

Effect of biosolid application to Mollisol Chilean soils on the bioavailability of heavy metals (Cu, Cr, Ni, and Zn) as assessed by bioassays with sunflower (*Helianthus annuus*) and DGT measurements

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Received: 3 September 2013 / Accepted: 29 December 2013 / Published online: 21 January 2014
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Abstract

Purpose This study assessed the effect of biosolid application on the bioavailable fraction of some trace elements (Cu, Cr, Ni, and Zn) using a bioassay with sunflower (*Helianthus annuus*) and a chemical assay, diffusion gradient in thin films (DGT).

Materials and methods Five surface soil samples (0–20 cm) were collected from an agricultural zone in Central Chile where biosolids are likely to be applied. Municipal biosolids were mixed with the soil at concentrations of 0, 30, 90, and 200 Mg ha⁻¹. The experiment to determine the bioavailability of metals in the soil using the bioassay was performed using sunflower. The DGT technique and Community Bureau of Reference (BCR) sequential extraction were used to determine the bioavailable fractions of the metals.

Results and discussion The application of biosolids increased the phytoavailability of Zn, Ni, and Cr in most of the soils, as indicated by the increasing concentrations in sunflower plants as the biosolid application rate increased. In two of the soils, Codigua and Pelvín, this increase peaked at an application rate of 90 Mg ha⁻¹. Decreases in the bioavailable fractions of Zn, Ni, and Cr were observed with higher biosolid application

rates. The bioavailability of metals was estimated through multiple linear regression models between the metals in the sunflower plants and the different chemical fractions of metals in the soils treated with different biosolid rates, which displayed a positive contribution of the labile (water soluble, carbonate, and exchangeable), oxide, and organic metal forms in the soil, particularly with respect to Ni and Zn at application rates of 30 and 90 Mg ha⁻¹. The bioavailable fraction of metals was determined in soils using the DGT technique. The effective concentration (C_E) results were compared with those in sunflower plants. The DGT technique could effectively predict the bioavailable fractions of Cr, Ni, and Zn in the Taqueral soil but only that of Zn in the Polpaico soil.

Conclusions The application of biosolids significantly increased the labile fraction of most of the metals in the studied soils, particularly at the highest biosolid application rate. C_E increased as the concentration of biosolids increased for most of the metals. The effectiveness of the DGT technique for predicting the bioavailability of metals was dependent on the soil type and the metal. However, the C_E for soil Cu was not related to plant Cu for all soils studied.

Keywords Biosolid · DGT · heavy metal bioavailability · sunflower

Responsible editor: Hailong Wang

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1 Introduction

In Chile, expanded wastewater treatment has resulted in significant improvements in water quality in rivers receiving

treated effluent. However, improvements in wastewater treatment have resulted in the generation of greater quantities of biosolids. These materials require adequate management to ensure that they are used in an environmentally sustainable manner. The land application of biosolids to agricultural soils has been recognized as one of the best options for recycling these materials. The high organic matter content and the macronutrient and micronutrient contents of the biosolids may be advantageously used to fertilize agricultural soils or reclaim degraded soils. However, these substrates may also contain inorganic and organic contaminants; thus, they must be appropriately used to avoid the risks associated with the transfer of these contaminants through the food chain. Evaluating the potential impact of the biosolids on the total content of trace metals in the receiving soils is also important.

The risk associated with heavy metal contamination in soils has been assessed through measurement of total metal concentrations or by simple or sequential extraction procedures. These extractions attempt to differentiate between the total metal concentration and bioavailable fraction. In Chile, current regulations on biosolids are based on the total metal content of both the biosolids and receiving soil. To predict the potential mobility of trace elements in the soil, the development of a reliable chemical method that is well correlated with biological methods is necessary to determine the available fraction of total metals that are phytoavailable. Previous studies have indicated that plant assays provide reliable information on the assessment of metal availability in contaminated soils (Remon et al. 2013). Identifying predictive methods for environmental assessment using laboratory extractions is important because plant assays can be time consuming and identifying appropriate plant species can be problematic. For this purpose, simple and sequential extraction methods have been utilized (Kennedy et al. 1997; Zimmerman and Weindorf 2010). Although these methods have been criticized, they remain an important tool for assessing the metal availability in soil–plant systems (Filgueiras et al. 2002).

