Bull. Environ. Contam. Toxicol. (1998) 61:44-50

Bulletin of Environmental Contamination and Toxicology

Growth Responses of Radish Plants to Soil Cadmium and Lead Contamination

M. S. Zaman, F. Zereen

Department of Biological Sciences and Department of Chemistry, Alcorn State University, Lorman, MS 39096, USA

Received: 27 February 1998/Accepted: 30 April 1998

Cadmium (Cd) and lead (Pb) are ubiquitous and potentially hazardous contaminants in the biosphere. These metals tend to accumulate in the soil plow layers. In addition, lead is tightly bound in most soils (Kabata-Pendias and Pendias 1989; Kumar et al. 1995). It is important to study the chemical forms, mobility, and distribution of these toxic metals in contaminated soils since these metals may eventually be translocated to plant tissues (Brown et al. 1996; Dudka et al. 1996) and may pose a threat to human health.

Various analytical methods are used to detect the existence of toxic metals in soil. However, these chemical measurements may not provide an actual measure of toxicity under field conditions (Cheung et al. 1989). Chemical doses can be translated into a quantifiable biological response by using bioassays (Cheung et al. 1989). The use of biological organisms as indicators of metal toxicity has been considered a relatively inexpensive, simple, and reliable alternative to chemical analyses (Hernandez et al. 1987).

The radish is considered to be a model crop (Kostka-Rick and Manning 1993) that is easily grown under a wide range of climates. The radish has been widely used as an indicator plant (Davies 1993) in experiments involving acid rain (Olson et al. 1987; Jacobson et al. 1988) ozone (Miyake et al. 1989) sulfur dioxide (Coleman et al. 1990), carbon dioxide (Idso et al. 1988), and heavy metal pollution (Khan and Frankland 1983). The objectives of this study were to evaluate the responses and tolerance of radish plants to various soil Cd and Pb levels, and to assess if the radish can be used as a biomonitor of Cd and Pb contamination of the environment.

MATERIALS AND METHODS

Experiments were conducted on Scarlet White Tip, a spring variety radish *(Raphanus sativus* L.). Seeds were purchased from a local seed store. Spring radishes have an exceptionally short growth

Correspondence to: M. S. Zaman

cycle (25-28 days) and can be grown under a wide range of climatic conditions (Kostka-Rick and Manning 1993).

Plants were grown on Memphis silt loam soil collected from an undisturbed, uncultivated forest area of Claiborne county in southeast Mississippi. This is a well characterized soil containing about 70% silt, 20% clay, 9% sand, and 1% organic matter with a pH of 6.9 (Panicker 1992).

Radish seeds were sown in plastic planters with four small holes at the bottom. Each planter contained 145 g of soil (dry weight). Approximately a 2-cm depression was made in the middle of the soil, and one pregerminated seed (radicle length about 3 mm) was placed into each depression. The seeds were covered with just enough soil to make them no longer visible. Twelve planters were placed in a 16 x 12 x 1 in. reservoir tray. Distilled water and nutrient solution were added to the reservoir tray throughout the study period. Water was added every other day or as needed. Once a week, full strength Hoagland solution (Hoagland and Arnon 1950) was added to the reservoir tray. Separate trays were maintained for different treatment groups. The plants were maintained in a glasshouse at 22.8 ± 1.2° C day time temperature, and 49.7 ± 2.6% relative humidity.

In experiment 1, plants were divided into one control group, three Cd treated groups (soil containing 100 ppm, 500 ppm, and 1000 ppm Cd), and three Pb treated groups (soil containing 100 ppm, 500 ppm, and 1000 ppm Pb). In experiment 2, plants were divided into one control group, three Cd treated groups (soil containing 50 ppm, 100 ppm and 500 ppm Cd), and four Pb treated groups (soil containing 100 ppm, 500 ppm, 1000 ppm and 2000 ppm Pb). In experiment 3, plants were divided into one control group, three Cd treated groups (soil containing 50 ppm, 100 ppm and 500 ppm Cd), and two Pb treated groups (soil containing 100 ppm and 500 ppm Pb). Cadmium or Pb was mixed with the soil in the form of $Cd(NO_3)_2$ or $Pb(NO_3)_2$ (Fisher Scientific, Fair Lawn, New Jersey, USA). In all experiments, each group consisted of 12 plants and a separate planter was used for each plant.

Plants were harvested on day 30 of the experiment. The hypocotyls and shoots were completely dehydrated at 70° C for 7 days. Dry weights of plant parts (hypocotyl and shoot) and the total plant for each group were determined. On the basis of the currently accepted protocol for phytotoxicity testing (Leita et al. 1993), the Grade of Growth Inhibition (GGI) was evaluated. All treatments were compared to the control group (control GGI = 0, i.e., 100% growth).

