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# Effect of EDTA on Phytoremediation of Copper–Polluted Soils

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Efficiency of additive agents was studied to remove copper from Cu–mixed soils by *Brassica juncea* and *Zea mays* L. The plants were grown in decomposed granite soil (Regosol) and volcanic ash soil (Andosol) for 3 weeks. To simulate actually–contaminated soil, copper contents of 25 $\mu$g Cu g$^{-1}$ and 250 $\mu$g Cu g$^{-1}$ were prepared for Regosol and Andosol, respectively. EDTA was found to be an excellent additive agent to raise the availability of Cu in soils. The effect of EDTA on Cu absorption by plants was limited in Regosol. On the other hand, in Andosol the Cu accumulation in shoot of *Z. mays* for the 100 m*M* EDTA treatment were 3.7 times larger than that for the 0 m*M* EDTA treatment. The Cu absorption by *B. juncea* was not affected by the EDTA addition to Andosol. These results suggest that the effect of EDTA on the Cu absorption by plants significantly changes with the additive concentration and with the types of soil and plant.

## INTRODUCTION

More than 150 metal mines are distributed all over Japan (Kitagishi and Yamane, 1981). About half of them have been producing ore containing copper (Cu). As most of the mines are located in mountain area (Taniyama, 1991), Cu contamination has extended from there to the down–streams. Water collected from the rivers is often used for the irrigation in the paddy fields developing in the low land areas. Although Cu is definitely an essential element for plants, excessive uptake of the element would cause damage to the plants (Kabata–Pendias, 2001).

A lot of task, cost, and time must be required in the conventional techniques for remediating the soil contaminated by heavy metal. Phytoremediation is becoming popular as a novel method that could restore the soils using the conventional crop cultivation. Usually, the cost of phytoremediation is lower than that of the engineering techniques where the excavation and landfilling technology can be used in a large area of the polluted lands (Glass, 2000). For the phytoremediation, lower concentration of heavy metal in soil solution may be preferable to prevent a heavy metal hazard to plants. Plant ability for remediating soils may be evaluated by the biomass of plant as well as by uptake of heavy metals by the plant.

Ebbs and Kochian (1997) reported that the zinc accumulation capability of *Brassica juncea* is high in hydroponics and its concentration in the shoot is about 1,000 $\mu$g g$^{-1}$ in two–week cultivation. Quartacci et al. (2001) also observed that *B. juncea* indicates the highest degree of cadmium translocation from root to shoot among four *Brassica* speacies. However, the biomass production of *B. juncea* was extremely lower (about 0.5 g plant$^{-1}$ in dry weight) than that of *Raphanus sativus* L. (Quartacci et al., 2001). The removal of heavy metal from the polluted soils would be promoted by enhancing the metal accumulation in *B. juncea*, or by making the plants produce higher biomass.

Addition of chemicals, such as a chelating agent, to soils has been used as a method to enhance the extraction of heavy metal by plants. For example, Huang et al. (1997) found that the lead absorption of *Pisum sativum* L. increases with addition of EDTA. The increase of selenium absorption in *Hordeum vulgare* L. and *Triticum aestivum* L. was also observed with additions of organic acids such as ascorbic acid (Blaylock and James, 1994).

The purpose of the present study is to investigate the efficiency of EDTA application for removing Cu from the contaminated soils by *B. juncea* and to compare the Cu removal efficiency of *B. juncea* with that of *Zea mays* L. having high biomass production.

## MATERIALS AND METHODS

### Soils

Samples of two types of soils were used. One is volcanic ash soil (Andosol) sampled from the field at the National Agricultural Reseach Center for Kyushu Okinawa Region in Nishigoushi, Kumamoto, and the other is decomposed granite soil (Regosol) which is commercially available. The soils were mixed with the powder of anhydrous copper (II) sulfate. The pH ($H_2O$) of the soils was determined by the glass electrode method, organic matter content by the ignition loss method (Nakano et al., 1995), cation exchange capacity (CEC) by the method of Wada and Harada (1969), and field water capacity by the general method (Cassel and Nielsen, 1986).

### Extractions of Cu from the soils

Total Cu content in soils was determined by the $HClO_4$ digestion method (Baker and Amacher, 1982), and the Cu content extracted by 0.1 *M* HCl was also measured by the method of Baker and Amacher (1982). The additive of EDTA2Na, citric acid or $(NH_4)_2SO_4$ was dosed in a 50 mL tube containing 5 g of Cu-mixed soil at the rate of 0.1, 1, 10, or 100 m*M* per 25 mL of solution. Then the tube was shaken for 1 hour at 30 °C. The extractant from the solution was analyzed for quantifying copper content by the atomic absorption spectrometry (Shimazu Co., Ltd., AA-670).

