals analyses were conducted via an atomic absorption spectrophotometry (AAS, Perkin Elmer).

## 2 d) Data and Statistical Analysis

Data were evaluated relative to the control to understand their statistical variation. Metal concentration of plants was recorded as mg of metal per kilogram of dry biomass. A triplicate of water and a sediment samples from each treatment were recorded and used for statistical analyses. Plant metal concentration was recorded as mg of metal per kilogram of dry biomass. Bioaccumulation coefficient (BCF; Croots/Ca soil or water) calculated as the metal concentration in plant divided by the heavy metal concentration in the solution or a soil for hydroponic and pot experiments, respectively. TF (shoots/Croots) was depicted as the ratio of concentration of metal in shoot to its concentration in root. It was calculated by dividing the metal concentration in shoot by the metal concentration in root. Schematic diagram of pot experiment and BCF and TF are shown in Fig. 1. Statistical significance was assessed using mean comparison test. Differences between treatment concentration means of parameters were determined by Student's t test. A level of  $p < 0.05$  considered statistically significant was used in all comparisons. Means are reported  $\pm$  standard deviation. One-way ANOVA wee employed to inference the difference among treatments. All statistical analyses were performed with Microsoft Office EXCEL 2007.

## 3 III.

## 4 Results and Discussion

### 5 a) Pot Experiment Results

The conceptual setup of the pot experiment is shown in Fig. 1 i. The background and metal fraction results

The background properties of a sediment was pH, organic matter were  $6.58 \pm 0.44$ ,  $3.43 \pm 0.13$  %, respectively. The background Cu, Zn, and Pb a sediment concentrations were  $3.26 \pm 4.72$ ,  $121.55 \pm 6.34$ , and  $76.55 \pm 12.68$  mg/kg, respectively. Total metal leaves were  $898.35 \pm 15.70$ ,  $5933.96 \pm 51.09$ , and  $3109.26 \pm 60.37$  mg/kg?respectively which was around 1.5 to 3 folds (Cu ? 400 mg/kg, Zn ? 2000 mg/kg, Pb?2000 mg/kg). The a sediment particle size of sand, silt, and clay were 5.7%, 82.2%, and 12.1% which is common for most farmland a soil properties.

## 6 ii. Sequential Fraction Results

Sequential extraction was performed to illumine the adsorption fraction; namely exchangeable, ionic adsorp organic bound, Fe-Mn bound, and sulfide bound portions were in the descending order. Zn demonstrated the worst growth which is rapid to wilt. Cu+EDTA was  $1.26 + 0.80$  which was 21 folds relative to Zn only. For BCF Zn performed the best for with or without EDTA were 2.84 and 1.41 folds, respectively. The vetiver root: stem: leaf weight was 2:1.5:1 Cu and Pb with addition were 3.62 and 3.55 folds relative to control. EDTA demonstrated prominent Cu and Zn vetiver uptake and translocation. No EDTA toxic effects had been observed.

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## 7 ? 2nd stage a sediment and a soil analysis c) The growth and toxicity symptoms of vetiver in pot experiments

The increased heights of vetiver for three chelators of pot experiment are shown in Table ???. The growth of vetiver was observed for 24 days to study the toxic effect of chelators. The initial average length and weight of vetiver was approximately 30cm and 18 g, respectively. For Cu, the increased height of vetiver for control, EDDS, citric acid, and EDTA were 14.4, 1.2, 9.0 and 0.7 cm, respectively. For Zn, the increased height of vetiver for control, EDDS, citric acid, and EDTA were 0.3, 0.2, 0.5 and 0.2 cm, respectively. For Pb, the increased height of vetiver for control, EDDS, citric acid, and EDTA were 1.3, 2.0, 2.3 and 0.8 cm, respectively. The results of Zn did not present significant growth in chelator amended a sediments.

Cu+EDDS and Cu+EDTA both presented yellowing and chlorosis of leaves at the 12th day. The control and citric acid presented less toxic symptom. For Zn, the control, Zn+EDDS, Zn+citric acid, Zn+EDTA all showed the yellowing at the 8th day of treatment. All Zn treated vetiver were presented serious chlorosis and wilt symptom at the 14th day. For Pb, all treatments presented yellowing at 10th day and the toxic effect was the in the order of EDTA> citric acid> EDDS.

According to the aforementioned results, the toxic effect of EDTA was more significant than which of other two chelators.