GGI = [(C-T) / C] x 100 where C and T represent the dry weight of tissues of control (C) and metal-treated plants (T).

Chlorophyll concentration was determined in experiment 3 using the methods of Einhellig and Rasmussen (1979). Following harvesting, leaves from an individual plant were immersed in 30 ml of 95% ethanol for 24 hr in a test tube. Ethanol-chlorophyll solution was decanted to a second container, and leaves were soaked for a second 24 hr period. Ethanol-chlorophyll solutions were pooled from each extraction. Extracted leaves were dried at 70° C for 96 h to determine dry weight. Test tubes were sealed with parafilm and ethanol-chlorophyll solutions were stored in darkness at room temperature. The absorbance (A) of the extract was determined at 665 and 649 nm on a spectrophotometer (Perkin-Elmer, Lambda 3A, UV/VIS spectrophotometer). Chlorophyll concentrations (µg chlorophyll/mg dry weight of leaf) and total chlorophyll content of each plant were calculated using the following equations (Einhellig and Rasmussen 1979):

$$\mu g \text{ chlorophyll a/mL solution} = (13.70)\ (A_{665\ nm}) - (5.76)\ (A_{649\ nm})$$
$$\mu g \text{ chlorophyll b/mL solution} = (25.80)\ (A_{649\ nm}) - (7.60)\ (A_{665\ nm})$$

Data obtained in this study were analyzed using one-way ANOVA and Tukey test. All values are reported as mean ± SEM.

RESULTS AND DISCUSSION

In experiment 1, plants treated with 500 ppm Cd and 1000 ppm Cd did not survive the toxicity, and therefore, they could not be used for statistical evaluation.

Table 1. The Grade of Growth Inhibition (GGI) of radish plants grown in soil containing varying concentrations of Cd or Pb for 30 days - experiment 1

Treatment	Hypocotyl (mean ± SEM)	Shoot (mean ± SEM)	Whole Plant (mean ± SEM)
100 ppm Pb	42.6 ± 9.2^{c}	5.4 ± 9.8^{b}	10.3 ± 9.1^{b,c}
500 ppm Pb	60.9 ± 5.0^{b,c}	2.0 ± 9.7^{b}	23.6 ± 6.6^{a,c}
1000 ppm Pb	70.9 ± 5.4^{b}	20.8 ± 8.4^{b}	39.1 ± 7.1^{a}
100 ppm Cd	92.7 ± 0.8^{a}	73.9 ± 4.7^{a}	81.3 ± 4.1^{d}
500 ppm Cd	Plants did not survive		
1000 ppm Cd	Plants did not survive		

Means followed by the same letter between the treatment groups are not significantly different at the $p < 0.05$ level: Tukey test.

A significant shoot GGI was observed in plants treated with 100 ppm Cd and 1000 ppm Pb. A significant hypocotyl GGI was observed in all Cd and Pb treated groups. A significant total plant (shoot plus hypocotyl) GGI was observed in plants treated with 500 ppm Pb, 1000 ppm Pb, and 100 ppm Cd. These effects were dose

related and the Cd induced more growth inhibition compared to Pb (Table 1).

In experiment 2, a significant shoot, hypocotyl, and total plant (shoot plus hypocotyl) GGI were observed in all Cd and Pb treated groups (Table 2).

Table 2. The Grade of Growth Inhibition (GGI) of radish plants grown in soil containing varying concentrations of Cd or Pb for 30 days - experiment 2

Treatment	Hypocotyl (mean ± SEM)	Shoot (mean ± SEM)	Whole Plant (mean ± SEM)
100 ppm Pb	45.1 ±12.5^{c}	49.9 ± 5.2^{b}	48.5 ± 6.9^{a,e}
500 ppm Pb	81.9 ± 4.5^{d}	67.4 ± 4.1^{d}	71.8 ± 4.0^{d}
1000 ppm Pb	79.8 ± 4.3^{d,b}	66.6 ± 4.6^{d}	70.6 ± 4.0^{d}
2000 ppm Pb	89.1 ± 1.4^{a}	81.4 ± 3.3^{a}	83.8 ± 2.7^{b}
50 ppm Cd	51.2 ± 4.8^{c,e}	32.0 ± 6.9^{c}	37.8 ± 5.0^{c,e}
100 ppm Cd	68.9 ± 6.2^{b,e}	51.6 ± 5.7^{b}	56.9 ± 5.5^{a}
500 ppm Cd	94.3 ± 1.1^{a}	81.2 ± 5.4^{a}	85.2 ± 4.0^{b}

Means followed by the same letter between the treatment groups are not significantly different at the $p < 0.05$ level: Tukey test.

