EFFECTS OF SELECTED TRACE METALS ON GERMINATING SEEDS OF SIX PLANT SPECIES

CLAIRE L. CARLSON*, DOMY C. ADRIANO, KENNETH S. SAJWAN, STEVEN L. ABELS, DAVID P. THOMA, and JOSEPH T. DRIVER

Biogeochemistry Division, Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802, U.S.A.

(Received July 2, 1990; revised January 21, 1991)

Abstract. Seeds of cabbage, lettuce, millet, radish, turnip, and wheat were treated with solutions containing Be, Ni, Tl, or V, and subsequent effects on seed germination and radicle elongation were measured after three days. Treatment with low concentrations of Be, Ni, or V stimulated root elongation in most species. Higher concentrations of these elements and all treatment with Tl caused reductions in root elongation. In general, turnip and lettuce were the most sensitive of the plants studied to the metals tested, while wheat and millet were the least sensitive.

1. Introduction

Beryllium, Ni, Tl, and V are potentially phytotoxic trace elements which are released into the environment from coal combustion (Peterson and Girling, 1981; Schoer, 1984; Adriano, 1986). While the toxic effects of Ni on plants have been fairly well-documented, relatively little is known about the toxicity of Be, Tl, and V. The few studies which have been conducted using Be salts have indicated that Be is very toxic, but at low concentrations the metal can stimulate growth (Williams and Le Riche, 1968; Peterson and Girling, 1981; Adriano, 1986). Nickel has also been reported to stimulate growth at low concentrations, while producing toxic effects at high concentrations (Mishra and Kar, 1974; Das and Mishra, 1978; Singh, 1984, 1985).

Thallium and V have both been shown to be very phytotoxic, with significant growth reductions observed in lettuce at solution concentrations of 0.05 mg V L^{-1} (Lepp, 1977) and chlorosis observed in tobacco plants at solution concentrations of 0.04 mg Tl L^{-1} (Smith and Carson, 1977). Growth stimulation upon treatment with V has also been reported in some plants, including lettuce, corn, tomato, barley, and rice (Basiouny, 1984).

Measurement of the inhibition of seed germination and root elongation is a U.S. Environmental Protection Agency (EPA)-approved method of determining the toxicity of wastes to plants (Brusick and Young, 1982). Although both parameters are acceptable, root elongation inhibition is the preferred endpoint (Brusick and Young, 1982). Radish, wheat, lettuce, and cabbage are among the plant species

* Author for all correspondence; current address of senior author: Research Services Office, 120 McFall Center, Bowling Green State University, Bowling Green, Ohio 43403-0180.

Water, Air, and Soil Pollution **59**: 231–240, 1991.

suggested by the EPA for testing (Wang, 1987). Turnip and millet have also been suggested as possible test species (Wang, 1987).

This study was undertaken to assess the relative toxicity of Be, Ni, Tl, and V to cabbage, lettuce, millet, radish, turnip, and wheat, six agronomic species known to vary in their tolerance to trace elements (Wang, 1987; Carlson *et al.*, 1989). While the toxicity of Ni has been fairly well established in previous studies, it was included in this study to provide a point of comparison for the less well-studied elements.

2. Methods

Experimental solutions were prepared using $NiSO_4 \cdot 6H_2O$, Tl_2SO_4, $VOSO_4 \cdot 3H_2O$, and $BeSO_4 \cdot 4H_2O$ salts (99.9% pure) in 0.01 M $Ca(NO_3)_2$, to obtain concentrations of 0, 0.25, 0.5, 1, 2, 4, 8, 12, 16, and 20 mg Ni L^{-1} and 0, 0.5, 1, 2.5, 5, 7.5, 10, 20, 30, and 40 mg L^{-1} for Be, Tl, and V. The calcium nitrate was used as a background solution to maintain constant ionic strength across all concentrations of the metals.

