Bull. Environ. Contam. Toxicol. (1998) 60:285-291

Bulletin of Environmental Contamination and Toxicology

Lead Uptake and Effects on Seed Germination and Plant Growth in a Pb Hyperaccumulator *Brassica pekinensis* Rupr.

Z.-T. Xiong

Department of Environmental Science, Wuhan University, Wuhan, Hubei 430072, People's Republic of China

Received: 18 September 1997/Accepted: 1 December 1997

Heavy metal contamination of soil, water and air has caused serious environmental hazard in the biosphere due to rapid industrialization and urbanization. Lead is probably one of the most frequently encountered heavy metals in polluted environment. The primary sources of this metal include mining and smelting of metalliferous ores, burning of leaded gasoline, disposal of municipal sewage and industrial wastes enriched in lead as well as using of lead-based paint (Kabata-Pendias and Pendias 1984; Seaward and Richardson 1990). Relatively high levels of lead concentration have been reported at some severely contaminated sites. For example, lead concentrations were recorded as high as 7000 μg g^{-1} in roadside soil (Kabata-Pendias and Pendias 1984) and 13380 μg g^{-1} in mining district soil (Wickland 1990).

Lead is among those heavy metals which have no known biological function. Nevertheless, numerous investigations show that plants can accumulate lead via root and shoot, and that the lead concentrations in plant tissues are significantly related to the lead levels in environment (Kabata-Pendias and Pendias 1984; Nwosu et al. 1995; Sawidis et al. 1995; Xiong 1997b). Excessive lead accumulated in plant tissue can be toxic to most plants, leading to decrease in seed germination, root elongation and biomass, inhibition of chlorophyll biosynthesis, as well as cell disturbance and chromosome lieson (Balsberg Pahlsson 1989; Kumar et al. 1991; Fargasova 1994; Xiong 1997c). In lead and other heavy metal-contaminatad sites, vegetation structure and biodiversity are usually reduced, barren patches of soil occur, and trees are sparse or absent (Wickland 1990).

In recent years, it has been reported that some plant species known as hyperaccumulators , which are usually derived from heavy metal-contaminated areas, have the ability to accumulate unusually high content of heavy metals without dramatically being impacted in their growth and development (Reeves and Brooks 1983; Brooks and Malaisse 1985; Baker and Brooks 1989; Xiong 1997a). This raises the suggestion that these hyperaccumulators may provide the basis for phytoremediation of heavy metal-contaminated sites (Baker et al. 1991; Salt et al. 1995). Phytoremediation potential of a few such species for heavy metal-contaminated soil and water have recently been detected (Brown et al. 1994; Kumar et al. 1995; Dushenkov et al. 1995; Huang et al. 1997; Blaylock et al. 1997).

Until recently, however, no heavy metal hyperaccumulating plants have been reported in China, though some investigations into a number of wild plant species have been conducted (e. g. Xiong 1997 b, c). In screening for heavy metal hyperaccumulators in Chinese-endemic crop species, we found that *Brassica pekinensis* (Cruciferae) , a very popullar vegetable with high yield, was able to accumulate unusually high content of lead in its root and shoot tissues. The present paper reports the data of lead uptake and translocation in the plant. To determine the ability of the species to tolerate elevated lead concentration in environment, seed germination and plant growth in lead-containing growth medium are also studied.

MATERIALS AND METHODS

Brassica pekinensis Rupr. seeds of cultivar JF-1 were purchased from Wuhan Vegetable Seed Company, Wuhan, Hubei. Quality seeds were immerged in 3% formalin for 5 min to prevent fungal growth, washed with deionized water, and soaked in water for 12 h. The soaked seeds were placed in plastic dishes of 10 cm diameter with a layer of filter paper on the bottom. In each dish, 10 seeds were evenly placed on the surface of the filter paper, and 10 mL analytical grade Pb $(NO_3)_2$ aqueous solution with certain Pb^{2+} concentration was added. The Pb^{2+} concentrations in the solutions were as follows : 0, 125, 250, 500 and 1000 μg mL^{-1}. Each treatment had three replicates. Exposure lasted 72 h under dark condition at 25°C. Then the germination rate, root and shoot length were recorded.