Biosolid applications to soil may alter the forms of metals as a result of both the organic and inorganic components of the biosolids. Thus, Chang et al. (1982) demonstrated that biosolid application increased the Cd and Zn concentrations in plant tissues. Shuman (1988) also found similar results when assessing the Mn, Cu, Fe, and Zn distributions in biosolid-treated soils; Mn and Fe changed from their less soluble forms to their more labile forms. To date, no long-term studies have identified any concerns associated with biosolid applications (McGrath et al. 1994; Evanylo et al. 2008; Brown and Cotton 2011). Studies performed by Ahumada et al. (2004) using soils from the central zone of Chile with biosolids added at different rates and with different incubation times found that the distribution of the labile, potentially labile, and inert forms varied based on the soil type and were metal specific. Although biosolid application increased the total metal

concentrations, the labile forms of many metals remained unchanged, and an increase in labile Zn was observed. This increase in the labile fraction was confirmed using assays with ryegrass and clover. In most cases, a direct correlation was observed between the available fraction and Zn bioavailable fraction as determined by plant assays (Guerra et al. 2007).

Several processes affect the supply of metals to plants, including diffusion and convective transport to the root. In addition, the microenvironment of the root is difficult to copy using simple chemical extractions. For example, the rate of resupply of ions to the soil solution from the solid phase will vary based on soil and plant factors (Zhang et al. 2001). Depletion of an ion in the rhizosphere due to plant uptake will result in a decreased concentration immediately adjacent to the plant root, which will subsequently result in a diffusion gradient with resupply of the ion from the solid to the solution phase. The rate at which this depletion occurs will depend on the nature of the solid phase. If the solid phase is able to resupply ions to the solution consistently and rapidly, the solid phase is considered kinetically labile. Some plants can actively mobilize nutrients, such as Fe, Cu, and Zn, from the solid phase under deficiency conditions. In contrast, under conditions of excess metals, soil solid phase factors will determine the soil solution concentrations of ions (Zhang et al. 2001).

Simple extractions have traditionally been used to determine metal bioavailability in the soil (Fuentes et al. 2004; Feng et al. 2005). Dilute salts have been used to evaluate the available metals under conditions of potential toxicity, whereas more aggressive extractions have been used in cases of potential deficiency. Although these procedures characterize the soil at the moment of the extraction, they do not provide information about the potentially soluble fraction of metals available to plants and microorganisms over time. The diffusion gradient in thin film (DGT) technique has proven to be a useful tool to assess heavy metal kinetics and bioavailability to plants over time (Black et al. 2011; Huynh et al. 2010). It also appears to provide useful information on redox-sensitive elements in soils (Mundus et al. 2012). DGT devices are similar to plant roots in that they cause a localized gradient in the concentration near the edge of the device and allow for diffusive transport from the soil solution and the solid phase to bring the solution into equilibrium. The kinetics of this transport determines the metal availability to plant roots (Zhang et al. 2001 and Nowack et al. 2004).

DGT devices provide information on the concentration of metals that are actually available for uptake by plant through a calculation of effective concentration (C_E). This new concept of C_E is based on research on diffusion processes in the rhizosphere (Zhang et al. 2001). Davison and Zhang (1994) and Davison et al. (2000) estimated the Zn concentration in a water system using this technique and found that in principle, it could be applied to any diffusing species. In addition, Zhang et al. (2001) demonstrated that C_E permitted the adequate

estimation of the Cu content in plants across a broad range of soil types with varied total Cu concentrations. C_E predicted the Cu concentration in *Leucanthemum heterophyllum* more accurately than traditional measures, including EDTA extractable, total, and soil solution Cu. DGT devices have also been used to predict the Zn phytoavailability in soil (Zhang et al. 2004). However, Almas et al. (2006) demonstrated that the response of the soil microbial community to Cd and Zn in the soil was as effectively predicted by the pore water concentrations of these elements as by the C_E . Nolan et al. (2005) evaluated traditional extraction methods alongside DGT in contaminated soils to predict Zn, Cd, Pb, and Cu uptake by wheat plants and found that the Zn and Cd concentrations in plants were closely related to C_E . In contrast, the other extractions, with the exception of dilute CaCl_2 , were not predictive of the plant uptake of these elements. These results suggest that the kinetically labile solid phase, as measured by the DGT device, plays an important role in Zn and Cd uptake by wheat.

