

Bioavailability of copper, cadmium, zinc, and lead in tropical savanna soils assessed by diffusive gradient in thin films (DGT) and ion exchange resin membranes

John O. Agbenin · Gerhard Welp

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Abstract The technique of diffusion gradient in thin films (DGT) for assessing bioavailable metals has not been tested under field conditions. We assessed the relationships of DGT- and cation exchange resin-membrane-measured concentrations of Cd, Cu, Pb, and Zn with plant uptake of the metals under greenhouse and field conditions. In the greenhouse, the effective concentrations of Cu, Pb, and Zn by DGT correlated significantly with uptake by sorghum (*Sorghum bicolor*), but cation exchange resin-membrane-measured concentrations of Cd, Pb, and Zn did not correlate with sorghum uptake. In the field, the DGT-measured concentrations of Cd, Pb, and Zn were

not linearly related to uptake Cd, Pb, and Zn by lettuce (*Lactuca sativa*) except for Cu uptake ($r = 0.87$, $p < 0.05$). Similarly, it was only the resin-membrane-extractable Pb that correlated with Pb uptake by lettuce ($r = 0.77$; $p < 0.05$). However, fitting non-linear regression models improved the plant metal uptake predictions by DGT-measured bioavailable Cd, Cu, Pb, and Zn under field conditions. In conclusion, the DGT technique was fairly predictive of bioavailability in the greenhouse, but not in the field.

Keywords DGT device · Cation exchange resin membrane · Effective concentration (CE) · Lettuce · Metal uptake · Sorghum

J. O. Agbenin
Department of Soil Science, Ahmadu Bello
University, PMB 1044, Zaria, Nigeria

G. Welp
Institute of Crop Sciences and Resource
Conservation, Division of Soil Science, University of
Bonn, Nussalle 13, 53115, Bonn, Germany

Present Address:
J. O. Agbenin (✉)
Department of Crop Science and Production,
Botswana College of Agriculture, University of
Botswana, Gaborone, Botswana
e-mail: joagbenin@yahoo.com

Introduction

A standard method for assessing bioavailability of metals in terrestrial ecosystems has not been universally established. Several methods have been reported in literature including extraction with 1.0 M NH_4NO_3 solution (Prueß 1997), ion activities in soil solution (Sauve et al. 1996, 1998), extraction with 0.1 M CaCl_2 solution (Young et al. 2000), and operational speciation of metals (Zeien and Bruemmer 1989).

The technique called diffusion gradient in thin films (DGT) was introduced to assess bioavailability of trace metals in terrestrial and aquatic

ecosystems (Zhang and Davison 1995). Several investigators have concluded that metals accumulated by DGT devices are strongly correlated with bioassay (bioconcentration in plants) in a number of studies across Europe, North America, and Australia (Harper et al. 1998; Hooda et al. 1999; Zhang et al. 2001; Nowack et al. 2004; Nolan et al. 2005).

The DGT method works with the principle of depleting soil solution concentrations of metals, thereby stimulating metal re-supply from the solid phase species principally by diffusion (Nolan et al. 2005). If the re-supply mechanism in a soil is wholly or predominantly diffusion controlled, it is expected, therefore, that metals accumulated by the DGT would correlate strongly with plant uptake. However, Koster et al. (2005) and Sonmez and Pierzynski (2005) did not find any superiority of DGT over conventional chemical extraction for characterizing bioavailable metal pools in soils.

An advantage of the DGT method is its ability to integrate metal supply kinetics from the solid phase with the bioavailable concentration in soil solution (Nolan et al. 2005). Thus, metals accumulated by DGT (C_{DGT}) is the metal supplied to the resin from both the solution and solid phases, provided the labile fraction at the solid phase adjacent the DGT device or assembly is not substantially depleted. Although, widely acclaimed as a theoretically useful technique for assessing metal bioavailability, little is known about its effectiveness under field conditions and in weathered tropical soils dominated by variable charge colloids, consisting of Al and Fe oxides to which metals are strongly occluded.

