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Investigation of heavy metal accumulation in *Polygonum thunbergii* for phytoextraction

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"Capsule": *Polygonum thunbergii* is useful to remove heavy metal from soil and water.

Abstract

In this study, cadmium (II), lead (II), copper (II) and zinc (II) were determined in *Polygonum thunbergii* and soil from the Mankyung River watershed, Korea. Soil samples contained detectable lead (<17.5 μg g^{-1}), copper (<8.4 μg g^{-1}) and zinc (<24.5 μg g^{-1}), whereas cadmium was undetectable. Whole plants of *P. thunbergii* contained detectable lead (<320.8 μg g^{-1}), copper (<863.2 μg g^{-1}) and zinc (<2427.3 μg g^{-1}), whereas cadmium was detectable only in the stem (<7.4 μg g^{-1}) and root (<10.1 μg g^{-1}). Whole plant concentrations were very different for each metal, particularly in the case of zinc. The mean content of heavy metal in the whole plants increased in the order of cadmium (8.5 μg g^{-1}) < lead (183.3 μg g^{-1}) < copper (548.1 μg g^{-1}) < zinc (1506.7 μg g^{-1}). Soil lead, copper and zinc were correlated with each metal's accumulation in the plants (lead, $r=0.841$, $P<0.005$; copper, $r=0.874$, $P<0.001$; zinc, $r=0.770$, $P<0.005$). Lead content in roots and leaves was highly correlated ($r=0.5529$, $P<0.001$), as was lead content in roots and stems ($r=0.5425$, $P<0.001$). Mean bioconcentration factors for the aboveground tissues were 4.2 (lead), 14.8 (copper) and 27.7 (zinc), and for the underground tissues, were 22.2 (lead), 92.9 (copper) and 62.7 (zinc). After hydroponic growth, bioaccumulation coefficients were 2.0 (cadmium), 3.2 (lead), 17.2 (copper) and 13.1 (zinc) for whole plants. We considered these results as indicative of the ability of *P. thunbergii* plants to take up metal ions from a soil matrix contaminated with heavy metals.

Keywords: Phytoextraction; *Polygonum thunbergii*; Heavy metals; Bioconcentration factor; Bioaccumulation coefficient

1. Introduction

In biological aspect, heavy metal is a connotation of toxicity as contaminant in ecosystem. The major industrial heavy metal contaminants, that is, cadmium, mercury, lead, chrome, copper and zinc, remain as dissolved phase in water and soil. Heavy metal contamination commonly results from anthropogenic activities such as mining and smelting, metalliferous electroplating, internal combustion engine operating, energy and fuel production, fertilizer and pesticide application, and the generation of municipal waste. Moreover, these activities have concentrated some of heavy metals in certain areas up to the dangerous levels of living organism (Chatterjee and Chatterjee, 2000). On the one hand heavy metals absorbed through the root systems induce chlorosis of leaf, deficiency of essential elements, and inhibition of root penetration and growth, but on the other hand heavy metals are safely accumulated by binding to cysteine-rich proteins or peptides (e.g. phytochelatins) in plants. The accumulation of heavy metals through the root systems and subsequent release of metals during decomposition represents a recycling of heavy metals in the ecosystem. Such a pathway could have an important effect on the level of toxic metals in surface soil and water. The mode of accumulation of heavy metals by a variety of plant species has been studied by a number of investigators. These studies have focused on the uptake and phytotoxic effects of heavy metals (Jain et al., 1989a, b, 1990; Sen and Mondal, 1990; Scott, 1992), the correlation between the content of heavy metals in the soil and the amount absorbed by

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bush beans (Jackson et al., 1990) and the relationship between the partitioning of heavy metals in lake sediment and their availability to the yellow water lily (Campbell et al., 1985). Recently there has been considerable interest in the use of plants as a green technology for the remediation of surface soil and water contaminated with toxic metals, and the potential of heavy metal-accumulating plants for environmental remediation has been fully realized. This technology is termed phytoextraction (Nanda Kumar et al., 1995; Salt et al., 1995; Blaylock et al., 1996). The process of phytoextraction generally requires the translocation of heavy metals to the easily harvestable plant parts. This study aims (1) to illuminate the relationship between the content of heavy metal accumulated by *Polygonum thunbergii* which was naturally grown along the riversides, and heavy metal content in the surface soil of the habitat; (2) to assess the bioavailability of heavy metals within this plants, thus allowing assessment of the potential use for the elimination of heavy metals from the surface soil of the riverside (i. e. phytoextraction).

