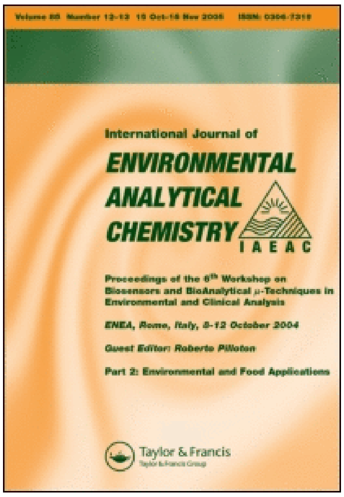

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Development of Resin Membranes as a Sensitive Indicator of Heavy Metal Toxicity in the Soil Environment

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DEVELOPMENT OF RESIN MEMBRANES AS A SENSITIVE INDICATOR OF HEAVY METAL TOXICITY IN THE SOIL ENVIRONMENT

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Ion exchange resins in contact with soil can act as a sink for metal cations, thereby simulate the action of plant roots. Ion exchange resins in membrane form offer additional advantages in ease of use and handling. A procedure was developed to assess the bioavailability of four heavy metals Cd, Cr, Ni and Pb via direct in soil burial. A growth chamber experiment with three representative crops (oats, radish and lettuce) was set up to determine the phytotoxic levels of the four heavy metals. The critical levels varied widely from crop to crop, and soil to soil. Lettuce was most sensitive to high concentration of metals. The toxic effects are more pronounced on sandier textured soils.

KEY WORDS: Heavy metals, phytotoxicity, resin membrane, soil test, burial in situ.

INTRODUCTION

Ion exchange resins in bead form have been used in assessing plant available nutrients for decades[1]. Ion exchange resin in membrane form was first used by Saunders[2]. He found that the anion exchange membrane could be used to predict phosphorus availability in soil as well as resin in the bead form. Later on, the resin membrane technique was developed and refined for soil testing for phosphorus availability[3,4] and as a multi-element soil test[5]. More recently, this method has been developed into a routine soil test for nutrient availability[6]. Ion exchange membranes may be thought of as plant root simulator since their mode of action of ion removal, when placed in the soil, closely resembles that of a plant root.

The movement of a nutrient ion from the soil solid phase to a plant root is a dynamic process. Nutrients in soil solution reach the root surface by means of mass flow and diffusion processes through spaces in the soil matrix, and also directly by root interception and contact. Processes of uptake and release of nutrients in the soil-solution-root system are multi-element dependent, selective and based on equilibria or gradient established when plant roots absorb nutrients from solution[7]. A buried membrane strip in situ will accumulate all nutrients that soil can deliver to a sink in response to a nutrient-specific or -selective and interdependent dynamic gradient established in soil-solution-sink continuum. This simulates the action of nutrient movement to a plant root in each specific soil under field conditions.

No known studies have used ion exchange membranes to evaluate the availability of metals and other trace elements in soil. Recently, there has been much concern about the accumulation in soil of heavy metals which are not known to be essential for plant growth such as Cd, Cr, Ni and Pb. Assessment of heavy metal pollution can be approached by assessing the degree of soil or crop pollution, and subsequentially predicting the likely effect on plant and animal health. It is generally accepted that the prediction of trace metal bioavailability and mobility is best carried out by analysis of soil solution or something closely related to it[8]. The difficulty with this approach is in knowing which particular ionic species to measure and then to analyze the low concentrations involved. Dilute acids, chelating agents, and neutral salts have been commonly used to extract metals from soils and provide a measure of availability and therefore the toxicity and accumulation potential for plants. Unfortunately, none of the existing methods are suitable for all metals and all situations. Ion exchange membranes saturated with chelating agents and buried in the natural soil environment may have a selective ability to adsorb metal ions, and mimic to the action of plant roots. Development of such methods could permit environmental monitoring for predicting the potential toxicity and mobility of heavy metals in the environment for a wide range of soil types and metals. The objective of this study, therefore, was to develop and evaluate the use of resin membranes as a sensitive indicator for heavy metal toxicity in the soil environment.

MATERIALS AND METHODS

It is important to determine the level of contamination above which crop yield and quality, animal or human health may be affected. A plant growth experiment in a controlled environment was carried out to provide information on the relationship between the extractable levels of heavy metals in the soil, and uptake of these metals by oats (cereal), radishes (root crop) and lettuce (leafy vegetable) and their toxicity to the plant. The results of such studies can provide a guide to the possible effects of soil contamination with these metals. The results obtained with oat, radish and lettuce will have some relevance for cereal, root and leafy vegetable crops in general.

