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Chapter 18

ACCUMULATION OF HEAVY METALS BY CUCUMBER AND *BRASSICA JUNCEA* UNDER DIFFERENT CULTIVATION CONDITIONS

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Abstract: Pollution by heavy metals from industries, the storage of polluted wastes, and agricultural fertilizer pose a serious threat to human health. These pollutants may pass into the soil where plant uptake or leaching to groundwater can contaminate the food chain. Phytoremediation is the technique for removing contaminants in the environment by plants, and is being currently researched world-wide. Evapotranspiration is also responsible for moving contamination into the plant shoots. Because contamination is translocated from the roots to the shoots, which are harvested, contamination is removed while leaving the original soil undisturbed. Some plants that are used in phytoextraction strategies are termed "hyperaccumulators", which are plants that achieve a shoot-to-root metal-concentration ratio greater than one. *Brassica juncea* is well-known as Pb hyperaccumulator. Cucumber is also requently used to study the transportation mechanism of heavy metals, because its sap is easy to collect. This study focused on whether or not the cultivation temperature of the plants relates to the transportation of heavy metal. Using Cucumber and *Brassica juncea*, plants were cultivated under hydroponics with Pb (60 to 600 ppm). The cultivation temperature was changed from 8 degrees to 30 degrees for 96hours. We measured Pb concentration and GSH concentration and protein concentration every 24 hours. Pb concentration was measured by AAS after being ashed with a microwave system. GSH and protein concentrations were measured after crushing with liquid nitrogen by HPLC. At 25 degrees, the amount of absorption of lead in the cucumber was at maximum. However for *Brassica juncea*, the amount of absorption of lead was at maximum at 12 degrees. This result shows that the heavy-metal accumulation time differs in each plant. It was shown also that GSH and protein concentration were related closely to the accumulation of heavy metal and to the cultivation temperature.

Key words: Phytoremediation; heavy metals; Cucumber; *Brassica juncea*; Pb; GSH; Amino acid; Hly

1. INTRODUCTION

Phytoremediation is the general term for the environmental purification technology that uses plants to remediate soils. Phytoextraction is a particular remediation method used to remove heavy metal from polluted soils. There are two methods of phytoextraction (accumulation of heavy metal by a plant). In the first method, a chelating agent is added to the soil when the biomass of the plant body has almost matured at which time the chelating agent can absorb the heavy metals rapidly. In the second method, the plant is allowed to continuously absorb heavy metals depending on the growth stage (Salt et al., 1998).

The first method using chelating agents is effective for heavy metals (lead or cadmium) which are insoluble in a conventional soil environment. Chelating agents used are citric acid of an organic acid as well as EDTA or EGTA. The addition of a chelating agent can increase the accumulation ability in plants where the natural accumulation rate is comparatively low. For example, the soil can absorb 0.01-0.06% lead in a dry weight without a chelating agent: with the addition of a chelating agent, it can suck up lead more than 1% lead in a dry weight (Huang et al., 1997, 1996; Blaylock et al., 1997). However, it is dangerous to add excessive amounts of chelating agents as they allow heavy metal immobilized by rain to invade ground water.

The second method of phytoextraction, which allows the plant to continuously absorb heavy metals, often uses a hyperaccumulator to accumulate heavy metal.

As has been shown, the accumulation stage of heavy metal in plants is important for phytoextraction. Both cultivation condition and temperature are thought to be important factors in the accumulation of vegetal heavy metal in particular. For example, a seasonal variation is suggested for *Artemisia princeps*, given the migration pattern of copper from subterranean root to above-ground shoot. (Takeda et al., 2004).

In this study, we investigated how cultivation conditions were related to the accumulation of heavy metals by using *Brassica juncea* and cucumber. From the early spring to the beginning of summer, *Brassica juncea* is a viable lead accumulation plant. In contrast, the cucumber is a non-accumulation plant, but the collection of sieve-tube fluid and excretory-duct fluid is unproblematic and the heavy metal displacement configuration from subterranean root to above-ground plant is easy to study.

2. MATERIAL AND METHODS

2.1 Plants

A cucumber (*Cucumis sativus*, Takii Seeds, Kyoto, Japan) was germinated with 0.5mM CaCl2 solution, and pre-incubated for one week at 25 degrees Celsius, in a dark period of 12 hours and a light period of 12 hours. *Brassica juncea* (obtained from the Kizu River) was germinated with vermiculite, and pre-incubated by using Hoagland solution (Hoagland et al., 1938) for one week at 25 degrees Celsius, in a dark period for 12 hours and a light period for 12 hours.