2. Materials and methods

2.1. Study area and sample collection

The Mankyung River watershed is located in west Korea, and includes Jeonju (26.3 km), Samchon (10.4 km), Bongdong (11.4 km), Soyang (7.5 km) stream and Mankyung (25.3 km) River. The geographical position is 36°00′ N, 127°00′ E, and the total area of this watershed is 1047 km^2. Particularly, Jeonju stream passes through the industrial area and the downtown of Jeonju city, and its upper area contains an abandoned mine, Duckon mine (Fig. 1). This mine had been digging for

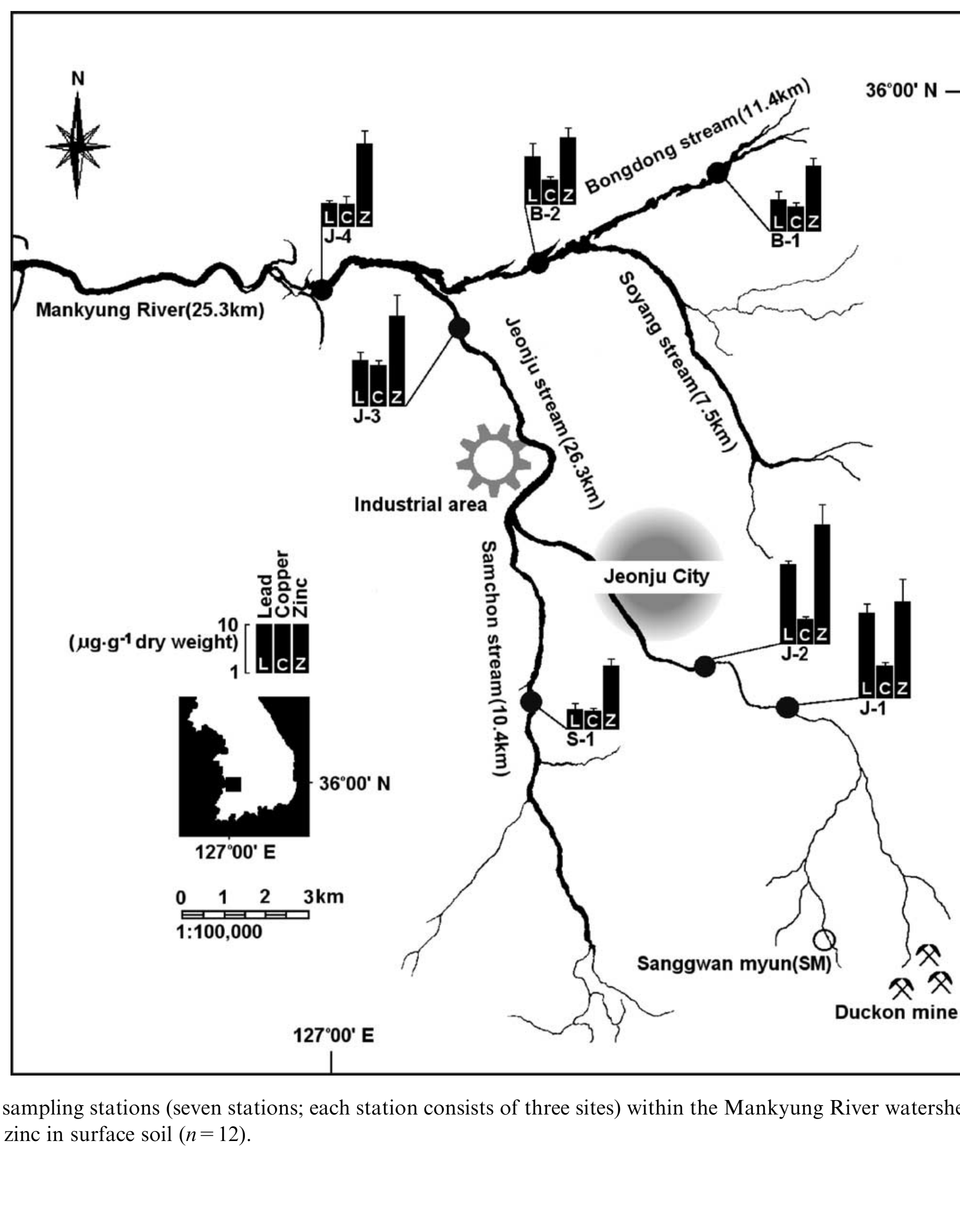

Fig. 1. Location of sampling stations (seven stations; each station consists of three sites) within the Mankyung River watershed. Bars indicate levels of lead, copper and zinc in surface soil ($n = 12$).

