Phytoremediation of Copper Contaminated Soil by *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var. *radiata*

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The efficiency of copper removal from soil by *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* was studied. Both plants were grown for 15 days before transferring to experimental pots. Copper sulfate ($CuSO_4 \cdot 5H_2O$) solution was added to the soil in each experimental pot at 0, 50, 100, 150 and 200 mg Cu/kg soil. Plants were observed for their growth and harvested after 65 days. Copper accumulation in roots and shoots was analyzed. The results showed that the maximum concentrations of copper of *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* were 3,771 and 879 mg/kg (dry weight) in experimental pots with 150 mg Cu/kg soil. Copper accumulation in both species and copper concentration in the soil were significantly different at the 95% confidence level. The statistical analysis indicated that copper accumulations between shoots and roots of *Brassica juncea* (L.) Czern were not significantly different when Cu was added at 0 and 50 mg. However, in the experimental pots amended with 100, 150 and 200 mg Cu/kg, copper concentration in the roots was greater than those in the shoots. For *Bidens alba* (L.) DC. var *radiata*, copper accumulation was higher in the roots than in the shoots in every composition. The highest accumulation efficiency of *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* was 1.61% and 0.14% in the pot with 150 mg Cu/kg soil.

Key words: Phytoremediation, copper, *Brassica juncea* and *Bidens alba*.

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การฟื้นฟูดินที่ปนเปื้อนทองแดงด้วยผักกาดเขียวปลี (*Brassica juncea* (L.) Czern) และก้นจ้ำขาว (*Bidens alba* (L.) DC. var. *radiata*)

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วารสารวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย **31 (1)**

การศึกษาประสิทธิภาพในการกำจัดทองแดงที่ปนเปื้อนในดินด้วยผักกาดเขียวปลี (*Brassica juncea* (L.) Czern) และก้นจ้ำขาว (*Bidens alba* (L.) DC. var. *radiata*) โดยการปลูกพืชทั้งสองชนิดในกระถางทดลองเป็นเวลา 15 วัน แล้วจึงเติมสารละลายคอปเปอร์ซัลเฟต ($CuSO_4 \cdot 5H_2O$) ที่มีความเข้มข้นของทองแดง 0, 50, 100, 150 และ 200 มิลลิกรัมต่อดิน 1 กิโลกรัม สังเกตดูการเจริญเติบโตของพืชและเก็บเกี่ยวพืชทั้งสองชนิดเมื่อมีอายุครบ 65 วัน จากนั้นทำการวิเคราะห์หาปริมาณทองแดงในรากและต้น ผลการศึกษาพบว่าผักกาดเขียวปลีและก้นจ้ำขาวในกระถางที่เติมทองแดงความเข้มข้น 150 มิลลิกรัมต่อกิโลกรัม สามารถสะสมทองแดงได้มากที่สุดเท่ากับ 3,771 และ 879 มิลลิกรัมต่อกิโลกรัม (น้ำหนักแห้ง) ปริมาณทองแดงที่สะสมในพืชทั้งสองชนิดที่ระดับความเข้มข้นของทองแดงต่างๆ กัน จะมีความแตกต่างกันอย่างมีนัยสำคัญที่ระดับความเชื่อมั่นร้อยละ 95 ผลการวิเคราะห์ทางสถิติพบว่าปริมาณทองแดงที่สะสมในต้นและรากของผักกาดเขียวปลีในกระถางที่เติมทองแดง 0 และ 50 มิลลิกรัมไม่มีความแตกต่างกันอย่างมีนัยสำคัญ อย่างไรก็ตามพบว่าผักกาดเขียวปลีในกระถางที่เติมทองแดง 100, 150 และ 200 มิลลิกรัม จะมีการสะสมของทองแดงในรากมากกว่าในต้น ส่วนก้นจ้ำขาวพบว่าจะมีการสะสมทองแดงในรากมากกว่าในต้นทุกระดับความเข้มข้นของทองแดง ประสิทธิภาพสูงสุดในการสะสมทองแดงของผักกาดเขียวปลีและก้นจ้ำขาวมีค่าเท่ากับร้อยละ 1.61 และ 0.14 ในกระถางที่เติมทองแดงความเข้มข้น 150 มิลลิกรัมต่อกิโลกรัม