Seeds of cabbage (*Brassica oleracea* var. *capitata* L.), lettuce (*Lactuca sativa* L.), millet (*Panicum miliaceum* L.), radish (*Raphanus sativus* var. *radicula* Perzoon.), turnip (*Brassica rapa* L.), and wheat (*Triticum aestivum* L. var. Florida 301) were obtained from a certified seed dealer. Seeds of a given species were placed, 10 seeds per dish, in 15-cm polyethylene Petri dishes containing one piece of Whatman No. 1 filter paper. Each Petri dish was then treated with 4 mL of the appropriate solution, wrapped with Parafilm™, and placed in an environmental chamber in a randomized, complete-block design. Each treatment was replicated ten times, for a total of 100 dishes per plant species for each metal. The environmental chamber was maintained on a 16/8 hr day/night photoperiod, with day and night temperatures of 28 °C and 24 °C, respectively. Illumination was supplied from cool white fluorescent lamps providing 64 $\mu E\ m^{-2}\ s^{-1}$.

After 3 days, the number of seeds germinated for each dish and the radicle length for each seedling in a dish were measured. The radicle lengths for a given Petri dish were averaged to obtain a mean length per dish. These averages were used for the statistical analysis. Percent germination was also calculated and used in the statistical analysis. Linear regression and multiple comparisons were performed using the SAS statistical package (SAS Institute, Cary, NC). To allow comparisons among plant species, radicle lengths expressed as a percent of the control were calculated. These percentages were then used to determine the relative sensitivity of the plants to the four metals.

3. Results and Discussion

Treatment with Ni at concentrations up to 20 mg Ni L^{-1} or with Be, Tl, or V at concentrations up to 40 mg L^{-1} did not significantly ($P > 0.05$) affect seed germination for any of the species studied. However, significant ($P < 0.05$) effects on seedling radicle elongation were observed. Because plant response varied among metals, effects on seedling radicle elongation will be discussed separately for each metal.

3.1. Beryllium

Of the plants studied, turnip and lettuce were the most sensitive to Be, with reductions

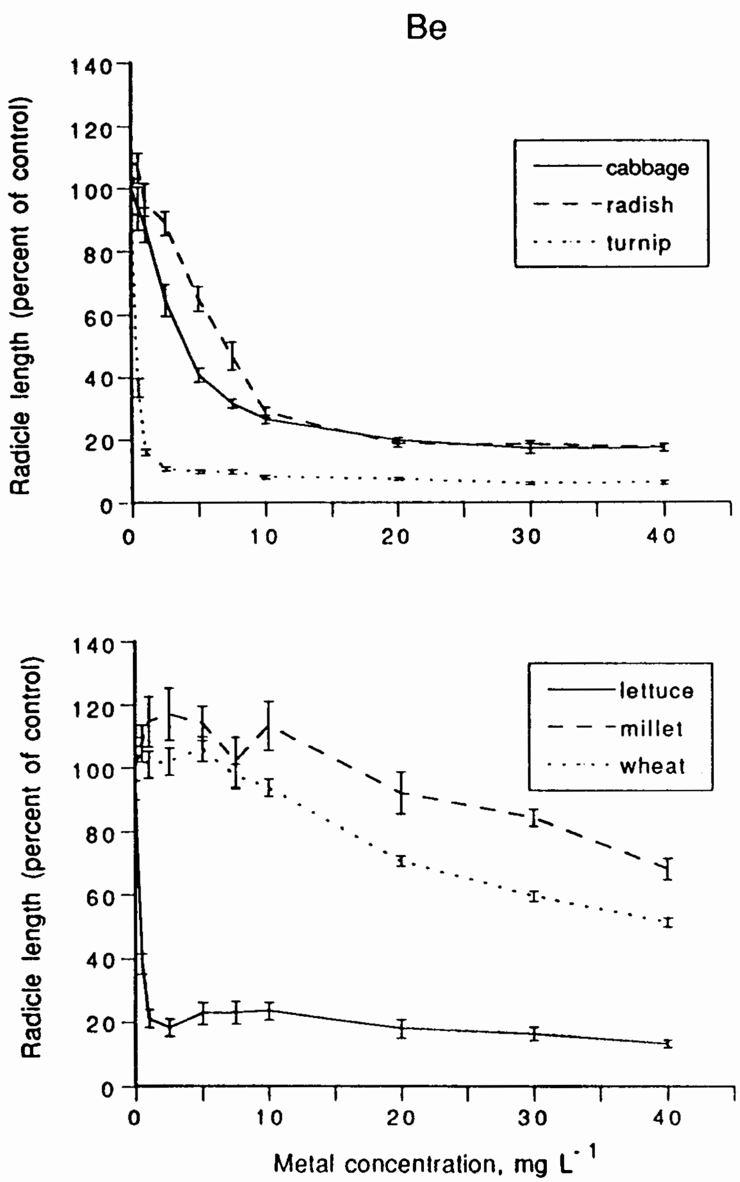

Fig. 1. Effects of Be on root elongation of cabbage, radish, turnip, lettuce, millet, and wheat. Values given are means plus or minus 1 SE.