Two soils were collected from the Saskatchewan agricultural region to represent a light (low clay content) and heavy (high clay content) textured soil with low levels of the heavy metals concerned (Table 1). Soil samples collected from the field were air-dried, and passed through a 2-mm polyethylene sieve. The air-dried soil was spiked with metal in solution form, air-dried again, and then mixed in a mixing machine for 0.5 hours. The

Table 1 Some properties of the two soils used.

Soil	*pH (1:2)*	*Organic matter (%)*	*CEC cmol kg^{-1}*	*Clay (%)*
Light	7.5	2.3	14.6	8.9
Heavy	8.3	3.0	34.7	44.6
		Total concentration (mg kg^{-1})		
	Cd	*Cr*	*Ni*	*Pb*
Light	0.25	21.7	8.3	4.8
Heavy	0.45	46.8	27.2	19.3

well mixed sample was then watered to field capacity, and incubated for two months before placing into pots. The two month incubation should allow time for any slower metal adsorption or precipitation reactions that would affect the fate of the metal in a natural soil system to take place.

The experiment was a factorial design, with four levels of each metal: 0, 5, 10, 20 mg kg^{-1} added Cd, and 0, 40, 80, 160 mg kg^{-1} added Cr, Ni, and Pb. Each treatment was replicated three times. Five hundred grams of spiked soil was placed in a plastic pot. Eight oat seeds (var. Cascade) and 5 radish (var. Cherry Bell) and lettuce (var. Slobolt) seeds were seeded into each pot and thinned to 4 plants for oat, 3 plants for radish and 1 plant for lettuce after establishment of seedling. Macro- and micro-nutrients were added to each pot to ensure that nutritional deficiency did not hinder plant growth. All pots were watered once a day to keep soil moisture at 85-90% of field capacity. The growth chamber temperature was set at 25°C daytime and 12°C at night. Oats were allowed to grow to maturity, radish for 30 days and lettuce for 40 days. At harvest, the radish plants were divided into leaves and edible bulbs. Fibrous roots were cut away and discarded. The leaves and bulbs were then carefully washed with distilled deionized water. The lettuce leaves were cut above soil surface, washed carefully with distilled deionized water after removing the damaged and dirty outer leaves. Oats were cut 5 mm above the soil surface, and manually separated into grain and straw after drying. The harvested plant materials were dried at 60°C for one week and weighed for dry matter yield determination.

Soil analyses prior to metal spiking included: pH in 1:1 soil to water suspension; organic carbon by a modified Walkley-Black procedure; exchangeable bases by equilibration with 1 M NH_4OAc and particle size analysis by the pipette methods; total soil metal content was determined using the microwave dissolution procedure[9]. The ground plant samples were digested in sulfuric acid-peroxide using a temperature controlled digestion block and determined by AAS for heavy metal concentration[10]. Accuracy was checked using certified reference materials (total soil contents and plant contents). The quality of the analysis was satisfactory.

Conventional DTPA extraction procedure: DTPA-extractable metals were extracted by the method of Lindsay and Norvell[11]. The DTPA extracting solution contained 0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M TEA. The pH of the final solution was adjusted to 7.3 with dilute HCl. Ten gram soil and 20 ml DTPA solution were placed into acid washed 125 ml conical flask and shaken at room temperature for 2 hours. The resulting extracts were filtered through Whatman #42 filter paper prior to determination of metal concentration by AAS.

AEM-chelate burial (membrane) procedure: Anion exchange membrane (AEM) (B.D.H product no. 55164) has a structure of polystyrene cross-linked with divinylbenzene with attached quaternary ammonium groups, thickness of 0.16 mm, exchange capacity of 10 μeq cm^{-2}, and high mechanical strength (3-4 kg cm^{-2}). To fit the requirement for soil direct burial, the membrane sheets (12 × 12 cm) were cut into rectangular strip sizes of 2 × 6 cm. Strips of AEM were placed in a 0.01 M DTPA + 0.02 M NaOH solution to saturate the positively charged functional groups on the membrane with DTPA. The strips were then washed with deionized water and then buried into 70 grams of soil in a plastic vial. The soil was saturated with deionized water and the buried membranes were allowed to sit for 60 min. Then the membrane strips were removed from soil, and washed free of soil with deionized water. The membrane was then shaken with 20 mL of 1 N HCl for 2 hours on an end on end shaker (200 rpm) in a 50 ml centrifuge tube. Following elution, the membrane was removed from the tube, and the metal content in the 1 N HCl eluents was determined by AAS. This procedure can be used in the field directly. The membranes are reusable and are made ready for reuse by washing again in the DTPA solution.