คำสำคัญ การฟื้นฟูสิ่งแวดล้อมโดยใช้พืช ทองแดง ผักกาดเขียวปลี ก้นจ้ำขาว

INTRODUCTION

Heavy metal contamination is caused by various sources, such as industrial processes, manufacturing, disposal of industrial and domestic refuse, and agricultural practices.[1] Campbell *et al.* compared natural and anthropogenic quantities of trace metals emitted to the atmosphere and showed that around 15 times more Cd, 100 times more Pb, 13 times more Cu and 21 times more Zn are emitted by human activities than by natural processes.[2] The main copper contamination sources in soils are pig and poultry manures, pesticides and metal finishing and microelectronics by products. In Thailand, 150-250 ppm copper, in the form of copper sulfate, is added to pig feed as a growth rate regulator. In some cases, zinc is also mixed with copper which causes an increase of these elements in pig waste (approximately 400 ppm zinc and 800 ppm copper).[3] Using contaminated pig manure as a soil amendment could cause soil pollution and the accumulation of heavy metal to a toxic level through out the food chain.

Phytoremediation is proposed as a cost-effective alternative for the treatment of contaminated soils. Topsoil would be preserved and the amount of hazardous materials reduced significantly.[4] The main factors controlling the ability of phytoextraction are plant species, metal availability to plant roots, metal uptake by roots, metal translocation from roots to shoots and plant tolerance to toxic metals. There are many types of plants currently used in phytoextraction, such as *Thlaspi carerulescens, Alyssum murale, A. lesbiacum, and A. tenium,* which can accumulate high levels of Zn and Cd in shoots. However, the remediation potential may be limited due to the slow growth and low biomass of these plants.[5]

Recently phytoremediation researchers have discovered that Indian mustard (*Brassica juncea* (L.) Czern) can accumulate high levels of metals, including Zn and Se. The metal accumulating ability of this plant, coupled with the potential to rapidly produce large quantities of shoot biomass, makes this plant ideal for phytoextraction.[6] Beggar's tick (*Bidens alba* (L.) DC. var *radiata*) has been introduced in Thailand for use by bee farms for producing good quality honey. This plant grows well and shows no signs of toxicity caused by elevated levels of As, Cu and Cr (196, 112 and 67 mg/kg, respectively).[7]

In Thailand, research on the metal-accumulating efficiency of crop plants and weeds is still limited. In this study, *Brassica juncea* (L.) Czern (crop plant) and *Bidens alba* (L.) DC. var *radiata* (weed) were selected because they have desirable characteristics such as high shoot biomass, metals tolerance, short life cycle and handling ease. The objectives of this research were to study the accumulation of copper in the shoots and roots of *Brassica juncea* (L.) Czern (Brassicaceae) and *Bidens alba* (L.) DC. var *radiata* (Asteraceae) and to compare the efficiency of copper removal of the two species.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse. Seeds of *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* were obtained from the Plant Protection Research and Development Office, Department of Agriculture, Thailand. The experimental pot was plastic with a 25 cm upper diameter, 20 cm lower diameter and 30 cm height. The two species of plants were grown in 5 different Cu concentrations; 0, 50, 100, 150 and 200 mg Cu/kg soil. Copper was directly added to the pot and thoroughly mixed with the soil. During the experiment, watering was not allowed to exceed the water holding capacity of the soil in order to prevent the leakage of copper from the pot. Initial copper content in the soil was analyzed prior to the experiment.

Soil and Plant Preparation

Soil samples were collected from topsoils at Moo 5, Bantasharap, Tambon Srisathong, Ampur Nakornchisri, Nakorn Pathom Province. The soil was air-dried, sieved through 0.5 and 2 mm screens and thoroughly mixed before use. Physical and chemical properties (*i.e.*, soil texture, moisture content, soil pH, cation exchange capacity, organic matter, total nitrogen, available phosphorus and available potassium) were analyzed.

The experimental pots (50 pots) were set up by adding 1.5 kg of soil sample and 25 seeds of either *Brassica juncea* (L.) Czern or *Bidens alba* (L.) DC. var *radiata* into each pot. The experimental pots were arranged in a way that a Completely Randomized Block Design could be conducted. After the first cotyledon appeared, small and unhealthy plants were removed. Further culling was done to obtain 12 plants in each pot. After 15 days of growth, copper sulfate ($CuSO_4.5H_2O$) solutions were added at 5 different concentrations; 0, 50, 100, 150 and 200 mg Cu/kg and 5 replicates were done for each composition. Physical changes of plants in each pot were observed daily.

Copper accumulation in plants and soils

Plants were harvested after 65 days of plantation. Each plant was rinsed, cut, and group selected into shoots and roots. Each part was dried in an oven at 65^{o} C for 72 hours. Both wet and dry weight were recorded. All dried parts were ground using mortar, mixed thoroughly, and digested with 0.1 N HCl. Sample solutions were analyzed for copper by flame atomic absorption on a Perkin Elmer AAnalyst 200 Spectrophotometer.