The ability of oxidic materials to re-supply metals to soil solution, on depletion, will determine the success of DGT technique over conventional chemical extraction in weathered soils. A previous study using 1 M NH_4NO_3 extraction to assess bioavailable trace metals in the soils did not yield satisfactory results (Agbenin et al. 2009). Our objective was to assess the predictive capacity of DGT and a simple cation exchange resin membrane for assessing bioavailable Cd, Cu, Pb, and Zn in a tropical savanna soil.

Materials and methods

Greenhouse pot experiment

The soil for the pot experiment was obtained from a fallow field at Samaru latitude $11^{\circ}11'$ N longitude $7^{\circ}38'$ E in the northern Guinea savanna of Nigeria. The soil was classified as Isohyperthermic Typic Haplustalf in the USDA Soil Taxonomy or Orthic Acrisol in the FAO Soil Classification System (Valette and Ibanga 1984). Surface soil samples were collected in the fallow field at 0–15 cm depth, air-dried, and screened through a 2-mm sieve. Unsorted and decomposing solid municipal wastes were collected from two dumpsites (Tudun Wada and Gaskiya) in the Zaria Metropolis and carefully sorted to remove food remnants, papers, glasses, plastics polyethylene bags, and a variety of non-decomposed materials. Between 33% and 45% of the solid wastes were non-soil materials. Subsistence farmers engaged in urban farming activities collect the decomposing wastes and apply them as soil amendments for raising high-priced vegetables during the dry season (November–April) and to maize (*Zea mays*), sorghum (*Sorghum bicolor*), and cowpeas (*Vigna unguiculata*) during the rainy season (May–October).

The decomposed solid waste material was air-dried and screened through a 2-mm sieve and incorporated into the soil in pots at the rate of 0%, 20%, 50%, and 100% on weight–weight basis and replicated four times to create a gradient of metal concentrations in the potted soil. The soil in the pot was thoroughly mixed and homogenized after removing root materials. Each pot held 3 kg soil–waste mixture. Prior to wetting, the water-holding capacity of the potted soil, with and without waste addition, was determined in a pressure membrane apparatus at -33 kPa soil moisture tension. The moisture retained by the soil at this moisture tension was determined gravimetrically. The pots were wetted to 80% field capacity and covered with polyethylene sheet and allowed to equilibrate for 28 days. Before sowing, sub-samples of the soil–waste mixture were taken to determine the concentrations of Cu, Cd, Pb, and Zn in the soils.

Sorghum seeds (*Sorghum bicolor* L) were sown in each pot at the rate of 18 seeds per pot and thinned to ten seedlings. All pots were maintained at 80% water-holding capacity by the replacement of moisture lost by evapotranspiration throughout the growth duration of the plants. Daylight temperatures varied from 28°C to 40°C while night temperatures varied between 20°C and 28°C at the glasshouse. Thirty-five days after sowing, the sorghum seedlings were cut at the soil-root level using a stainless steel blade. The shoots were immediately weighed, rinsed in deionized water, and oven-dried at 65°C for 48 h to a constant weight in order to determine the dry matter (DM) yield. The oven-dried sorghum DM was milled and stored in brown envelopes for analysis of Cu, Cd, Pb, and Zn.

Field study

Urban garden soils under long-term manurial treatment with municipal biosolids were sampled at six locations. At each site, three fields were sampled for soils (0–20 cm depth) and lettuce (*Lactuca sativa*) vegetables. Five stands each of lettuce (*L. sativa*) were cut at the soil-root level with a sharp stainless steel blade, rinsed in deionized water, and stored in nylon bags. The soil samples were air-dried and screened through a 2-mm sieve for metal analysis while the plant shoots were oven-dried to a constant weight, milled, and analyzed for Cu, Cd, Pb, and Zn.