All replicated treatments were subjected to analysis of variance. StatView SE + Graphics[12] program was used in the statistical analysis and a Fisher PLSD test was used to compare the means.

RESULTS

CADMIUM

Effect of enhancement of 'available' cadmium level

The effect of added Cd on dry matter yield of radish, lettuce and oats with corresponding soil DTPA-extractable and membrane extractable Cd is shown in Table 2. Both DTPA and membrane extractable Cd reflected the relative Cd contamination of soil very well, and acted as a suitable index of relative Cd bioavailability. One should note that in the case of the metal extracted by membrane, results are expressed as weight of metal removed per square centimeter of strip surface and are not quantitatively comparable with the DTPA extractable which is expressed as weight of metal per unit weight of soil.

Added Cd had no significant effect on yield of radish and oats, over the range employed. Lettuce leaves were noticeably darker in color at the two highest treatment levels. Addition of 5 mg kg^{-1} Cd significantly inhibited the growth of lettuce in the light textured soil, while a spike rate of 10 mg kg^{-1} was enough to reduce yield in the heavy textured soil. These results confirm the finding of Purves[13] that leafy vegetable are more sensitive to Cd toxicity and that the toxic effect are more pronounced in light textured soil. Soils with high clay contents are able to retain more of the added metal on the negatively charged exchange sites on the clay mineral surfaces where it is less toxic than

Table 2 Effect of added Cd as $CdSO_4$ on dry matter yield of radish, lettuce and oats (average of three pots).

Spike rate (mg kg^{-1})	*0*	*5*	*10*	*20*
		Light soil		
DTPA-Cd (mg kg^{-1})	0.1	3.9	6.9	16.1
Membrane Cd (μg cm^{-2})	0.01	0.04	0.07	0.13
DMY(g)/radish	2.8_a	2.4_a	2.5_a	2.5_a
DMY(g)/lettuce	3.5_a	2.2_b	1.7_{bc}	1.3_c
DMY(g)/oats	15_a	15_a	13_b	14_{ab}
		Heavy soil		
DTPA-Cd (mg kg^{-1})	0.1	4.4	8.0	17.9
Membrane Cd (μg cm^{-2})	0.01	0.04	0.08	0.14
DMY(g)/radish	3.0_a	2.7_a	3.1_a	3.4_a
DMY(g)/lettuce	4.0_a	3.2_{ab}	2.6_b	1.5_c
DMY(g)/oats	15_a	15_a	15_a	14_a

Values followed by a different letter are significantly different at $p = 0.05$ for each crop.

Table 3 Coefficient of determination (r^2) for relationship between Cd in plant and Cd added rate, DTPA-Cd, and membrane-Cd.

Cd in plant	*Added rate* r^2	*DTPA-Cd* r^2	*Membrane-Cd* r^2
Radish uptake	0.988*	0.986*	0.989*
Lettuce conc.	0.980*	0.986*	0.980*
Oat uptake	0.985*	0.989*	0.994**

*, **, Significant at the 0.05, 0.01 levels, respectively.

in solution. Cadmium concentration in plant materials (data not shown) indicated that Cd is very likely to be concentrated in the leaves and roots. This confirms the observation that leafy and root vegetables can be a significant route of Cd supply to man.

Effectiveness of DTPA and membrane burial in predicting Cd uptake

The relationships between Cd uptake by the three crops and Cd spike rate, DTPA-, and membrane extractable are given in Table 3. Cadmium uptake by radish and oats, as well as Cd concentration was significantly correlated with spike rate. This suggests that this highly toxic element can be readily taken up by plants to produce quite high concentrations in the plant without the appearance of phytotoxicity, so that an apparently normal crop may be unsafe for human and animal consumption. The regression coefficients in Table 3 demonstrate that membrane extractable Cd predicts Cd bioavailability better than or equal to the conventional DTPA method.

