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Growth Responses of Indian Mustard [*Brassica juncea* (L.) Czern.] and Its Phytoextraction of Lead from a Contaminated Soil

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Pollution of the environment with toxic metals has increased dramatically since the onset of the industrial revolution (Nriagu 1979). The main sources of this pollution are fossil fuel burning, mining and smelting of metalliferous ores, municipal wastes, landfill leachates, fertilizers, pesticides, and sewage (Forstner 1995). In 1986 there were approximately 1000 national priority or superfund sites in the U. S., 40% of which reported metal problems such as lead, cadmium, chromium and arsenic (Forstner 1995). Toxic metal contamination of soil, streams, and ground water poses a major environmental and human health risk. The threat that heavy metals pose to human and animal health is aggravated by their long-term persistence in the environment (Shaw 1990).

In spite of the ever-growing number of toxic metal-contaminated sites, the most commonly used method of cleaning a heavy-metal polluted site is excavation and burial. Recent estimates revealed that cleanup of U.S. sites contaminated with heavy metals alone can cost $7.1 billion while mixtures of heavy metals and organics bear an additional $35.4 billion (Salt et al. 1995). Recently however, the value of metal-accumulating plants for environmental cleanup has been vigorously pursued (Brown et al. 1995; Salt et al. 1995), giving birth to a specific area of phytoremediation termed phytoextraction (Kumar et al. 1995). The process of phytoextraction generally requires the translocation of heavy metals to easily harvestable shoots. In the phytoextraction process, several hyperaccumulating plants may be used in a cropping scheme to reduce heavy metal concentrations of contaminated sites to environmentally acceptable levels.

The success of any phytoremediation technique depends upon the identification of suitable plant species that hyperaccumulate heavy metals and produce large amounts of biomass using established crop production and management practices. Indian mustard was chosen because it is related to a well known heavy-metal hyperaccumulator, *Thlaspi caerulescens* (Baker et al. 1994), and it produces at least 20 times

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more biomass than *T. caerulescens* under field conditions (Salt et al. 1995). Moreover, Banuelos and Meek (1990) showed under field conditions that when Indian mustard was planted over several years and managed with minimal irrigation, selenium levels were reduced up to 50% within a soil depth of one meter. The specific objective of this experiment, therefore, was to determine the suitability of Indian mustard as a phytoextraction species based on its growth responses and Pb uptake when grown on a Pb-contaminated soil.

MATERIALS AND METHODS

Plants were grown at the Jackson State University glasshouse in the spring of 1996. The daylight period was extended to 12 hrs using high intensity super halide lamps (1000 W H.Y. Lites Horizontal System, High Yield, Inc., Camas, WA). The photosynthetically active radiation (PAR; 400-700 nm) measured at the canopy level was no less than 1800 µmol photons m^{-2} s^{-1}. Indian mustard *[Brassica juncea* (L.) Czern. cv. Florida Broadleaf] seeds were obtained from BWI Companies, Inc., Texarkana, TX 75505 USA. Unless otherwise specified, seeds were sown in 1 L plastic pots containing equal volumes of horticultural grade, coarse perlite and vermiculite. Based upon a preplanting germination test, a pre-determined number of pre-soaked seeds were planted per pot. Emerged seedlings were thinned out to a desired population density (2 plants per pot) at 10 days after planting. Four concentrations (0, 100, 250, and 500 µg / mL) of Pb (supplied as aqueous solutions of lead nitrate) were applied to the surface of the growth medium immediately after thinning. Plants were watered every 2 to 3 d, depending on the evaporative demand, with full strength, modified nutrient solution (Begonia 1997). Each lead treatment consisted of a row of ten pots (2 plants per pot) giving a total of 20 plants per treatment. Rows of pots were arranged in a randomized complete block design with four replications. Excess soil moisture draining from perforations at the bottom of each pot was trapped in a 10-cm plastic saucer placed below each pot to prevent leaching and cross contamination among pots.

Any symptoms of metal toxicity (e.g., discoloration, pigmentation, yellowing, stunting) exhibited by plants were visually noted during the experimental period. Just before harvest, total leaf area for each plant was measured. Subsamples of twenty 0.38 cm^2 leaf discs were randomly obtained from various leaves of each plant using 0.7 cm inside diameter cork borer. The specific leaf area (area / dry weight, cm^2 / g) of the leaf discs was used to convert leaf biomass into leaf area. All plants were at the early flowering stage when they were harvested at eight wks after planting. During harvest shoots and roots were separated, washed with tap water to remove any adhering debris, then oven-dried at 70°C for 48 hrs. Dried samples were weighed and ground in a Wiley

mill equipped with a 425 µm (40-mesh) screen. Pb contents of each 200 mg dry plant tissue were extracted using modified nitric acid-hydrogen peroxide procedures (Begonia 1997). Pb concentrations were quantified using inductively coupled argon plasma spectroscopy (Perkin Elmer Optima 6000) and expressed as µg Pb/g dry wt of plant tissue. This analytical system had a 97.3% recovery efficiency and detection limit of 5 ppb Pb.

RESULTS AND DISCUSSION

Lead applied at 100 µg/mL to the growth medium did not have a significant deleterious effect on total leaf area per plant (Table 1). There was an inverse relationship between total leaf area and concentration of applied Pb. Compared to the control plants, total leaf area was reduced by 25% and 47%, respectively, in plants treated with 250 and 500 µg/mL Pb. In addition to reduced leaf area expansion, Pb-treated plants exhibited anthocyanin pigmentation or purplish coloration, which developed within 14 d of initial Pb treatment. These pigmentations became more severe toward harvest time.

