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Cadmium phytoavailability in soils and evaluation of extractant effectiveness using an isotope technique

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ABSTRACT: Large areas of land are nowadays contaminated by heavy metals and, it is therefore, important to monitor their levels in soils. Vegetables act as transfer mechanisms of such contaminants from soils to higher levels in the food chain. In this study, we aimed to evaluate the effectiveness of chemical extractants by the L-value method for Cd phytoavailability using the ^{109}Cd radionuclide. In a greenhouse experiment, rocket plants (*Eruca sativa* L.) were cultivated in pots with samples from Typic Hapludox and Typic Quartzipsamment soils. Cadmium concentrations ranging from 0 to 16 mg kg^{-1} were added to a 200 mL solution containing 148 kBq ^{109}Cd. The available Cd in the soil was extracted by DTPA, Mehlich-1, Mehlich-3, and a mixture of organic acids (acetic, citric, lactic, and oxalic acids). Cd concentrations were determined by atomic absorption spectrophotometry, and ^{109}Cd radionuclide activity was measured by low-level β-counting. The dry matter yield was not influenced by Cd rates, but the Cd content and accumulation in shoots had a positive linear correlation. Generally, Cd was extracted in higher quantities by Mehlich-1 followed by DTPA, Mehlich-3, and organic acids. A linear correlation was found between the chemical extractants and Cd accumulation in shoots for both soils. According to the L Ratio, the extractants based on strong acids and chelating agents presented low efficiency regarding Cd phytoavailability. The organic acids, which presented values close to the L-value, may provide a promising method for evaluating environmental contaminants.

Keywords: Potentially toxic element, L-value, radioactive tracer, weathered tropical soils, soil contamination

Introduction

Heavy metals (HMs) are among the pollutants of greatest concern around the world. Some of them, however, act as essential nutrients, such as Cu and Zn (Thuy et al., 2007). There are several anthropogenic sources of HMs in the soil and the most important include mining residues, agricultural inputs, sewage sludge, fossil fuels, metallurgy, and chemical industries (Kabata-Pendias and Pendias 2001).

Cadmium (Cd), a non-essential element for organisms with high toxic potential, is one of the contaminants that concerns the scientific community, because it is easily absorbed by plant roots and accumulates in the shoots in concentrations that could adversely affect the food chain (Koleli et al., 2004; Santona et al., 2006). This concern has resulted in the development of numerous analytical procedures used to determine the total and available concentrations of this element and other HMs (Zimmerman and Weindorf, 2010). However, the main criticism regarding chemical extractions is that under the acidic conditions of most weathered tropical soils the results are inconclusive for predicting phytoavailability (Fontes and Santos, 2010).

One alternative, which is considered the most rigorous method used to evaluate plant-available forms of an element in the soil, is the direct measurement of its labile portion (L-value) using the isotopic dilution technique, with the corresponding radioisotope, when available (Almas and Singh, 2001; Stanhope et al., 2000). This approach may be the most appropriate method of measurement, because plants are the best indicators of the availability of an element in the soil, and the evaluation is conducted by using only the element itself in the form of its isotope (Stacey et al., 2001). The L-value is determined by adding a certain amount of the elemental isotope to the soil, which does not quantitatively alter its content. As it grows, the test plant uptakes the element and its isotope in a ratio according to its availability in the soil; thus, it is possible to determine the content of the element in the soil from its plant-available form (Dileep et al., 2013).

This method has been used to quantify the availability of different elements such as phosphorus (P) (Dileep et al., 2013), and more recently, its use has been extended to nickel (Ni), Cd, and zinc (Zn) (Rosén et al., 2012; Stacey et al., 2001). The objective of the current study was to evaluate the effectiveness of chemical extractants by the L-value method for Cd phytoavailability using the ^{109}Cd radionuclide.