Soil and waste characterization

The particle size distribution of the soil and waste matter was determined by the hydrometer method after dispersing the soil with sodium hexametaphosphate solution (Gee and Bauder 1982). The soil pH was determined in 0.01 M CaCl₂. Organic C in the soils and wastes was determined by dichromate oxidation (Nelson and Sommers 1982). The cation exchange capacity (CEC) of the soil and wastes was determined by the neutral ammonium acetate method (Rhoades 1982). The concentration of metals in the soil was determined by “aqua regia” digestion (HNO₃ +

HCl). Two grams of <2 mm sieved wastes or soil were weighed into digestion tubes to which were added 20 mL of concentrated HCl and 10 mL of HNO₃ and digested at 150°C for 16 h or overnight. The digests were diluted to 100 mL mark and filtered. The concentrations of Cu, Cd, Pb, and Zn in the digests were analyzed in inductively coupled plasma atomic emission spectroscopy (ICP-AES) spectrophotometer. The concentrations of Cd, Cu, Pb, and Zn in plant samples were determined by digesting an oven-dried and milled plant material in concentrated HNO₃ (Zarcinas et al. 1983). To 0.5 g oven-dried plant matter weighed into digestion tubes were added 4 mL of concentrated HNO₃ and digested for 16 h at a temperature of 150°C. The concentrations of Cu, Cd, Pb, and Zn in the plant digests were determined in ICP-AES.

Ion exchange resin membrane extraction

Cation exchange resin membranes measuring 500 × 1,500 mm (Product 551654W, VWR Ltd, England) were procured and sliced into 12.5 × 125 mm strips. The membrane strips were pre-treated by soaking in 2 M HNO₃ solution for 2 days to remove impurities from the resin membrane. On the first day, the washing solution was renewed after every 3 h and then allowed to stay overnight or for 16 h. On the second day, the membrane strips were transferred to 0.1 M NaNO₃ solution and the strips were rinsed repeatedly with this solution until the pH of the washing solution was 4.5 ± 0.2. Five grams of <2-mm sieved soil were weighed into 50 mL centrifuge with tight screw caps to which 25 ml of double-deionized water were added to maintain soil/solution ratio of 1:5. With a pair of pincers, two strips of the ion exchange resin membrane were introduced to the soil suspension and shaken for 16 h or overnight. The resin strips were removed and washed clean of adhering soil particles with a jet stream of double-deionized water and placed into acid-washed clean extraction bottles. Twenty milliliters of 2 M HNO₃ were added to the resin in the extraction bottles to elute the metals by shaking for 16 h. The concentrations of Cd, Cu,

Pb, and Zn in the eluent were determined either in ICP-OES or graphite furnace

The DGT deployment to the soil

The DGT devices used in the study were procured from DGT Research Ltd, Lancaster, England. The devices consisted of diffusive and chelex gels made from acrylamide with crosslinker (DGT Research Ltd). The device, housed in a plastic assembly, had a layer of resin embedded in gel and then overlain by a layer of diffusive gel and a protective filter through which metal ions diffuse freely. The surface area (A) of the DGT device in contact with the soil was 3.14 cm^2 . The diffusive gel thickness was 0.1 cm . Before deploying the DGT device to the soil, 25 g soil were weighed into grooves made into Perspex solid specifically designed to hold between 25 and 30 g of soil. The soils were wetted to 80% water holding capacity (WHC) and allowed to equilibrate for 72 h at this moisture content. One DGT device was gently pressed into the soil to maintain maximum contact between the soil surface and the DGT device on a laboratory bench at a temperature of 20°C and a deployment time of 24 h . All experiments were replicated three times. After 24 h , the devices were retrieved and rinsed with a jet stream of double-deionized water to remove any adhering soil particles to the device. The resin gel was retrieved from the plastic assembly and placed into a clean vial and eluted with 0.75 ml of 1 M HNO_3 for 24 h to determine Cu, Cd, Pb, and Zn accumulated by the resin. The concentration of Cu, Cd, Pb, and Zn accumulated by the resin was determined in ICP-mass spectrometer at the University of Lancaster, England.