Table 1. Total leaf area (mean ± s.e.), shoot dry biomass, and root dry biomass of Indian mustard 8 weeks after planting as affected by various concentrations of soil-applied lead. Means followed by a common letter are not significantly different from each other using LSD test (P= 0.05).

Lead treatment (µg Pb/mL)	Total leaf area (cm^2/plant)	Shoot dry biomass (mg/plant)	Root dry biomas (mg/plant)
0	20.91a ±3.29	176.89a±29.2	134.12a ±20.6
100	23.33a ±2.76	211.12a±39.9	157.88a ±17.2
250	15.61bc ±1.88	212.45a±21.4	105.81b ±12.1
500	11.08c ±0.63	165.66a±11.3	75.53c ± 6.0

Shoot growth of Indian mustard was not inhibited by any concentration of applied Pb (Table 1).

The roots of Pb-treated Indian mustard were purplish, in contrast to the dirty white roots of untreated plants. Aside from the visual differences in color, root growths of plants treated with 250 and 500 µg/mL Pb were reduced 21% and 44%, respectively as compared to the untreated plants (Table 1). However, roots of plants treated with the lowest concentration (100 µg/mL Pb) were not affected by the metal.

Stunting is a commonly observed growth response in a wide range of plants grown in metal-laden soils. The reduction in growth, expressed as reduced leaf area and decreased root biomass (Table 1) of Pb-treated

plants can be due to specific toxicity of the metal to the plant, antagonism with other nutrients in the plant, or inhibition of the root penetration in the soil (Begonia 1997). In this study, there was not much Pb translocated to the shoot (Table 2). It is possible that the small amount of Pb translocated to the shoot was not enough to illicit a reduction in shoot biomass. A previous phytoextraction study showed that various varieties of Indian mustard exhibited differential uptake of Pb applied to the growth medium (Kumar et al. 1995). Perhaps, the variety used in this study was not one of those efficient translocators of Pb.

Although the nutrient solution and aqueous lead nitrate were applied separately, it is possible that the reduced leaf area and anthocyanin pigmentation in leaves of Pb-treated plants can be ascribed to a deficiency of an element like phosphorous. Lead had been shown to form insoluble complexes with phosphorous. One of the known symptoms of phosphorous deficiency in plants is the accumulation of anthocyanin in leaves (Salisbury and Ross 1992).

Shoots of Indian mustard accumulated different amounts of Pb depending on the concentration of Pb applied to the growth medium (Table 2). Generally, the amount of accumulated Pb in the shoot increased with increasing concentration of applied Pb. More specifically, no Pb was detected in the shoot when no lead (0 µg / mL Pb) was applied to the growth medium. However, the application of 100, 250, and 500 µg Pb / mL to the growth medium resulted in the shoot uptake of 72, 149, and 258 µg Pb / g dry biomass, respectively.

Table 2. Lead contents (mean ± s.e.) in shoots and roots of Indian mustard after eight weeks of growth in a lead-contaminated soil. Means followed by a common letter are not significantly different from each other using LSD test (P=0.05).

Lead treatment (µg Pb / ml)	Shoot lead content (µg Pb / g dry tissue)	Root lead content (µg Pb / g dry tissue)
0	0.0d	0.0d
100	72.5c±14.9	494.4c± 53.3
250	148.8b± 5.3	1,481.9b±160.1
500	258.1a±31.1	2,675.4a±l69.9

Roots of Indian mustard accumulated substantial amounts of soil-applied Pb (Table 2). As noted for shoots, the amounts of accumulated Pb increased with increasing concentration of applied Pb. No lead uptake was observed in roots of plants that received 0 µg / mL Pb. However, plants grown in soil contaminated with 100, 250, and 500

µg/mL Pb showed Pb uptake of 494, 1482, and 2675 µg Pb/g dry biomass, respectively. When averaged across treatments, accumulation of Pb in roots was almost ten times more than in the shoots (Table 2).

Tight binding of Pb to soils and plant materials partially explains the relatively low mobility of Pb in soils and plants. Lead binding to clay and organic matter and its inclusion in insoluble precipitates make a significant fraction of lead unavailable for root uptake by field-grown plants. While plants are known to concentrate Pb in the roots, Pb translocation to the shoots is normally very low. For example, in most Pb-contaminated soils studied by Kumar et al. (1995), Pb in soil solution was usually less than 0.15 %. Thus, Pb availability to plants was limited. It was also shown that in plants growing in the above Pb-contaminated soils, Pb translocation from roots to shoots was less than 30% even for the best Pb-translocating variety. Moreover, actively growing roots provide a barrier which restricts the movement of Pb to the above-ground parts of plants (Jones et al. 1973). This restricted movement of Pb may explain why Pb concentrations in shoots were relatively less than in the roots. This point is further substantiated by previous findings (Kumar et al. 1995) which showed that significant Pb translocation to the shoots of Indian mustard and other species was observed only at relatively high concentrations of Pb in the hydroponic solution, and only after the Pb-binding capacity of the roots was partially saturated.

Recent studies (Huang et al. 1997; Blaylock et al. 1997) demonstrated that addition of chelates to a Pb-contaminated soil increased shoot Pb concentrations to as high as 140-fold in corn *(Zea mays* L.) and pea *(Pisum sativum* L.). The surge in Pb accumulation in both plant species was associated with the surge in Pb level in the solution due to the addition of chelates to the soil. Chelate-assisted metal phytoextraction efforts must be further pursued in the future to enhance the effectiveness of phytoextraction as a decontamination strategy for metal-laden soils.

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