The purpose of this study was to assess the effect of biosolid application to the soil on the bioavailable fraction of some trace elements (Cu, Cr, Ni, and Zn) through an assay with sunflower (*Helianthus annuus*) plants and determine whether the plant uptake of these elements was correlated with C_E as measured using the DGT technique.

2 Materials and methods

Six composite surface soil samples (0–20 cm) were collected from an agricultural zone in Central Chile where biosolids are likely to be applied. The soils were Mollisols and are referred to by their series names: Codihua, Lampa, Pelvín, Polpaico, and Taqueral. Each soil sample was air dried and sieved through a 2-mm mesh polyethylene sieve. The soils were stored in plastic jars at room temperature. A sample of anaerobically stabilized biosolids was collected from a monofill at a sewage treatment plant in Santiago. The biosolids were sieved as described above for soils. The biosolids and soils were characterized for pH using a 1:2.5 soil-to-water ratio. Organic matter was determined by the spectrophotometric method, and the cation exchange capacity (CEC) was determined by the acetate procedure at pH 7 (Sadzawka et al. 2006). The total metal concentration was determined after acid digestion (4 mL of HNO_3 , 4 mL of HF, and 2 mL of H_2O_2) of 200-mg soil samples using a Milestone/mls Mega microwave oven. The resulting solution was taken to a final volume of 10 mL with 1 M HCl. The metals were determined by flame atomic absorption spectroscopy (FAAS). A certified reference material (Montana Soil, NIST 2710) was included in the analysis to verify the accuracy of the extractions. The experimental values were similar to the certified values ($p < 0.05$).

2.1 Greenhouse experiment

Biosolids were mixed thoroughly into the soils at rates equivalent to 0, 30, 90, and 200 Mg ha^{-1} . Each treatment was replicated three times. Pots containing the equivalent to 500 g of soil were irrigated to field capacity and allowed to stand for 15 days before sowing with sunflowers. Five grams of seeds were added to each pot. The experiment was conducted under controlled conditions, with 14/10-h light/dark periods at 20 ± 5 °C. The irrigation quantities were determined by weight at two-thirds of the water-holding capacity (33 kPa) of each soil. The seedlings were watered daily with distilled water to maintain the soils at field capacity conditions for 30 days after seed sowing.

The sunflower seedlings were harvested at day 30. The plant samples were washed with distilled water and dried at 30 °C. The plant samples were ground and homogenized for each treatment. Sunflower has been shown to be tolerant to heavy metals and has been suggested as a potential phytoremediation crop (Solhi et al. 2005).

Five hundred milligrams of plant tissue was digested with a mixture of 2 mL of H_2O_2 and 4 mL of HNO_3 in a microwave oven. The resulting solution was taken to a final volume of 10 mL with Milli-Q water. The metals were determined by FAAS or ICP-mass spectrometry (Thermo Fisher Scientific Xserie II).

2.2 Soil sequential extraction

The soil samples were subjected to sequential extraction using the Community Bureau of Reference (BCR) scheme (Ure et al. 1993), with specific reagents used to obtain the following operationally defined fractions for trace metals in soils: labile metal fraction (F1), corresponding to soluble, exchangeable and bound to carbonate, and acetic acid-soluble (20 mL of 0.11 M HOAc for 16 h); metal bound to oxides (F2), corresponding to soluble under reducing conditions (20 mL of 0.50 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ adjusted to pH 2 with HNO_3 for 16 h); metal bound to organic matter (F3), corresponding to soluble under oxidizing conditions (digestion with 10 mL of 30 % H_2O_2 and extraction with 25 mL of 1 M NH_4OAc pH 5 for 16 h); and the residual fraction (10 mL of aqua regia). Depending on the concentration, metal determination in the extracts was performed either through FAAS or ICP-MS. Approximately 0.5 g of soil from each treatment was weighed into a 50-mL centrifuge tube. Each treatment was analyzed in triplicate. Each reagent was added to the soil. The samples were then set on a shaker and centrifuged. The supernatant was separated, filtered, and stored in polyethylene bottles at 4 °C until AAS or ICP-MS analysis. The residues were washed with 10 mL of distilled water, and the rinsate was discarded. The centrifuge tubes were weighed for volume correction after each step. For the oxidizable fraction (step

3), 10 mL of hydrogen peroxide (30 %) was added to the residue from step 2, and the samples were refrigerated. Next, the samples were placed uncovered in a water bath and brought to near dryness at 85 °C. Forty milliliters of 0.1 M ammonium acetate (adjusted to pH 2 with nitric acid) was added to the cooled residue, which was then shaken for 16 h at room temperature. To determine the residual fraction, the recommended extraction protocol with aqua regia (ISO11466) was adapted for microwave oven use. The residue oxidizable fraction was digested with a concentrated mixture of HNO₃ and HCl (1:3 v/v) using a Milestone/mls 1,200 M microwave oven. The sequential extraction procedure method was validated using certified reference material CRM 483; the experimental results were statistically similar to the results reported by BCR.