DGT metal fluxes

The mass of metal (M) accumulated by DGT was calculated by Eq. 1

$$M = C_e (V_{\text{HNO}_3}) + V_{\text{gel}}/f_e \quad (1)$$

where C_e is the concentration of the metal in the eluent ($\mu\text{g L}^{-1}$), V_{HNO_3} is the volume of 1 M HNO_3 added to the resin gel (1 ml), V_{gel} is the volume of the resin gel ($0.15 \text{ }\mu\text{l}$) and f_e is the elution factor given as 0.8 (Zhang and Davison

1995). The DGT measured concentration of the metal in soil (C_{DGT}) was calculated by Eq. 2

$$C_{\text{DGT}} = M\Delta g / (A\Delta t) \quad (2)$$

where M is the mass of metal accumulated by resin gel ($\mu\text{mol cm}^{-2}$), Δg is the thickness of the diffusion layer or path of the device (0.094 cm), A is the interfacial or surface area of the DGT device (3.14 cm^2) (Zhang and Davison 1995), D is the diffusion coefficient of the metal, and t is the deployment time. The C_{DGT} can be converted to an effective concentration (CE) of the metal by use of Eq. 3

$$\text{CE} = C_{\text{DGT}}/R_{\text{diff}} \quad (3)$$

where R_{diff} is a measure of the extent of depletion of the concentration at the interface of the DGT device and the soil for the diffusion-only-case. For the DGT devices used in this study, R_{diff} was given as 0.08 (Harper et al. 1998).

Statistical analysis

Correlations and regression analyses were used to explore the relationships between measurements of bioavailability of the metals in soils and the concentrations of the metals in plant tissues. The fit of the regression models to the experimental data was assessed on the basis of coefficient of determination (R^2) that explains the variance in plant metal uptake accounted for by DGT effective concentration of the metals in soil and resin extractable concentrations of the metals. All statistical analyses were conducted with SAS (SAS 1999–2001).

Results and discussion

Some properties of the soils and wastes

The sorted municipal wastes contained high sand content compared to the experimental soils (Table 1). The organic carbon (OC) concentration in the municipal wastes was almost five times greater than the OC concentration in the soil used for the pot experiment. The pH and CEC of the sorted municipal wastes were greater than the

Table 1 Selected soil properties and the concentration of cadmium, copper, lead, and zinc in the experimental soils

Soil/waste	Sand %	Silt	Clay	OC g kg ⁻¹	pH	CEC CaCl ₂ mmolc kg ⁻¹	Cd mg kg ⁻¹	Cu	Pb	Zn
Soil	46.2	40.0	13.8	10.0	5.5	90	0.02	1.4	5.8	13.8
T/wada waste	70.0	20.2	9.8	52.5	7.8	193	0.40	46.9	60.6	388
Gaskiya waste	64.2	24.0	11.8	52.8	8.6	188	1.1	48.2	78.2	512

experimental soils. The pH of the sorted municipal wastes was alkaline, while the experimental soils had acid to slightly acid pH. The concentrations of Cd, Cu, Pb, and Zn were far lower than the concentrations in the municipal wastes by several orders of magnitude because of the relatively high concentration of these metals in the municipal wastes. Zinc concentration in the municipal wastes was more than two times greater than 200 mg kg⁻¹ considered the maximum allowable limit for agricultural soils (McGrath et al. 1994). Table 2 shows the range of soil properties and metal concentrations in the potted soil prior to sowing the test crop. The range of soil properties and metal concentrations in the potted soil was much greater than the range in the fields. Nonetheless, the range of metal concentrations in the soils provided an adequate setting for evaluating the performance of DGT under greenhouse and field conditions.

DGT measured metal concentration and plant uptake

In the pot experiment correlation analyses between metal uptake by the plants and bioavailable measurements indicated that CE Cu, Pb, and Zn were linearly correlated with sorghum uptake in the greenhouse study, but not for Cd uptake (Table 3). However, metal uptake predictions were best made by either an exponential model for Cd ($R^2 = 0.84$) and Zn ($R^2 = 0.90$) or by a

quadratic polynomial for Cu ($R^2 = 0.64$) and Pb ($R^2 = 0.81$; Fig. 1). The concentration of Cd, Cu, Zn, and Pb in the sorghum tissues reached a maximum concentration and thereafter remained unchanged with increasing CE. This uptake pattern of the metals with increasing CE is indicative of the internal capacity of the plant to exercise control over the translocation of metals from the root to the shoot and from the soil to the root with a view to maintaining appropriate concentration of the metals in cellular compartments (Nolan et al. 2005; Sonmez and Pierzynski 2005), even though metal uptake by the DGT device increased. Growing plants can exercise control over metal uptake by inducing changes in their rhizosphere chemistry (pH and redox changes) or make physiological changes to restrict translocation from root to shoot. All of these changes are not captured by the DGT sink, thus making the relationship between CE measurements and metal uptake by plants to be non-linear.

