Effect of EDTA and EDDS on phytoremediation of Pb- and Zn-contaminated soil by Brassica Juncea

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Abstract. Effect of EDTA and EDDS on phytoremediation of Pb- and Zn- contaminated soil by Brassica Juncea was investigated in this work. Especially, the effect of the kind and the method of adding chelating agent was investigated during the plant growth. Plants were grown in an environmental control system. The biomass of the whole plant was weighed, and the uptake of Pb and Zn in shoot and root were determined using ICP-AES. Consequently, the following matters have been obtained: (1) Both EDTA and EDDS significantly enhanced the translocation of metals (Pb and Zn) in soil from root to shoot. Furthermore, the two chelating agents resulted in a sharply biomass loss for more than 30% of the control. As a result, the total uptake amount of metals by Brassica Juncea was decreased (except the uptake of Pb with the addition of 3.0 mmol kg^{-1} EDTA). (2) EDDS showed the higher inhibition for the growth of Brassica Juncea than EDTA. (3) The method for adding EDTA and EDDS at several times separately did not necessarily increase the uptake of heavy metals.

Introduction

With the rapid development of industry and agriculture, the soil pollution is becoming more and more serious in Japan. For example, the number of the polluted soil caused by lead (Pb) is increased from 1205 cases in 2005 to 1928 cases in 2007 based on the survey from Ministry of the Environment of Government of Japan [1]. In case of Niigata Prefecture, there are several reports about the polluted soil by heavy metals such as Pb, arsenic (As) and mercury (Hg) recently [2]. Then it is particularly important and urgent to remediate the polluted soil because metals do not degrade and thus persist almost indefinitely in the environment.

Phytoremediation of heavy metals, which has the advantages of low-cost, environmental-friendly and minimal soil disturbance, has attracted more and more interest in recent years [3-6]. Phytoremediation makes use of the harvestable part of plants to remove pollutants. Any plant can uptake metals from soil theoretically, however, the overwhelming majority of plants generally remove only a small percentage of heavy metals from contaminated soil due to the low biomass and/or the weak transloction from root to shoot. Therefore, using plant with high biomass yields (such as Brassica juncea) along with adding chemically enhanced agent (such as EDTA) has been widely applied as a viable strategy for removing heavy metals from soils over a reasonable time frame [7-9].

The objectives of this research are to confirm the effectiveness of Brassica Juncea for phytoremediation of Pb and zinc (Zn), and to investigate the ability of the two synthetic chelators such as ethylenediaminetetraacetic acid disodium salt (EDTA) and (S,S)-ethylenediamine-N, N-disuccinic acid trisodium salt (EDDS) for enhancing the phytoremediation of Pb- and Zn-contaminated soil by Brassica juncea. Futhermore, a key goal is to ascertain if the effect of the phytoremediation varies with the kind and the method of adding chelating agent. The different additional dosage and times of the two chelators were studied to elevate metal uptake and translocation from root to shoot. The amount of metals uptaken by Brassica juncea was determined in order to evaluate whether the chelators may represent an effective tool for the removal of metals from a Pb- and Zn- polluted soil for practical use.

Experimental

Apparatus and Reagents. A plant environmental control system (LPH-220N, Nippon Medical & Chemical Instruments Co. LTD, Japan) was used to cultivate plant. An inductively coupled plasma atomic emission spectrophotometer (ICP-AES) instrument (SPS1500, Seiko Instruments Inc., Japan) was employed to determine the concentrations of Pb and Zn. The operating conditions of ICP-AES are based on our other previous paper [10]. Plant and soil samples were digested with a microwave digestion system (Speedwave4, ACTAC. Co. LTD, Japan).

Metallic salts such as $Pb(NO_3)_2$ and $Zn(NO_3)_2·6H_2O$ were purchased from Kanto Chemical Co., Inc. (Japan). Ethylenediaminetetraacetic acid disodium salt (EDTA) and (S,S)-ethylenediamine-N, N-disuccinic acid trisodium salt (EDDS) were purchased from Kanto Chemical Co., Inc. (Japan) and Sigma-Aldrich, Inc. (Germany), respectively. Pb and Zn standard solutions used for making the calibration curve were prepared by diluting the standard solutions ($1000mg·dm^{-3}$ in 6% HNO_3 solution) purchased from Merck Co. LTD (Japan). All chemical reagents used were of analytical grade. Water (>18.2 MΩ), which was treated by an ultrapure water system (Advantec aquarius: RFU 424TA), was used throughout the work.

