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Uptake and Tolerance to Lead in *Populus alba* × *glandulosa* and *Betula Schmidtii*[1]

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현사시와 박달나무의 Pb 吸收能力 및 耐性[1]

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ABSTRACT

This study was conducted to estimate the capability of *Populus alba* × *glandulosa* and *Betula schmidtii* for the uptake of Pb from the lead-contaminated soil and their tolerance to lead. Rooted cuttings in the former species and germinated seedlings in the latter species were planted in pots and irrigated with Pb-containing water for 60 days. In both tree species, growth inhibition was observed in 800, and 1,500ppm of $Pb(NO_3)_2$. Most Pb was accumulated in plant roots and only a small portion was transported to the shoots. The translocation rates of Pb for *B. schmidtii* and *P. alba* × *glandulosa* were 1.6～2.6% and 1.2～1.6%, respectively. The maximum Pb content accumulated in shoots was 468.0mg/kg d.w. in *P. alba* × *glandulosa*, and 602.0mg/kg d.w. in *B. schmidtii*. Although tolerance to lead was generally higher in *B. schmidtii* than *P. alba* × *glandulosa*, the highest tolerance to lead was observed in *P. alba* × *glandulosa* clone, 72-16. Finally, we discussed the applicability of *P. alba* × *glandulosa* and *B. schmidtii* for phytoextraction based on their Pb uptake ability, high biomass production, and easiness in large-scale cultivation.

Key words : lead, phytoextraction, tolerance, Populus alba × glandulosa, Betula schmidtii

要 約

본 연구는 Pb로 오염된 토양의 phytoextraction을 위해 현사시(*Populus alba* × *glandulosa*)와 박달나무(*Betula schmidtii*)의 Pb의 흡수능력 및 이에 대한 내성을 조사하였다. 종자로 양묘한 박달나무 및 삽목으로 증식한 현사시 묘목을 화분에 식재 후 Pb를 함유한 수용액으로 관수하였다. 이들 2수종은 $Pb(NO_3)_2$ 농도 800ppm이상의 처리구에서 생장량 감소가 관찰되었다. 대부분의 Pb는 식물체의 뿌리에 축적되었고, 극히 일부분만이 식물체의 지상부위로 이동되었다. 박달나무와 현사시의 Pb의 지상부 전위율은 각각 1.6～2.6%와 1.2～1.6%로 나타났다. 지상부 수체의 최대 Pb 함량은 현사시의 경우 468.0mg/kg, 박달나무는 602.0mg/kg이었다. Pb에 대한 내성은 박달나무가 현사시에 비해 일반적으로 높은 경향이었으나, 현사시 72-16호 클론은 전체 클론 중에서 가장 높은 내성을 보였다. Biomass 생산과 Pb 흡수능력을 고려하여 이들 두 수종의 phytoextraction을 위한 가능성을 제시하였다.

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INTRODUCTION

Soil contamination with heavy metal such as Pb is widely observed at industrial and mining areas in the world. It is generally known that soil contamination with lead is caused by industrial activities such as the use of paints, gasoline, expolsives, and anti-spark linings containing lead and the disposal of municipal sewage sludges enriched in Pb. The damage of heavy metals to the environment is aggravated by their long-lasting precipitation in the soil. For example, lead is known to be retained in the soil for 150 to 5,000 years (Kumar et al., 1995).

In Korea, the average Pb concentration in monitoring sites of all over the country is 6.25mg/kg. Although this value is lower than the suggested standard of paddy/farm soils (100mg/kg), it is higher than that of ordinary soils (5.38mg/kg). On the other hand, maximum Pb concentrations of some rice fields and waste landfill areas in Korea are reported to be 159.0mg/kg and 266.7mg/kg, respectively (Ministry of Environment, 1999). The concentration of Pb in tailings averaged over three mine areas, Gubong, Daebong, and Samgwang, is 870mg/kg. Among these areas, Gubong showed the highest value of 1,510mg/kg (Kim et al., 1998). From above results, we could deduce that the soil contamination with lead in Korea, on average, is not that serious, but in some areas such as mines, the degree of contamination is very high.