The soil was air-dried, sieved through a 2.0 mm screen and digested with 0.1 N HCl. Analysis of copper was conducted by the same procedure described above.

The Efficiency of Copper Removal

Total copper accumulation in the plants was determined and compared with the total amount of copper in each experimental pot. The efficiency of copper removal was calculated using the equation below:

$$\text{Efficiency of copper removal (\%)} = \frac{\text{(Cu in shoots + Cu in roots) (mg)}}{\text{Total Cu in pot (mg)}} \times 100$$

Then, copper removal efficiency was analyzed using one-way ANOVA (SPSS program version 12.0 for Windows) at 95% significance.

RESULTS AND DISCUSSION

Soil Properties and Availability of Copper

It was necessary to determine the physical and chemical properties of soil which govern both availability and relative toxicity of metal contaminants such as soil pH, clay content, organic matter content and the nutritional status.[8] The physical and chemical properties of soil used in this study are shown in Table 1.

Soil pH affects the solubility and mobility of copper. At soil pH below 7.0, most copper was found in the form of cuprous ion rather than of cupric ion. The solubility of cuprous ion was greater than cupric ion. Moreover, at low pH, copper which forms chelating compounds with organic matter is easily separated. The pH of soil used in this study was 5.25. Therefore, the concentration of free and exchangeable copper ions were high and, thus, increased the amount of copper uptake by the plants.

In general, a fertile, loamy top soil has an average organic matter content of only 5%. In less fertile soils and in subsoils, the organic matter content is lower than 5% by volume. The clay and organic matter contents of soil used in this study were high (34.94% and 7.34%) resulting in high cation exchange capacity of the soil (36.45 $cmol_c$/kg). Copper ions could be adsorbed by negatively charged surfaces of the soil colloids and organic matter.

Nitrogen is an essential nutrient for plant growth. It is taken up by plants in large amounts, whereas its concentration in soils is frequently very small. The nitrogen content of the Ap horizons of most cultivated soils is estimated to range between 0.05-0.4% N. The total nitrogen content of soil used in this experiment was slightly low.

The amount of copper in the soil used in this study was 19 ppm. Generally, the total copper concentration in soil and the critical level of copper is in the range of 10-80 and 100 ppm.[9]

Table 1. The physical and chemical properties of soil studied.

Parameters	Value	Analyzed Method
Soil texture sand:silt:clay	Clay Loam 38.50:26.56:34.94	Hydrometer Method
Moisture content	20.73 %	Gravimetric Method
pH	5.25	Potentiometric Method (soil:distilled water = 1:1)
Cation exchange capacity	36.45 $cmol_c$/kg	Ammonium Acetate Method
Organic matter	7.34 %	Walkley-Black Method
Total nitrogen	0.137 %	Kjeldahl Method
Available phosphorus	530 ppm	Mehlich’s No.1 Method
Available potassium	265 ppm	Atomic Absorption Spectrophotometer
Total copper	19 ppm	Atomic Absorption Spectrophotometer

Growth Observation

The growths of *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* were different in each composition. It was observed that *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* grew well in experimental pots amended with 50, 100 and 150 mg Cu/kg, respectively. For both species, small and chlorosis in stem and leaves were found in the pots amended with 200 mg Cu/kg.

Copper accumulation in plant

Normal concentration of copper in plant tissues is approximately 5-25 mg/kg. Plant copper concentrations are controlled within a remarkably narrow range and plant copper concentrations above 100 mg/kg are rare even in the presence in high soil copper. The variation in copper accumulation may be related to soil pH, soil moisture, the season of the year, individual genotypic variability and varying degrees of soil contamination.[10]