### Cultivation

The plants were cultivated in a phytotron glass room (temperature, 25 °C; humidity, 70%) during November 2000 to January 2002. Indian mustard (*Brassica juncea* Coss. cv. Hakarashina) and corn (*Zea mays* L. cv. Pioneer 33G26) were grown in 350 mL pot for 3 weeks. Andosol was not fertilized because of continuous application of manure before the cultivation. To Regosol, 100 kg N $ha^{-1}$, 43.6 kg P $ha^{-1}$, and 83 kg K $ha^{-1}$ of nutrients were applied. During the growing period, the soil water content was kept the field water capacity by adjusting the application of distilled water.

### EDTA application

Ten mL of 0, 0.1, 1, 10, or 100 m*M* EDTA2Na solution was applied to soil surface by a syringe. Shoots and roots (only Andosol) were sampled 3 weeks after the sowing. Sampled shoots and roots were dried at 70 °C and milled. The milled samples were ashed at 500 °C, and then dissolved in dilute HCl and $HNO_3$ solution. Copper concentration of the sample solutions was measured by the atomic absorption spectrometry.

**Statistical analysis**

Each experiment was performed in triplicate. The variance for each results was analyzed and the differences between the averaged values was tested by the Tukey's method.

## RESULTS AND DISCUSSION

**Soil properties**

Both organic matter content and CEC of Andosol were higher than those of Regosol (Table 1). The total Cu concentration of Andosol was 10 times as much as that of Regosol, while Cu extracted by 0.1 *M* HCl in Andosol was almost the same as that in Regosol. This suggests that the components of Andosol strongly absorb Cu. The soil pH of Andosol (pH 5.6) was lower than that of Regosol (pH 6.4). Despite the difference, soil pH was not adjusted during the experiments since pH has little influence on the cultivation.

**Table 1.** Soil properties

| Soil | pH ($H_2O$) | OM# % | CEC $cmol_c\ kg^{-1}$ | FWC## $m^3\ m^{-3}$ | Total Cu $\mu g$ Cu $g^{-1}$ | 0.1 MHCl Cu $\mu g$ Cu $g^{-1}$ |
|---|---|---|---|---|---|---|
| Regosol | 6.4 | 3.4 | 0.5 | 0.23 | 4.3 | 0.14 |
| Andosol | 5.6 | 23.3 | 31.2 | 0.60 | 42.2 | 0.15 |

# OM: Organic matter content.
## FWC: Field water capacity.

**Examination of the Cu addition**

Initially, we need to know the moderate concentration of Cu in soil, at that concentration plants can absorb Cu suitably and the plants grow normally. Figure 1 shows the Cu concentration, dry weight, and Cu absorption of the plants grown in the Cu–mixed Regosol. Since *B. juncea* could not grow in Regosol of 100 $\mu g$ Cu $g^{-1}$ in the preliminary experiment, only four levels (0, 25, 50, and 75 $\mu g$ Cu $g^{-1}$) of the addition are compared in the figure. Copper concentration in the shoots increased with the Cu addition to the soil. The Cu concentration of *B. juncea* was 3.1 to 8.6 times higher than that of *Z. mays* even in the same Cu addition. Shoot dry weight of *B. juncea* for the Cu addition of 50 $\mu g$ $g^{-1}$ was about 1/10 of that for no treatment (0 $\mu g$ Cu $g^{-1}$). For *Z. mays*, the dry weight for the addition of 75 $\mu g$ Cu $g^{-1}$ was about one fifth of that for no treatment. The Cu concentrations in both shoots of *B. juncea* and *Z. mays* sharply increased with Cu addition. The increase in Cu concentration brought about decrease in dry weight of plant. The two types of plants did not suffer damage by Copper in 25 $\mu g$ Cu $g^{-1}$ soil. Thus, the soil added with 25 $\mu g$ Cu $g^{-1}$ was used for the experiments in Regosol.