silver, copper and lead for 6 years (from 1971 to 1976), and after that it would be leaved without follow-up measures (from Korea Institute of Geoscience and Mineral Resources). The average of the total precipitation of this area in a year during the past 30 years was 1286 mm and the average temperature was 13.0 °C. Generally most of the precipitation comes in the rain period from July to September (from Korea Meteorological Administration). We selected this watershed because there are a variety of possible heavy metal sources on one side and agricultural areas on the other side of this watershed as above stated. In four tributaries of Mankyung River watershed, dominant aquatic plants were *Polygonum thunbergii*, *Phragmites japonica*, *Trapa japonica* and *Nymphoides peltata*, and especially *Polygonum thunbergii* was widely distributed over the riparian zones of Jeonju stream passing through the downtown area and the industrial area of Jeonju City (Kil, 1992). We selected *Polygonum thunbergii* (Polygonaceae). We choose this species because it grows ubiquitously and vigorously in this polluted area. *P. thunbergii* also has a relatively fast growth rate and a high productivity. Moreover, this species can easily regenerate from the parts of parental plant. Plants (about 11.5–47.2 g/individual) of *P. thunbergii* were carefully uprooted, and a sample of surface soil (after removal of the top 3 cm) was collected weekly from each plant's habitat. The plant and soil samples were collected from each sampling station from July to October 1994. Collected samples were immediately frozen with dry ice and transferred to the laboratory for heavy metal analysis. For the bioaccumulation experiment, plant samples (about 5.75–6.74 g/individual) of *P. thunbergii* were collected at Sanggwan myun (SM; Fig. 1), a relatively pristine area in this watershed, and acclimated in modified Hoagland's solution [macronutrients 5mM KNO_2/5 mM $Ca(NO_3)_2{\cdot}4H_2O$/2 mM $MgSO_4{\cdot}7H_2O$/1 mM KH_2PO_4, micronutrients 2.4 μM $MnCl_2{\cdot}2H_2O$/9 μM H_3BO_3/1.5 μM KI/1.2 μM $Na_2MnO_4{\cdot}H_2O$/13 μM $Fe_2(C_4H_4O_6)_3$ and pH 6.50] for 2 weeks, before beginning the bioaccumulation experiment.

2.2. Bioaccumulation experiment

To compare and assess the bioaccumulation of cadmium, lead, copper and zinc in *P. thunbergii* exposed to these metals under laboratory conditions, *P. thunbergii* were selected for uniformity from Sanggwan myun (SM, Fig. 1) streamsides, and were hydroponically grown in modified Hoagland's solution (pH 6.50) containing one of the following heavy metals: cadmium [44 mg l^{-1}, supplied as $Cd(NO_3)_2{\cdot}4H_2O$], lead [82mg l^{-1}, supplied as $Pb(NO_3)_2$], copper [26mg l^{-1}, supplied as $Cu(NO_3)_2{\cdot}3H_2O$] and zinc [25 mg l^{-1}, supplied as $Zn(NO_3)_2{\cdot}6H_2O$]. Plants were grown in a growth chamber (temperature; 25–30 °C, relative humidity; 60–70%, photon fluxes; 1200–1300 μmol·m^{-2} s^{-1}, daylength: 15 h light/9 h dark) for 6 days. Heavy metal contents were measured at 24 h intervals.

2.3. Heavy metal analysis

Plant samples were dried at 180 °C for 12 h and ground with a mortar and pestle. Plant samples (2 g dry weight) were digested with 20 ml nitric acid and 10 ml hydrogen peroxide, and impurities were removed by filtration. Soil samples were dried at 70 °C for 24 h and passed through a 600 μm nylon sieve. Ten grams of dry soil were then digested with 20 ml nitric acid, 10 ml hydrogen peroxide, 5 ml hydrochloric acid and 5 ml sulfuric acid, and impurities were removed by filtration. The final volume of each sample solution was made up with deionized water, and analyzed by Trace Element Analyzer 3000 (TEA 3000, Camtronics Co. Ltd., Australia) using the stripping voltametric method (Mann and Lintern, 1983, 1984; Sambamoorthy et al., 1985; Florence et al., 1987). For the statistic analysis of data, we used the SAS statistic software package (Release 8.02), and an index of the association of two quantitative variables was expressed by Pearson product–moment correlation coefficient (r).

3. Results

3.1. Field investigation

3.1.1. Heavy metal content in surface soil

All soil samples from the Mankyung River watershed had detectable lead (Pb^{2+}), copper (Cu^{2+}) and zinc (Zn^{2+}) whereas cadmium (Cd^{2+}) was not detected in study sites (Table 1). The mean zinc content (17.2±1.95 μg g^{-1}) was higher than lead content (9.8±1.67 μg g^{-1}) or copper content (5.5±0.59 μg g^{-1}). The highest lead (17.5±1.81 μg g^{-1}) and zinc (24.5±3.05 μg g^{-1}) levels were detected in the surface soil of station J-1 and J-2, and the copper content was highest (8.4±0.90 μg g^{-1}) at station J-3 along the Jeonju stream (Fig. 1). These heavy metal levels were relatively high at the stations of the Jeonju stream compared with the stations of the other stream areas.