## 8 d) The impact of chelator on the uptake and translocation of metals in pot tests

The results of the uptake and translocation of metals are shown in Table ??? For Cu, total metal accumulation concentrations of EDDS, citric acid, and EDTA were 14, 4, and 12 folds ( $p = 0.002, 0.02$ , and  $3.5 \times 10^{-6}$ ) increase compared to the control, respectively. The translocation to aerial parts were significant for EDDS, citric acid, and EDTA showing in shoot Cu concentrations raised 151, 6 and 84 folds ( $p = 0.004, 4.76 \times 10^{-5}$ , and 0.002) compared to control, respectively. The results demonstrated which EDDS and EDTA statistically significant increased total metal concentration and metal in aerial parts of vetiver. In particular, the shoot concentration of Cu+EDDS was  $936 \pm 274$  mg/kg which was around the hyperaccumulator level (1,000 mg/kg).

For Zn, the whole plant accumulation concentrations of EDDS, citric acid, and EDTA were 1.2, 1.1, and 1.1 folds compared to control, respectively. The statistical analysis compared with the control did not present significant difference ( $p = 0.52, 0.88$ , and 0.77) for three chelators. However, the aerial parts Zn concentration all achieved hyperaccumulator levels for three chelator treatment (10,000 mg/kg). For Pb, the whole plant accumulation concentrations of EDDS, citric acid, and EDTA were 1.1, 1.3, and 1.6 folds ( $p = 0.55, 0.128$ , and 0.045) increase relative to control plants, respectively. EDTA presented significant difference ( $p < 0.05$ ) with respect to the control. The other two chelators did not show clear uptake enhancement. EDTA also improved Pb uptake in aerial parts to reach the hyperaccumulator levels (1,000 mg/kg). The prominent uptake of Pb by EDTA can be explained by the stability constant ( $\log K_s = 17.88$ ) with Pb while the constants for biodegradable chelators EDDS and citric acid with were  $\log K_s = 18.4$  and  $\log K_s = 6.5$ , respectively. The critical results of our current research were the achievement of vetiver as a hyperaccumulator.

Another similar research showed which prominent metal uptake and translocation of Pb with EDTA. They explained by its effect on enhancing the solubility of Pb and absorption of the Pb-EDTA complex by the plant *Brassica napus* ???Zaier et al., 2010). In Lin's study, a sediment was applied with EDTA by using sunflower. Pb concentration in the shoot of plants was found directly proportional to the amount of EDTA added to a sediment. The a sediment concentration of soluble Pb was correlated with the Pb concentration in plants grown on the a sediment (Lin et al., 2009). Another investigation also demonstrated which EDTA bound Pb was less toxic to free Pb ions and might induce less stress on plants. Pb complexes with phytochelatin were the possible Pb tolerance mechanisms.

The results showed which vetiver accumulated 19,800 and 3350 mg/kg in root and shoot tissues, respectively (Andra et al., 2009). A discrepancy study demonstrated which EDDS caused in 2.54, 2.74, and 4.3 fold increase in Cd, Zn, and Pb shoot metal concentration, respectively as compared to control plants. In their study also reported which EDTA induced 1.77, 1.11, and 1.87 fold increase in Cd, Zn, and Pb shoot metal concentration, respectively, as compared to control plants. Their results demonstrated which EDDS was more effective than EDTA in stimulation the translocation of metals from roots to shoots (Santos et al., 2006).

Research has reported which the treatment with 5 mmole/kg EDDS, a sediment resulted in accumulation of 157, 129, and 122 mg/kg of Cu, Zn, and Pb in whole plant, respectively.

The concentration in *Brassica carinata* shoots with 2 to 4 fold increase compared to control. Comparing to NTA, the results showed which EDDS in a sediment degraded rapidly, reducing the risks associated with the leaching of metals to the groundwater (Quartacci et al., 2007). Other research studied the EDDS enhancement phytoextraction of Cu, Zn, and Pb by maize. The results indicated which a sediment treated with EDDS significantly increased the concentration of metal in maize shoots (increments of 66%, 169%, and 23% for Cu, Zn, and Pb with respect to the control (Salati et al., 2010). Wang et al. (2009) suggested which phytoremediation of high Pb a sediment, EDDS would be better at concentration of 5 mmole in a single dosage. Citric acid showed less obvious effect might be related to its easy biodegraded in the a sediment in their study.

Rescarach demonstrated which the accumulation of metals in the plant fractions was in descending sequence  $\text{Cr} > \text{Zn} > \text{Cu} > \text{Pb}$ . The presence of either compost or *B. licheniformis* BLMB1 strain enhanced metal by *B. napus* accumulation, Cr in particular, in the experimental conditions used (Brunetti, et al, 2011).