Under field conditions, the correlations between metal uptake by lettuce and their measured concentrations in soil by the DGT were poor (Table 3). However, CE Cu linearly correlated with Cu uptake by the lettuce ($r = 0.87, p < 0.05$). Fitting non-linear regression did not improve Cd uptake from CE Cd ($R^2 = 0.43$) or Pb uptake from CE Pb (Fig. 2). The uptake of Cu and Zn by lettuce showed negative linear relations with CE (Fig. 2). This relationship can either be explained by an apparent restricted plant uptake of Cu and

Table 2 The range of selected properties and concentration of cadmium, copper, lead, and zinc in potted soil–waste mixtures and field soil samples

Soil	OC g kg ⁻¹	pH CaCl ₂	WHC ^a %	Clay	ECEC mmolc kg ⁻¹	Cd mg kg ⁻¹	Cu	Pb	Zn
Pot ($n = 9$)	10–53	5.5–8.7	26–46	9.8–13.8	90–188	0.03–1.06	1.4–46.9	5.8–78.2	13.8–388
Field ($n = 6$)	4–13	5.9–6.9	32–40	9.6–42.2	113–200	1.18–2.80	14.0–37.6	85–155	181–259

^aWater-holding capacity of potted soil (w/w basis)

Table 3 The correlation of DGT, aqua regia, and resin membrane measured concentrations of Cd, Cu, Pb, and Zn with bioconcentrations of the metals in plant tissues

Bioavailability measurement	Bioconcentration in plant tissues			
	Cd	Cu	Pb	Zn
	<i>r</i>			
Greenhouse study				
DGT-CE	0.54	0.75*	0.85*	0.75*
Resin membrane	0.62	0.29	–	0.49
Field study				
DGT-CE	0.48	0.87**	0.24	0.47
Resin membrane	0.32	0.36	0.77	0.42

Zn with increasing availability of the metals in the soil due probably to metal toxicity to the plant root. Metal uptake by plants subjected to toxic concentration had often decreased, whereas metal binding to DGT resin might not (Almas et al. 2006), thereby leading to negative and complicated relationship between CE and plant metal uptake. As a passive sink in soil, the DGT may not be effective in capturing the dynamics of nu-

trient uptake which is an active process (Buzier et al. 2006; Cattani et al. 2006). As an infinite sink for metals, metal binding to the DGT device could continue uninhibited, whereas in adverse conditions such as metal toxicity in soil, already alluded to, metal uptake by plants can be regulated through the mechanism of homeostasis. There is also some evidence showing that DGT could accumulate some amounts of inert organic complexes