2.2 A variation of lead concentration with culture temperature

For four days, we cultivated one of each plant (Light 12 hours, Dark 12 hours), each in a lead solution (a cucumber, 60ppm $Pb(NO_3)_2$, *Brassica juncea* 600ppm $Pb(NO_3)_2$ component 1/2Hoagland aqueous solution) at 8 degrees Celsius; 12 degrees Celsius; 25 degrees Celsius; and 30 degrees Celsius. We measured lead concentration in each plant body every day. We then used nitric acid and H_2O_2 and did wet ashing in a microwave oven after drying the plants in dry oven at 80 degrees Celsius for 24 hours. The lead concentration was measured using atomic absorption spectrophotometry.

2.3 The measurement of GSH (reduced glutathione) concentration and amino acid concentration of a cucumber

We added 10 mM Tris-Buffer (pH8.5) and crushed root at 25 degrees Celsius and 30 degrees Celsius after freezing it in liquid nitrogen, then collected the supernatant by centrifugation (1. 1200g, 15 minutes, 4 degrees Celsius; 2. 1200g, 5 minutes, 4 degrees Celsius). The supernatant GSH and amino acids were measured using 100 μM 5,5'-dithiobis-(2-nitrobenzoic acid) solution for GSH, and an Ez faast Amino Acid Kit for the amino acid.

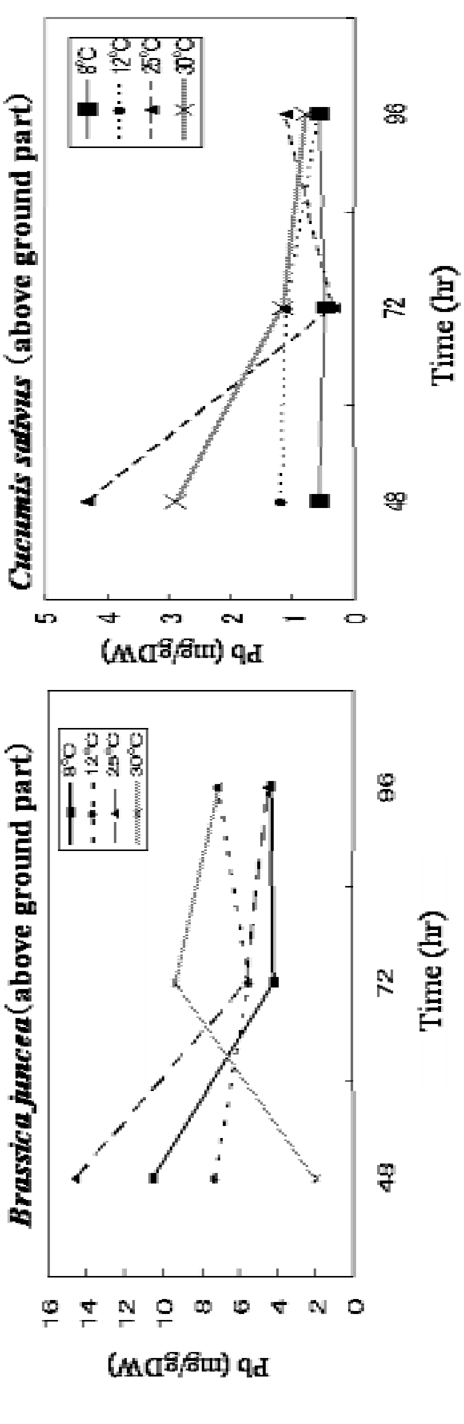

Figure1. Variation of lead concentration with culture temperature lead concentration.

Brassica juncea **600 ppm;** ***Cucumis sativus*** **60 ppm.**

3. RESULTS AND DISCUSSION

3.1 A variation of lead concentration with culture temperature

After 48 hours, at 25-degrees Celsius, both plants show a high accumulation of lead. At 30-degrees Celsius, the cucumber showed a higher accumulation. After 96 hours, the cucumber is the same temperature in all four plants. It is thought that the mechanism, which does not absorb lead intentionally by having shown approximately the same quantity of accumulation works. Unlike the cucumber, *Brassica juncea* showed a high accumulation at a temperature level of 8 degrees Celsius and at 12 degrees Celsius. By 72 hours at 30 degrees Celsius, accumualtion gradually increased in *Brassica juncea*. Both the cucumber and *brassica juncea* had a similar accumulation and in both plants the accumulation decreased over time. But *Brassica juncea* maintained some lead accumulation. Also, there was a variation in the quantity of lead accumulation at 12 degrees Celsius area through the experimental period. For foreign material such as lead, an accumulation plant, such as *Brassica juncea*, does detoxication positively to understand it from two kinds of vegetal differences, and the mechanism that can store some lead is present in the above ground part. In contrast, it is thought that only the discharge, or the non-absoption mechanism, works with a non-accumulation plant such as a cucumber. In addition, it is suggested that temperature can become an important factor in the detoxication mechanism of an accumulation plant. See Figure 1.