Our results for EDTA addition revealed the concentration of Cu, Zn, and Pb of 521, 11233, and 1125 mg/kg in shoot, respectively. The discrepancy compared to other studies might be due to the variation of plant species, initial total metal concentration, and metal bound fraction in a sediment. In particular, the metal concentration in a sediment was higher than most of research reported in our study. e) BCF, TF, and PEF factors in pot-cultural experiments BCF, TF and PEF in pot experiments of different treatment conditions are depicted in Fig. ?? BCF values in the pot experiment can be referenced to evaluate vetiver accumulation and adsorption at its root rhizosphere. For Cu, the values EDDS, citric acid, and EDTA were 1.97, 0.88, and 2.22 equivalent to 9, 4, 10 times of control, respectively. Based on t test analysis, the variation between control and three chelators presented significant difference ( $p = 0.00084$ ,  $0.022$ , and  $8 \times 10^{-7}$ ). Three chelators all showed the significant enhancement of root Cu uptake. For Zn, the BCF values of control, EDDS, citric acid, EDTA were 2.24, 1.95, 1.67, and 1.50, respectively. Three chelators did not presented statistical difference compared to control ( $p = 0.48$ ,  $0.22$ , and  $0.09$ ). For Pb, the BCF values of control, EDDS, citric acid, EDTA were 0.51, 0.63, 0.67, and 0.58, respectively. Similar to Cu results, Pb with three chelators treatment also did not presented statistical difference compared with control ( $p = 0.296$ ,  $0.1$ , and  $0.29$ ). Three tested chelator only has significant effect on Cu. The variation might be due to the metal complex property with chelators and total metal concentration in a sediment.

TF ratio can be used to evaluate the translocation effects in vetiver.

High TF can be explained as prominent transfer from root to aerial parts of plant. For Cu, TF of the control, EDDS, citric acid, EDTA were 0.03, 0.51, 0.04, and 0.2. EDDS, citric acid, and EDTA treatments were equivalent to 17, 1.3, 8 folds ( $p = 0.0003$ ,  $0.18$ , and  $0.0022$ ) TF increase relative to the control treatment, respectively. For Zn, TF values of the control, EDDS, citric acid, and EDTA were 0.64, 0.74, 0.82, and 0.86 which indicated the TF of EDDS, citric acid, EDTS equivalent to 1.2, 1.3, 1.3 times of control ( $p = 0.027$ ,  $0.034$ , and  $0.05$ ), respectively. EDDS, citric acid, and EDTA all revealed statistical difference relative to control ( $p < 0.05$ ). For Pb, the TF values of the control, EDDS, citric acid, and EDTA were 0.02, 0.06, 0.05, and 0.24. These TF values of EDDS, citric acid, and EDTA were equivalent to 3, 2.5, 12 folds ( $p = 0.08$ ,  $0.1$ , and  $5 \times 10^{-5}$ ) increase to the control, respectively. Only EDTA revealed statistical difference when compared with the control. PEF was calculated by the concentrations and weights of a sediment and shoot. The p values of EDDS, citric acid, and EDAT compared to the control were Cu:  $0.004$ ,  $4 \times 10^{-5}$ , and  $0.001$ , for Zn:  $0.17$ ,  $0.19$ , and  $0.39$ , and for Pb:  $0.025$ ,  $0.04$ , and  $0.0007$ , respectively. Our results compared with previous are depicted in Table which demonstrated which our results were conformed with those researches.

In our study, the critical finding is which vetiver has been demonstrated as a hyperaccumulator for treatment of EDDS with Cu; EDDS, citric acid, and EDTA with Zn; and EDTA with Pb. The other important message is using PEF value to predict the required duration for a sediment remediation. The remediation time required for phytoextraction reference to PEF can be predicted by the following formula. Phytoextraction time (yr) = (metal concentration (mg/kg) in a sediment needed to decrease  $\times$  a sediment mass (kg)) / (metal concentration in plant shoot (mg/kg)  $\times$  plant shoot biomass  $\times$  the frequency of harvested (number of harvest/yr)). This information is paramount crucial for a project engineer to design an in-situ phytoremediation. In this study, EDTA has been showed to be an effective chelator though its toxic effect and possible leaching to subsurface to induce groundwater contamination. EDDS has the comparable effect with EDTA, but it might be more pricy than EDTA. The alternative might chose biodegradable EDDS if groundwater leaching was a major concern. Future study should be focused on the synergistic effect of multi-metal contamination which is more realistic for the real word application.

IV.

## 9 Conclusion

EDTA significantly increased a sediment mobility and further enhance vetiver plant uptake, Pb performed the best among four metals while and underground and aboveground were increased 6. 9