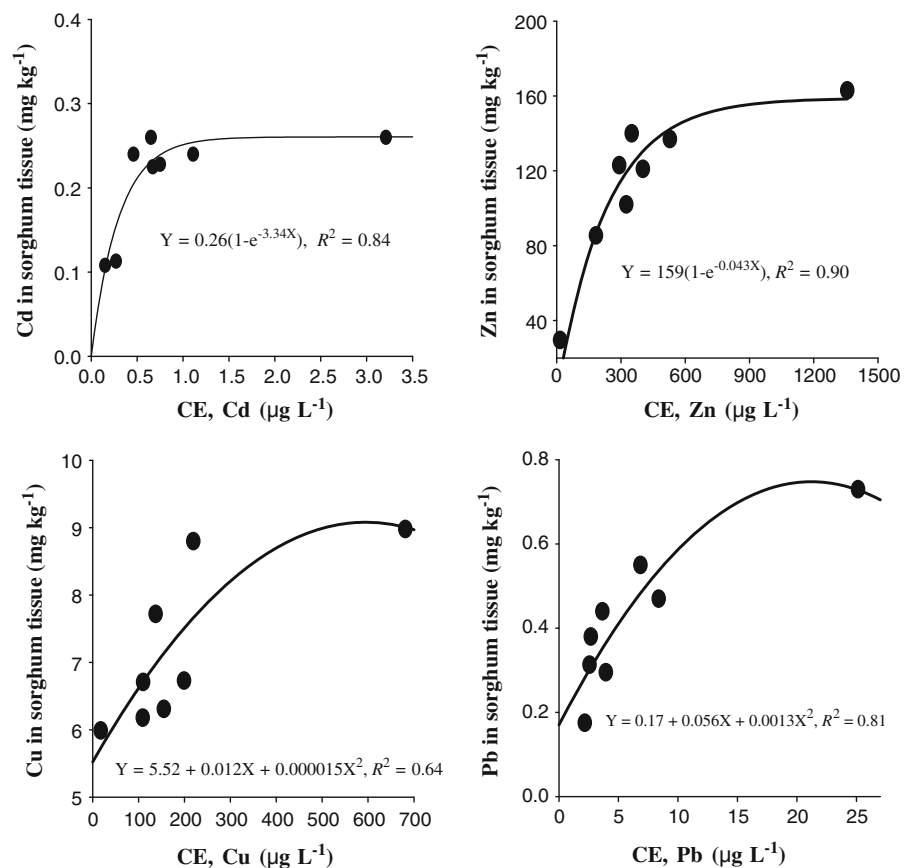
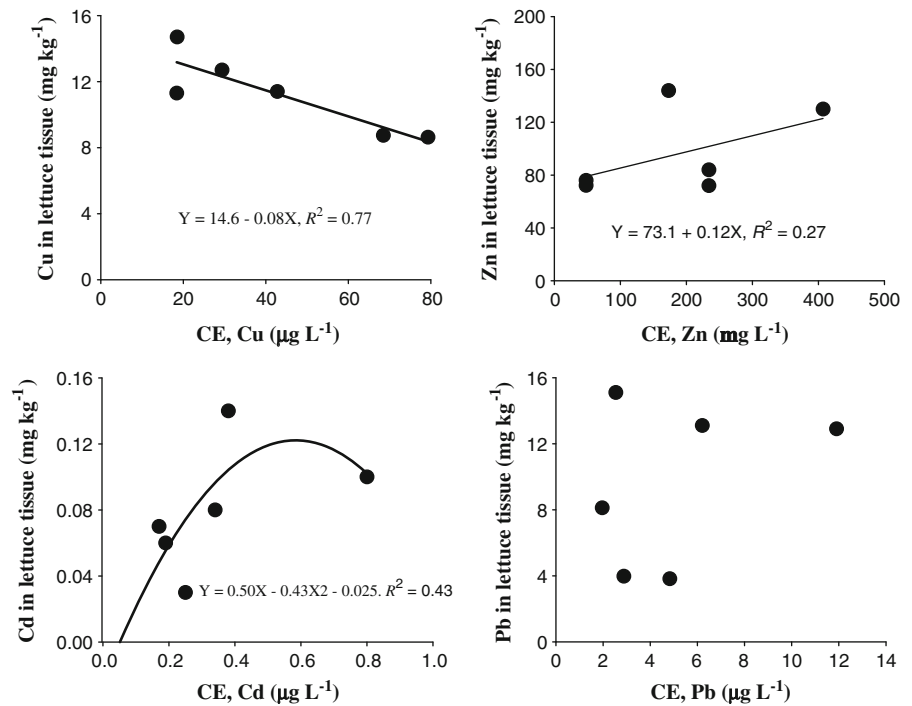
Fig. 1 The relationship between effective concentration (CE) of Cu, Cd, Pb, and Zn as determined by DGT device and the bioconcentration of the metals in sorghum seedling

Fig. 2 The relationship between effective concentration (CE) of Cu, Cd, Pb, and Zn as determined by DGT device and the bioconcentration of the metals in lettuce (*Lactuca sativa*)



of metals (Buzier et al. 2006), as is possible in these garden fields irrigated with wastewater, and with relatively high OC. Such organic complexes of the metals are not readily bioavailable as the inorganic complexes. The DGT device does not guarantee that only the inorganic metal specie is measured.