The nutrient solution was prepared by dissolving “Otsuka House No.1 and No.2” (Otsuka AfriTechno Co., Ltd., Japan) in ultrapure water, then used as fertilizer during the period of the plant cultivation [11]. The composition of the nutrient solution was shown in Table 1.

Table 1 Nutrient solution composition*)

Parameters	Concentration [$mg·kg^{-1}$]
Total nitrogen	260
NH_4^+-N	23
NO_3^--N	233
P_2O_5	120
K_2O	405
CaO	230
MgO	60
MnO	1.5
B_2O_3	1.5
Fe	2.7
Cu	0.03
Zn	0.09
Mo	0.03
EC[$dS·m^{-1}$]	2.6

*)The data are taken from [11]

Preparation of Pb- and Zn-contaminated Soil. The contaminated soil used in the present study was prepared by adding the solution containing two kinds of metallic salts ($Zn(NO_3)_2·6H_2O$ (5mmol kg^{-1}) and $Pb(NO_3)_2$ (5mmol kg^{-1})) to a soil “ Expanded Vermiculite”. The soil was purchased commercially from Takamura Co. LTD (Japan), which has high moisture retention capability, air capacity and nutrient preserving capability. Physical and chemical characteristics of the Expanded Vermiculite are listed in Table 2. Their measure methods are the same as shown in our previous paper [12].

Table 2 Physical and chemical characteristics of the Expanded Vermiculite

Parameters	Expanded Vermiculite
pH(H_2O)	7.20
pH(KCl)	5.17
EC [$\mu S·cm^{-1}$]	23.8
Moisture content [%]	0.241

Organic matter content [%]	1.40
Cation exchange capacity (CEC) [$cmol \cdot kg^{-1}$]	11.5
Pb [$mg\ kg^{-1}$]	Not detected
Zn [$mg\ kg^{-1}$]	193

Uptake of metals (Pb and Zn) by Brassica Juncea. The contaminated soil was used to fill 500ml plastic pots (140 g soil per pot) and moistened with ultrapure water to reach approximately 80% water holding capacity. Each pot was planted with five Brassica Juncea seeds, and germinated in the plant environmental control system under the controlled conditions as shown in Table 3. The nutrient solution above-mentioned was used as basal fertilizers to supply nitrogen (N), phosphate (P) and potassium (K) for plants. Pots were watered every 2 days with 20 ml of the nutrient solution and ultrapure water according to water loss by weight to maintain 80% of water holding capacity. Following seedling emergence, the pots were thinned to one plant per pot. After 25 days of growth, EDTA and EDDS were added at 0.5, 1.5 and 3.0 mmol kg^{-1} soil, then 0.5 mmol EDTA and EDDS per kg soil were added again in Pot No.1 and Pot No.4 in next two weeks (one time every week), respectively. On the other hand, no chelators were amended in Pot No.7 (contaminated by Pb and Zn) and Pot No.8 (without contamination) which were kept as a control and as a blank, respectively (Table 4). Each treatment was performed in duplicate with a random block design; thus, 16 pots were used. Chelators were applied to the soil surface as solutions. Following the application of chelators, watering were performed into soils everyday.

Table 3 Conditions of plant growth in the plant environmental control system

	Temperature [℃]	Relarive humidity [%]	Photon flux density [lx]	Photoperiod [h]
Day	27	60	7500	12
Night	22	60	0	12

Table 4 Conditions of pot experiments

Pot [No.]	Pb, Zn [$mmol \cdot kg^{-1}$]	EDTA [$mmol \cdot kg^{-1}$]	EDDS [$mmol \cdot kg^{-1}$]	Chelator-adding time after seeding [day]
1	5	0.5, 0.5, 0.5	0	25, 32, 39
2	5	1.5	0	25
3	5	3.0	0	25
4	5	0	0.5, 0.5, 0.5	25, 32, 39
5	5	0	1.5	25
6	5	0	3.0	25
7	5	0	0	-
8	0	0	0	-

"-" means no addition of any chelators.

Extraction of Plant Samples and Determination of Pb and Zn. Plants were harvested after 8 weeks growth by cutting stems 1 cm above the soil surface. The above-ground parts were considered as shoot and the parts below the ground were as root. Shoot and root samples were washed carefully with ultrapure water after tap water to remove any soil splash, and were oven-dried at 80℃ for 24 h, and then ground in a mortar. Dried plant samples (0.1g of each sample) were digested with 10 cm^3 conc. HNO_3 (61%) and 3.0 cm^3 conc. HF (46%) in a microwave digestion system for metal analysis. The digested samples were diluted to 100 cm^3, then Pb and Zn were determined by ICP-AES.