Table 3. Chlorophyll concentration (µg chlorophyll/mg leaf dry wt) of radish plants grown in soil containing varying concentrations of Cd or Pb for 30 days - experiment 3

Treatment	Chl. a (mean ± SEM)	Chl. b (mean ± SEM)	Total Chl. (a+b) (mean ± SEM)
Control	6.7 ± 0.2^{c}	4.0 ± 0.1^{b,d}	10.7 ± 0.2^{b}
500 ppm Pb	5.9 ± 0.1^{b}	3.3 ± 0.0^{d,e}	9.2 ± 0.1^{e}
1000 ppm Pb	5.3 ± 0.1^{b}	3.1 ± 0.2^{c}	8.4 ± 0.3^{a,d}
50 ppm Cd	6.3 ± 0.3^{c}	3.3 ± 0.2^{c,e}	9.6 ± 0.5^{b,e}
100 ppm Cd	5.8 ± 0.2^{b}	3.1 ± 0.1^{c}	8.8 ± 0.2^{d}
500 ppm Cd	1.0 ± 0.4^{a}	1.5 ± 0.5^{a}	2.5 ± 0.2^{c}

Means followed by the same letter between the treatment groups are not significantly different at the $p < 0.05$ level: Tukey test.

Chlorophyll concentration (µg chlorophyll/mg leaf dry wt) was found to be significantly reduced in most metal treated groups. Significant differences of chlorophyll a, chlorophyll b, and chlorophyll a+b concentrations between different treatment groups are presented in Table 3.

Chlorophyll content (total chlorophyll in µg) was found to be reduced in all Cd treated plants. No significant reduction in total chlorophyll content was observed in Pb treated plants (Table 4).

The chlorophyll a+b content of 500 ppm Cd treated plants was significantly lower than all other groups. The chlorophyll a+b content of 100 ppm Cd treated plants was significantly lower than that of the control, 500 ppm Pb, and 1000 ppm Pb treated plants. The chlorophyll a+b of 50 ppm Cd treated plants was significantly lower than that of the control, 500 ppm Pb, and 1000 ppm Pb treated plants (Table 4).

Table 4. Total chlorophyll content (µg) of radish plants grown in soil containing varying concentrations of Cd or Pb for 30 days - experiment 3

Treatment	Chl. a (mean ± SEM)	Chl. b (mean ± SEM)	Total Chl. (a+b) (mean ± SEM)
Control	755 ± 33.1^{c}	449 ± 33.0^{c}	1200 ± 65.9^{c}
500 ppm Pb	672 ± 19.3^{c,d}	383 ± 14.6^{c}	1050 ± 33.2^{c}
1000 ppm Pb	753 ± 39.9^{c}	441 ± 43.9^{c}	1190 ± 83.5^{c}
50 ppm Cd	234 ± 20.1^{d}	122 ± 10.3^{b}	356 ± 30.3^{b}
100 ppm Cd	336 ± 43.3^{b}	175 ± 18.4^{b}	511 ± 61.3^{b}
500 ppm Cd	3.1 ± 1.2^{a}	2.8 ± 0.5^{a}	5.9 ± 0.7^{a}

Means followed by the same letter between the treatment groups are not significantly different at the $p < 0.05$ level: Tukey test.

In experiment 1, plants treated with 500 and 1000 ppm Cd did not survive. In experiment 2, although the 500 ppm Cd treated plants did survive, the plant growth was inhibited about 85 percent (Tables 1 and 2). The shoot GGI of 100 and 500 ppm Pb treated groups was statistically significant in experiment 2, but not in experiment 1 (Tables 1 and 2). A similar situation was also observed when whole plant GC1 of 100 ppm Pb treated groups in experiments 1 and 2 (Tables 1 and 2) were considered. All other comparisons in plant growth were statistically significant both in experiments 1 and 2 (Tables 1 and 2).

Begonia (1997) reported that presence of Pb in artificial soil media produced dose-related biomass inhibition in several plant species. The present series of experiments demonstrate that the presence of Cd or Pb in soils produces dose-related growth inhibition in radish plants. These experiments also indicate that Cd and Pb affect the development of radish plants to different degrees. Plant growth was more inhibited in the Cd contaminated soils than in the Pb contaminated soils probably for the following reasons: (1) Pb is more bound to the soil particles (Kabata-Pendias and Pendias 1989; Kumar et al. 1995), and therefore, less available to plant tissue, or (2) Cd is more mobile than Pb within the plant tissues (Khan and Frankland 1983; Dushenkov et al. 1995), and therefore, is capable of exerting more toxic effects. Further experiments are required to address these issues.

Plants that demonstrated growth inhibition, also demonstrated low chlorophyll concentration/content in the leaf tissue. Since there is a correlation between chlorophyll content and leaf area photosynthetic rate (Buttery and Buzzell 1977), it is possible that reduction in chlorophyll in treated plants caused an overall inhibition in plant growth due to inadequate photosynthesis.