in radicle length of 63 and 62%, respectively, when exposed to 0.5 mg Be L^{-1} (Figure 1). Radish and cabbage were less sensitive to Be, but still exhibited >50% reduction in radicle length at a solution concentration of 7.5 mg Be L^{-1}. Wheat and millet were the least sensitive of the plants studied to Be, with millet exhibiting improved radicle elongation in the presence of up to 10 mg Be L^{-1}. The order of sensitivity to Be (based on radicle length reduction) was: turnip > lettuce > cabbage = radish > wheat > millet.

The Be concentrations associated with reduced root elongation in cabbage, lettuce, radish, and turnip in this study are similar to values reported in the literature. Romney and Childress (1965) reported that concentrations greater than 2 mg Be L^{-1} in nutrient solution significantly reduced the growth of lettuce, beans, and clover. Peterson and Girling (1981) also found that concentrations of 1 to 2 mg Be L^{-1} in nutrient solution were toxic to crop plants. Romney *et al.* (1962) found that 3 mg Be L^{-1} in nutrient solution retarded root development of bush beans.

The response of wheat and millet in this study was surprising, however. Reduced root elongation was not observed for these two species until the concentration exceeded 10 mg Be L^{-1}. Romney and Childress (1965) reported reduced growth of wheat at solution concentrations of 4 mg Be L^{-1}. Their study was conducted using 10-day-old seedlings in nutrient solution. Because the root is the first organ to be affected by Be, the earliest stage of plant development, i.e., germination, should be the most sensitive to Be toxicity (Yopp *et al.*, 1974). However, less sensitivity was observed in this study using germinating seeds than was observed by Romney and Childress (1965) using 10-day-old seedlings. The wheat cultivar used in this study must have been less sensitive to Be than the one used by Romney and Childress (1965). This variability among species, and among varieties within a species, in response to Be should be kept in mind when using plants species for toxicity testing.

The stimulatory effect of Be at low concentrations has been reported by previous investigators (Williams and Le Roche, 1968; Peterson and Girling, 1981). Hoagland (1952) demonstrated that Be inhibits the splitting of ATP, and hypothesized that this was responsible for the stimulatory effect. The effects of Be on enzyme activity and the ability of Be to substitute for Mg have also been proposed as the cause of this effect (Williams and Le Riche, 1968; Yopp *et al.*, 1974; Adriano, 1986).

3.2. Nickel

Turnip and lettuce were also the most sensitive of the plants studied to Ni, with radicle lengths reduced by 72 and 59%, respectively, at 2 mg Ni L^{-1} (Figure 2). Cabbage, wheat, and radish were intermediate in sensitivity to Ni, with millet again the least sensitive of the species studied. Cabbage, lettuce, and millet exhibited increased radicle elongation when treated with low concentrations of Ni. This effect was most pronounced in the case of millet, in which greater than 4 mg Ni L^{-1} was necessary to reduce radicle lengths to significantly less than that of the controls. The order of sensitivity to Ni was: turnip > lettuce > cabbage > wheat = radish > millet.