A pot study was conducted to determine lead bioaccumulation and tolerance of the plants under controlled conditions. Quality seeds were immerged in 3% formalin for 5 min, washed with deionized water, and soaked in water for 12 h. The soaked seeds were sown in 180 g (DW) of acid-washed 3 : 2 (w/w) mixture of fine sand (<l mm) and crushed stone (<5 mm) placed in round plastic pots of 12 cm diameter, each pot with 15 seeds. The pots were watered daily until the seeds germinated. Then the young seedlings were fertilized daily with full-strength Hoagland's solution for 3 weeks. 8 uniform seedlings were retained in each pot and the others eliminated. the pots were randomly (by random number determining method) divided into 5 groups (control and treatments), each group with 3 pots (replicates). Aqueous solutions of chemical grade $Pb(NO_3)_2$ with different Pb^{2+} concentrations were supplemented to the growth media at the beginning of the treatment period. The Pb^{2+} concentrations in the growth media were 0, 125, 250, 500 and 1000 μg g^{-1}(DW) for the control and the treatments respectively. The seedlings were placed in a greenhouse with 14 h, 25°C/10h, 18°C day/night regime. During the treatment period, the seedlings were fertilized with Hoagland's solution omitted phosphates and sulfates in order to prevent precipitation of lead. Excess growth medium moisture leached out from pot was trapped in plastic saucers placed below each pot and returned to the pot. The plants were harvested 2 weeks after the lead treatment. The roots were carefully washed with tap water to get rid of the sand, and then washed with deionized water. The plants were seperated into roots, stems and leaves, and dried in an oven at 85°C for 2 days. Then the biomass (dry weight) was determined.

Dried sample was ground, digested in concentrated HNO_3 for 14 h, 12 h at room tem-

perature first, then 2 h at 60°C, and further digested with concentrated HNO_3-$HClO_4$ (3 : 2, v/v) for 3 h at 140-160°C. After cooling, the extract was diluted with 1 *N* HCI and made up to 25 mL. Regent blanks were prepared by carrying out the whole extraction procedure but in the absent of sample. lead content of the extract was determined with flame atomic absorption spectrometer (AAS) (Shimadzu AA-680).

Student's t-test was performed to test the significance of differences of germination rate, root and shoot length between control and the treatments. One-way and two-way analysis of variance (ANOVA) were used to examine differences of biomass and differences of lead concentration in the plant respectively.

RESULTS AND DISCUSSION

The effects of various concentrations of lead on seed germination, root and shoot length of *B. pekinensis* cultivar JF-1 are shown in Table 1. In general, the results demonstrate a concentration-dependent inhibition of the seed germination, root and shoot length. Significant decreases ($P<0.01$) of these parameters with the increasing Pb concentration in the solution have been observed. However, response to Pb treatment varies among the seed germination, root and shoot length. The germination rate at 125 μg m L^{-1} treatment is the same with that of the control. Even at the highest treatment (1000 μg mL^{-1}), i t is as high as 43. 33% of the control. This is not the case for root length. At 125 μg m^{L-1} treatment, the root length is significantly different from the control, decreasing to a half of the control. At 1000 μg mL^{-1} treatment, the root length is only as long as one tenth of the control. According to the length difference of the root and shoot between the lowest treatment (125 μg mL^{-1}) and the control, highly significantly vs. non-significantly, the root seems to be more sensitive to lead than the shoot. Similar results have also been observed in *Sesamum indicum* (Kumar et al. 1991), *Sinapis alba* (Fargasova 1994) and lettuce and radish (Nwosu et al. 1995). One of the explanations for roots to be more responsive to toxic metals in environment might be that roots were the specialized absorptive organs so that they were affected earlier and subjected to accumulation of more heavy metals than any of the other organs. This could also be the main reason that root length was usually used as a measure for determining heavy metal-tolerant ability of plant.