Materials and Methods

The experiment was developed in a greenhouse in Piracicaba, in the state of São Paulo (SP), Brazil (22°42'30" S; 47°38'01" W; 554 m asl). Rocket plants (*Eruca sativa* L.) were cultivated in pots with soil from the surface layer (0–0.2 m) of a Typic Hapludox (TH) and a Typic Quartzipsamment (TQ) soil (Soil Survey Staff, 2010) collected in Piracicaba and São Pedro, SP (22°34'41.5" S;

47°53'29.2" W). Pots of 3.0 L capacity were filled with 2 kg of soil. The samples were not packed directly into pots but into plastic bags for future use with the radioactive material. The soil chemical characteristics are presented in Table 1. The values for the textural analysis of the TH soil were 150, 80, and 770 g kg^{-1}, while those for the TQ were 30, 20, and 950 g kg^{-1} for sand, silt, and clay, respectively.

Procedures and experimental design

Soil samples were first thoroughly mixed with lime to raise base saturation to 80 %, which is the recommended level for adequate crop growth, and incubated for 30 days. Soil water content was maintained at 70 % of maximum water retention capacity by watering. After air-drying, the soil in each pot was homogeneously remixed. The pots were arranged in a randomized complete block design with three replicates for each treatment. The treatments were 0.0, 2.0, 4.0, 8.0, and 16.0 mg kg^{-1} Cd ($CdCl_2$), as a solution, based on the guideline values of the current soil Cd Intervention Values for the state of São Paulo (CETESB, 2014) and other studies (Domínguez et al., 2009; Ok et al., 2004). Total Cd contents less than 3.6 and 14.0 mg kg^{-1} of soil are not considered to be contaminated for agricultural and residential sites, respectively.

Before applying the radioactive material, all nutrients were added in quantities adequate for rocket plant growth as follows: 50, 100, and 25 mg kg^{-1} of N, P and K for the TQ soil; and 50, 100, and 50 mg kg^{-1} of N, P, and K for the TH soil, respectively, as mono-ammonium phosphate, urea and potassium chloride. The micronutrients were applied in the form of a solution at a concentration of 4 mg kg^{-1} Fe [iron chloride (III), hexa-hydrate], 4 mg kg^{-1} Zn (zinc sulfate, hepta-hydrate), 2 mg kg^{-1} B (boric acid), 2 mg kg^{-1} Cu (copper sulfate, penta-hydrate), and 1 mg kg^{-1} Mo (ammonium molybdate, tetra-hydrate) for both soils. The isotopic ratio of the soil samples was altered by a 200-mL solution containing 148 kBq ^{109}Cd. The samples were then watered to 70 % of maximum water retention capacity and maintained for 10 days so as to reach isotopic equilibrium (Gray et al., 2004; Stacey et al., 2001; Young et al., 2000).

After the incubation period, the soil in each pot was homogeneously remixed and a small amount (~100 g) of representative soil from each pot was taken for chemical extractant analysis. Rocket plant seeds were sown and thinned to two plants per pot ten days after emergence. During plant growth, the soil water content was maintained at ~70 % of the maximum water retention capacity.

Chemical analysis of soil samples

The soil available Cd was extracted by four chemical methods using DTPA - 0.05 mol L^{-1} diethylenetriaminepentaacetic acid at pH 7.3 (Lindsay and Norvell, 1978), Mehlich-1 (0.04 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4) (Mehlich, 1978), Mehlich-3 (0.2 mol L^{-1} acetic acid + 0.25 mol L^{-1} NH_4NO_3 + 0.013 mol L^{-1} HNO_3 + 0.015 mol L^{-1} NH_4F + 0.001 mol L^{-1} EDTA - ethylenediaminetetraacetic acid) (Mehlich, 1984), and a mixture of organic acids (acetic, citric, lactic, and oxalic acids at concentrations of 1.00, 0.72, 0.49, and 0.12 mol L^{-1}, respectively) (Pires et al., 2004). The Cd concentrations in the extracts were determined by atomic absorption spectrophotometry (Varian-Spectra AA 140).