3.1.2. Heavy metal content in P. thunbergii

The levels of cadmium, lead, copper and zinc accumulated by *P. thunbergii* are presented in Table 1. Whole plants of *P. thunbergii* throughout the Mankyung River watershed contained detectable lead, copper and zinc, whereas cadmium was detected only in the stem and root samples from Station J-1, J-2, J-3, J-4 and B-1. Levels of cadmium, lead, copper and zinc contents in *P. thunbergii* ranged from 4.0 to 17.5 μg g^{-1}, 101.6 to

Table 1
Heavy metal contents in the surface soil and the leaf, stem and root of *P. thunbergii*, and bioconcentration factors (BCFs) determined ($n = 12$) (μg heavy metal g^{-1} dry weight)

Station	Heavy metal	Aboveground		Underground	ΣHeavy	Surface	BCF[b] underground
		Leaf	Stem	Root	metals[a]	Soil	(aboveground)
J-1	Cd^{2+}	ND	1.6±0.35	2.4±1.01	1.6	ND	–
	Pb^{2+}	21.6±6.41	13.8±3.21	100.4±22.50	35.4	17.5±1.81	5.7 (2.0)
	Cu^{2+}	29.7±12.48	47.8±10.86	270.9±73.80	77.5	6.6±0.72	41.0 (11.7)
	Zn^{2+}	176.9±5.29	119.4±29.56	439.9±114.93	296.3	20.0±1.21	22.0 (14.8)
J-2	Cd^{2+}	ND	7.4±2.85	10.1±1.61	7.4	ND	–
	Pb^{2+}	17.7±5.86	8.2±3.32	75.7±18.12	25.9	16.4±0.95	4.6 (1.6)
	Cu^{2+}	24.3±2.35	35.7±10.80	364.6±51.50	60.0	5.1±0.47	71.5 (11.8)
	Zn^{2+}	256.9±98.13	139.1±22.49	683.3±188.88	396.0	24.5±3.05	27.9 (16.2)
J-3	Cd^{2+}	ND	2.2±0.62	2.7±0.81	2.2	ND	–
	Pb^{2+}	14.6±2.55	13.2±2.97	110.4±31.57	27.8	9.4±1.21	11.7 (3.0)
	Cu^{2+}	37.3±9.77	68.2±14.57	468.3±149.80	105.5	8.4±0.90	55.8 (12.6)
	Zn^{2+}	418.8±76.74	320.6±114.4	1687.9±584.35	739.4	18.5±1.16	91.2 (40.0)
J-4	Cd^{2+}	ND	3.5±1.25	5.4±1.70	3.5	ND	–
	Pb^{2+}	24.9±6.08	25.6±7.78	270.3±115.87	50.5	4.7±0.59	57.5 (10.7)
	Cu^{2+}	35.0±7.50	98.1±7.19	614.6±194.94	133.1	4.6±0.23	133.6 (28.9)
	Zn^{2+}	502.0±114.29	443.9±133.31	657.2±75.43	945.9	17.0±2.39	38.7 (55.6)
B-1	Cd^{2+}	ND	3.0±1.11	4.0±1.99	3.0	ND	–
	Pb^{2+}	14.2±5.39	8.5±3.32	113.3±23.20	22.7	6.5±1.64	17.4 (3.5)
	Cu^{2+}	34.2±11.40	37.3±10.99	333.7±93.09	71.5	4.9±0.57	68.1 (14.6)
	Zn^{2+}	179.3±63.53	131.7±35.37	503.4±170.46	311.0	13.5–2.63	37.3 (23.0)
B-2	Cd^{2+}	ND	ND	ND	–[c]	ND	
	Pb^{2+}	22.9±7.36	17.3±6.17	244.6±86.68	40.2	9.8±4.88	22.9 (4.1)
	Cu^{2+}	28.7±10.75	49.6±10.28	784.9±120.26	78.3	4.9±0.70	160.2 (16.0)
	Zn^{2+}	223.6±35.15	163.3±45.43	1866.3±481.21	386.9	13.7±1.95	136.2 (28.2)
S-1	Cd^{2+}	ND	ND	ND	–	ND	–
	Pb^{2+}	15.3±4.79	4.2±0.85	146.3±34.60	19.5	4.1±0.62	35.7 (4.8)
	Cu^{2+}	22.0±9.97	7.8±1.59	444.1±82.15	29.8	3.7±0.56	120.0 (8.1)
	Zn^{2+}	164.6±65.18	50.7±19.84	1117.9±308.22	215.3	13.1±1.25	85.3 (16.4)

ND: nondetectable.
[a] The sum of the content of each heavy metal content in the leaf and stem of *P. thunbergii*.
[b] BCF is the ratio microgram of heavy metal/g dry weight of plant to microgram of heavy metal/g dry weight of soil.
[c] Not calculated.