Table 1. Seed germination, root and shoot length of *Brassica pekinensis* after 72 h of lead treatment

Pb treatment (μg mL^{-1})	Germination rate (%)	Root length (mm)	Shoot length (mm)
0	100. 00±0. 00a	17. 85±11. 32a	9. 10±5. 02a
125	100. 00±0. 00a	8. 37±4. 53b	6. 23±3. 87a
250	66. 67±5. 77b	2. 56±1. 63c	1. 45±0. 94b
500	56. 67±5. 77c	2. 21±0. 86c	1. 00±1. 00b
1000	43. 33±5. 77c	1. 77±0. 83c	0. 62±0. 87b

Results are means±SD. Means with different letters are significantly different from one another ($P<0.01$) according to Student's t-test

The plant biomass after two-weeks culture in the pots supplemented with various content of lead are presented in Table 2. In contrast with the seed germination and root and shoot length data shown above, the meam biomass after two-weeks culture in the Pb-supplied medium is not significantly different from the control as well as from each other ($P>0.05$). It is interesting to note that the plant biomass shows a increasing tendency instead of decreasing as it is expected with increasing of lead content in the growth media (Table 2). The response pattern of the plants to the supplied $Pb(NO_3)_2$, according to the biomass, seems to be a combination of some stimulating and inhibiting effects. The biomass reaches its maximum at 500 μg g^{-1} treatment, and then declines at 1000 μg g^{-1} treatment. This might suggest that the stimulating effect to the plant growth had been of dominance over the inhibiting before the lead concentration in the growth media got up to 1000 μg g^{-1}, and thereafter the inhibiting effect became stronger. Similar stimulating phenomena of lead supplements to plant growth were reported occasionally in the literature (e. g. Dou and Hu 1987; see Kabata-Pendias and Pendias 1984). It had been believed by some researchers that lead was probably the stimulating factor, while others suggested that it was not lead itself but some other substances that were responsible for the stimulation of plant growth (see Kabata-Pendias and Pendias 1984). No matter which mechanism is involved in the stimulation of plant growth under the condition of Pb $(NO_3)_2$ supplement in the growth media, *B. pekinensis* could be considered as a lead-tolerant species, since even at the highest treatment (1000 μg g^{-1}), the biomass is, though not significantly, higher than that of the control.

Table 2. Biomass (DW) of *Brassica pekinensis* after two-weeks of culture in Pb-enriched medium

Pb treatment (μg g^{-1})	Root (mg)	Shoot (mg)	Total (mg)
0	6.3±1.5	22.0±6.2	28.3±7.6
125	6.0±2.0	24.3±3.2	30.3±4.0
250	6.7±0.6	29.3±4.0	36.0±4.4
500	10.0±2.6	40.0±14.7	50.0±17.3
1000	7.0±1.7	35.7±11.6	42.7±13.8
F ratio	2.333	2.0531	2.1353
P value	>0.05	>0.05	>0.05

Biomass results are means±SD (n=3). F ratios are the results of one-way ANOVA

The lead concentrations in the roots, stems and leaves of *B. pekinensis* grown in sand / crushed stone mixture supplemented with Pb $(NO_3)_2$ are listed in Table 3. Lead concentration in the plant is significantly affected by both the lead content supplied in the growth medium and the plant tissue as well as by the interaction between the two factors ($P<0.001$; Table 4). In all the tissues, the mean Pb concentrations increase without exception as the Pb contents in the growth medium increase. The lead concentration in the root is largely directly proportional to the lead content in the growth medium. When the lead supplied in the growth medium doubles, the lead accumulated in the root also almost exactly doubles (compare the lead concentrations in the root between any pair of the vicinal treatments in Table 3). However, no such double-increasing pattern of lead concentration is observed in the cases of stem and leaf.

Table 3. Lead concentrations in root, stem and leaf of *Brassica pekinensis* after two-weeks of culture in Pb-enriched medium

Pb treatment ($\mu g\ g^{-1}$)	Root ($\mu g\ g^{-1}$)	Stem ($\mu g\ g^{-1}$)	Leaf ($\mu g\ g^{-1}$)
0	251±133	61±58	26±14
125	3443±561	84±59	55±14
250	6662±1539	979±906	466±117
500	15287±1965	2468±1712	1220±152
1000	33647±8123	7358±7024	2670±937

Results are means±SD (n=3)

Table 4. Two-way ANOVA summary for lead concentrations in root, stem and leaf of *Brassica pekinensis* after two-weeks of culture in Pb-enriched medium

Source	df	SS	MS	F	P
Pb treatment	4	1372684343	343171086	42.0287	<0.001
Plant tissue	2	1039626402	519813201	64.0441	<0.001
Pb tr. × Pl. tis.	8	902376919	112797115	13.8973	<0.001
Error	34	275960600	8116488		

