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BRIEF COMMUNICATION

Hyperaccumulation of lead by roots, hypocotyls, and shoots of *Brassica juncea*

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Abstract

The effects of different concentrations of lead nitrate (10^{-5} to 10^{-3} M) on root, hypocotyl, and shoot growth of Indian mustard (*Brassica juncea* L. var. *megarrhiza*), and the uptake and accumulation of Pb^{2+} by its roots, hypocotyls, and shoots were investigated. Lead had no significant inhibitory effect on the root growth at concentrations of 10^{-5} to 10^{-4} M during the entire treatment, while at 10^{-3} M, Pb slightly inhibited the root and shoot growth. *B. juncea* has ability to take up Pb from solutions and accumulate it in its roots, and transport and concentrate it. The Pb contents in the parts of plants treated with 10^{-3} M Pb were greater than those of untreated plants, by factors of 230 in the roots, 170 in the hypocotyls, and 3 in the shoots.

Additional key words: growth, Indian mustard, lead uptake.

Lead (Pb) exists naturally in many forms throughout the world and has a soil retention time of 150 - 5 000 years (Friedland 1990). It has been demonstrated that Pb toxicity does not appear when organic matter and other mineral nutrients are in abundant supply (Baumhardt and Welch 1972), and that its toxicity occurs most commonly on waste heaps from mining operations where the organic matter and nutrient content of the soil are low (Woolhouse 1983). Lead toxicity in many nontolerant plants is reported to be associated with the disturbance of mitosis (Levan 1945, Ahlberg *et al.* 1972, Ramel 1973, Wierzbicka 1989, 1994), induction of leaf chlorosis (Johnson *et al.* 1977, Johnson and Proctor 1977), inhibition of enzyme activities (Hampp *et al.* 1973), depression of photosynthetic rate (Bazzaz *et al.* 1974) and inhibition of root elongation (Lane and Martin 1980).

The idea of using plants which hyperaccumulate metals to selectively remove and recycle excessive soil metals was introduced by Chaney (1983). Only recently has the value of metal-accumulating plants for environmental remediation been fully realized (Baker *et al.* 1994, Raskin *et al.* 1994, Salt *et al.* 1996). Most of the metal-accumulating plants, such as duckweed (*Lemna minor*) and water velvet (*Azolla pinnata*) are small, slow growing, and produce a small amount of biomass. However, Salt *et al.* (1996) reported that certain producing high biomass crop, cultivars of *B. juncea* can tolerate and accumulate unusually high amounts of heavy metals in their roots and shoots.

B. juncea var. *megarrhiza* can be thought to be a kind of plants grown in the soils contaminated by metals in Tianjin, China. The aim of this investigation was to increase our understanding of the effects of different concentrations of Pb^{2+} on the roots, hypocotyls, and shoots growth of *B. juncea* and the uptake and accumulation of Pb^{2+} by these plant parts.

Seeds of *B. juncea* L. var. *megarrhiza* kindly provided by the Centre of Popularizing Agriculture Technique of Tianjin, P.R. China were germinated and grown in a plastic cylinder (8 cm tall, 6 cm in diameter) containing a 7-cm deep layer of vermiculite. Plants were grown under a light/dark cycle of 14/10 h at temperature of 25/21 °C and irradiation of 360 W m^{-2}. The seedlings were fertilized every 3 d with Hoagland's nutrient solution containing 5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1 mM KH_2PO_4, 50 μM H_3BO_3, 4.5 μM $MnCl_2$, 3.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$ and 0.1 mM $(NH_4)_6Mo_7O_{24}$.

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Fifteen-day-old seedlings were selected for uniformity and their roots were rinsed in deionised water in order to remove traces of nutrient solution. Selected seedlings were grown hydroponically in the containers for 4 d. Each container was filled with 2000 dm^3 of aerated Pb solutions made up from lead nitrate, ranging from 10^{-3} to 10^{-5} M Pb. The Hoagland's nutrient solution was used for the control treatment.

Ten seedlings from each treatment and control were harvested after 4 d and were divided into roots, hypocotyls and shoots, respectively. The roots were washed thoroughly with deionised water. The samples were dried for 3 d at 40 °C and dried again for 6 h at 105 °C in oven, and ashed for 10 h at 600 °C. Pb was determined in ash by a *180-80* polarized *Zeeman* (Tokyo, Japan) atomic absorption spectrophotometer, using the procedure described by Hou (1991). All treatments were replicated 3 times.