Severe soil contamination with Pb may cause a variety of environmental problems, including a loss of vegetation, contamination of groundwater, and toxic effects on plants, animals, as well as human beings (Huang et al., 1997). In Korea, lead compounds are regarded as toxic chemicals and the level of lead concentration in drinking water is strictly restricted to the maximum value of 0.05mg/ℓ (Ministry of Environment, 1999). Generally, the remediation of Pb-contaminated soils is very expensive. So far, the remediation has been carried out mainly through the engineering-based techniques (Huang et al., 1997). Recently, phytoremediation has gained attention as an alternative approach for soil remediation. The phytoremediation takes the advantage of the fact that a living plant can extract particular elements from the environments and accumulate them in its body. The plants rich in accumulated contaminant can be harvested and processed for eliminating detrimental elements through drying, ashing, or composting in relatively easy and safe ways (Raskin et al., 1997).

Heavy metals, including lead, are not essential for plant growth, but affect plant metabolism such as photosynthesis (Baszynsky et al., 1980; Stefanov et al., 1993), DNA synthesis(Gabara et al., 1992; Liu et al., 1994), chlorophyll biosynthesis(Van Assche and Clijster, 1990), and seed germination (Wierzbicka and Obidzinska, 1998). Baker and Brooks(1989) reported that plants having a high ability of uptake and accumulation of heavy metals can be effectively used for the soil remediation. However, in this case, the biomass of the candidate plants is very critical to the effective remediation. In other words, low biomass is not efficient in successful remediation. Additionally, the probability of the candidate plants for a large-scale cultivation should be taken into consideration. Regarding this, recent researches have focused interests on searching and/or breeding plants with high biomass as well as easiness for large-scale cultivation (Raskin et al., 1997).

Populus is one of the most well-known tree species used for phytoremediation plantings, because it is easy to establish on a large scale and grows fast. Its high transpiration rate and wide-spreading root system make it ideal to intercept, absorb, degrade, and detoxify soil contaminants (Dix et al., 1997). Hybrid poplar has been used for phytoremediation of soils contaminated with atrazine (Burken and Schnoor, 1997). On the other hand, the genus *Betula* is often found in areas contaminated with large amount of various heavy metals (Eltrop et al., 1991). So, it seems to be tolerant to heavy metals.

The objectives of this study were to investigate the ability of *Populus alba* × *glandulosa* and *Betula schmidtti* in the uptake of lead and their tolerance to lead and to check if these plants can be used for the phytoremediation of contaminated soil.

MATERIALS AND METHODS

For *P. alba* × *P. glandulosa*, 5 clones (65-22-4, 72-16, 72-17, 72-19, and 72-30) were used for this study, which were selected based on growth performance and biomass production. Five open pollinated families (BS9, BS12, BS14, BS15, and BS17) of *B. schmidtii* was also used. Those open-pollinated seeds were collected from B. schmidtii trees growing at abandoned coal mine areas in Taebaek Province. The collected seeds were germinated and then one-year-old seedlings were finally used. On the other hand, in the case of *P. alba* × *P. glandulosa,* cuttings from one year old shoots were rooted into the pot filled with quartz sand in March, 1996. They were cultivated with groundwater in a greenhouse for 2 months. Then, the plantings were irrigated with water containing different four levels (0, 200, 800, and 1,500ppm) of $Pb(NO_3)_2$ for 60 days. The solution was regularly supplied through a hole in the bottom of pots two times a day. The $Pb(NO_3)_2$ was dissolved in deionized water without any other nutrients in order to prevent it from the formation of precipitates. The water containing $Pb(NO_3)_2$ and groundwater for control were replaced every 15 days. Three cuttings of *P. alba* × *P. glandulosa* and three seedlings of *B. schmidtii* were cultivated in each lead treatment. Planting height, leaf area and total chlorophyll content were measured every 15 days. At the end of the experiment, leaves, shoots and roots were collected from each treatment as well as the control which was not provided with lead-containing solution and washed thoroughly with running tap water. The dry matter accumulated in shoot and root was measured after plant tissues were dried for 3 days at 80℃. Tolerance to lead was determined with relative dry matter production of each Pb treatment to control. Dried tissues were dissolved in a mixture of HNO_3 and H_2O_2 (1 : 1) with microwave digestion unit. Lead content of the acid extract was measured with atomic absorption spectrophotometer, Model AA-6701F(Shimadzu, Japan).