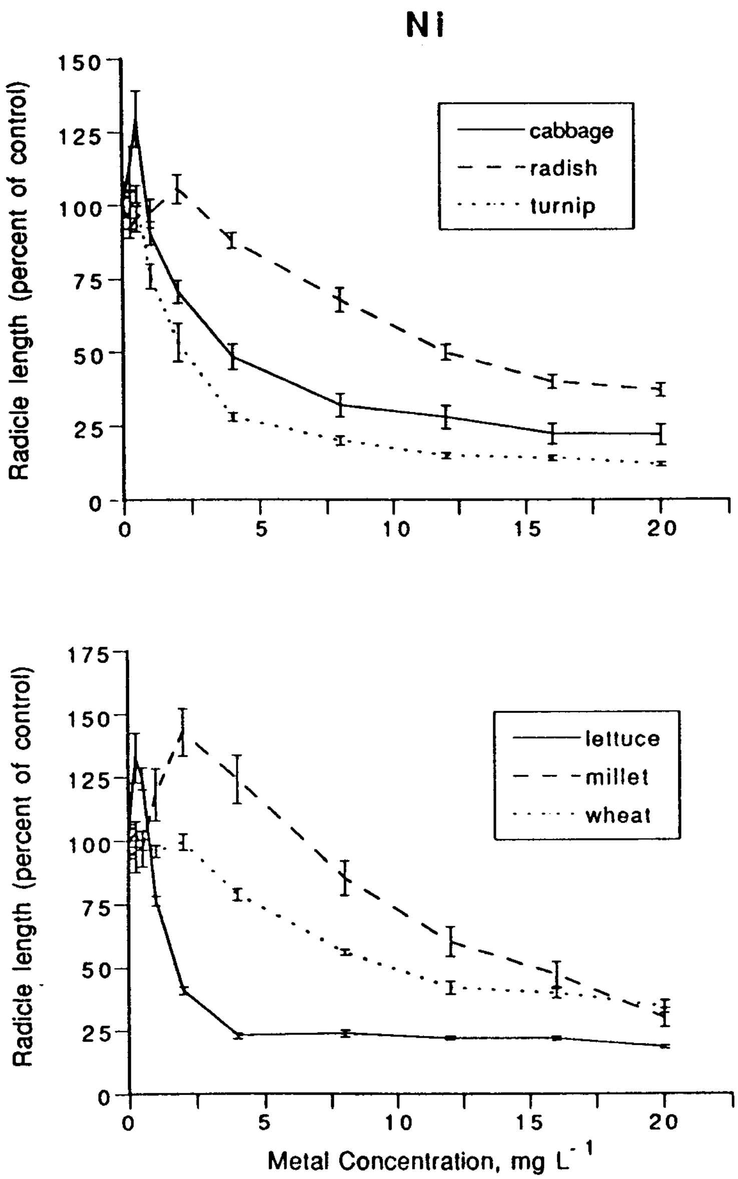

Fig. 2. Effects of Ni on root elongation of cabbage, radish, turnip, lettuce, millet, and wheat. Values given are means plus or minus 1 SE.

Previous investigators have also reported that lettuce is very sensitive to Ni (Berry, 1978; Wang, 1987). However, in both studies, concentrations of approximately 0.85 mg Ni L^{-1} were associated with a 50% reduction in the radicle length of germinating lettuce seeds. In this study, concentrations greater than 1 mg Ni L^{-1} were required to cause a 50% reduction. In fact, at a concentration of 0.5 mg Ni L^{-1}, radicle elongation was stimulated. There appears to be a narrow range between beneficial and toxic concentrations of Ni for this species. The stimulatory effect of low concentrations of Ni has been well-documented for a variety of species (Mishra and Kar, 1974; Davis *et al.*, 1978; Singh, 1985; Adriano, 1986).