Resin membrane extraction

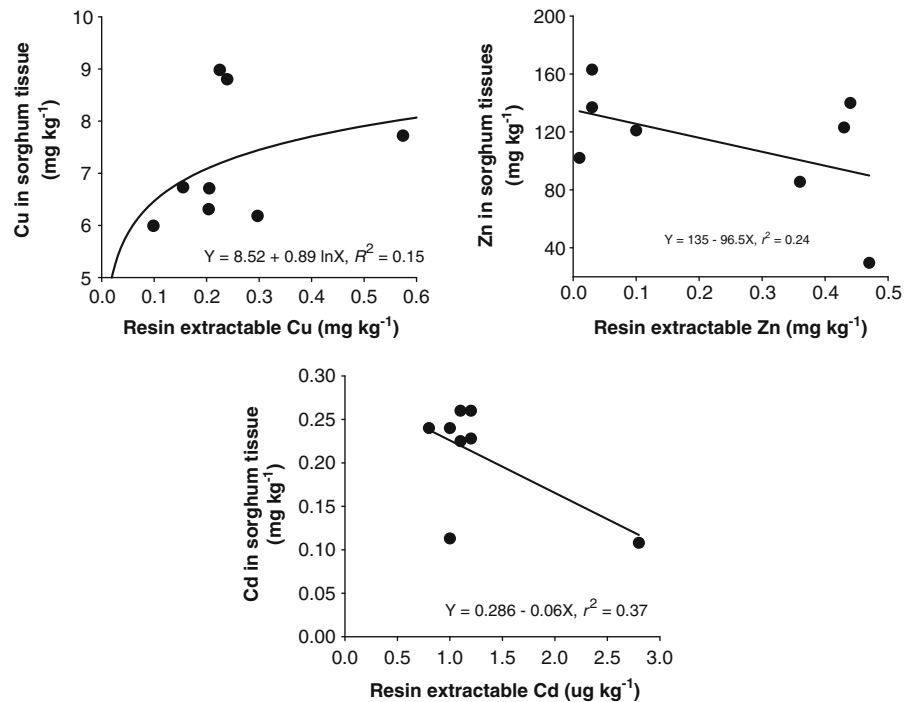
The cation exchange resin membrane extractable concentrations of Cd, Cu, and Zn did not correlate with sorghum uptake (Table 3). Fitting non-linear regression did not improve prediction of sorghum uptake of Cu, Cd, and Zn from cation exchange resin-membrane-extractable concentrations of the metals (Fig. 3). In the field study, however, cation exchange resin-membrane-extractable Pb and Zn were related to their concentrations in lettuce (Fig. 4). Non-linear relationship between metal uptake and bioavailable measurements in soils is usually caused by the complexity of factors affecting nutrient uptake by plant roots and subsequent translocation to the shoot.

The mechanisms through which plants acquire metal ions in soils are usually the interplay of

three basic processes of mass flow, diffusion, and root interception (Barber 1995). However, the ability of plant roots to solubilize metals through pH and redox modifications of their rhizospheres further complicates metal uptake mechanisms by plants (Barber 1995; Nolan et al. 2005), thereby making direct or simple straightforward correlations between measurements of bioavailability of metals in soils and plant uptake unlikely. Except, perhaps, there can be a measurement technique that integrates these complex processes into one holistic assessment that will make possible a direct, simple correlation between soil metal bioavailable measurements and plant uptake. Neither the DGT device nor exchange resin membrane evaluated in this study was able to achieve this integration.

Clearly, the cation exchange resin membrane was a poorer predictor of metal uptake by lettuce and sorghum than DGT device, and the DGT technique gave better overall prediction of plant uptake of the metals in both the pot experiment and field study than the resin membrane. The DGT-measured Cd, Cu, Pb, and Zn did not correlate with cation resin-membrane-extractable concentrations of the metals (Table 4), but total

Fig. 3 The relationship between cation exchange resin membrane extractable Cu, Cd, Pb, and Zn and the bioconcentration of the metals in sorghum seedlings



concentrations of Zn and Cd correlated significantly with the DGT-measured Cd and Zn (Table 4), suggesting that high proportions of total

Zn and Cd concentration in the soil were in the kinetically labile solid phases in contrast to total Pb and Cu in the soils.