RESULTS AND DISCUSSION

1. Lead uptake

All the test plants accumulated lead in their roots (over 90%) and only a small amount of lead was transported to the shoots (Figure 1). This result is in good agreement with that of previous studies (Berti and Cunningham, 1994; Kumar et al., 1995). The result may be closely related to the evolu-

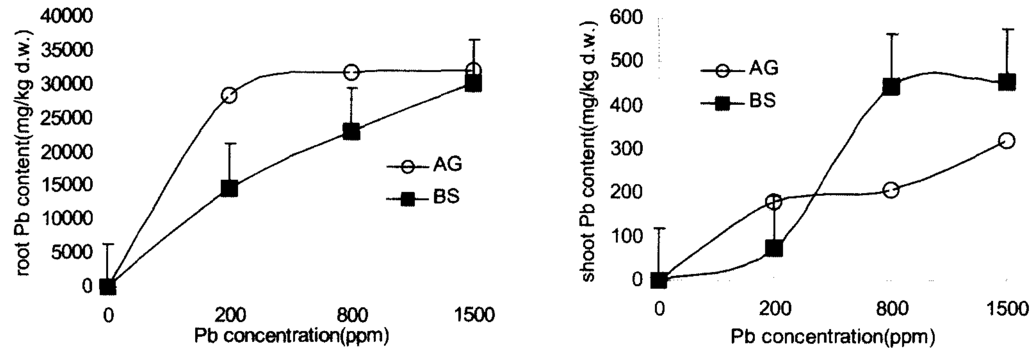

Figure 1. The lead contents in roots and shoots of *P. alba* × *glandulosa*(AG) and *B. schmidtii*(BS) at different lead concentrations.

tionary process for protecting shoots from toxins which are absorbed into roots (Landberg and Greger, 1996). Since *P. alba* × *glandulosa* and *B. schmidtii* accumulated the most amount of lead in their roots, they could be classified into excluders. At the same lead treatment, there was no statistically significant difference in lead uptake among clones or seedlings in the same species. However, we observed the difference in lead uptake between species at each lead treatment (Figure 1).

1) Lead content of roots

The Pb contents in roots of *P. alba* × *glandulosa* and *B. schmidtii* increased with the increase of lead concentrations (Figure 1). However, the increasing pattern of Pb content was very different between *P. alba* × *glandulosa* and *B. schmidtii*. In *P. alba* × *glandulosa*, the Pb content in roots was sharply increased at 200ppm, reaching 88.3% of the maximum lead uptake. By contrast, *B. schmidtii* increased gradually with the increase of Pb concentrations.

This difference may be caused by different growth patterns of *P. alba* × *glandulosa* and *B. schmidtii*. *P. alba* × *glandulosa* is a fast growing tree species and requires more water and nutrients at the early growth stage than *Betula* (Eltrop et al., 1991). On the other hand, the speed of Pb removal from irrigating solution is known to be directly proportional to the root biomass (Dushenkov, 1995). The root biomass of *P. alba* × *glandulosa* was 1.2~1.5 times larger than *B. schmidtii*. Additionally, the leaf area of *P. alba* × *glandulosa* was 3.3~5.6 times larger than *B. schmidtii*. Thus, the transpiration rate of *P. alba* × *glandulosa* might be much higher than that of *B. schmidtii* so that this led to more Pb uptake in *P. alba* × *glandulosa* than in *B. schmidtii*.

Bioaccumulation coefficient is the ratio of metal concentration in root tissue (mg/g d.w.) to initial metal concentration in solution (mg/ℓ) (Duschenkov et al., 1995). Bioaccumulation coefficient of root decreased with the increase of initial Pb concentrations in solution (Table 1). Bioaccumulation coefficient of root of *P. alba* × *glandulosa* was higher than that of *B. schmidtii* in all treatments. This indicates that *P. alba* × *glandulosa* is more efficient in removal of Pb than *B. schmidtii*.

Table 1. Bioaccumulation coefficients and translocations rate of *P. alba* × *glandulosa* and *B. schmidtii* cultivated in different Pb concentrations for 60 days.