320.8 μg g^{-1}, 348.4 to 863.2 μg g^{-1} and 736.2 to 2427.3 μg g^{-1}, respectively (Fig. 2). The highest cadmium, lead and zinc contents were observed in plants from Jeonju stream (station J-2, J-4 and J-3) and the copper content was highest at Bongdong stream (station B-2). Mean levels of lead and zinc contents in *P. thunbergii* increased in the order of stem < leaf < root whereas in the case of copper, increased in the order of leaf < stem < root (Fig. 3). Mean content of cadmium in the stem and root of *P. thunbergii* were 3.5±1.24 and 4.9±1.42 μg g^{-1}, but was not detected in the leaf (Table 1). Translocation ratios ($[HM_{Leaf\ or\ Stem}]/[HM_{Root}]$) of copper and zinc from root to stem or leaf were calculated for each heavy metal (HM). In the case of stem, the translocation ratios ranged from 0.03 to 0.14 (mean±S.D.; 0.11±0.021) for lead, 0.02 to 0.18 (0.14±0.033) for copper and 0.05 to 0.68 (0.32±0.202) for zinc, and in the case of leaf, 0.09 to 0.23 (0.16±0.061) for lead, 0.04 to 0.10 (0.08±0.023) for copper and 0.12 to 0.76 (0.43±0.196) for zinc. However, we were unable to calculate the translocation ratio of cadmium from root to leaf because cadmium was not detected in leaves.

3.1.3. *Relationships between heavy metal contents in soil and* P. thunbergii

Correlation coefficients between lead, copper and zinc in surface soil and in *P. thunbergii* were calculated from the field data. Significantly high positive correlations were found between the lead, copper and zinc contents in *P. thunbergii* and the levels of these metals in surface soil from station B-2 as follows: for whole plants, lead

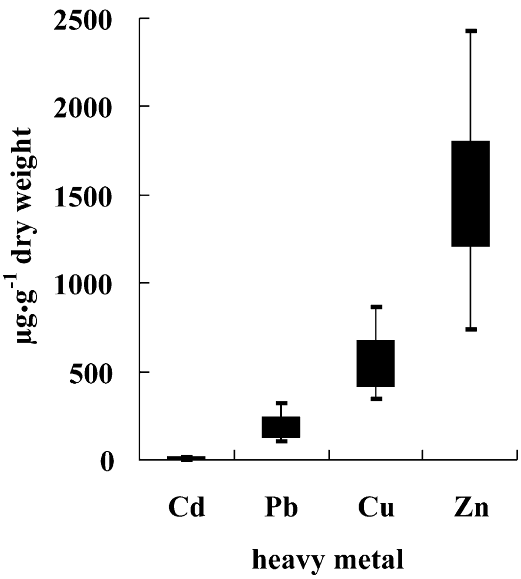

Fig. 2. Range of levels of cadmium, lead, copper and zinc in whole plants of *P. thunbergii* from sampling stations in the Mankyung River watershed ($n = 80$).

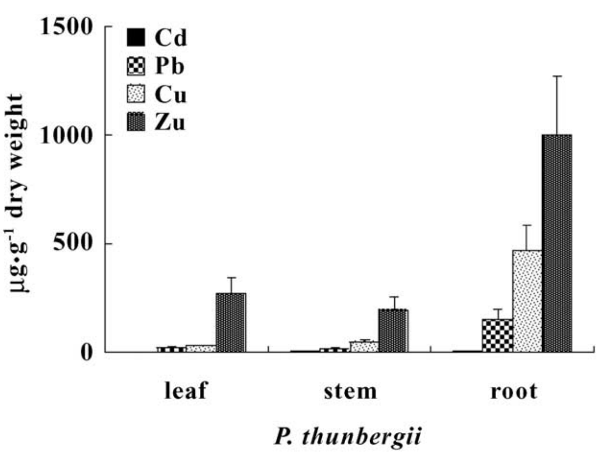

Fig. 3. Comparison of levels of cadmium, lead, copper and zinc in the leaf, stem and root of *P. thunbergii* from sampling stations in the Mankyung River watershed ($n = 80$).