2.3 Determination of trace metal bioavailability by DGT

Water was added to the soil samples and soil–biosolid mixtures to form a saturated paste with three replicates for each treatment. The paste was allowed to incubate for 3 days at 25 °C. Each DGT device was prepared by placing the Chelex-100 disc on the support, followed by a diffusive gel disc (DGT Research Ltd., Lancaster, UK), and then by a membrane filter. The upper cover, with a window exposed to the sample, was then fixed (Zhang and Davison 1995; Zhang et al. 2001). After 3 days, the devices were removed, washed with Milli-Q water, and dried with absorbent paper. The resin gel was recovered by breaking the DGT cover and then placed in test tubes with 5 mL of 1 M HNO₃ for 24 h, and the solution was analyzed by ICP-MS.

After the DGT device was removed from the saturated paste, a portion of the paste was centrifuged at 3,000 rpm for 20 min. The supernatant was filtered and stored for ICP-MS analysis to determine the metal concentration in pore water.

The following equation was used to determine the effective metal concentration (M) in the resin:

$$M = C_{\text{elu}} \cdot (V_{\text{acid}} + V_{\text{gel}}) / f_e$$

C_{elu}	concentration of metals in the HNO ₃ (μg/L) solution
V_{acid}	volume of HNO ₃ acid added to the resin
V_{gel}	volume of resin gel
f_e	elution factor of each metal

Concentration of the metal measured by DGT (C_{DGT}) was calculated by

$$C_{\text{DGT}} = M \cdot \Delta g / D \cdot t \cdot A$$

Δg	diffusion layer thickness (diffusive gel+membrane filter)
D	diffusion coefficient of the metal in the resin gel

t	deployment time
A	area of the exposure window

The conversion of C_{DGT} to C_{E} , the effective available concentration of metal from the soil solution, was performed using the following equation:

$$C_{\text{E}} = C_{\text{DGT}} / R_{\text{diff}}$$

R_{diff} is the ratio of the concentration at the DGT interface to the concentration in the bulk soil solution, with only the diffusive case being considered. This parameter was calculated using the computer numerical model of the DGT soil, the DGT-induced fluxes in sediments (DIFS) system. The particle concentration (P_c) and porosity (ϕ) must be known to calculate R_{diff} .

2.4 Statistical analysis

The results were analyzed using an analysis of variance (ANOVA) procedure. When significant F values were observed, differences between the means were tested using the LSD test for data from the sequential extraction. Linear correlation tests were used to assess the relationship between the plant metal concentrations and C_{E} from the DGT analysis. The bioavailable fraction of metals was estimated through the multiple linear regression models between the metal total concentration in the sunflower plants and the different chemical fractions of metals in soils treated with different rates of biosolids. All statistical tests were conducted using Statgraphic 5.0 software.

3 Results and discussion

The general properties of the biosolids and soils are shown in Table 1. The biosolids had a high organic carbon concentration, high CEC, and neutral pH. The soils had a total C content of 1.4–2.1 %. The soil pH values ranged from slightly acid for the Codigua and Pelvín soils to neutral for the Polpaico and Lampa soils and alkaline for the Taqueral soil. The CEC was similar across all soils, ranging from 21 to 23 cmol kg⁻¹; only the Polpaico soil exhibited a higher CEC value (39.2 cmol kg⁻¹).