Species	Pb concentration in solution (ppm)	Bioaccumulation coefficient* of root	Translocation rate** (%)
P. alba × *glandulosa*	200	143.3±17.3 a	1.3±0.1 b
	800	40.5±3.9 b	1.6±0.3 a
	1,500	21.5±1.8 c	1.2±0.1 b
B. schmidtii	200	74.7±13.0 a	1.6±0.6 b
	800	29.0±4.4 b	2.6±0.4 a
	1,500	20.2±0.3 c	2.0±0.3 b

* Bioaccumulation coefficient was expressed as the plant/solution ratio of lead content

** Translocation rate was expressed as the shoot/root ratio of lead content

2) Lead content of shoot

The Pb content of shoot increased with the increase of lead concentrations. However, the changing pattern was very different from that of root (Figure 1). In *P. alba* × *glandulosa*, the Pb content of shoot increased gradually with the increase of lead concentrations. By contrast, the Pb content in shoots of *B. schmidtii* sharply increased at 800ppm, which was 6 times larger than that of 200ppm.

It may show that the translocation of Pb from root to shoot is not directly proportional to the degree of Pb uptake in root. The translocation rates in *P. alba* × *glandulosa* and *B. schmidtii* ranged between 1.2~1.6% and 1.6~2.6%, respectively (Table 1). The translocation rate in *B. schmidtii* seemed to be higher than that in *P. alba* × *glandulosa*. The translocation rate of *P. alba* × *glandulosa* and *B. schmidtii* averaged over three different treatments was 1.4% and 2.1%, respectively.

The translocation of heavy metals to shoot is one of the important factors in determining the possibility for phytoextraction because shoot is a major part of plants that can be picked for phytoextraction (Kumar et al., 1995; Raskin et al., 1997). Among heavy metal ions, lead is known to be hardly transported to the shoot. The transport is known to be both apoplastic and symplastic in the root tissue (Landberg, 1996). Pb translocation rate (or phytoextraction coefficient) was reported to vary greatly with plant species and even cultivars of the same species (Kumar et al., 1995; Wierzbicka, 1999). In the case of *Brassica juncea* cultivars, phytoextraction coefficient fell within the range of 0.7% to 55.2% (Kumar et al., 1995). The estimation of phytoextraction could be also influenced by experimental conditions, particularly Pb concentration and exposure time even though the same plant species is used as materials. For example, phytoextraction coefficient of *Zea mays* was calculated as 1.3% in Kumar et al.'s study (1995), whereas it was estimated as 4.1% in another study (Wierzbicka, 1999). Accordingly, we have to be very cautious when we compare one result with the other ones. Regardless, average Pb content accumulated in shoot of *P. alba* × *glandulosa* and *B. schmidtii* seemed to be higher compared with those of other plant species. This shows that *P. alba* × *glandulosa* and *B. schmidtii* may belong to the group of high accumulators.

2. Toxicity

Numerous studies have shown the detrimental toxic effects of lead on plant metabolic processes (Wierzbicka, 1999). In this study, the total chlorophyll content of leaves under the lead treatments was not significantly different from that of control. Although Pb toxicity varied depending on plant species (Wierzbicka, 1999), Pb was nearly exclusively bound to the cell wall and affected the plasticity and the rigidity of the cell wall (Landberg, 1996). In our case, Pb was unlikely to have a direct effect on the metabolism as in the case of lichens (Chettri et al., 1998). However, the number of leaves came into after treatment significantly decreased with the increase of lead concentrations as already shown in Eltrop et al., (1991). Leaf area and relative growth rate of shoot were also reduced by lead treatment. Kumar et al.(1995) reported that all the Pb-treated plants showed stunted growth and reduced leaf expansion. These symptoms could be interpreted as lead toxicity (Eltrop et al., 1991).