Fig. 4 The relationship between cation exchange resin membrane extractable concentration of Cu, Cd, Pb, and Zn and the bioconcentration of the metals in lettuce (*Lactuca sativa*)

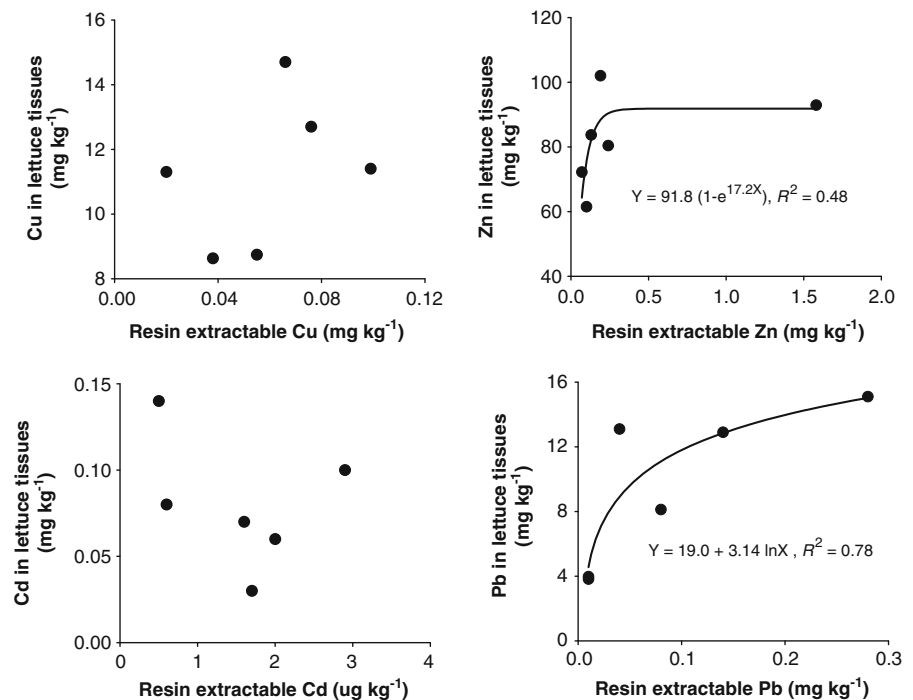


Table 4 The correlation of DGT effective concentration (CE) of the Cd, Cu, Pb, and Zn with total and resin membrane extractable concentrations of the metals

DGT-CE (n = 16)	Total r	Resin membrane
Cd	0.83 ^a	0.14
Cu	0.28	0.32
Pb	0.09	0.02
Zn	0.54 ^b	0.54 ^b

^aSignificant at 1% probability levels

^bSignificant at 5% probability levels

At this juncture, it is appropriate to comment on the apparent failure of the resin membrane to predict plant uptake of the metals. One plausible explanation for this is the possibility of accumulation of significant amounts of larger and inert molecules of organic metal complexes from the soil by the cation exchange membrane as compared to the DGT device that was equipped with a hydrogel layer that restricts the diffusion of large molecules of organic metal complexes to the resin layer in the DGT device. In our opinion, this singular characteristic difference between the resin membrane and the DGT device could account for their differential effectiveness for predicting plant uptake of the metals, and the poor correlation between the DGT and the cation exchange resin membrane.

The performance of the DGT method was, however, not entirely satisfactory to be recommended as a routine method for bioavailable Cu, Cd, Pb, and Zn for this type of soil. None of the metals assessed by DGT bioavailability measurement (CE) correlated strongly and linearly with uptake, thus implying that re-supply from the kinetically labile solid phases of Cd, Cu, Pb, and Zn was not fully controlling their phytoavailability in these soils.

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