Chemical and radioisotopic analysis of plant samples

Plants were cut at the soil surface 35 days after sowing, air-dried at 65 °C, weighed, and finely ground in a Willey mill. A portion of 1 g of each sample was digested in a digestion block, with nitric acid : perchloric acid (3:1) solution in a Pyrex tube. Samples were cooled and diluted to a final volume of 25 mL with deionized water. The Cd content in this solution was determined by atomic absorption spectrophotometry (Varian-SpectrAA 140), and the ^{109}Cd radionuclide activity was measured by low-level β-counting (WALLAC 1409 LSC). The distribution pattern of absorbed ^{109}Cd was studied by autoradiography, which is a technique that allows the visualization of HM absorption and translocation within a plant. After the sowing, the plants were pressed using a herbarium press and dried at 70 °C in an oven for 30 min. Pressed and dried plants were kept in contact with an X-ray film under safelight conditions and after an exposure period of two weeks, positive prints were taken (George et al., 1992; Wehtje et al., 2007).

The L-value method

The isotopically exchangeable Cd (L-value) was calculated as (Stacey et al., 2001): $L\text{-}value = (Y\ C_a)/C_p$, in which Y is the standard activity (dpm per pot), C_a is the accumulated Cd in the plant tissue (mg Cd per pot), and C_p is the total Cd activity in the plant (dpm per pot). The *L-value* was divided by 2 (i.e., 2 kg of soil) to convert mg per pot to mg kg^{-1}.

Table 1 – Chemical characteristics of the soil samples.

Soil	pH[1] 0.01 mol L^{-1} $CaCl_2$	pH[2]	SOM[3]	P[4]	K[4]	Ca[4]	Mg[4]	H+Al	CEC[5]	SB[6]	V[7]	Cd[8]
			g dm^{-3}	mg dm^{-3}	mmol$_c$ dm^{-3}						%	mg kg^{-1}
TH[9]	4.8	5.6	23	8	1.2	18	11	25	55.2	30.2	55	0.05
TQ[10]	4.8	5.5	7	3	0.2	5	3	11	19.2	8.2	43	0.07

[1]Before lime; [2]after lime; [3]SOM = soil organic matter; [4]P = phosphorus, K = potassium, Ca = calcium and Mg = magnesium extracted by resin; [5]CEC = cation exchange capacity; [6]SB = sum of basis; [7]V = basis saturation; [8]Available cadmium (DTPA pH 7.3); [9]Typic Quartzipsamment; [10]Typic Hapludox

Data Analysis

Analysis of variance (ANOVA) and regression (n = three replicates) were conducted. The efficiency of the chemical extractants was evaluated by the L Ratio, (the ratio between the L-value and extractants), which is considered the most accurate assessment of Cd phytoavailability and chemical extractants: *L Ratio = L-value / Extractant,* in which the *L-value* and *Extractant* units are calculated as mg kg^{-1}; the closer to 1 (one) the ratio for an extractant is, the more efficient it is considered. The extractant's effectiveness was compared for each Cd rate.

Results and Discussion

There was no correlation between Cd rate and dry matter (DM) (Figure 1A). According to Prasad (1995), HM tolerance is related to physiological mechanisms that allow plants to experience typical growth patterns, despite high HM concentrations. The role played by intracellular ligants such as phytochelatins reduces HM cytoplasmic toxicity by complexation, in cases where the phytochelatin-Cd complex is less toxic to the cellular metabolism of plants than the free metal ion. The average DM differed between the soils, with 5.59 and 2.54 g of production for the TH and TQ soils, respectively, and this probably occurred due to the greater fertility level of the TH soil, as in the cases of, for example, SOM and CEC (Table 1).

The Cd content in the rocket plants increased as a function of the Cd levels in both soils, and lower levels were found in plants grown in the TH soil (Figure 1B). Vegetables present different developmental growth patterns and other reactions in response to high Cd concentrations in soils. The toxicity of an element to plants must be monitored when reduced growth or harvest, visual symptoms, and/or abnormal concentration in the tissues occur (Beckett, 1991). The rocket plant leaves became, with increasing severity of toxicity, chlorotic, mainly in the TQ soil. On some occasions, plants may have been contaminated but showed no visual signs of toxicity (Jiang et al., 2010). Lettuce (*Lactuca sativa* L.), for instance, is known as a Cd "accumulating" plant, because it is not sensitive to the toxic effects of Cd at low and medium concentrations in the soil (Alexander et al., 2006; Melo et al., 2012).