Phytotoxicity threshold

Up to 20 mg kg^{-1} added Cd only significantly inhibited the growth of lettuce. The cause-and-effect relationships between leaf tissue concentration of Cd and percent growth retardation for lettuce was best described by a quadratic exponential function model (Figure 1). Growth retardation is defined as the percentage of growth reduction for plants grown on metal-treated substrates when their total biomass is measured against that of an experimental control[14]. If plant growth is enhanced by the presence of metals, the growth retardation is assumed to be zero. From the equation in Figure 1, Cd concentration in lettuce leaf tissue corresponding to 50, 20, 10% growth retardation (PT_{50}, PT_{20} and PT_{10}) is computed as 42, 14.5 and 7.5 mg kg^{-1}, respectively. The DTPA-extractable and membrane Cd corresponding to PT_{50} are 7 mg kg^{-1} and 0.08 μg cm^{-2} for light textured soil and 11 mg kg^{-1} and 0.10 μg cm^{-2} for heavy soil, respectively.

CHROMIUM

Effect of added Cr on plant dry matter yield

The effect of added Cr on dry matter yield of radish, lettuce and oat along with DTPA- and membrane extractable Cr are shown in Table 4. Both conventional DTPA- and

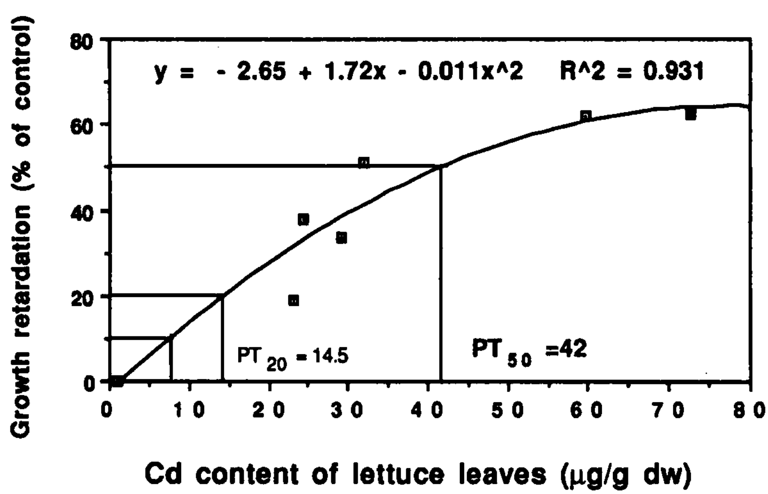

Figure 1 Cause-and-effect relationship between lettuce tissue Cd concentration and growth retardation.

Table 4 Effect of added Cr as $CrCl_3$ on dry matter yield of radish, lettuce and oats (average of three pots).

Spike rate (mg kg⁻¹)	*0*	*40*	*80*	*160*
		Light soil		
DTPA-Cr (mg kg^{-1})	0.01	0.11	0.17	0.34
Membrane-Cr (µg cm^{-2})	0.00	0.01	0.02	0.04
DMY(g)/radish	2.8_a	3.0_a	3.2_a	2.1_b
DMY(g)/lettuce	3.5_a	2.7_b	2.7_a	1.4_b
DMY(g)/oats	15_a	15_a	16_b	13_b
		Heavy soil		
DTPA-Cr (mg kg^{-1})	0.01	0.06	0.08	0.12
Membrane Cr (µg cm^{-2})	0.00	0.01	0.02	0.04
DMY(g)/radish	3.0_a	3.0_a	3.5_a	3.5_a
DMY(g)/lettuce	4.0_a	3.4_{ab}	3.2_b	3.7_c
DMY(g)/oats	15_a	15_a	15_a	15_a

Values followed by a different letter are significantly different at $p = 0.05$ for each crop.

membrane extractable Cr reflect the Cr spiking very well. In this experiment, the highest treatment level (160 mg kg^{-1}), corresponding to determined levels of DTPA-extractable Cr of greater than 0.3 mg kg^{-1} and of membrane Cr of greater than 0.04 µg cm^{-2}, produced a significant yield reduction in the three crops grown in the light textured soil. However, no significant effects were observed on yield of these three crops grown in heavy textured soil over the range of Cr rate employed. This suggests that soils with high metal binding capacity (high clay content) are more tolerable to metal contamination.

Table 5 Coefficient of determination (r^2) for relationship between Cr in plant and Cr added rate, DTPA-Cr, and membrane-Cr.

Cr in plant	Added rate r^2	DTPA-Cd r^2	Membrane-Cd r^2
		Light soil	
Radish uptake	1.00***	0.995**	0.998***
Lettuce conc.	0.997**	0.998***	0.987*
Oats straw conc.	0.915	0.965*	0.977*

*, **, ***, Significant at the 0.05, 0.01, 0.001 levels, respectively.