3. Tolerance to lead

Tolerance to heavy metals is a relative measure, meaning that tolerance is expressed as a comparision (Landberg, 1996). Tolerance index is defined as the percent of measurements of treated plants to untreated plants. The measurements include weight, length, number of roots or shoots of plants (Landberg and Greger, 1996). In this study, tolerance to lead was determined by relative dry weight of roots

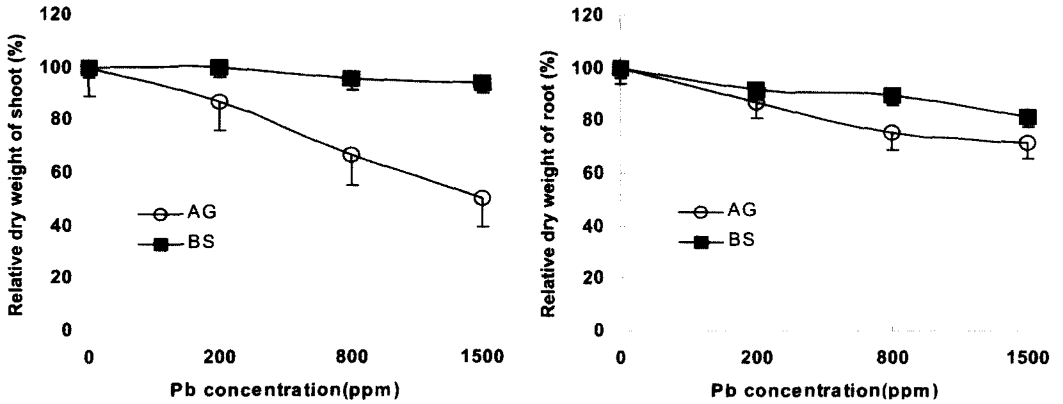

Figure 2. Relative dry weight of shoots and roots of *P. alba* × *glandulosa*(AG) and *B. schmidtii* (BS) at different lead concentrations.

and shoots in lead-treated plants to those of controls (Figure 2, 3). An ANOVA indicated that the relative shoot and root dry weights of *P. alba* × *glandulosa* decreased with the increase of lead concentrations ($P<0.01$ and $P<0.002$, respectively). Also, the same patterns were observed in shoot ($P<0.05$) and root dry weight ($P<0.01$) of *B. schmidtii* (Figure 2).

In Korea, the lead concentration in severely polluted areas has been rarely investigated with the exception of several mining areas. The highest lead concentration of tailings in three mining areas is 1,510 μg/g (Kim et al., 1998). The highest lead concentration applied for the present study was 1,500ppm. Therefore the growth response of the plants to lead investigated here may provide valuable information on selecting tree species and/or clones which can be planted for the phytoextraction of lead. At 1,500ppm lead, dry weights of shoot and root in *P. alba* × *glandulosa* were significantly different among clones ($P<0.0001$) while *B. schmidtii* showed no difference among different familys (Figure 3). Average reduction rates of shoot dry weight in *P. alba* × *glandulosa* and *B. schmidtii* were 49.9% and 6.0%, respectively. Average reduction rates of root dry weight in *P. alba* × *glandulosa* and *B. schmidtii* were 28.4% and 18.7%, respectively. It showed that dry weight of *P. alba* × *glandulosa* was more affected by lead treatment than that of *B. schmidtii*. Wierzbicka (1999) reported that lead has rather a minor effect on the growth of above-ground plant part. In *P. alba* × *glandulosa*, however, it was observed that the growth of shoot was more affected by lead treatments than that of root (Figure 2). This difference might be resulted from peculiar growth characteristics and nutrients demand of *P. alba* × *glandulosa*.

Tolerance index of clones based on total dry weight relative to that of control was represented in Figure 3. In this study, no correlation was found between Pb content of plant tissue and the degree of tolerance to lead, as earlier suggested by Kumar et al.(1995) and Wierzbicka(1999). Baker and Walker(1990) reported that differences in tolerance to metals cannot be explained as differences in uptake and transportation. It seems that the lack of such a relationship is due to differences among species in the capacity of root cortex cell walls which are the main storage site of lead(Wierzbicka, 1998). The highest tolerance to lead was found in 72-16 clone, although it was not significantly different from *B. schmidtii* families. The remnant clones of *P. alba* × *glandulosa* showed lower tolerance to lead than *B. schmidtii* families. From this result, we could conclude that the lead-tolerance of *B. schmidtii* was higher than that of *P. alba* × *glandulosa*. However, it was worthy of noting that tolerance index of *P. alba* × *glandulosa* clones was more variable than *B. schmidtii* families. However, considering the facts that *P. alba* × *glandulosa* had wide range of tolerance index and the highest tolerance index was observed in a *P. alba* ×