In the preliminary experiment for Andosol, the plant growth for the 500 $\mu g$ Cu $g^{-1}$ was

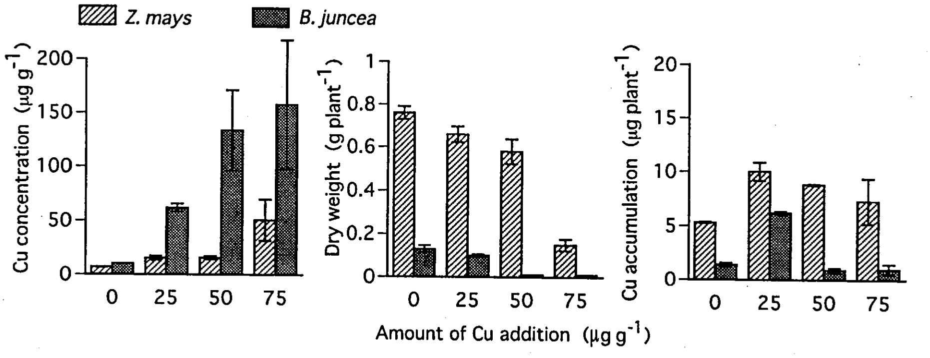


**Fig. 1.** Copper concentration, dry weight of shoot, and Cu accumulation of the plants in the Cu-mixed Regosol.

almost the same as that for no treatment. Therefore, it can be expected that the plant available Cu of Regosol is different from that of Andosol when the same amount of Cu is added to two soils. The amount of Cu extracted by 0.1 *M* HCl from Andosol was lower than that from Regosol (Fig. 2). The amount for the addition of 1,000 $\mu$g Cu $g^{-1}$ was 900, and 215 $\mu$g Cu $g^{-1}$ in Regosol and Andosol, respectively. Since the organic matter content in Andosol was high (Table 1), the amount of Cu adsorbed on the surface of organic matter in the soil may be more than that in Regosol in which the extraction of Cu by HCl was limited. The Cu extraction by HCl from Regosol for the addition of 25 $\mu$g Cu $g^{-1}$ was almost similar to that of Andosol for the addition of 250 $\mu$g Cu $g^{-1}$. Thus, the soil added with 250 $\mu$g Cu $g^{-1}$ was used for the experiments in Andosol.

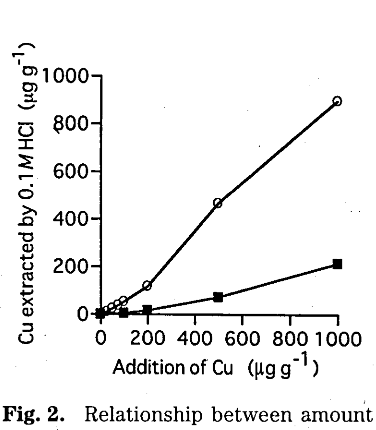


**Fig. 2.** Relationship between amount of addition of Cu to soil and Cu extracted by 0.1 M HCl. ○ Regosol, ■, Andosol.

**Enhancement of Cu extraction by the additives**

Huang et al. (1997) described that the lead absorption rate of a pea (*Pisum sativum* L.) increases with addition of EDTA. It was also reported that the uranium concentration in *B. juncea* increases with citric acid (Huang et al., 1998), and cesium–137 concentration in *B. juncea* and *Z. mays* increases with ammonium sulfate (Blaylock and Huang, 2000). In the present study, the Cu extraction from the soils by three sorts of the additive agents (EDTA, citric acid, and ammonium sulfate) with different concentrations was investigated (Fig. 3). In Regosol, since there was little difference in Cu concentration between two cases of 10 and 100 m*M* of EDTA additives, the Cu extraction from the soil could reach a maximum for 10 m*M* EDTA. In Andosol, the amount of Cu extraction for EDTA additive corresponded to 7.1 and 183 times as much as those for citric acid and ammonium sulfate additives, respectively, for 100 m*M* solution. Thus, EDTA was selected as an excellent additive agent to raise the availablity of Cu in soils.

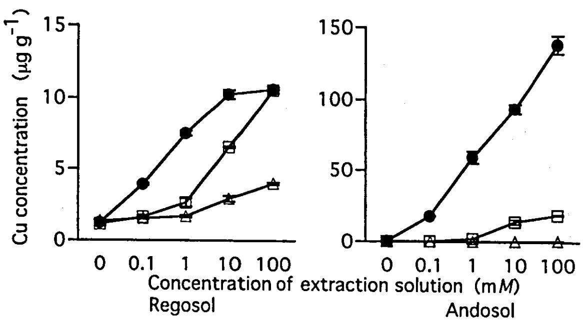


**Fig. 3.** Comparison of the amount of extracted copper among three additives under the different additive concentration. Cu concentration in soil: Regosol, 25 μg $g^{-1}$; Andosol, 250 μg $g^{-1}$. ●, EDTA; □, citric acid; △, ammonium sulfate.