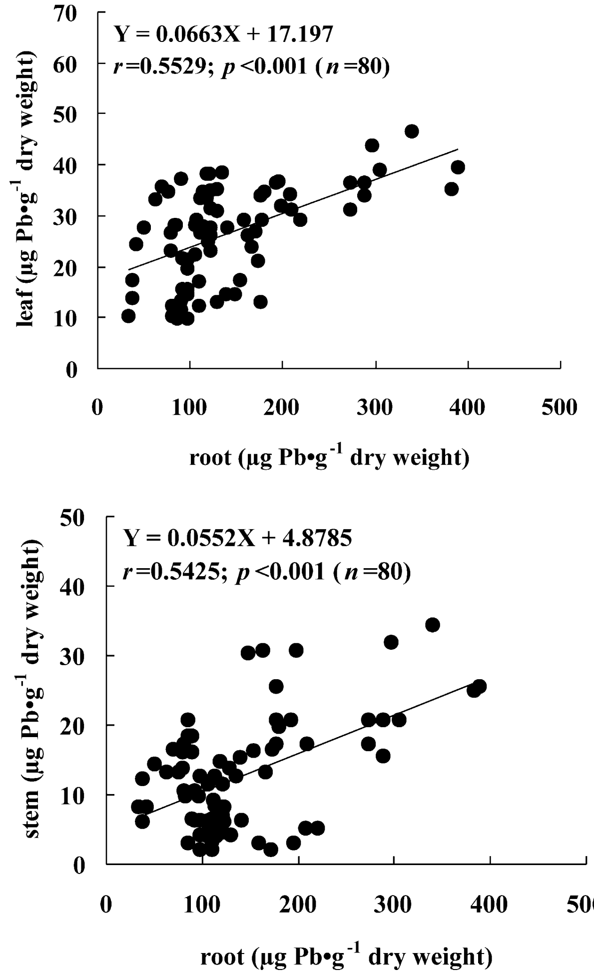

Fig. 4. Relationship between lead content in the root of *P. thunbergii* and in the leaf and stem of *P. thunbergii*.

($r = 0.841$, $P < 0.005$), copper ($r = 0.874$, $P < 0.001$) and zinc ($r = 0.770$, $P < 0.005$) were calculated, and in the case of root, lead ($r = 0.823$, $P < 0.001$), copper ($r = 0.766$, $P < 0.005$) and zinc ($r = 0.789$, $P < 0.005$) were calculated. However, low or non-significant correlations were found from the sites of the other stations (data not shown). Positive correlations were observed between lead content in the root and this metal content in the leaf ($r = 0.5529$, $P < 0.001$, $n = 80$) and the stem ($r = 0.5425$, $P < 0.001$, $n = 80$) (Fig. 4). Whereas no correlation was found between copper and zinc content in the root and these metal contents in the other organ, leaf and stem.

3.1.4. *Bioconcentration factor of* P. thunbergii

The bioconcentration factor (BCF), also known as the plant to soil uptake factor, is the ratio of heavy metal content in the plant or the part of plant (μg g^{-1} dry weight tissue) to soil levels of the heavy metal (μg g^{-1} dry weight soil). The bioconcentration factors of lead, copper and zinc in the aboveground tissues (leaf and stem) ranged respectively from 1.6 to 10.7 (mean; 4.2), 8.1 to 28.9 (14.8) and 14.8 to 55.6 (27.7), and in the underground tissues (roots), from 4.6 to 57.5 (mean 22.2), 41.0 to 160.2 (92.9) and 22.0 to 136.2 (62.7), respectively (Table 1).

3.2. *Laboratory investigation*

3.2.1. *Heavy metal content in* P. thunbergii *after metal treatment*

P. thunbergii did not show visible phytotoxicity for the duration of heavy metal treatment. Metal accumulation by *P. thunbergii* is summarized in Table 2. Cadmium (II), lead (II), copper (II) and zinc (II) accumulation by *P. thunbergii* increased in the sequence

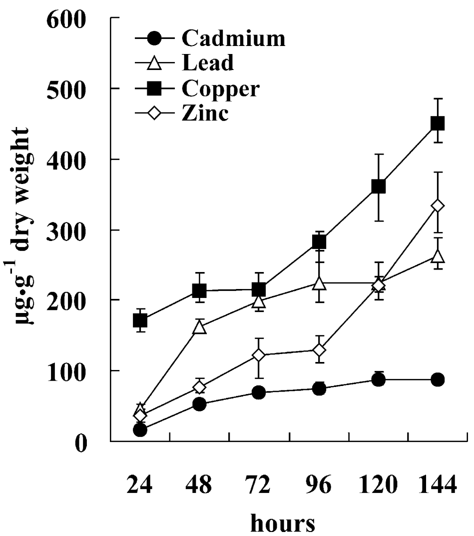

Fig. 5. The time course of heavy metal accumulation in *P. thunbergii* exposed to cadmium (44mg l^{-1} as Cd^{2+}), lead (82 mg l^{-1} as Pb^{2+}), copper (25 mg l^{-1} as Cu^{2+}) and zinc (26 mg l^{-1} as Zn^{2+}) for 144 h. Vertical bars represent standard deviation ($n=3$).

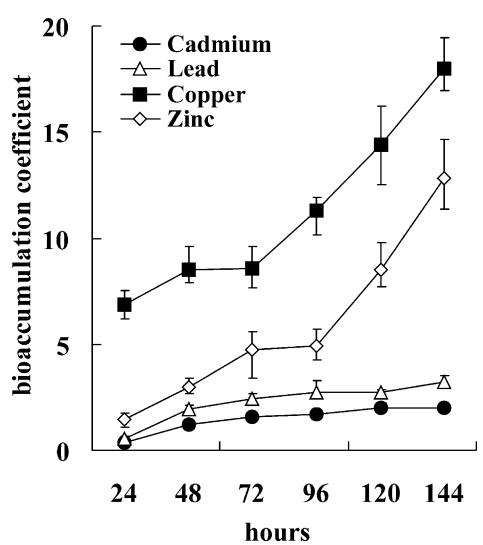

Fig. 6. The time course of bioaccumulation coefficients for cadmium, lead, copper and zinc in *P. thunbergii* exposed to cadmium, lead, copper and zinc supplied in the hydroponics medium for 144 h. Vertical bars represent standard deviation ($n=3$).