The Zn concentration was highest in the biosolids compared with Cr, Cu, and Ni. McLaren and Clucas (2001) found values in the same order of magnitude in a similar material for Zn, Cu, and Ni. Except for Ni, these concentrations were lower than the values recommended by the Chilean regulations for application to agricultural soils (DS-4 2009). In the soils, Cu and Zn occurred at higher concentrations than Cr and Ni. Copper ranged from 55 to 141 mg kg⁻¹ for the Pelvín and Codigua soils, respectively. Zinc ranged from 26 to 161 mg kg⁻¹ for the Pelvín and Taqueral soils, respectively. The soil Cr

Table 1 Some properties of the biosolids and soils under study

		Biosolid	Taqueral	Polpaico	Codigua	Pelvín	Lampa
OC	(%)	27.8	1.42	1.81	1.51	2.09	1.79
pH	H ₂ O 1:2.5	6.97	8.21	7.21	6.14	6.18	7.16
CEC	cmol kg ⁻¹	71.6	22.8	39.2	20.6	22.9	22.66
		Total metal					
Cr	mg kg ⁻¹	558±3.5 ^a	17.1±2.1	11.5±5.1	25.7±1.2	35.2±4.6	34.8±3.7
Cu	mg kg ⁻¹	508±3.2	79.5±0.3	79.9±1.0	54.9±3.3	140.6±9.1	94.9±13.9
Ni	mg kg ⁻¹	112±5.4	16.5±6.1	20.1±5.6	28.5±7.8	28.0±1.7	32.8±4.5
Zn	mg kg ⁻¹	1,879±2.0	26.0±17.9	58.2±15.3	129.4±1.5	160.9±2.6	131.5±18.7

OC organic carbon, CEC cation exchange capacity

^a Standard deviation

concentration ranged from 17 to 35 mg kg⁻¹, and Ni ranged from 17 to 35 mg kg⁻¹. The highest concentrations of Cr and Ni were found in the Pelvín and Lampa soils.

3.1 Chemical fractionation of Cr, Cu, Ni, and Zn

The results from the sequential extraction indicate that in the amended soils, all metals were present principally in

the residual form. A small content corresponding to soluble, exchangeable, or bound-to-carbonate fractions was found. This fraction would correspond to the labile or phytoavailable fraction of the total metal (Ahumada et al. 2009).

The biosolid addition at all rates included in the study modified the residual or inert fraction of the metals in the soils, with a subsequent change in the relative concentrations

Table 2 Cr and Cu fractionation (mg kg⁻¹) of the Codigua, Lampa, Pelvín, Polpaico, and Taqueral soils treated with biosolids at rates of 0, 30, 90, and 200 Mg ha⁻¹. Values with the same letter are not significantly different at $p < 0.05$ within treatments of each metal for each soil

Soils	Biosolid Mg ha ⁻¹	Fractions ^a				Fractions			
		I Cr (mg kg ⁻¹)	II	III	Res	I Cu (mg kg ⁻¹)	II	III	Res
Codigua	0	1.10 a	1.24 a	10.3 a	8.50 a	4.58 a	21.4 a	10.7 a	32.2 d
	30	1.26 ab	2.20 ab	18.5 b	73.7 b	5.15 a	20.8 a	13.7 a	12.8 a
	90	1.53 ab	4.40 ab	28.5 c	89.1 d	5.27 a	20.9 a	20.1 b	23.3 c
	200	1.64 b	8.34 b	46.9 d	86.2 c	7.10 b	20.9 a	34.5 c	32.2 d
Lampa	0	0.19 a	0.71 a	4.81 a	83.1 a	4.81 a	28.8 c	23.0 a	12.3 a
	30	0.37 a	1.37 a	11.8 b	96.0 c	4.87 a	27.4 b	20.4 a	21.7 c
	90	0.64 a	3.32 ab	23.6 c	97.3 d	7.62 b	25.1 a	24.5 a	22.1 d
	200	1.40 b	6.53 b	44.7 d	87.3 b	9.80 c	25.2 a	38.5 b	20.5 b
Pelvín	0	0.14 a	3.67 a	2.21 a	2.50 a	8.93 a	70.8 d	35.1 a	89.9 d
	30	0.68 a	4.09 a	7.12 b	36.6 b	12.8 b	66.1 c	41.4 a	55.2 a
	90	1.34 b	6.36 a	18.8 c	57.3 c	16.0 c	58.8 b	48.5 b	59.8 c
	200	2.48 c	9.32 a	41.6 d	71.4 d	17.8 c	51.1 a	63.9 c	56.6 b
Polpaico	0	0.26 a	0.42 a	1.19 a	9.64 c	0.35 a	4.27 a	7.15 a	60.6 a
	30	0.26 a	0.82 a	9.42 b	9.44 c	0.45 b	4.70 b	8.05 a	59.9 a
	90	0.68 b	2.08 ab	24.8 c	6.74 b	0.89 c	5.73 c	13.6 b	64.1 ab
	200	0.94 c	4.12 b	43.7 d	3.31 a	1.77 d	6.98 d	16.6 b	69.5 b
Taqueral	0	0.13 a	0.53 a	1.74 a	12.6 d	0.34 a	4.53 a	17.1 a	53.8 a
	30	0.32 b	1.17 a	9.94 b	5.80 b	0.51 b	5.24 b	18.5 a	51.5 a
	90	0.77 c	2.70 ab	22.0 c	10.5 c	0.86 c	6.45 c	24.8 b	56.1 a
	200	1.12 d	5.23 b	43.8 d	3.29 a	1.65 d	8.88 d	34.8 c	53.8 a