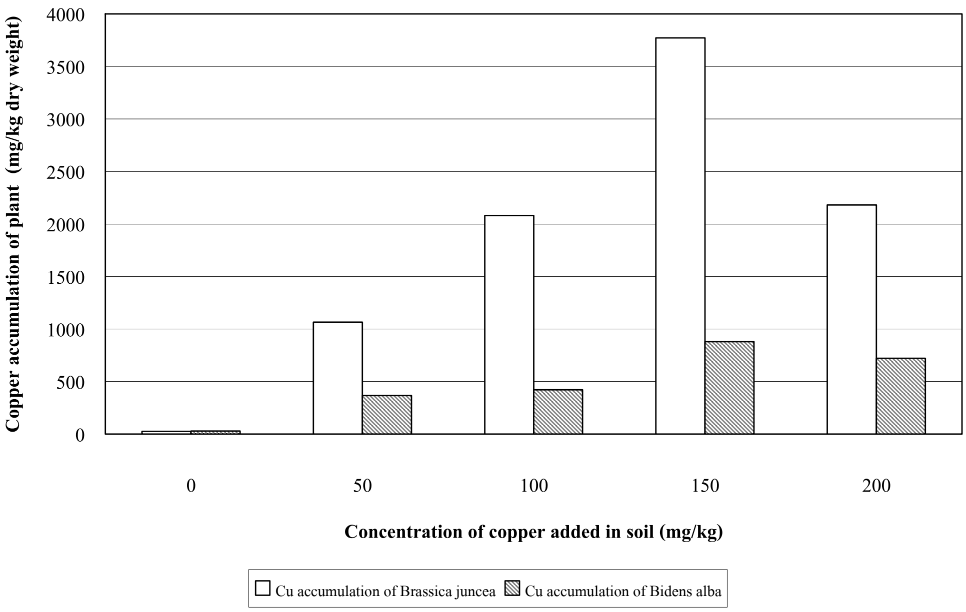

Figure 1. Copper accumulation of *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* at different concentrations.

The results showed that the maximum concentrations of copper of *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* were 3,771 and 879 mg/kg in the experimental pots with 150 mg Cu/kg (Figure 1). For the 200 mg Cu/kg amended soils, the copper accumulation of both species decreased due to the lower biomass of plants. In addition, there was a significant difference between copper accumulation in both species and the copper concentration in soil at the 95% confidence level.

In recent years, researchers discovered that *Brassica juncea* can accumulate moderate levels of environmentally important metals including Zn and Se. Montes-Bayon *et al.* reported that *Brassica juncea* can accumulate Se up to hundreds of ppm in various parts of the plant.(6)

Maximum concentrations of copper accumulated by plants in Family Asteraceae, such as *Anisopappus chinensis* and *Vernomia petersii,* were 1,657 and 1,555 mg/kg.(11) Rockwood *et al.* reported that *Bidens alba* (L.) DC. var *radiata* grew well and showed no sign of toxicity at the studied Cu contaminated site (112 mg/kg).(7)

The statistical analysis showed that copper accumulation between shoots and roots of *Brassica juncea* (L.) Czern were not significantly different in soil when amended with 0 and 50 mg of Cu. At low concentration, the level of copper was not toxic to *Brassica juncea* (L.) Czern. Therefore, it could be that copper translocated from roots to shoots. However, in the experimental pot with 100, 150 and 200 mg Cu/kg, copper concentrations in the roots were greater than in the shoots (Figure 2). For *Bidens alba* (L.) DC. var *radiata*, copper accumulation was higher in the roots than in its shoots in every composition (Figure 3). For the 150 mg Cu/kg amended soil, the highest copper accumulation in shoots of *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* were 587 and 180 mg Cu/kg (in shoots dry matter), whereas in roots, the amount of copper accumulated were 3,184 and 699 mg Cu/kg (in roots dry matter), respectively. These results were very similar to the study performed by Morishima and Oka.(12) The copper tolerant weed in paddy fields contains more copper in its roots than in the leaves. The limiting factor is a long distance translocation from roots to shoots. Once the divalent cations are transported into roots cells, they are either precipitated in the cell or chelated with an organic compound. For long distance transport, the toxic metals are chelated with organic compounds available inside the cell.(13)

The Efficiency of Copper Removal

The copper removal efficiency of *Brassica juncea* (L.) Czern was 11 times greater than *Bidens alba* (L.) DC. var *radiata*. The highest efficiency of *Brassica juncea* (L.) Czern and *Bidens alba* (L.) DC. var *radiata* were 1.61% and 0.14% in the experimental pot with 150 mg Cu/kg soil, respectively. Both species showed a significant difference between efficiency of copper removal and copper concentrations at the 95% confidence level.