**Effect of EDTA additive on the Cu absorption**

In Regosol, the Cu accumulation in shoots of *Z. mays* was not varied with the EDTA concentrations (Fig. 4). The case of 100 m*M* EDTA for *Z. mays* was not treated in Regosol. This is because the plants in the 100 m*M* EDTA treatment did not grow probably due to salt injury. For *B. juncea*, the Cu accumulation in shoots in 100 m*M* EDTA treatment was higher than that in 0 m*M* EDTA treatment. However, in 100 m*M* EDTA treatment, the shoot dry weight was low, resulting in low Cu absorption (Fig. 5).

In Andosol, the Cu concentration in shoots of *Z. mays* in the 100 m*M* treatment was higher than that in the 0 m*M* EDTA treatment, but there was no significant difference. However, the Cu accumulation in shoots of *Z. mays* in the 100 m*M* EDTA treatment was 3.7 times larger than that in the 0 m*M* EDTA treatment ($p<0.01$) (Fig. 6). In contrast, no effect of EDTA on the Cu accumulation was found for *B. juncea* (Fig. 7).

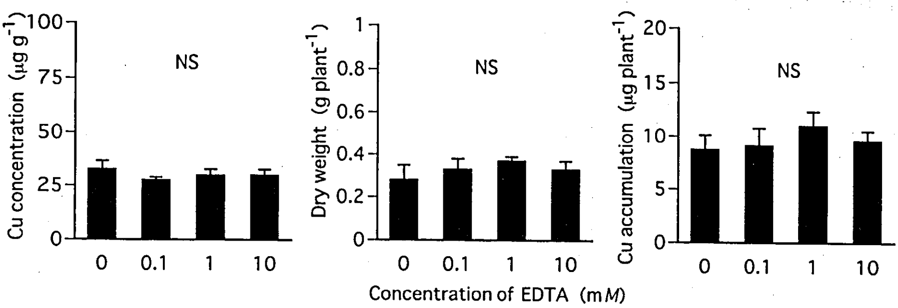


**Fig. 4.** Copper concentration, dry weight of shoot, and Cu accumulation of *Z. mays* in Regosol adding EDTA. NS shows no significant difference.

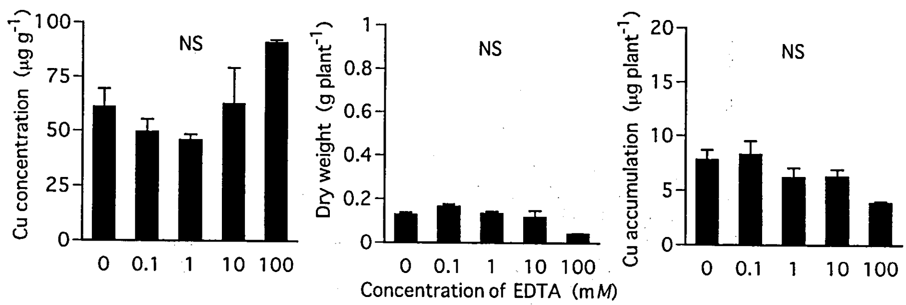


**Fig. 5.** Copper concentration, dry weight of shoot, and Cu accumulation of *B. juncea* in Regosol adding EDTA. NS shows no significant difference.

For *Z. mays*, after absorption by roots Cu translocation from roots to shoots could be restricted. The roots grown in Andosol actually accumulated Cu of 45 $\mu$g plant$^{-1}$ for the 0 m*M* EDTA treatment. On the other hand, in the 100 m*M* EDTA treatment, the Cu accumulations in roots were about 29 $\mu$g plant$^{-1}$. Two reasons can be considered for the high accumulation in shoots. One is that the Cu accumulated by the root may be transformed to the EDTA–Cu complex by addition of EDTA, and loaded to the shoots. The other is that Cu combined with EDTA in the soil passed through the root to the shoot, causing the increase in Cu accumulation in shoots.

Unlike *Z. mays*, the translocations of the Cu to shoot were high even in the 0 m*M* EDTA plots in *B. juncea*. Quartacci et al. (2001) found that *B. juncea* carried larger amounts of cadmium to the shoot than the other plants among *Brassica* species. Actually, the Cu accumulation in the root of *B. juncea* in Andosol was about 0.7 times that in the shoot, and differed greatly from *Z. mays* (12 times than the 0 m*M* EDTA treatment). There was no effect of EDTA on the Cu accumulation a whole plant of *Z.*