## 10 Table Captions

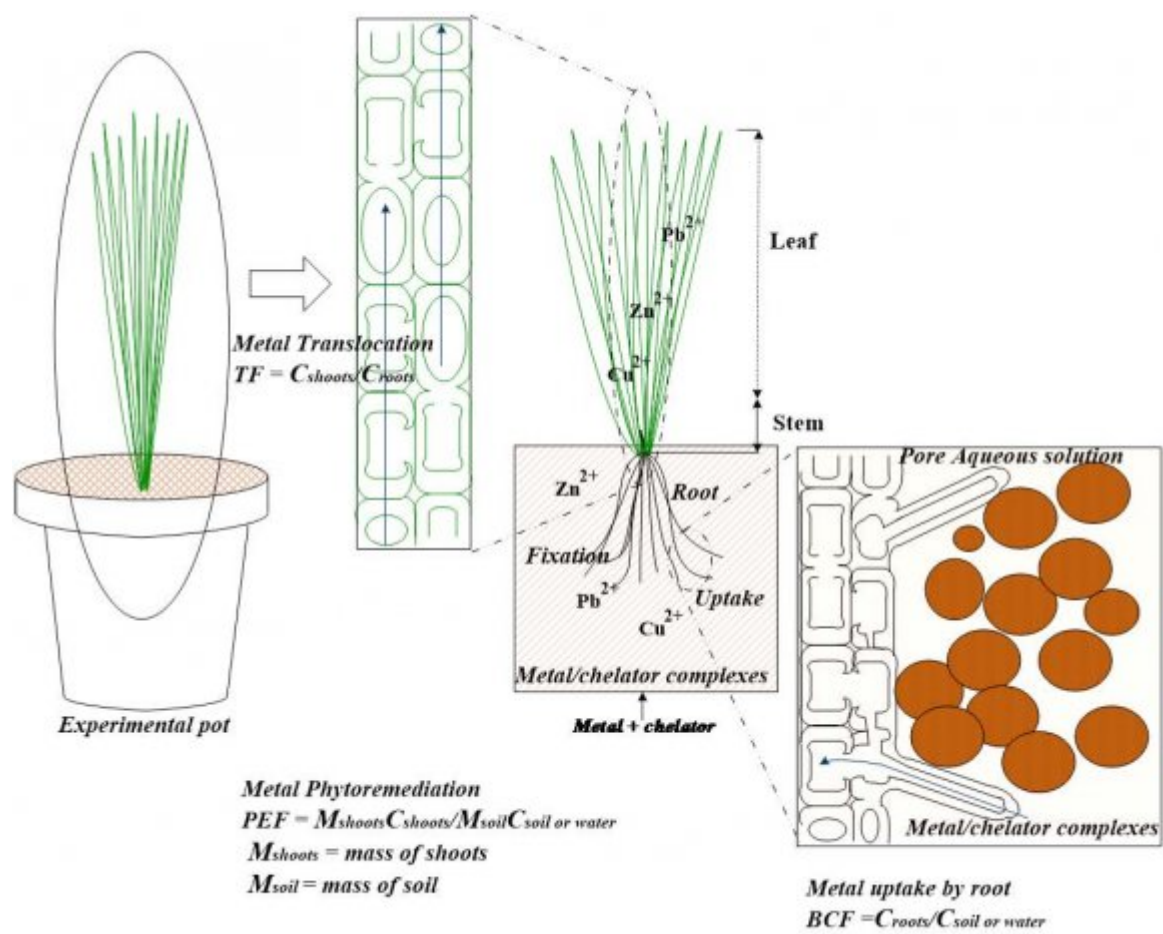
? Table ?? Plant uptake and transportation in recent study.

Figure Captions

1



Figure 1: ? Fig. 1



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Figure 2: Figure 1 :Fig. 2 :

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## II. Materials and Methods

### a. Cu uptake enhancement results

For Cu, sequential extraction results are depicted in Fig, Exchangeable fraction was 44 folds while adsorped fraction increased 1.98 folds relative to control while the organic bound and carbonated bound fraction were decreasing. The results indicated which EDTA significant enhanced Cu mobility which transferred from stable to loosely bound fractions from 1.49 to 23.47% which might facilitate future plant uptake.

### iii. Zn Fractionation Results

Zn fraction results are depicted in Fig. Initial exchangeable, adsorped bound, organic bound, carbonate bound, and sulfide bound fraction of Zn concentration  $519.25 \pm 29.80$  mg/kg,  $1.08 \pm 1.27$  mg/kg,  $1.08 \pm 1.27$  mg/kg,  $1091.41 \pm 13.78$  mg/kg,  $2587.85 \pm 84.80$  mg/kg,  $294.84 \pm 24.17$  mg/kg, respectively. After addition EDTA the fractions were exchange able, adsorped bound, organic bound, carbonate bound and sulfide bound fraction of Zn concentrate on  $771.07 \pm 37.20$  mg/kg,  $21.38 \pm 3.33$  mg/kg,  $1.08 \pm 1.27$  mg/kg,  $1651.88 \pm 19.49$  mg/kg,  $2968.19 \pm 9.09$  mg/kg,  $208.16 \pm 41.97$  mg/kg respectively. Stable adsorp factions increased 19.80 as EDTA addition which depicted which EDTA enhanced metal transfer to loosely bound from 11.57% to 14.1% which will further increase vetiver uptake.

[Note: a]

Figure 3:

Figure 4:





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