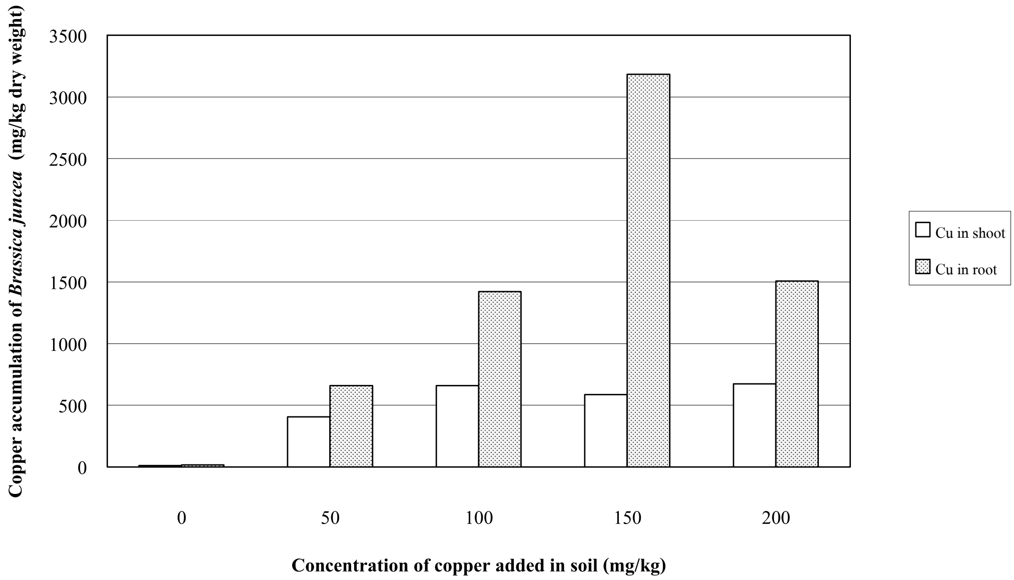

Figure 2. Copper accumulation in the shoot and root of *Brassica juncea* (L.) Czern.

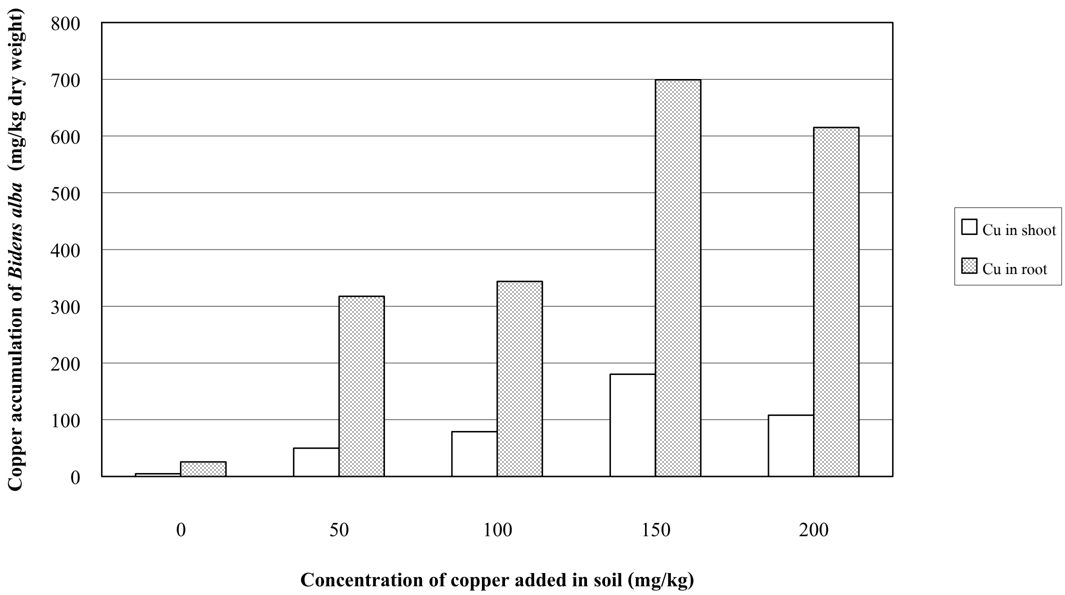

Figure 3. Copper accumulation in the shoot and root of *Bidens alba* (L.) DC. var *radiata*.

CONCLUSIONS

Although the copper concentration in shoots of *Brassica juncea* (L.) Czern should not exceed 1,000 mg/kg dry weight, the total amount of copper accumulated in shoots and roots was high (3,771 mg/kg). Therefore, *Brassica juncea* (L.) Czern could be regarded as a hyperaccumulator for copper remediation in contaminated soils. The copper concentration of *Bidens alba* (L.) DC. var *radiata* was low (879 mg/kg). However, there was no sign of copper toxicity in this plant when testing in moderately contaminated soil (150 mg Cu/kg soil). Thus, it could be classified as a copper tolerant species. For *Bidens alba* (L.) DC. var *radiata*, chelates, organic acids and certain chemical compounds should be applied to increase the solubility of copper in the soil solution and copper accumulation in the plants.

ACKNOWLEDGMENT

The authors gratefully acknowledge the financial support of the National Research Center for Environmental and Hazardous Waste Management (NRC-EHWM) for this study.

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Received: October 10, 2005
Accepted: January 16, 2006