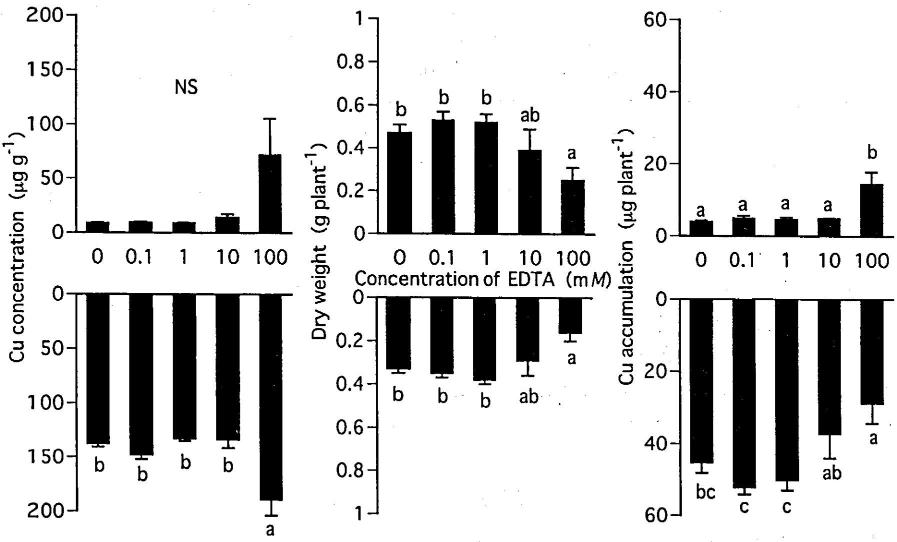


**Fig. 6.** Copper concentration, dry weight of shoot, and Cu accumulation of *Z. mays* in Andosol adding EDTA. NS shows no significant difference. Different letters shows a 5% significant difference.

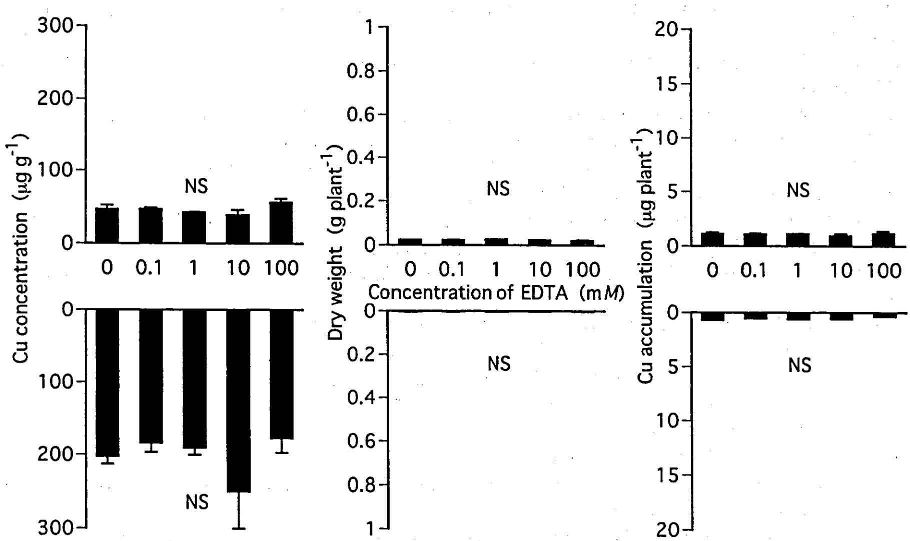


**Fig. 7.** Copper concentration, dry weight of shoot, and the Cu accumulation of *B. juncea* in Andosol adding EDTA. NS shows no significant difference.

*mays* and *B. juncea* (Fig. 6, and 7). Thus, EDTA may obviously affect the Cu translocation to the shoot as well as the Cu extraction from the soil.

The effect of EDTA on Cu absorption of the shoots was limited in Regosol but not in Andosol. Most of the Cu element could exist in Andosol in the form of adsorbed Cu on the surface of organic matters and the oxide minerals, and could be desorbed by adding EDTA. Thus, *Z. mays* cultivated in Andosol could easily absorbe Cu (EDTA–Cu) in the soil solution. Dissolved Cu existed little in Regosol even under the addition of EDTA. This implies no effect of EDTA on Cu accumulation in shoot in Regosol.

In short, the present study indicated that the effect of EDTA on the Cu absorption by plants changes with the additive concentration and with the types of soil and plant. We should note that the addition of the chelating agent to the soil polluted by heavy metal might cause the secondary contamination to groundwater. Therefore, to succeed in removing heavy metals, it is necessary to contemplate the amount of the chelating agent, the soil characteristic of polluted area, and the plant species.

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