A linear pattern was observed in response to increasing Cd contents in the rocket plants for both soils. Leaf vegetables are unable to regulate the amount of elements taken up and accumulated in the plant (Khan et al., 2010; Li et al., 2010) and may acquire concentrations that could be harmful to the continuity of their physiological cycle, known as luxury consumption (Epstein and Bloom, 2004). There was a uniform distribution of Cd labeled with ^{109}Cd (Figure 2), which clearly indicates that rocket plants and other leafy vegetables (Khan et al., 2010; Li et al., 2010) may not have a specific avoidance mechanism related to the absorption of Cd. Consequently, they may represent a high risk because of their adverse affects on human health.

The rocket shoots accumulated about twice as much Cd in the TQ soil than in the TH soil (Figure 1C). This difference in Cd uptake by the rocket plants is related to differences in clay, SOM and Fe and Al oxide

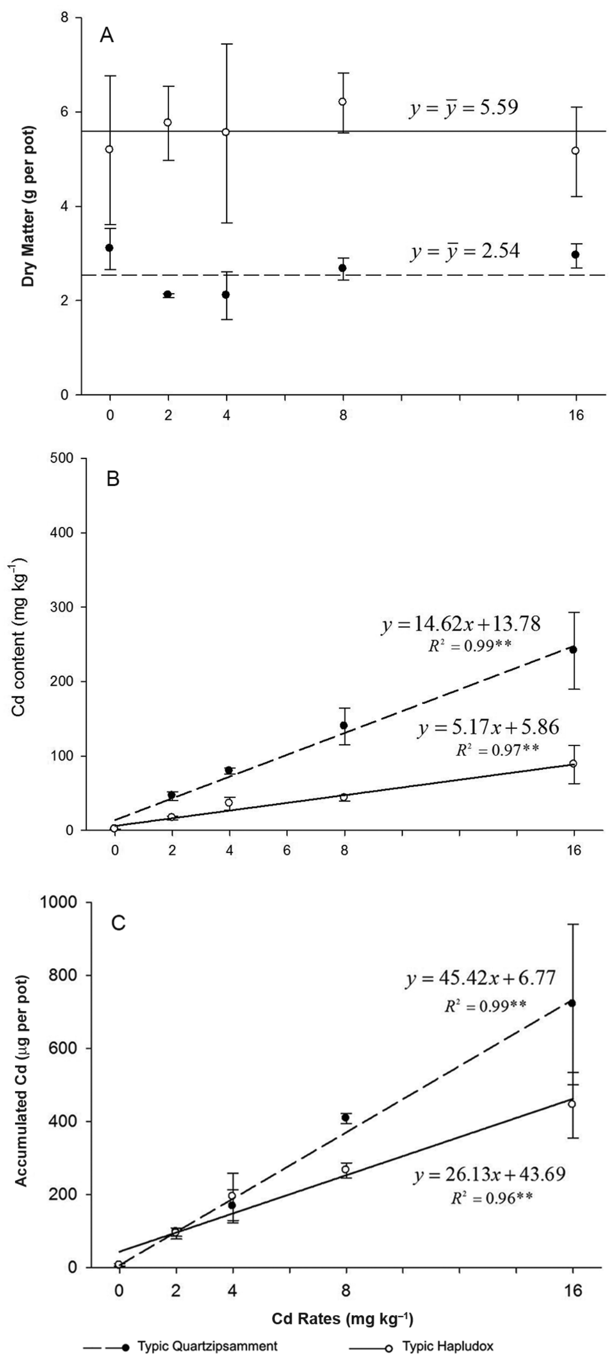

Figure 1 – Shoot dry matter production (A), rocket cadmium (Cd) content (B), and Cd accumulation in the rocket shoots (C) as a function of Cd rates. **Significant at 1 %.

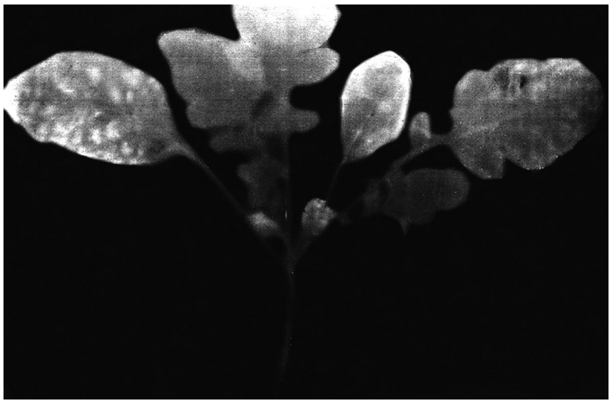

Figure 2 – Autoradiograph of the rocket plant showing a uniform distribution of Cd labeled with ^{109}Cd. White color represents the activity of ^{109}Cd.

