Bull. Environ. Contam. Toxicol. (1994) 52:452–456

Environmental Contamination and Toxicology

Effect of Pb, Cd, Hg, As, and Cr on Germination and Root Growth of *Sinapis alba* Seeds

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Received: 6 April 1993/Accepted: 9 July 1993

Heavy metals have been widely recognized as highly toxic and dangerous. Plants, algae and bacteria respond to heavy metal toxicity by inducing different enzymes, creating ion influx/efflux for ionic balance and synthesizing small peptides. These peptides bind metal ions and reduce toxicity (Reddy and Prasad 1990). Metals come from the natural weathering processes of the earth's crust, industrial discharge, pest or disease control agents applied to plants, urban run-off, mining, soil erosion, sewage effluents, air pollution fallout and other sources.

Plants can be affected directly by air pollutants, as well as indirectly through the contamination of soil and water. At the same time, plant is a member of the food chain and may create a risk for man and animals through contamination of food supplies. In recent years a considerable progress has been made in the assay of trace elements in environmental plant samples.

For higher plants, the accumulation of metals, especially cadmium, was tested when plants grew on sewage sludge-amended soils or in soils of cadmium residues from phosphate fertilizers (Williams and David 1976, Lund et al. 1981, Adema and Henzen 1989). No reports were accessible to us on the direct effect of tested metals (Pb, Hg, Cr, As, Cd) on seed germination and root growth. The paucity of literature initiated our present work. In this study, an attempt has been made to investigate the acute toxicity of five metals (Cr^{6+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, As^{5+}) which are widely spread in the environment and are widely recognized as highly toxic and dangerous. As the testing subject, mustard seeds (Sinapis alba) were used and their germination and root growth were observed.

MATERIALS AND METHODS

Seeds of Sinapis alba were used as the testing subject. Metals were dissolved in various concentrations in dilution water (Bringmann and Kühn 1982) with the following physicochemical properties (mg/L): calcium 3.26; magnesium 0.486; sodium 0.708; potassium 0.120; chloride 5.781; sulfate 1.921; hydrogencarbonate 1.881; pH=6.67, water temperature 20 °C. The following metal ions were tested: Hg^{2+} [$HgCl_2$], Cd^{2+} [$CdCl_2.2.5H_2O$], As^{5+} [$Na_2HAsO_4.7H_2O$], Pb^{2+} [$Pb(CH_3COO)_2.3H_2O$], Cr^{6+}(1) [CrO_3] and Cr^{6+}(2) [$(NH_4)_2CrO_4$]. Test solutions of metal ions were prepared in dilution water and their pH values were measured. The seeds were placed in Petri dishes with a 14 cm diameter and filter paper on the bottom. The temperature in the lab was 25 °C and dishes were not situated in the direct sunlight. In each Petri dish, 50 seeds were evenly displayed on the surface of filter paper and the amount of solution used was 10 mL per dish. Each concentration was duplicated three times. Exposure lasted 72 hr and then the germination (%) and root length (cm) was determined.

The LC50 values were determined for germination [LC50(G)] and root-growth inhibition [EC50(I)] by using probit analysis and 95 % confidence limits were calculated by the moving average angle method (Harris 1959). Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The effects of various concentrations of Hg^{2+}, Cd^{2+}, As^{5+}, Pb^{2+}, Cr^{6+}(1) and Cr^{6+}(2) on germination and root growth of Sinapis alba seeds as LC50, EC50 values and their 95 % confidence limits are summarized in Table 1. There were great differences in the LC50 and EC50 values affecting seed germination and root growth inhibition, with LC50 values being several times higher for seed germination. The greatest differences between LC50(G) and EC50(I) values were with Cr^{6+}(1) (24.55-times) and the least with Cr^{6+}(2) (2.18-times).

The results of this study demonstrated that after 72 hr the most toxic metal for seed germination was As^{5+} and the least toxic was Pb^{2+}. The resulting rank order of toxicity for metal ions on germination was $As^{5+}>Cr^{6+}(2)>Cr^{6+}(1)=Hg^{2+}>Cd^{2+}>Pb^{2+}$. The LC50 (G) values were in some cases extremely high (Pb^{2+}, Cd^{2+}). We do not expect to find such high concentrations in the environment, but these values are estimated for comparing with other metal ions tested. On the basis of these comparisons, these metal ions can be placed in the rank order of toxicity.