3.3. THALLIUM

Lettuce was the most sensitive species to Tl, with radicle length reduced by 67%

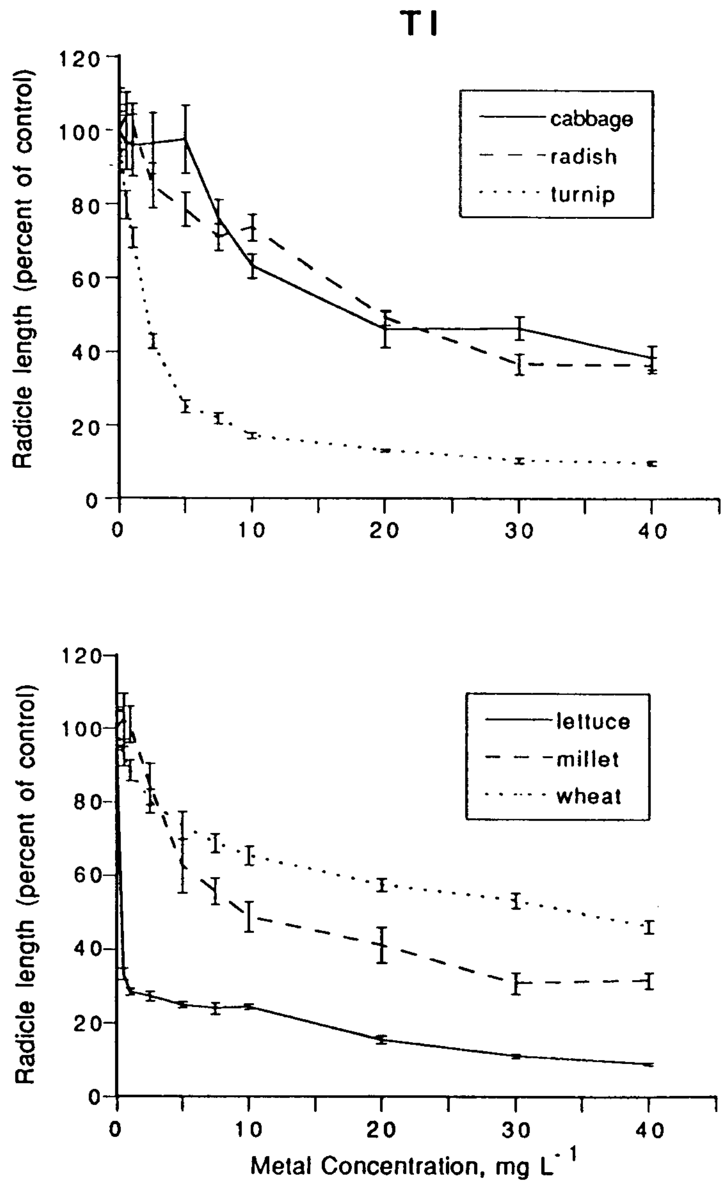

Fig. 3. Effects of Tl on root elongation of cabbage, radish, turnip, lettuce, millet, and wheat. Values given are means plus or minus 1 SE.

at a concentration of 0.5 mg Tl L^{-1} (Figure 3). Turnip was also quite sensitive to Tl, with a reduction in radicle length of 57% at a concentration of 2.5 mg Tl L^{-1}. Millet was less sensitive to Tl than lettuce and turnip, but still exhibited radicle length reductions of 37% at 5 mg Tl L^{-1}. Wheat, radish, and cabbage were the least sensitive to Tl. None of the plants studied exhibited a stimulatory effect of Tl on radicle elongation. The order of sensitivity to Tl was: lettuce > turnip > millet > wheat = radish = cabbage.

Thallium is considered a very phytotoxic element, with toxicity associated with solution concentrations of less than 1 mg Tl L^{-1} (Yopp *et al.*, 1974; Adriano, 1986). Schoer (1984) reported that lettuce and radish are not very susceptible to Tl toxicity. However, in this study, lettuce showed a very high sensitivity to this element. Schoer (1984) also reported that cabbage species display a high resistance to Tl. This is certainly supported by the data from this study - cabbage showed less sensitivity to Tl than to any of the other elements studied. The reasons for the differences in response among species is not known (Allus *et al.*, 1987). Thallium was the only one of the four elements studied which did not produce a stimulatory effect at low concentrations. This is consistent with previous studies (Yopp *et al.*, 1974; Schoer, 1984; Adriano, 1986; Allus *et al.*, 1987).

3.4. Vanadium

Turnip and cabbage were the most sensitive to V, with radicle lengths reduced by 49 and 42%, respectively, at a concentration of 2.5 mg V L^{-1} (Figure 4). Lettuce was less sensitive, with radicle length reduced by 27% at 2.5 mg V L^{-1}, after exhibiting increased radicle elongation at the 0.5 and 1 mg V L^{-1} treatments. Radish and wheat were intermediate in sensitivity, with millet again the least sensitive of the plants tested.