Results and Discussion

Concentrations of Zn in Plant. The concentrations of Zn in shoot and root are shown in Fig. 1. By comparing each other, it can be found that adding chelating agents significantly enhanced the concentrations in shoot (except Pot No.4 when adding 0.5 mmol kg^{-1} EDDS separately for three times) and reduced the concentrations in root. Furthermore, the Zn content in root of the control is highest. It suggests that chelating agents (EDTA and EDDS) have the excellent translocation from root to shoot for Zn. The concentrations of Zn were larger when adding equivalent chelating agents at one time (one-time-addition) than when adding at three times separately (three-time-addition) although they were nearly varied in shoot between two additional methods for EDTA. It is considered that the proportion of Zn, existed as the soluble state (the proportion of Zn absorbed by plant) in soil, is higher when one-time-addition method was applied. For this reason, it seems that one-time-addition of chelating agents is more favorable for Zn uptake by plants. In addition, the concentrations of Zn in Pots adding 1.5 and 3.0 mmol kg^{-1} EDDS are higher than those in Pots adding the same level EDTA in shoot, whereas it is opposite in the case of root. It may be considered that EDDS has a stronger translocation for Zn than EDTA.

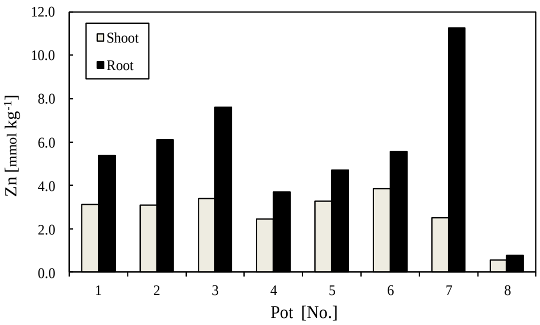

Fig. 1 Concentration of Zn in shoot and root

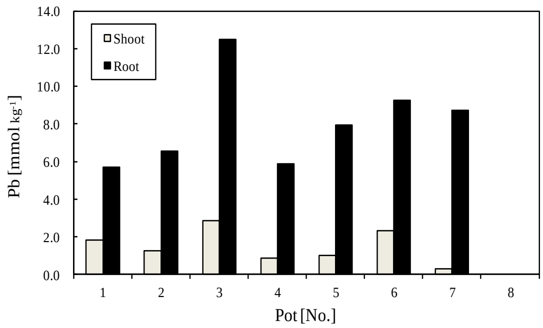

Fig. 2 Concentration of Pb in shoot and root

Concentrations of Pb in Plant. The concentrations of Pb in shoot and root of Brassica Juncea are shown in Fig. 2. As observed in Fig. 2, Pb concentrations in shoot adding 1.5 and 3.0 mmol kg^{-1} EDTA increased by 1.2-fold of those in shoot adding the same level EDDS, which may be due to the difference of the stability constants between two chelating agents with Pb (i.e., $\log K_{Pb\text{-}EDTA}$=17.88 and $\log K_{Pb\text{-}EDDS}$=12.70) [13]. In other words, EDTA has the stronger extractable ability for Pb from soil than EDDS. Considering the uptake effect only, EDTA is more effective for the uptake of Pb in soil, but EDTA-heavy metal complexes have low biodegradation rates, and are toxic to plants and soil microorganisms [8, 14]. In order to achieve phytoremediation techniques with lower environmental risk, EDDS is a more practical chelating agent for phytoremediation of Pb because of its biodegradable and eco-friendly property [15]. The concentrations of Pb in root increased in the following order of adding 3.0 mmol kg^{-1} (Pot No.3 or Pot No.6) > the control (Pot No.7) > adding 1.5 mmol kg^{-1} (Pot No.2 or Pot No.5) > adding 0.5 mmol kg^{-1} separately for three times (Pot No.1 or Pot No.4) for both chelating agents, respectively. As the case of Zn, the concentrations of Pb were generally larger when adding chelating agents at one time (one-time-addition) than when adding at three times separately (three-time-addition) except for that in shoot by three-time-addition in case of EDTA. In addition, the translocation of Pb from root to shoot is also observed clearly when chelating agents were amended.

Comparing Fig. 1 with Fig. 2, it is found that the uptake of heavy metals may depend on the dose of chelating agents. That is, the uptake of Pb and Zn is high when the dose of each chelating agent is large. The concentrations of Pb in root were remarkably high and those in shoot were low, comparing with those of Zn in case of adding the same level chelating agents. It is suggested that Pb is more difficulty of being translocated by Brassica Juncea than Zn with the addition of EDTA or EDDS.