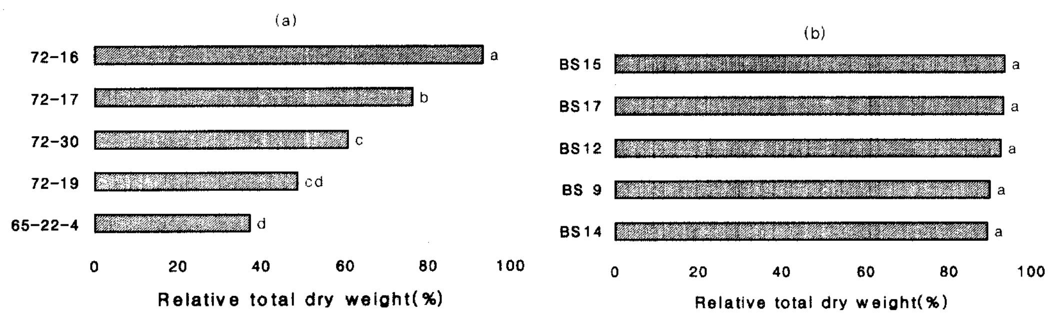

Figure 3. Total dry weights of *P. alba* × *glandulosa*(a) and *B. schmidtii* clones(b) at 1,500ppm treatment relative to the control. Same letters on the right side of the bar indicate no significant differencs at $P<0.05$.

glandulosa clone (72-16), we could consider that *P. alba* × *glandulosa* would be a good material to be selected as a lead-tolerant tree species.

4. Implications for phytoextraction

There are two important factors which should be considered when you select candidate plant species for phytoextraction; translocation of heavy metals from root to shoot and biomass production of shoot (Berti and Cunningham, 1994; Kumar et al., 1995; Raskin et al., 1997). In this study, *P. alba* × *glandulosa* and *B. schmidtii* showed the lower level of Pb accumulation and the slower degree of translocation rate compared with those of other species known as hyperaccumulator (Baker and Brooks, 1989). However, generally, the plant species which have been classified into hyperaccumulators so far have low biomass production and low competitive ability in non-polluted environments (Greger and Landberg, 1999). On the other hand, high accumulators are known to accumulate lower level of metals than hyperaccumulators do, but much more than normal plants. They usually have high level of biomass production (Greger and Landberg, 1999). Thus, high accumulators with high level of biomass would be an alternative to hyperaccumulators for phytoextraction, because they can be easily cultivated using already-established agronomic practices (Raskin et al., 1997).

Considering their high level of biomass production and broad range of adaptability, *P. alba* × *glandulosa* and *B. schmidtii* may be good candidate plant species for phytoextraction. *P. alba* × *glandulosa* would be more suitable for planting in an urban area polluted with the low level of lead. In view of human contact, the risk posed by lead contaminated soils was more severe in polluted urban areas(Body et al., 1991). Because *P. alba* × *glandulosa* is a fast growing tree species with high biomass as well as can be easily regenerated by sprouts and/or root suckers, regeneration time is shorter than other tree species and consequently it is less costly from a management point of view. On the other hand, in forest lands, the adaptability of *B. schmidtii* is much higher than that of *P. alba* × *glandulosa* (Eltrop et al., 1991), although the level of biomass production and the growth rate of *B. schmidtii* are lower than *P. alba* × *glandulosa*. As a consequence, *B. schmidtii* might be recommended as a good candidate tree species for phytoextraction in mountainous mining areas contaminated with high concentration of lead.

Phytoremediation is an environmentally friendly and cost-effective technique(Raskin et al., 1997). This technique has been more focused as the environmental pollution has become more serious. Consequently, the results presented here could provide very valuable information when we set up a plan for phytoremediation of heavy metal pollutants. This kind of studies should be extended to more tree species which can be easily cultivated using already-established agronomic practices (Raskin et al., 1997) as well as may have high abilities for phytoextraction of pollutants.

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