Effectiveness of DTPA and membrane burial in predicting Cr bioavailability

The relationships between Cr in the plant and DTPA-extractable and membrane Cr are given in Table 5. Radish Cr uptake and lettuce Cr concentration are significantly correlated with Cr spike rate, DTPA-extractable and membrane Cr. Chromium concentration in oat straw was found to be highest in the control and at the 160 mg kg^{-1} spike rate. The mechanism for this is not understood, but Cr interaction with other major elements in the soil has been suggested[15]. Generally, both the conventional DTPA method and the membrane burial method predict Cr bioavailability for radish and lettuce very well.

NICKEL

Effect of added Ni on plant dry matter yield

The effects on dry matter yield of increasing Ni spike rate up to 160 mg kg^{-1} in soil are illustrated in Table 6. The DTPA- and membrane extractable Ni represent the rate of Ni application very well. Dry matter yield response to spiked Ni varied widely from crop to crop, and soil to soil. Lettuce is more sensitive than the other crops with lettuce dry matter declining with increasing Ni spike rate on both soils, the decline starting at rates above 40 mg kg^{-1}. Dry matter yield of radish and oats declined only at the highest treatment level in the light textured soil. All three crops grown in light textured soil showed symptom of Ni phytotoxicity. Radish leaves were small and reddish-brown, with the leaf margin curled inside. Lettuce leaves showed chlorotic spots in the early stages which later extended to whole leaf. Oats showed high Ni levels in the form of pronounced interveinal chlorosis in new leaves. It appears that the light textured soils has low soil buffering and fixation capacity for metals and therefore can tolerate less metal contamination before phytotoxicity sets in. The results confirm the findings of Willaert and Verloo[16], and Abdel-Sabour[17] that high buffering and fixation capacity in the soil can protect plant growth from excess toxic Ni. Lower levels of DTPA and membrane extractable Ni in the heavy textured soil found equivalent spike rates reflect the lower toxicity of a given rate of Ni addition in a heavy textured soil. The mechanism of Ni toxicity to plant is not well understood. However, it was reported that high Ni concentration inhibits the photosynthesis and transpiration process in plants[18].

Table 6 Effect of added Ni as $NiSO_4$ on dry matter yield of radish, lettuce and oats (average of three pots).

Spike rate (mg kg⁻¹)	*0*	*40*	*80*	*160*
		Light soil		
DTPA-Ni (mg kg^{-1})	0.9	32.5	63.4	139.4
Membrane-Ni (μg cm^{-2})	0.01	2.4	4.5	11.0
DMY(g)/radish	2.8_a	2.9_a	2.6_a	0.3_b
DMY(g)/lettuce	3.5_a	2.5_b	2.2_b	0.2_c
DMY(g)/oats	15_a	15_a	14_{ab}	11_b
		Heavy soil		
DTPA-Ni (mg kg^{-1})	2.0	23.2	54.8	96.2
Membrane-Ni (μg cm^{-2})	0.02	1.2	2.6	5.0
DMY(g)/radish	3.0_a	3.1_a	3.6_a	3.3_a
DMY(g)/lettuce	4.0_a	3.2_{ab}	2.6_{bc}	2.1_c
DMY(g)/oats	15_a	15_a	15_a	14_a

Values followed by a different letter are significantly different at $p = 0.05$ for each crop.

Effectiveness of DTPA and membrane burial in predicting Ni bioavailability

Nickel uptake by radish and lettuce and Ni concentration in oat grain increased linearly with Ni spike rate, DTPA-extractable and membrane Ni (Table 7). Both DTPA-extractable and membrane burial can predict plant Ni uptake or concentration very well (regression coefficients are highly significant). It can be concluded that ion exchange membrane burial is a good predictor for Ni bioavailability.

LEAD

Effect of added Pb on plant dry matter yield

Both soil availability indices, DTPA and membrane burial, reflect Pb spike rate (Table 8). No obvious deleterious effects on the growth of radish, lettuce and oats were observed where soluble Pb was added up to a level of 160 mg kg^{-1} in the heavy textured soil. This is a high rate of contamination, since uncontaminated soil normally contain around 1 mg

Table 7 Coefficient of determination (r^2) for relationship between Ni in plant and Ni added rate, DTPA-Ni, and membrane-Ni.

Ni in plant	*Added rate* r^2	*DTPA-Ni* r^2	*Membrane-Ni* r^2
		Light soil	
Radish uptake	1.00***	1.00***	1.00***
Lettuce uptake	1.00***	1.00***	1.00***
Oat grain conc.	0.986*	0.985*	0.979*

*, ***, Significant at the 0.05, 0.001 levels, respectively.