Numerous studies demonstrate that heavy metal concentration in plant is a function of heavy metal content in the environment. When the soil is heavy metal-contaminated, the plants in it will take up the metal via root system (Kabata-Pendias and Pendias 1984; Nwosu et al. 1995; Xiong 1997c). In the air polluted environment, plant leaves can play an important role in trapping and accumulating heavy metals, resulting in a relatively high level of heavy metal concentration in the aboveground tissues (Tumi et al. 1990; Sawidis et al. 1995; Xiong 1997b). These results suggest that direct contect of plant tissue with heavy metal contaminants in environment is important in determining the contaminant concentration in that tissue. In the present case, the plant roots are no doubt directly subjected to lead contamination, while the stems and leaves not. Thus the lead concentration in the roots is more closely related to that of the growth medium than in the stems and leaves. The crucial factors determining Pb distribution in different plant tissues may lie in the Pb translocation process in plant. Several mechanisms, including anatomical, biochemical and physiological (Salt et al. 1995), can be involved in heavy metal translocation in plant, thus complicating the metal accumulation and distribution in aboveground tissues in the case of soil contamination.

The *B. pekinensis* plants accumulate an unusually high level of lead in their tissues during a short growth period (Table 3). The lead concentrations in the root, stem and leaf are 27- to 34, 4- to 7-, and 2- to 3-fold higher than those in the corresponding growth medium respectively, with only the exception for the stem and leaf at the lowest treatment (125 $\mu g\ g^{-1}$). This means that, as Salt et al. (1995) pointed out, the plants can be compared to solar driven pumps which can extract and concentrate lead from the growth medium. Theoretically, the higher the ratio of lead content in plant to that in growth medium is, the more powerful the "pump" will be. In the present study, *B. pekinensis* demonstrates a relatively powerful species in extraction of lead via root system, especially at the highest treatment (1000 $\mu g\ g^{-1}$). It could compare with the few

lead hyperaccumulators discovered already (Reeves and Brooks 1983; Kumar et al. 1995) in respect to the lead concentration in plant. As far as the Pb-tolerant ability concerned, *B. pekinensis* would also seem to be superior to them (Kumar et al. 1995), since it did not show inhibited symptoms during the Pb treatment, and its biomass was higher than that of the control even at the highest treatment (1000 μg g^{-1}) (Table 2). Furthermore, according to the double-increasing tendency of lead concentration in the root from 500 μg g^{-1} to 1000 μg g^{-1} treatment (i. e. 15287 μg g^{-1} to 33647 μg g^{-1}; see Table 3), the plant ability to accumulate lead would not seem to have reached its peak at the 1000 μg g^{-1} treatment. Higher Pb concentration in the plant would seem to be expected when the Pb content in the growth medium is further elevated.

A higher shoot/root ratio of heavy metal content in plant is important in practical phytoremediation of heavy metal-contaminated soils. This property can enable phytoremediation of the heavy metal-contaminated soils only by harvesting the aboveground parts of the plants, thus simplifying the agricultural practices. It has been shown that lead hyperaccumulating plants usually have a higher shoot/root ratio of lead content in plant (0.04 - 0.1) than the non-hyperaccumulators (see Table 1 in Kumar et al. 1995). In the present study, *B. pekinensis* shows a 0.09 shoot/root ratio of Pb content, comparing well with the reported ones of *B. juncea* and *B. nigra* (Kumar et al. 1995). Although this ratio is far below some desired level, say 1, as in the case of Zn accumulation (Brown et al. 1994), it demonstrates a superior ability to accumulate lead from soil to shoot to non-hyperaccumulating plants, as far as the immobile property of lead concerned. A comparison of the phytoextraction coefficients of shoots for Cr^{6+}, Cd, Ni, Zn, Cu, Pb and Cr^{3+} shows that lead is among those which are the most immobile heavy metals from soil to shoot (Kumar et al. 1995). Tight binding of lead to soils and plant materials would at least partially account for the low mobility of this metal in soils and plants (Kabata-Pendias and Pendias 1984). To make lead easier to translocate in soils and in plants, acidifier and chelator amendments in growth medium would most likely be workable (Salt et al. 1995; Huang et al. 1997; Blaylock et al. 1997).

Acknowledgment. We would like to thank Mr. Zhu Liping for his technical assistance.

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