^a Fractions: I (acetic acid-soluble), II (soluble in reducing condition), III (soluble in oxidizing conditions) Res (residual fraction)

of the other fractions. The results of the sequential extraction are presented in Tables 2 and 3. For Cr, the biosolid amendments increased the concentration of Cr in almost all fractions, with the most significant increase observed for the organically bound fraction of this metal. The labile fraction of Cr also increased for all soils at the highest biosolid loading rate. These observed changes were significant for Cr associated with organic matter for all soils and loading rates. For the II fraction of Cr, increases in this pool were only observed for some of the soils (Codihua, Lampa, Polpaico, and Taqueral) and only at the highest biosolid application rate.

Excluding the residual fraction, Cu was found predominantly bound to oxides in the Pelvín, Codigua, and Lampa soils. In the Taqueral and Polpaico soils, Cu was associated with organic matter, which increased in all soils with increasing biosolid rate. The labile fraction of this element increased significantly with biosolid application in the Pelvín, Polpaico, and Taqueral soils. For the Lampa and Codigua soils, this increase was only significant at the highest biosolid loading rate.

Of the elements studied, Ni was present at the lowest concentration, and without considering the residual fraction, this metal was bound primarily to oxides in most of the

soils. In the Codigua soil, the fraction of Ni corresponding to the soluble, exchangeable, and bound-to-carbonate fractions was also important and did not change significantly with biosolid application. In the other soils, this fraction increased significantly with the biosolid loading rate. Increases in the second fraction were observed for most of the soils as a result of biosolid addition. In all other soils, this pattern was significant only at the 200 Mg ha⁻¹ application rate. The fraction of Ni associated with organic matter varied significantly in the Polpaico and Taqueral soils only at the highest biosolid loading rate. This change in Ni partitioning was observed in the other soils only at the lowest biosolid rate.

Zinc was found to be associated with oxides in all soils without considering the residual fraction. All soils displayed an increase in Zn availability with biosolid application, as demonstrated by the significant increase in this metal in the acid-soluble fraction. This finding suggests that this increase would result in higher phytoavailable Zn for plants grown in the biosolid-amended soils. Similar results have been observed previously (Guerra et al. 2007). In addition, the fraction associated with oxides increased significantly with biosolid application. In contrast, the Zn form

Table 3 Ni and Zn fractionation (mg kg⁻¹) of the Codigua, Lampa, Pelvín, Polpaico, and Taqueral soils treated with biosolids at rates of 0, 30, 90, and 200 Mg ha⁻¹. Values with the same letter are not significantly different at *p*<0.05 within treatments of each metal for each soil