Since the Scarlet White Tip radish demonstrates different levels of sensitivity in response to various concentrations of Cd or Pb in soils, it is suggested that these plants may be used as biomonitors/bioindicators for Cd and Pb assessments in metal polluted soils. Moreover, since this variety of radish has the potential to survive in Cd and Pb contaminated soils, it is possible that these plants may be able to absorb such metals from soil and thus may be used in phytoremediation. The determination of plant tissue metal content will establish the correlation between metal concentration in soil and the optimum bioaccumulation of these metals in plant tissue. To explore these possibilities, this study must be extended using larger plant samples and soils with various metal concentrations. Further studies may be conducted to identify other metal-tolerant varieties of radish which might be more suitable for biomonitoring and phytoremediation studies.

Acknowledgments. This project was partly supported by a grant from DOE. The authors greatly appreciate Drs. Charles F. Rhyne, Susmita Ghosh, and Gregorio B. Begonia, Jackson State University, Jackson, Mississippi, USA, and Mr. Muhammad Rizvi and Mrs. Narjis Rizvi, Alcorn State University, Lorman, Mississippi, USA, for their support in this project.

REFERENCES

Begonia GB (1997) Comparative lead uptake and responses of some plants grown on lead contaminated soils. J Miss Acad Sci 42: 101-106

Brown SL, Chaney RL, Lloyd CA, Angle JS, Ryan JA (1996) Relative uptake of cadmium by garden vegetables and fruits grown on long-term biosolid-amended soils. Environ Sci Technol 30: 3508-3511

Buttery BR, Buzzell RI (1977) Relationship between chlorophyll content and rate of photosynthesis in soybeans. Can J Plant Sci 57: l-5

Cheung YH, Wong MH, Tam NFY (1989) Root and shoot elongation as an assessment of heavy metal toxicity and 'Zn equivalent value' of edible crops. Hydrobiologia 188/189: 377-383

Coleman JS, Mooney HA, Winner WE (1990) Anthropogenic stress and natural selection: variability in radish biomass accumulation increases with increasing SO_2 dose. Can J bot 68:102-106

Davies BE (1993) Radish as an indicator plant for derelict land: uptake of zinc at toxic concentrations. Comm Soil Sci Plant Anal 24:1883-1895

Dudka S, Piotrowska M, Terelak H (1996) Transfer of cadmium, lead, and zinc from industrially contaminated soil to crop plants: a field study. Environ Pollut 94:18l-188

Dushenkov V, Kumar PBAN, Motto H, Raskin I (1995) Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ Sci Technol 29:1239-1245

Einhellig FA, Rasmussen JA (1979) Effects of three phenolic acids on chlorophyll content and growth of soybean and grain sorghum seedlings. J Chem Ecol 5: 815-823

Hernandez LM, Gonzalez MC, Hernan MA (1987) Environmental contamination by lead and cadmium in plants from urban area of Madrid, Spain. Bull Environ Contam Toxicol 38: 203-208

Hoagland DR, Arnon DI (1950) The water culture method for growing plants without soil. Calif Agr Exp Sta 347

Idso SB, Kimball BA, Mauney JR (1988) Effects of atmospheric CO_2 enrichment on root: shoot ratios of carrot, radish, cotton and soybean. Agric Ecosys Environ 21: 293-299

Jacobson JS, Kuja A, Shriner D, Perrigan S, Irving PM, Lee J, Troiano J, Cullinan V (1988) A collaborative effort to model plant response to acidic ram. J Air Pollut Contr Assoc 38: 777-783

Kabata-Pendias A, Pendias H (1989) Trace elements in soils and plants. CRC Press, Boca Raton, FL

Khan DH, Frankland B (1983) Effects of cadmium and lead on radish plants with particular reference to movement of metals through soil profile and plant. Plant Soil 70: 335-345

Kostka-Rick R, Manning WJ (1993) Radish (Raphanus sativus L.) : a model for studying plant responses to air pollutants and other environmental stresses. Environ Pollut 82: 107-137

Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ Sci Technol 29: 1232-1238

Leita L, Nobili MD, Mondini C, Garcia MTB (1993) Response of Leguminosae to cadmium exposure. J Plant Nutr 16:200l-2012

Miyake H, Matsumura H, Fujinuma Y, Totsuka T (1989) Effects of low concentrations of ozone on the fine structure of radish leaves. New Phytol 111: 187-195

Olson RL, Winner WE, Moore LD (1987) Effects of 'pristine' and 'industrial' simulated acidic precipitation on greenhouse-grown radishes. Environ Exp Bot 27: 239-244

Panicker, GK (1992) The effects of pine needles, gypsum and polymers on soil crusting, seedling emergence, and yield of snap beans. M.S. Thesis, Alcorn State University