Table 8 Effect of added Pb as $PbCl_2$ on dry matter yield of radish, lettuce and oats (average of three pots).

Spike rate (mg kg⁻¹)	*0*	*40*	*80*	*160*
		Light soil		
DTPA-Pb (mg kg^{-1})	0.5	30.6	64.7	113.1
Membrane-Pb (μg cm^{-2})	0.02	2.6	4.8	8.7
DMY(g)/radish	2.8_{ab}	3.2_{a}	2.8_{ab}	2.3_{b}
DMY(g)/lettuce	3.5_{a}	3.0_{ab}	3.0_{ab}	2.3_{b}
DMY(g)/oats	15_{a}	15_{a}	16_{a}	15_{a}
		Heavy soil		
DTPA-Pb (mg kg^{-1})	1.5	33.2	61.3	106.4
Membrane Pb (μg cm^{-2})	0.14	2.9	6.4	11.4
DMY(g)/radish	3.0_{a}	2.5_{a}	3.2_{a}	2.6_{a}
DMY(g)/lettuce	4.0_{a}	3.1_{a}	3.2_{a}	2.9_{a}
DMY(g)/oats	15_{a}	15_{a}	15_{a}	14_{a}

Values followed by a different letter are significantly different at p = 0.05 for each crop.

kg^{-1} available Pb. This element, therefore, does not appear to be highly phytotoxic, even at levels in the soil characteristic of heavy contamination. Plants do not tend to accumulate Pb. This confirms the findings of Purves[13], Tjell *et al*[19] and Karamanos *et al*[20] that only a small proportion of the Pb in soil is available for uptake by plants.

Effectiveness of DTPA and membrane burial in predicting Pb bioavailability

Lead uptake by the three crops was not significantly correlated with Pb spike rate, DTPA-extractable and membrane Pb (Table 9). Only the lettuce Pb concentration was significantly correlated with DTPA-extractable and membrane Pb. This suggests that the relationship between lead contamination of soil and plant uptake is weak. Relatively little is known about factors which control the availability of Pb at the root/soil interface.

Table 9 Coefficient of determination (r^2) for relationship between Pb in plant and Pb added rate, DTPA-Pb, and membrane-Pb.

Pb in plant	*Added rate* r^2	*DTPA-Pb* r^2	*Membrane-Pb* r^2
		Heavy soil	
Radish uptake	0.617	0.656	0.670
Lettuce conc.	0.971*	0.973*	0.984*
Lettuce uptake	0.873	0.882	0.902

*, Significant at the 0.05 level.

DISCUSSION

A good bioavailability index should have several characteristics, including a high correlation with artificial spike rate, plant uptake, and with existing bioavailability indices. The burial of anion exchange membrane pretreated with DTPA was strongly correlated with spike rate, plant uptake and the conventional DTPA-extractable metals. The conventional DTPA or other chemical methods only measure soil nutrient pools, but not the diffusion component of bioavailability. The burial membrane also allows the diffusion component to be included.

The potential advantages using AEM pretreated with chelating agents in soil burial are twofold: (l) it is based on fundamental chemical and kinetic principles, operative in metal movement and adsorption in the rhizosphere; and (2) it can be measured in situ, and is simple and easy to use. Current preparation techniques for laboratory analysis can radically alter the chemistry of soil and subsequential metal solubilities. Ion exchange membrane burial in situ can avoid such changes associated with handling drying and grinding soil samples.

The membrane method, however, requires extensive additional testing to assess its general application. The method needs to be tested with more soils and crop species under field conditions. In addition, the effect of key soil factors, such as soil pH and CEC on phytotoxicity threshold should be evaluated as the thresholds will be plant and soil specific. Therefore a wide range of soils and crop types needs to be tested. We believe that this method may prove to be a superior index for heavy metal toxicity in soil environments.

SUMMARY

This study describes the development of a methodology for evaluating the bioavailability of heavy metals in soil environments. Ion exchange membranes saturated with chelated agents can be buried in situ to simulate the action of plant roots. Membrane burial will measure the metal cation pools as well as the diffusion to the sink, and therefore should provide better relationship with plant uptake. It is a simple and easy to use procedure. This paper present the results of offsite growth chamber experiments carried out on a limited range of artificially contaminated soils. Future work should involve testing of the method on a wide range of soils and crops species under field conditions.

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