Table 1. LC50 values (mg/L) of tested metal ions for germination (G) and EC50 values (mg/L) for root-growth inhibition (I) and their corresponding 95 % confidence limits

Metal ion	Germination		Root-growth inhibition	
	pH	LC50(G)+95 % CL	pH	EC50(I)+95 % CL
Hg^{2+}	7.09	128.82 (122.18-141.39)	7.70	9.33 (6.68-10.51)
Cd^{2+}	6.16	691.83 (630.88-717.23)	6.90	47.86 (34.56-51.83)
Pb^{2+}	5.10	1148.15 (957.19-1380.38)	5.80	263.03 (251.22-297.43)
As^{5+}	7.28	30.20 (27.61-35.10)	7.25	5.49 (3.15-7.23)
Cr^{6+}(1)	2.46	123.03 (116.38-142.89)	4.20	5.01 (4.20-6.93)
Cr^{6+}(2)	7.25	100.00 (89.76-103.85)	7.32	45.71 (38.65-50.46)

CL - confidence limit

The greatest inhibition of root growth was with Cr^{6+}(1) and As^{5+} and the least with Pb^{2+}. The rank order of toxicity for root-growth inhibition was $Cr^{6+}(1)=As^{5+}>Hg^{2+}>Cr^{6+}(2)=Cd^{2+}>Pb^{2+}$. From both rank orders of toxicity the results indicate that the most toxic metal ion for plant seeds (their germination and root growth) was As^{5+} and the least toxic was Pb^{2+}. Because of the paucity of literature on toxic effects of metals on higher plants, their seed germination and root-growth inhibition, there was no possibility to compare our rank orders of toxicity and LC50(G) and EC50(I) values with other authors.

Chromium was as toxic to mustard seeds for germination as well as for root growth. There were differences also between the toxic effects of both Cr^{6+}(1) and Cr^{6+}(2). During tests with Cr^{6+}(1), the pH value decreased very rapidly to 4.2 with the increase in concentration of the compound. This rapid decrease may correspond with a maximum uptake of Cr^{6+}(1) (Tripathi and Chandra 1991), thereby causing a great inhibition of root growth. A low pH (2.46) did not have such an intensive effect on seed germination. When Cr^{6+}(2) was used, the pH values changed only slightly. In this case the EC50(I) value was 9-times higher than Cr^{6+}(1). Perhaps in this case pH may had more of an effect than the metal. The LC50(G) value was not very different from the LC50(G) value for Cr^{6+}(1). This difference can be explained by the greater heavy metal absorption capacity of the metals in the roots (Lyngby and Brix 1984). Chromium and zinc occur mainly in plant fluids or are transported to the growing regions (Lyngby et al. 1982).

Koeppe (1977) reported that lead might be bound to the outer surface of plant roots, as crystalline or amorphous deposits, and could also be sequestrated in the cell walls or deposited in vesicles. This might explain the higher concentrations of lead in roots (Lyngby and Brix 1984) and can explain the low toxic effect of lead on mustard seeds.

Mercury is a common and potentially the most toxic metal contaminant and little work describes its effects on plants. The most information found pertained to about cadmium (Williams and David 1976, Outridge 1992). The literature reports on the accumulation, uptake and cadmium content of plants grown in soil with high cadmium levels. For all these processes pH is an important determinant (Williams and David 1976). In our tests the pH values changed only slightly with increasing concentrations. Cd^{2+} was not very toxic for germination and root growth of <u>Sinapis alba</u> seeds.

The results of the present study indicated that metals had very low toxic effects on seed germination. They were more likely to damage root growth, which is necessary for nutrient absorption and plant growth. When simultaneous determination of heavy metal concentrations in above- and underground parts of plants was described, the results indicated that the higher amounts of metals were found in the roots (Havránek et al. 1983). In the roots of some plants the cell wall accumulates heavy metals.It was found that in soil-less culture the EC50 values were much lower than those in soil culture. It is concluded that adsorption of the chemicals to soil particles is the main factor governing the results. In an additional experiment with Cd, it was demonstrated that the Cd content of reacting plants was about the same in tests carried out in soil and in nutrient solution, pointing to the obvious fact that the amount of chemical taken up is the basis for the toxic effect. In the interpretation of tests carried out with chemicals added directly to soil, this should be taken into account. It is recommended that soilless culture of terrestrial plants be seriously considered in ecotoxicological testing (Adema and Henzen 1989). That is why we decided to determine EC50(I) values for root-growth inhibition in soil-less culture.

In highly polluted areas plants show increased concentration of elements specific for impurities in air and water, and thus can serve as a long-term monitor of environmental pollution. An advantage of these bioindicators is that they are stationary, commonly available in large number and give relatively equal reactions.

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