Effect of Chelating agents on the Biomass. The shoot and root biomass (dry weight) of Brassica juncea in all Pots after 8 weeks growth are shown in Fig. 3. In the absence of chelating agents, the biomass of the control (Pot No.7) exhibit clearly reduction compared with that of the blank (Pot No.8), and the reduction amount of the shoot and root are about 16% and 33% of the blank biomass, respectively. It indicates that heavy metal (Pb and/or Zn) can inhibit the plant growth. From these experiments, it is also found that the addition of chelating agents along with heavy metals led to a severe reduction of the biomass. Moreover, it is suggested as follows. (1) The biomass in the Pots adding chelating agents are reduced for more than 30% relative to that of the control. The reduction amount of the shoot is almost similar to each other except the Pots adding 3.0 mmol kg^{-1} EDDS. (2) The shoot and root biomass of the Pots adding 3.0 mmol kg^{-1} EDDS is only about 1/3 and 1/5 of that of the control respectively; or 1/2 and 1/4 of that of adding the same concentration of EDTA, respectively. (3) When 1.5 mmol kg^{-1} of EDTA was added to soil, the difference of the biomass was not observed between the adding methods (i.e., Pot No.1 and Pot No.2); however, considering EDDS, the biomass of three-time-addition was slightly lower than that of one-time-addition regardless shoot and root. The similar inhibition was observed between EDTA and EDDS at the lower concentration. However, when the concentrations of chelating agents are higher, EDDS has larger inhibition than EDTA. This suggests that the biomass may mainly depend on the kind of chelating agent regardless of the method of adding chelating agent.

From our other preliminary experiment (Data in the experiment are not shown in this paper), it is confirmed that the addition of enhancing regulators, such as gibberellic acid (GA_3) and indole-3-acetic acid (IAA), improves the biomass in case of Brassica juncea as with the work of Hadi et al.[16] using maize (Zea mays L.). Then, the reduction of the biomass observed in this work can be settled by using regulators such as GA_3 and IAA.

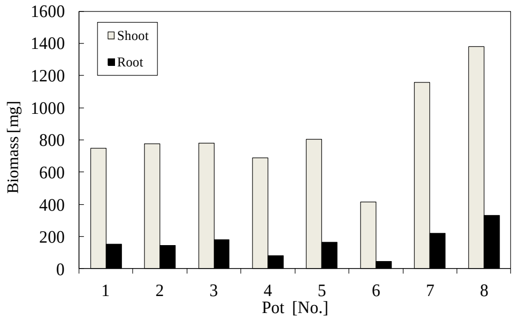

Fig. 3 The shoot and root biomass of Brassica Juncea

Total Uptake Amount of Pb and Zn in Plant. The total uptake amount of metals in shoot and root is calculated according to the following formula:

$$T = C \times M \quad (1)$$

T, the total uptake amount of metal in shoot (or root), μmol
C, the metal concentration in shoot (or root), mmol·kg^{-1}
M, the biomass of shoot (or root), mg

Total uptake amount of Pb and Zn in shoot and root are shown in Fig.4 and Fig. 5. The uptake amount of Zn in the control was highest, following that in the Pots adding EDTA, regardless of the part of plant (i.e., shoot or root). This may be mainly attributed to the high biomass of the control although the shoot concentrations of the Pots adding chelating agents were larger than that of the control. On the other hand, the uptake amount of Pb in the Pots adding 3.0 mmol kg^{-1} EDTA was biggest both in shoot and in root, and were 2.3-fold and 5.5-fold of that in the Pots adding 3.0 mmol kg^{-1} EDDS, respectively. These results suggest that EDTA is more effective for the accumulation of Pb and Zn from soil than EDDS as with the work of Tandy et al. [15]. The amount of Pb in shoot was obviously elevated in the Pots when adding EDTA at three times separately (three-time-addition) in contrast to adding at one time (one-time-addition). On the contrary, the result was opposite for EDDS. It is suggested that adding the same amount of EDTA in three divided doses is more beneficial for the translocation of Pb than adding at one time.