3.2 GSH (reduced glutathione) concentration and amino acid concentration of a cucumber

An increase of GSH in comparison with control in lead exposition area was revealed when quantity of GSH calculated increase rate according to an area for 1 in normal GSH (Figure 2). Table 1 showed the content of detected amino acid. The roots of control and experimental area of 25 degrees Celsius, and control and control of 30 degrees Celsius were measured. The glutamic acid and the cysteine which synthesized glutathione were not detected, but hydroxylysine (Hly) decreased in comparison with the control in the lead exposition experiment area. Hly is an amino acid constituting collagen. Hly was used with a part of defense action for lead, and it was suggested that it decreased.

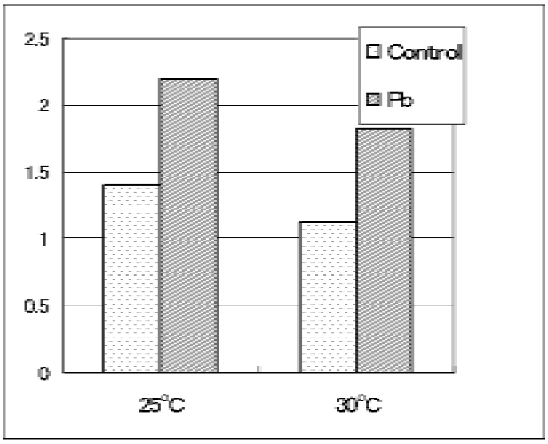

Figure 2. GSH concentration of a cucumber.

Table 1. Concentration of Amino acids in Cucumber roots incubating at 25 and 30 degrees Celsius for 72 hours

Amino acid nmol/g DW	**25°C**		**30°C**	
	control	**Pb**	**control**	**Pb**
THR	3150	916	8475	7261
SER	8961	3822	ND	4607
ASP	ND	ND	160051	ND
HIS	ND	ND	ND	4976
PRO	2708	2979	ND	2705
HLY	3717	2162	23016	9636
TLY	ND	ND	18054	ND

ND: Not detected
Control is incurvated with ½ Hoagland aqueous solution,
Pb is incuvated with 60 ppm $Pb(NO_3)_2$.

4. CONCLUSION

An absorption system of heavy metal works in the resistance mechanism of heavy metal and completely different pathway, and it is thought that hyperaccumulator and accumulator usually develop than a plant in detoxication mechanism of heavy metal. We suggested that cultivation temperature became an important factor by these investigational processes. Accumulation mechanism is not clarified, however, cannot absorb a plant easily because actually mobility of heavy metal in the soil is low. For example, *Thlaspi caerulescens* known as hyperaccumulator of Zn did not show a correlation between Zn content and plant accumulation Zn quantity in the soil (Knight et al., 1994). However, it is the dose which is important whether it let extracted quantity of Zn in soils and absorbed dose extract heavy metal how from the soil by being correlative (Romheld, 1991). We suggested that potential accumulation class was present in a herb plant (Takeda et al., 2005). The result can become a help to clarify an accumulation mechanism of heavy metal from such a point of view.

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REFERENCES

Salt, D.E., Smith, R.D., and Raskin, I., 1998, Phytoremediation, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49. 643-668.

Huang, J. W, Chenn, J. J., Berti, W. R., and Cunninghum, S. D., 1997, Phytoremediation of lead-containated soils: role of synthetic chelates in lead phytoextraction,, *Environ. Sci. Technol.* 31, 800-805.

Huang, J. W., and Cunninghum, S. D., 1996, Lead phytoextraction: species variation in lead uptake and translocation. *New Phytol.* 134: 75-84.

Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., 1997, Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technol.* 31, 860-865.

Takeda, R., Yoshimura, N., Matsumoto, S., Komemushi, S., and Sawabe, A., 2004, Accumulation of Heavy Metals by Japanese Weeds and their Seasonal Movement, *Contaminated soils, Sediments and Water: Science in the Real World*, Vol. 9, eds. by Calabrese E. J., Kostecki,, P. T. and Dragun, J., Springer, USA, pp. 349-359, ISBN 0-387-23036-X.

Hoagland, D. R., and Arnon, D. I., 1938, The water-culture method for growing plants without soil. *Univ. Calif. Coll. Agric. Exp. Sta. Circ.* Berkeley, CA, 347-353.

Knight, B, Zhao, F. J., McGrath, S. P., and Shen, Z. G., 1994, Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* in contaminated soils and its effects on the concentration and chemical speciation of metals in soil solution, *Plant* ***Soil*** 197**,** 71–78.

Romheld, V., 1991, The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: an ecological approach, *Plant* ***Soil*** 130, 127–134.

Takeda, R., Komemushi, S., and Sawabe, A., 2005, Phytoremediation- Investigation of a high plant of ability for environmental purification system-, *J. Environ. Cont. Tech.*, **.**23, 49-56 (in Japanese).