Soils	Biosolid Mg ha ⁻¹	Fractions ^a				Fractions			
		I Ni (mg kg ⁻¹)	II	III	Res	I Zn (mg kg ⁻¹)	II	III	Res
Codigua	0	3.36 a	3.12 ab	2.54 b	6.0 a	7.29 a	19.4 a	8.74 ab	92.4 d
	30	4.03 a	2.79 a	2.18 b	43.0 b	25.3 b	30.8 b	8.03 a	84.2 c
	90	3.93 a	4.13 b	1.12 a	52.0 c	37.4 c	56.0 c	9.64 c	80.2 b
	200	3.77 a	5.72 c	2.82 b	56.8 d	62.5 d	84.9 d	9.03 bc	52.3 a
Lampa	0	0.48 a	5.06 a	1.31 a	43.8 a	4.64 a	17.6 a	13.8 a	19.5
	30	1.80 b	5.15 ab	6.37 b	58.4 c	16.1 b	36.3 b	13.7 a	87.2
	90	4.21 c	5.86 ab	6.73 b	58.5 c	36.6 c	69.4 c	13.4 a	71.4 b
	200	4.56 b	7.15 b	6.63 b	52.9 b	88.6 d	113 d	17.0 b	19.5 a
Pelvín	0	1.29 a	2.40 a	0.90 a	6.20 a	17.7 a	36.7 a	14.6 ab	128 d
	30	1.53 a	1.24 a	5.56 c	16.3 b	53.7 b	54.4 b	14.5 ab	56.8 b
	90	2.67 b	4.20 b	3.20 b	29.0 c	85.0 c	72.9 c	14.4 a	58.5 c
	200	3.79 c	7.50 c	3.57 b	39.6 d	140 d	116 d	16.6 b	39.0 a
Polpaico	0	0.29 a	1.74 a	0.85 a	6.62 c	0.84 a	8.43 a	3.50 a	83.9 b
	30	0.52 a	2.58 b	1.00 a	4.87 b	3.30 b	16.6 b	4.14 a	85.0 b
	90	1.11 b	3.68 c	1.11 a	2.95 a	15.5 c	47.5 c	8.77 b	84.6 b
	200	2.28 c	6.04 d	2.54 b	2.38 a	37.4 d	84.9 d	9.03 b	53.1 a
Taqueral	0	0.41 a	0.98 a	0.66 a	2.80 b	0.99 a	10.1 a	4.47 a	38.0 a
	30	0.36 a	1.56 a	1.19 a	1.18 a	4.43 b	20.2 b	4.83 a	39.3 ab
	90	1.42 b	2.52 b	0.84 a	2.41 b	14.5 c	46.6 c	5.73 b	42.2 ab
	200	1.73 c	4.51 c	2.34 b	0.69 a	37.8 d	86.5 d	8.16 c	57.8 b

^a Fractions: I (acetic acid-soluble), II (soluble in reducing condition), III (soluble in oxidizing conditions) Res (residual fraction)

associated with organic matter only changed significantly at the highest biosolid application rate. Organic matter from biosolids plays a dual role in metal availability. Solid organic matter immobilizes metals by the formation of insoluble complexes, whereas dissolved organic matter promotes metal solubility by forming strong soluble complexes (Ashworth and Alloway 2008).

3.2 Trace metal bioavailability in crops of sunflower plants

Table 4 presents the total trace metal concentration of the sunflower plants. The plant uptake of Cu and Zn was higher than that of Cr and Ni. This result is likely also related to the high Zn concentration of the biosolids. In general, the metal uptake followed the order Zn>Cu>Cr>Ni. However, there were some differences in this pattern for each of the soils. In the Taqueral soil, the Cr phytoavailability increased with the biosolid application rate. For the other soils, higher biosolid loading rates decreased the Cr concentration in plants.

The biosolid application did not alter the plant uptake of Cu in the Taqueral, Polpaico, and Lampa soils compared with the control soil. In the Codigua and Pelvín soils, the plant Cu concentrations decreased at the highest biosolid loading rate. The patterns for plant Ni uptake were similar to those for Cr in the Taqueral soil with increases in the phytoavailable Ni with

Table 5 Multiple linear regression equation between the Cu and Zn contents in sunflower plants and the different chemical fractions of these metals in soil amended with 0, 30, 90, and 200 Mg ha⁻¹ biosolids (B)

Multiple linear regression equation	R ^{2a} (%)	C L ^b (%)
Cu		
Cu plant0B=107–21.3 F1+4.3 F2	85.9	95
Cu plant30B=64.7+3.5 F3	71.8	99
Cu plant90B=-0.58+5.1 F3	83.6	99
Cr		
Cr plant0B=3.72+3.1 F2	81.1	99
Cr plant30B=0.44+2.8 F2	88.0	99
Cr plant90B=-54.6+25.9 F1+1.74 F3	86.7	99
Cr plant200B=-19.8+0.62 F3	51.6	95
Ni		
Ni plant0B=-27.3+10.9 F2–25.0 F3	85.2	95
Ni plant30B=-2.45+9.50 F1+1.50 F3	97.0	95
Ni plant90B=-3.58+8.66 F1	73.4	99
Ni plant200B=-4.1+4.45 F3	83.0	99
Zn		
Zn plant0B=-1.03+5.2 F2+5.8 F3	96.7	95
Zn plant30B=69.0+3.8 F1+5.8 F3	94.9	90
Zn plant90B=-215+1.82 F1+6.70 F2	86.3	95
Zn plant200B=-364–2.64 F1+5.94 F2+15.7 F3	87.1	99