The effects of Pb^{2+} on root growth of *B. juncea* varied with the different concentrations of lead nitrate solutions used (Fig. 1). Lead had no significant inhibitory effect on root length at concentrations of 10^{-5} to 10^{-4} M, while seedlings exposed to 10^{-3} M Pb solution showed slight reduction in growth (Fig. 1*A*). Shoot growth was strongly affected by exposure to Pb. The shoot dry mass (d.m.) of plants treated with 10^{-5} M Pb and 10^{-3} M Pb were 0.48 g and 0.39 g, respectively, when compared to 0.54 g for the control plants (Fig. 1*D*). Exposure to the same concentrations of Pb did not affect the d.m. of root and hypocotyl (Fig. 1*B*,*C*). Therefore, the shoots were more sensitive to the Pb toxicity than the roots. The seedlings appeared wilted during the first 24-h period, but recovered after that. Some leaves appeared chlorotic after 4 d of treatment with Pb^{2+}.

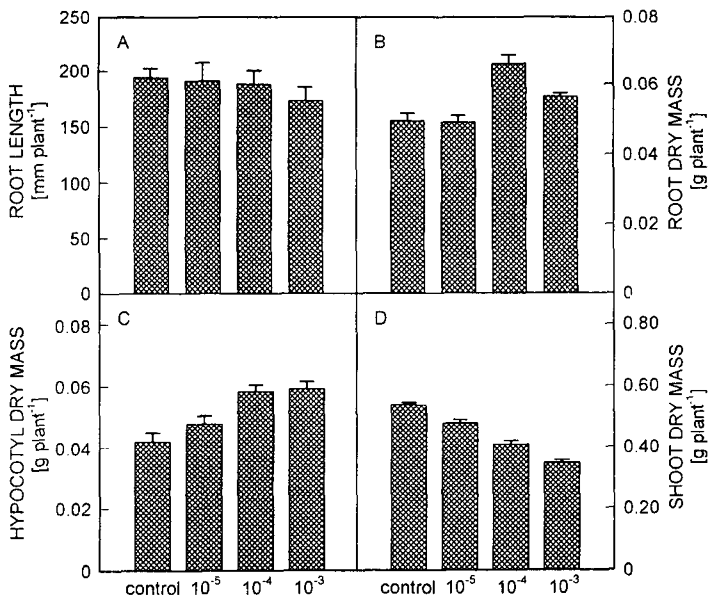

Fig. 1. Effect of Pb in different concentrations (10^{-5} to 10^{-3} M) on root length and root, hypocotyl, and shoot dry masses of *B. juncea*. *Vertical bars* denote SE (n = 10).

Uptake and accumulation of Pb in the roots, hypocotyls, and shoots varied with different Pb concentrations used. Pb contents in roots, hypocotyls, and shoots of *B. juncea* increased with increasing concentration of Pb^{2+} in the solution (Fig. 2). The Pb contents in the hypocotyls and the shoots of the different test groups were correlated with the accumulated Pb in the roots. The roots of plants exposed to 10^{-3} M Pb accumulated large amounts of Pb, and the Pb content was about 230-fold, greater than in the control. Also, the Pb content in the roots was about 6 and 17 times greater in 10^{-5} and 10^{-4} M Pb, respectively, in comparison to the control plants.

Accumulated Pb in the roots, hypocotyls, and shoots as a percentage of the total amount of absorbed Pb (Fig. 3) showed that in all the treated groups, except the treatment with 10^{-3} M Pb, the Pb ions accumulated mainly in the roots, and small amounts of Pb were translocated to the hypocotyls and the shoots. The hypocotyls of plants grown in 10^{-3} M Pb translocated about 45 % of absorbed Pb.