The response of wheat and millet to V was quite surprising. Concentrations up to 40 mg V L^{-1} did not significantly ($P>0.05$) reduce the radicle lengths of wheat below the control level. In the case of millet, treatment with V had a strong stimulatory effect on radicle elongation. At the 40 mg V L^{-1} treatment, radicle lengths were still greater than for the controls. The experiment was repeated, and produced the same results. The solutions were then checked for possible impurities, such as Fe or other micronutrients, which may have produced the stimulatory effect. No impurities were found. A third experiment was performed, using concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, and 100 mg V L^{-1}, to determine at what point V ceases to be stimulatory. The results of that experiment indicated that concentrations greater than 50 mg V L^{-1} were necessary to reduce the radicle length below the control level (Figure 4). The order of sensitivity to V (based on radicle length reductions) was: turnip > cabbage > lettuce > radish > wheat > millet.

The response of lettuce in this study as similar to that reported by Lepp (1977). In Lepp's study, root elongation was reduced by 37% at a solution concentration of 0.5 mg V L^{-1}, while in this study a 33% reduction was observed at that concentration. Lettuce is considered a V accumulator (Yopp *et al.*, 1974), and appears

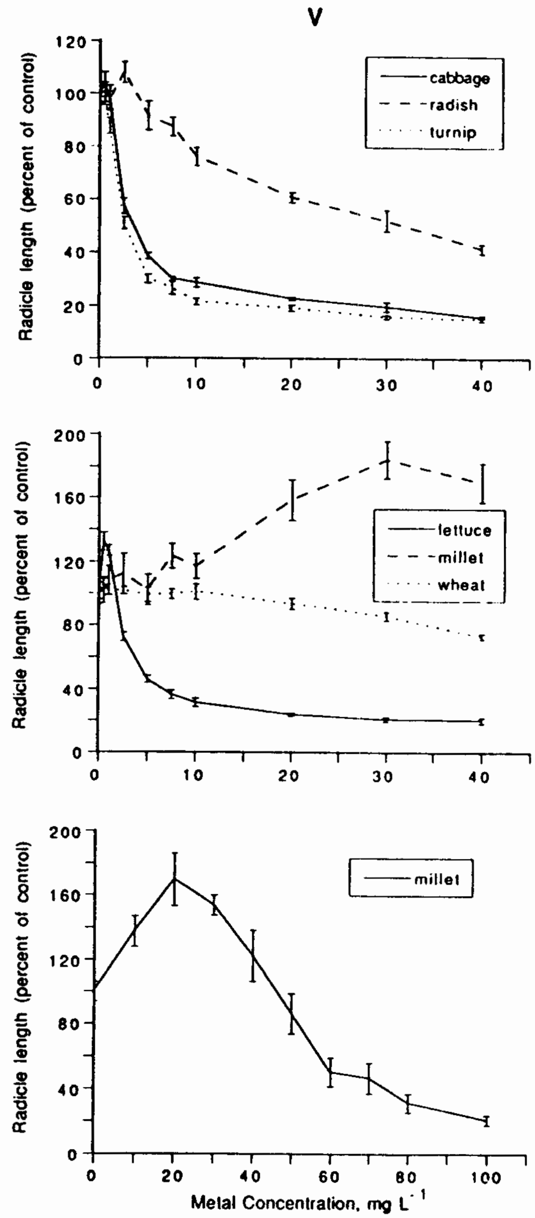

Fig. 4. Effects of V on root elongation of cabbage, radish, turnip, lettuce, millet, and wheat exposed to concentrations up to 40 mg V L^{-1}, and millet exposed to concentrations up to 100 mg V L^{-1}. Values given are means plus or minus 1 SE.

to have more resistance to its toxic effects than to those of the other elements tested. Berry (1978) found that V was more toxic to lettuce than Ni when comparing response on a microequivalent basis. When his results are converted to a ppm basis, however, Ni is the more toxic element. That was the case in this study as well.

Vanadium has been shown to be beneficial to some plants, including lettuce, tomato, asparagus, corn, barley, and rice (Canon, 1963; Basiouny, 1984; Adriano, 1986). This appears to be a result of the metal's ability to substitute for Mo and to enhance the absorption of Fe (Canon, 1963; Basiouny, 1984; Adriano, 1986). In this study, V at low concentrations had a stimulatory effect on all of the species studied except turnip. The stimulatory effects of V on wheat and millet were quite amazing. Catalina (1966) reported stimulation of seed germination by V for a variety of species, including wheat. The effect of V on root elongation for wheat and millet does not appear to have been reported previously. The reasons for stimulation over such a wide range of concentrations merits further study.