of cadmium < lead < zinc < copper, and metal contents in *P. thunbergii* increased continually with time. Specifically, cadmium increased from 16.0±2.05 to 87.7±4.03 μg g^{-1}, lead increased from 46.5±6.61 to 263.1±22.27 μg g^{-1}, copper increased from 171.7±16.26 to 450.2±31.38 μg g^{-1} and zinc increased from 37.2±9.18 to 333.7±43.73 μg g^{-1}, respectively (Fig. 5). Other laboratory studies (Jain et al., 1990; Nanda Kumar et al., 1995) have observed a similar pattern of metal accumulation as *P. thunbergii*.

3.2.2. Bioaccumulaiton coefficient of P. thunbergii *after metal treatment*

Bioaccumulation coefficients (Dushenkov et al., 1995; Oppelt, 2000), the ratio of heavy metal content in plant tissues (μg g^{-1} dry weight) to initial heavy metal concentration in culture solution (mg l^{-1}), varied significantly for each metal. At the each concentration used, copper had the greatest bioaccumulation coefficient, whereas cadmium had the lowest. These bioaccumulation coefficients were not proportional to the initial concentrations of each heavy metal in the *P. thunbergii* culture solution (Fig. 6).

4. Discussion

Our field investigations have shown that heavy metal levels at the Jeonju stream were higher than those of other stream areas. Stations J-1, J-2 and J-3 along the Jeonju stream are located at downstream from the Duckon mine, and the primary source of lead, copper and zinc in the Jeonju stream is thought to be effluent leached from this mine (Fig. 1). These surface distribution patterns of lead, copper and zinc in the Mankyung River watershed are similar with the results of other mining areas (Franzin and McFarlane, 1980; Mann and Lintern, 1983; Gratton et al., 2000). Mean levels of heavy metal in the plants of *P. thunbergii* naturally grown in sampling areas increased in the order of cadmium (5.9–11.1 μg g^{-1}) < lead (126.4–240.2 μg g^{-1}) < copper (420.1–676.1 μg g^{-1}) < zinc (1208.9–1804.4 μg g^{-1}) (Fig. 2). These results are similar to the bioaccumulation patterns of heavy metals within various plant species, including *Potamogeton crispus*, *P. perfloiatus* and *Myriophyllum alterniflorum* (Welsh and Denny, 1980), *Agrostis gigiantea* (Hogan and Rauser, 1981), *Phaseolus vulgaris* (Hardiman et al., 1984), *Lolium perenne* and *L. muliflorum* (Kovács, 1992), and *Triticum aestivum* (Nan et al., 1999). Other vascular plant species, including *Utricularia vulgaris*, *Myriophyllum exalbescens* and *Calla palustris* (Franzin and McFarlane, 1980), *Nuphar varigatum* (Campbell et al., 1985), and *Mimulus guttatus* (Samecka-Cymerman and Kempers, 1999) exhibit a similar pattern of increasing content as follows; Cd (0.77–1.21 mg·kg^{-1}) < Pb (4.6–10.5 mg·kg^{-1}) < Cu (8.9–11.3 mg·kg^{-1}) < Zn (72.0–255.0 mg·kg^{-1}). Moreover woodland moss *Hylocomium splendens* (Mäkinen, 1987), aquatic moss *Rhynchostegium ripariodes* (Kelly et al., 1987) and red algae *Lemanea fluviatilis* and *L. condensata* (Harding and Whitton, 1981) have a similar pattern of heavy metal accumulation.

Within site variability in levels of heavy metals is likely due to the existence of distinct microhabitats within the sampling sites (Harding and Whitton, 1981) and the sampling of plants at slightly different stages in their growth cycle (Larsen and Schierup, 1981). Specifically, we confirm that, as in other species, the bioaccumulation of cadmium, lead, copper and zinc are higher in the root than in the leaf and stem of *P. thunbergii*, and the distribution of these heavy metals within *P. thunbergii* is far from homogeneous, particularly in the case of zinc. Most heavy metals are transported from roots to shoots in terrestrial plants, though to different extents. Within a certain concentration range, copper and zinc extensively translocated, as they are essential to the plant metalloenzymes diamine oxidase, ascorbate oxidase, cytochrome *C* oxidase, superoxide dismutase and plastocyanin oxidase (Delhaize et al., 1985; Van Assche and Clijsters, 1990) and photosynthesis (Hsu and Lee, 1988). In contrast, cadmium and lead are apparently non-essential and can be toxic to photosynthetic activity (Skórzyñska-Polit and Baszyñski, 1997), chlorophyll synthesis (Stobart et al., 1985) and antioxidant enzymes (Somashekaraiah et al., 1992). Various translocation patterns of lead, copper and zinc in *P. thunbergii* were similar to the results from *Nuphar variegatum* (Hutchinson et al., 1975; Campbell et al., 1985). These results are probably related to differences in solubility and availability of each heavy metal ion. Variability of within-plant distribution of cadmium, lead, copper and zinc in *P. thunbergii* may also be due to compartmentalization and translocation in the vascular system. These mechanisms are poorly understood and need further study. However, several workers have reported the bioaccumulation of heavy metals in hydrovascular plants. For example, Muramoto and Oki (1983) reported Cd, Pb and Hg removal by *Eichhornia crassipes*, Jain et al. (1989a) reported the contents of lead (0.01–246.5 ppm), copper (13.2–270.1 ppm) and zinc (46.1–266.1 ppm) accumulated by *Azolla pinnata* after heavy metal treatment, and Miranda and Ilangovan (1996) reported uptake of lead by *Lemna gibba*. Generally, heavy metals are accumulated by plants at much higher concentrations than in the ambient soil or waters. Therefore, these results illustrate that plants, specifically *P. thunbergii*, play an important role in the bioaccumulation and phytoextraction of metal ions in the surface soil of riversides contaminated by toxic heavy metals.