Table 2 – Cd contents extracted by Mehlich.

Extractants	Rates[1]				
	0	2	4	8	16
	mg kg^{-1}				
	Typic Quartzipsamment				
Mehlich-1	0.15	1.99	3.87	7.89	15.89
Mehlich-3	0.08	1.48	3.51	5.93	12.08
DTPA	0.07	1.97	3.93	7.54	14.99
Organic acids	0.09	1.21	2.61	4.71	9.81
L Value	0.00	0.68	1.29	2.95	5.52
	Typic Hapludox				
Mehlich-1	0.03	1.95	4.01	7.74	16.04
Mehlich-3	0.07	1.80	3.65	6.71	13.04
DTPA	0.05	1.81	3.45	7.01	12.40
Organic acids	0.15	1.10	1.98	3.69	7.33
L Value	0.00	0.68	1.34	2.56	5.12

[1]Mean of three replicates.

contents, which act as Cd sorbents (Fontes and Santos, 2010; Havlin et al., 2005; Melo et al., 2012; Naidu et al., 1994).

Cd Phytoavailability by Chemical Extractants and L-value

For the TQ, Mehlich-1 extracted the highest amount of available Cd, followed by DTPA, Mehlich-3, organic acids, and the L-value. For the TH, Mehlich-1 also extracted the highest amount of available Cd, followed by Mehlich-3, DTPA, organic acids, and the L-value (Table 2). Basar (2009) reported that chelating extractants and strong acid compounds extracted higher amounts of HMs. For acid extractants, extraction levels close to the total content are due to their power to dissolve mineral structures that retain soil HMs (Taylor et al., 1993). The amounts of extractable Cd measured by Mehlich-1, Mehlich-3, DTPA, organic acids, and the L-value methods showed a linear correlation with the values of Cd content accumulated in rocket plants ($p < 0.01$) (Figure 3).

Many researchers have evaluated the effectiveness of chemical extractants in assessing the phytoavailability of HMs, including DTPA, Mehlich-1, and Mehlich-3 (Borkert et al., 1998; Cajuste et al., 2000; Gupta and Aten, 1993; Lee et al., 2009; Menzies et al., 2007) but with no consensus in the literature. This apparent conflict in reported effectiveness may be due not only to differences in SOM content, soil pH, the amount, source and form of the metal contaminant, but also to the expanded use of chemical extractants, like DTPA, for metals other than Fe, Zn, Mn and Cu (Menzies et al., 2007).

In this study, high regression coefficients for the extractions ($R^2 > 0.90$) could be justified by the Cd-soluble source added to the soil to obtain the Cd gradients. Thus, using this coefficient as a tool to verify the effectiveness of extractants in assessing phytoavailable Cd is not feasible, as was considered by Havlin et al. (2005). Therefore, the efficiency of the extractants was verified by the L Ratio.