4. Summary and Conclusions

The results of this study demonstrate the wide range of response exhibited by plant species to trace metals. In general, turnip and lettuce were the most sensitive of the species studied, and wheat and millet were the least sensitive. Care should therefore be taken in selecting plant species for use in toxicity testing. All of the species used in this study have been suggested as test species (Brusick and Young, 1982; Wang, 1987). The results of this study indicate the importance of using multiple species testing. Recommendations for these metals based on the use of turnip or lettuce as a test species would be quite different from those based on the use of millet or wheat.

Acknowledgments

This research was performed under the auspices of contract number DE-AC09-76SROO-819 between the U.S. Department of Energy and the University of Georgia. We thank B. Burton and M. Headden for their assistance in data collection.

References

Adriano, D. C.: 1986, *Trace Elements in the Terrestrial Environment*, Springer-Verlag, New York.

Allus, M. A., Martin, M. H., and Nickless, G.: 1987, *Chemosphere* **16**, 929.

Basiouny, F. M.: 1984, *J. Plant Nutr.* **7**, 1059.

Berry, W. L.: 1978, 'Comparative toxicity of VO_3^-, CrO_4^{2-}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+} to lettuce seedlings', in D. C. Adriano and I. L. Brisbin (eds.), *Environmental Chemistry and Cycling Processes*, U. S. Department of Energy, pp. 582–589.

Brusick, D. J. and Young, R. R.: 1982, *IERL-RTP Procedures Manual: Level 1. Environmental Assessment, Biological Tests*, Report No. EPA-600/S8-81-024, U. S. Environmental Protection Agency.

Canon, H. L.: 1963, *Soil Sci.* **96**, 196.

Carlson, C. L., Kaplan, D. I., and Adriano, D. C.: 1989, *Environ. Exper. Bot.* **29**, 493.
Catalina, L.: 1966, *Annals Edafol. Agrobiol. (Madrid)* **25**, 551.
Das, P. K. and Mishra, D.: 1978, *Z. Pflanzenphysiol. Bd.* **90**, 225.
Davis, R. D., Beckett, P. H. T., and Wolan, E.: 1978, *Plant Soil* **49**, 395.
Hoagland, M. B.: 1952, *Arch. Biochem. Biophys.* **35**, 259.
Lepp, N. W.: 1977, *Z. Pflanzenphysiol. Bd.* **83**, 185.
Mishra, D. and Kar, M.: 1974, *Bot. Rev.* **40**, 395.
Peterson, P. J. and Girling, C. A.: 1981, 'Other Trace Metals', in N. W. Lepp (ed.), *Effects of Heavy Metal Pollution on Plants*, Vol. 1, Applied Science Publishers, London, pp. 213–279.
Romney, E. M. and Childress, J. D.: 1965, *Soil Sci.* **100**, 210.
Romney, E. M., Childress, J. D., and Alexander, G. V.: 1962, *Science* **135**, 786.
Schoer, J.: 1984, 'Thallium', in O. Hatzinger (ed.), *The Handbook of Environmental Chemistry*, Vol. 3, Part C, Springer-Verlag, Berlin, pp. 143–220.
Singh, S. N.: 1984, *Trop. Ecol.* **25**, 90.
Singh, S. N.: 1985, *Indian J. Ecol.* **12**, 162.
Smith, I. C. and Carson, B. L.: 1977, *Trace Metals in the Environment*, Vol. 1. Thallium. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan.
Wang, W.: 1987, *Environ. Toxicol. Chem.* **6**, 409.
Williams, R. J. B. and Le Riche, H. H.: 1968, *Plant Soil* **29**, 317.
Yopp, J. H., Schmidt, W. E., and Holst, R. W.: 1974, *Determination of Maximum Permissible Levels of Selected Chemicals that Exert Toxic Effects on Plants of Economic Importance in Illinois*, Report No. 74–33, Illinois Institute for Environmental Quality, Springfield, Illinois.