Lead is not generally considered an essential element for the growth of plants, but appears to stimulate plant growth in some plants in small amounts (Dou 1988). *B. juncea* can be regarded as a kind of plant that accumulates high levels of Pb and other heavy metals without negative effect (Nanda Kumar *et al.* 1995). This species is valuable in our understanding of plant tolerance to Pb especially from the standpoint of cytology. Although Pb induced c-mitosis in root tip cells of *B. juncea*, the frequency was low in comparison with *Allium* species, *e.g. Allium cepa* (Jiang and Liu 1999, Liu

1994). *B. juncea* also possesses low damage of cell structure under the high accumulation for Pb (Dushenkov *et al.* 1995).

The results in the present investigation indicated that lead slightly inhibited the root growth of *B. juncea* only at the concentration of 10^{-3} M Pb^{2+}. *B. juncea* has large capacity to take up Pb from solutions and accumulate it in roots, with a relatively small proportion being transported and concentrated in hypocotyls and shoots. These differences in root and shoot uptake can possibly be explained by the fact that one of the normal functions of roots is to selectively acquire ions from the soil solution, whereas shoot tissue does not normally play this role (Salt *et al.* 1997). These results can support the findings of Nanda Kumar *et al.* (1995) and Dushenkov *et al.* (1995). However, *B. juncea* var. *megarrhiza* has less efficient than the cultivars used by Nanda Kumar *et al.* (1995) and Dushenkov *et al.* (1995) for accumulating Pb in the roots and shoots.

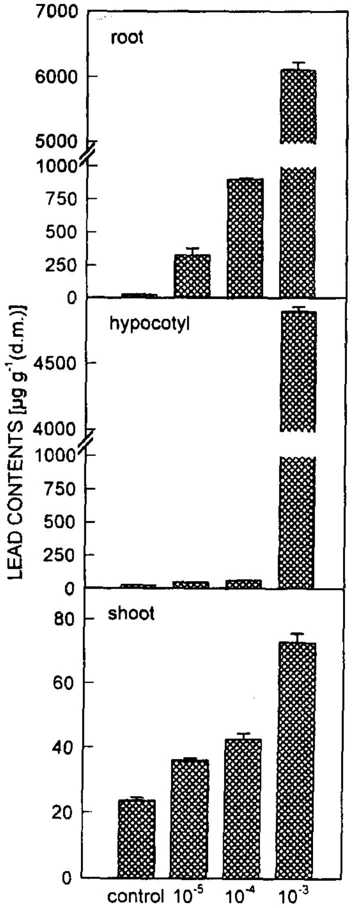

Fig. 2. Lead contents in *B. juncea* roots, hypocotyls and shoots after 4-d treatment with 10^{-5} to 10^{-3} M Pb. *Vertical bars* denote SE ($n = 4$).

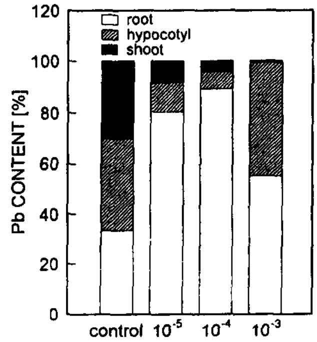

Fig. 3. The percentage of accumulated lead in root, hypocotyl, and shoot of *B. juncea* after treatment with different Pb concentrations.

The metabolic activity in the roots can return to normal level after the first 12 - 24 h treatment with Pb, although the total amount of lead in the roots keeps increasing (Wierzbicka 1995). This may explain the reason that the seedlings appeared wilted during the first 24-h of Pb^{2+} (10^{-3} M) treatment, but recovered after that. Many factors are involved in metal uptake, such as Ca and other elements content, presence of organic matter and clay fractions, pH, oxygen concentrations, and redox potential. The phytochelatins can be produced in roots of *B. juncea* exposed to Pb and involved in Pb detoxification (Salt *et al.* 1996). Baker (1981) considered tolerance as a constant low level shoot concentration of heavy metal until a critical soil concentration is reached when toxicity ensues and unrestricted metal transport appears. The mechanism on the high tolerance and accumulation, and detoxification of lead in the root tip cells of *B. juncea* remains to be explained.

Seedlings of *B. juncea* appear to have the potential to provide a novel method for the removal of metals from contaminated waters from various sources including sewage and groundwater used for irrigation, industrial wastes, and landfill leachates (Salt *et al.* 1997). Some of the fundamental questions about the tolerance of *B. juncea* to Pb, such as stress physiology and localization of metal ions in plant cells should be further answered.

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