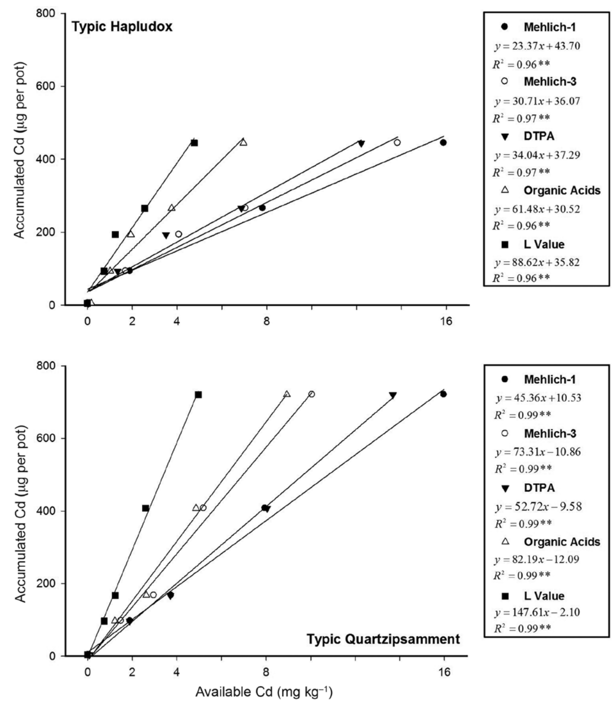

Figure 3 – Linear regression between Cd accumulation in the rocket shoots and Cd phytoavailability by Mehlich-1, Mehlich-3, DTPA, organic acids, and the L-value. **Significant at 1 %.

Extractant Effectiveness and the L Ratio

The most efficient extractant at all rates for the TQ were the organic acids, with an overall average of 0.55 (Table 3). However, there was no difference ($p < 0.05$) in the values for Mehlich-3 (0.49). The sequence for extraction efficiency was as follows: organic acids = Mehlich-3 > DTPA = Mehlich-1. For the TH soil, the most efficient extractant was also the organic acids (0.69) and the sequence was as follows: organic acids > DTPA

Table 3 – Extractant effectiveness in evaluating Cd phytoavailability as compared to the L Ratio.

Dose	L Ratio - OA	L Ratio - DTPA	L Ratio - M1	L Ratio - M3
		Typic Quartzipsamment		
2	0.62 a[1]	0.40 a	0.39 a	0.53 a
4	0.48 a	0.34 b	0.33 b	0.42 ab
8	0.53 a	0.32 b	0.33 b	0.51 a
16	0.57 a	0.37 b	0.32 b	0.50 ab
Mean	0.55 a	0.36 b	0.34 b	0.49 a
		Typic Hapludox		
2	0.73 a	0.45 b	0.39 b	0.44 b
4	0.64 a	0.36 b	0.30 b	0.30 b
8	0.69 a	0.37 b	0.33 b	0.36 b
16	0.69 a	0.40 b	0.30 b	0.34 b
Mean	0.69 a	0.40 b	0.33 b	0.36 b

[1]Means (n = 3) with the same letters, at the rows, do not differ (Tukey test, $p < 0.05$).

= Mehlich-3 = Mehlich-1. There are only a few studies that use the ratio of specific activities in plants and soils, but none of them pertaining to Cd. According to Mason et al (2013) and Muraoka et al (1983), a ratio for plant/extractants around 0.60 could be considered efficient.

Plants can serve as reference tools for assessing nutrient levels in the soil, thus reflecting actual nutrient availability. Thus, a good extractant must simulate plant root behavior (Havlin et al., 2005). Smolders et al. (1999) evaluated Cd extractants using the ratio between plant-specific activity and extractant-specific activity values (^{109}Cd/Cd). Rather than specific activity, we decided to use the L-value because, although it yields similar results, the unit of measurement (mg kg^{-1}) gives us a better comprehension of Cd phytoavailability. Many researchers have used the isotopic dilution technique to assess phytoavailability (Hutchinson et al., 2000; Rosén et al., 2012; Stacey et al., 2001) and the zooavailability of Cd in soils (Scheifler et al., 2003).

A likely reason for the greater efficiency of the organic acids as extractants (i.e., closest to the L-value) is that the method is based on a simulation of reactions that occur in the rhizosphere (Yang and Pan, 2013), which also affect the L-value. Low molecular weight organic acids present in the rhizosphere are effective in solubilizing soil-linked metals (Marschner,1995). With increasing concern related to environmental preservation, organic acids present a good alternative as Cd extractants because synthetic extractants commonly used to simulate HM phytoavailability may pollute the environment when used incorrectly (i.e., they have a low level of biodegradability). Organic acids, however, are natural chelating agents and are quickly degraded in the soil.

Conclusions

The extractants based on strong acids and chelating agents presented low efficiency. The organic acids´ extractant, which presented values closer to the L-value and better efficiency in the Typic Hapludox soil, may provide a promising method for evaluating environmental contaminants availability for crops.

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