A positive correlation coefficient was found between lead, copper and zinc contents in the *P. thunbergii* plant tissue and lead, copper and zinc contents in the habitat soil. Samecka-Cymerman and Kemper (1999)s demonstrated that the heavy metal content of the aquatic marcrophyte *Mimulus gutattus* was strongly correlated with heavy metal content in sediment and water. Herawati et al. (2000) reported a significant positive correlation between cadmium and zinc content in rice and levels in various soil types. These reports support our results from station B-2. On the basis statistical results from station B-2 we can therefore confirm that the soil type, that is, soil texture, particle size and composite stability etc. of station B-2 is more suitable for the root proliferation of *P. thunbergii* than the other stations. Statistical positive correlations were observed between lead content in the root of *P. thunbergii* and lead content in the leaf and stem of *P. thunbergii*, whereas no correlation was found between copper and zinc content in the root of *P. thunbergii* and these metal contents in the other organ, leaf and stem. However, Campbell et al. (1985) reported that the relationship between [Cu $_{\text{Stem}}$] and [Cu $_{\text{Root}}$] was statistically significant ($r = 0.72$, $P < 0.005$, $n = 21$) in the hydrovascular plant, *Nuphar varigatum*, whereas no apparent relationship between [Zn $_{\text{Stem}}$] and [Zn $_{\text{Root}}$]. These contrasting observations are likely due to complex interactions between kinds of metal ions and plant species, leading to variability in heavy metal absorption and assimilation by growing plants. Plant uptake of heavy metals from soil occurs passively with the mass flow of water into the roots or through active transport across the plasma membrane of root epidermal cells, and under normal growing conditions, plants can potentially accumulate certain metal ions an order of magnitude greater than the surrounding medium.

The data from our laboratory experiment showed that the accumulation patterns of cadmium, lead, copper and zinc in whole plants of *P. thunbergii* increased in the order of cadmium < lead < zinc < copper, and bioaccumulation coefficients increased in the order of cadmium (2.0) < lead (3.2) < zinc (13.1) < copper (17.2). Dushenkov et al. (1995) reported that the accumulation of heavy metal in the root of *Brassica juncea* grown using hydroponics increased in the order of Cd < Pb < Cu < Zn, whereas the bioaccumulation coefficients increased in the order of Zn < Cd < Cu < Pb. Nanda Kumar et al. (1995) reported that the accumulation of these metals in the shoot of *Brassica juncea* grown in a fertilized sand/perlite mixture increased in the order of Cu < Cd < Pb < Zn, whereas the phytoextraction coefficients, the ratio of metal concentration in the plant to the initial soil concentration of the metal, increased in the order of Pb < Cu < Zn < Cd. These results illustrate that discrepancy between the content ratio of heavy metal and the absorbency of heavy metal in plant is linked to variation in: (a) the concentration of heavy metals; (b) the form of heavy metal present; (c) other members of the soil solution; and (d) plant species present.

5. Conclusions

The plant of *P. thunbegii* is an annual weed and exhibits a rapid regeneration from the node. This species reaches maximum biomass around late September. Moreover, our field survey showed that the concentrations of heavy metals in this plant closely correlate with plant biomass (data not shown). We, based on our results, conclude that the plants of *P. thunbergii* can effectively accumulate cadmium (II), lead (II), copper (II) and zinc (II) in soils or aquatic media. It also actively removes heavy metals from soil matrix and solution, and translocates heavy metals from the roots to the shoots in a similar fashion to the mechanism suggested for heavy metal hyperaccumulator species. Therefore, we expect that *P. thunbergii* plants play a substantial role in bioaccumulation and phytoextraction. A rapid regrowth ability of *P. thunbergii* makes it possible that frequent cutting of the plant is necessary to remove the toxic metal ions from the polluted environment throughout the summer.

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