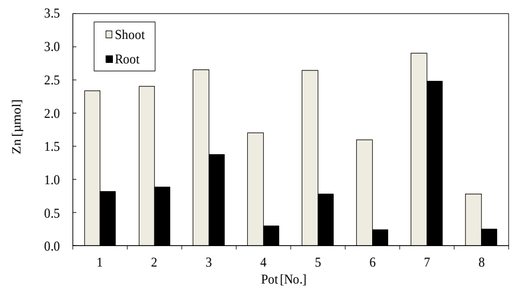

Fig. 4 Total uptake amount of Zn in shoot and root

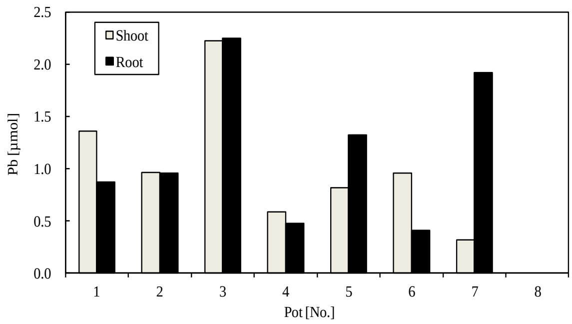

Fig. 5 Total uptake amount of Pb in shoot and root

Conclusions

Effect of EDTA and EDDS on phytoremediation of Pb and Zn in soil by Brassica Juncea was investigated. Consequently, the following matters have been obtained:

(1) Both EDTA and EDDS significantly enhanced the translocation of metals (Pb and Zn) in soil from root to shoot, especially the translocation of Pb by EDTA is remarkable. Furthermore, the two chelating agents led to sharply loss of biomass for more than 30%. As a result, the total uptake amount of metals by plant was decreased (except the uptake of Pb in the case of Pots adding 3.0 mmol kg^{-1} EDTA).

(2) EDDS exhibits the higher inhibition for the plant growth than EDTA. However, EDDS will be a good substitute for EDTA from the viewpoint of phytoremediation if the inhibition of the biomass is improved.

(3) Adding chelating agents at one time (one-time-addition) is generally more efficient than adding at three times separately (three-time-addition) for the uptake of Pb and Zn from soil, especially in case of using EDDS. It is suggested that adding at several times separately does not necessarily increase uptake of heavy metals.

The data obtained and the method used in this work can be useful for the remediation of metal polluted soils in future work.

References

[1] Information on http://www.env.go.jp/water/report/h19-04/02.pdf (In Japanese)
[2] Information on http://www.maroon.dti.ne.jp/bandaikw/news/metal/japan/japanmetal.htm (In Japanese)
[3] Mitch M. Lasat: Journal of Environmental Quality, Vol. 31 (2002), p. 109-120
[4] Changcun Lin, Jun Liu, Li Liu, Tingcheng Zhu, Lianxi Sheng and Deli Wang: Environmental and Experimental Botany, Vol. 65, No.2-3 (2009), p.410-416
[5] Y. Ma, M.N.V. Prasad , M. Rajkumar and H. Freitas: Biotechnology Advances, Vol. 29, No.2 (2011), p. 248-258
[6] Nicoletta Rascio and Flavia Navari-Izzo: Plant Science, Vol. 180, No.2 (2011), p.169-181
[7] E. Lombi, F.J. Zhao, S.J. Dunham and S.P. McGrath: J. Environ. Qual. Vol. 30 (2001), p.1919-1926
[8] L.H.Wu, Y.M. Luo, X.R. Xing and P. Christie: Agric. Ecosyst. Environ, Vol. 102 (2004), p.307-318
[9] A.K. Gupta and S. Sinha: J. Harzard. Mater. Vol. B136 (2006), p.371-378
[10] N. Kano, Y. Otsuki, H. Lu and H. Imaizumi: Journal of Ecotechnology Research, Vol. 13, No.2 (2007), p.79-84
[11]Information on http://www.otsuka.agritechno.jp/products/contents/x0121_con.html#YODOKOHIRYO (In Japanese)
[12] Lidi Gao, Naoki Kano, Yuichi Sato, Chong Li, Shuang Zhang and Hiroshi Imaizumi: Bioinorganic Chemistry and Applications, ID number: 173819, in press

[13] Z. Z. An, Z. C. Huang, M. Lei, X. Y. Liao, Y. M. Zheng and T. B. Chen: Chemosphere, Vol. 62 (2006), p.796
[14] Grcman, H., Vodnik, D., Velikonja-Bolta and S., Lestan: J. Environ. Qual. Vol. 32 (2003), p.500-506
[15] Susan Tandy, Rainer Schulin and Bernd Nowack: Chemosphere, Vol. 62 (2006), p.1454-1463
[16] Fazal Hadi, Asghari Bano, Michael P. Fuller: Chemosphere, Vol. 80 (2010), P.457-462