^a Determination coefficient of ^b confidence level

Table 4 Total metal (mg kg⁻¹) content in sunflower (*Helianthus annuus*) plants grown in the Taqueral, Polpaico, Codigua, Pelvín, and Lampa soils treated with biosolids at rates of 0, 30, 90, and 200 Mg ha⁻¹

Soil	Mg ha ⁻¹	Cr	RSD	Cu	RSD	Ni	RSD	Zn	RSD
Taqueral	0	3.78	9.82	139	3.23	2.65	3.01	66.7	8.06
	30	4.16	13.1	122	3.93	3.08	8.73	98.1	4.05
	90	7.68	7.31	134	2.49	4.09	3.09	138	18.6
	200	10.8	9.90	142	4.67	6.78	8.56	232	15.9
Polpaico	0	1.50	5.76	159	56.3	1.81	11.5	66.4	8.14
	30	4.28	6.66	120	6.71	2.50	2.72	86.5	17.0
	90	6.34	2.38	117	10.4	3.46	2.54	128	10.8
	200	3.79	4.33	127	4.43	2.85	7.95	163	11.7
Codigua	0	8.67	15.7	105	6.80	70.5	8.04	161	7.12
	30	9.69	7.62	118	8.77	34.9	3.88	249	4.35
	90	8.92	18.7	71.0	10.2	35.4	15.2	224	13.1
	200	2.75	17.5	31.2	1.56	8.80	21.2	97.2	2.04
Pelvín	0	18.4	17.2	224	6.94	34.9	5.30	272	7.83
	30	19.4	2.17	226	6.93	29.4	3.22	343	3.76
	90	31.6	3.23	253	12.4	33.1	0.78	471	2.57
	200	10.8	3.20	66.1	15.1	15.3	11.8	244	14.4
Lampa	0	7.97	2.93	100	8.79	25.8	7.28	174	4.94
	30	6.94	10.7	102	11.0	23.4	3.04	209	7.74
	90	17.9	12.2	112	9.98	28.5	13.0	315	14.8
	200	9.15	2.23	100	13.2	23.8	16.7	350	0.13

RSD relative standard deviation (%)

increasing biosolid application rates. In the Codigua and Pelvín soils, the plant Ni concentrations decreased with the biosolid application rate. Finally, biosolid amendment had no effect on the plant Ni concentrations in the Lampa soil.

The bioavailability of Zn in all soils increased with increasing biosolid application rate, but this increase in the bioavailable Zn fraction in the Codigua and Pelvín soils reached its peak when the soil was treated with 90 Mg ha⁻¹ biosolid, whereas a decrease in the bioavailable Zn fraction was observed with the highest biosolid application rate. This effect could be related to an increase in the conductivity values in the soils. Parkpian et al. (2002) found that heavy metal bioavailability in biosolid-treated soils reached a maximum when the medium exhibited conductivity values between 4 and 6 dS m⁻¹. A decrease in metal bioavailability was observed at higher conductivity values.

3.3 Relationship between metal chemical fractionation and metal bioavailability in sunflower plants

A multiple linear regression model was used to fit the plant uptake of Cr, Cu, Ni, and Zn as dependent variables and the

chemical fractions (F) of these metals in the soils as independent variables. As shown in Table 5, a consistently significant relationship between extractable metals and plant uptake was observed. The models were able to explain 52–88 % of the variability for Cr, 72–86 % for Cu, 73–97 % for Ni, and 86–97 % for Zn. The most significant factor for predicting the metal uptake by cultivated plants in the control soil was the metal associated with oxides. In the case of Zn, the fraction of the metal associated with organic matter was also important. There was no significant relationship between the soil fraction of the metal and plant Cu at the highest biosolid rate in the biosolid-treated soils. F2 also contributed to the bioavailability of Cr and Zn in soils treated with 30 and 90 Mg ha⁻¹ biosolids, respectively. For the lower biosolid application rates, the Cu associated with the organic fraction of the soil contributed to Cu bioavailability. For Cr, Ni, and Zn, in addition to the metal fraction associated with organic matter, the labile fraction of these metals, corresponding to F1, contributed to their bioavailable fraction. These results indicate that the phytoavailable fraction of an element is not limited to the labile fraction (F1) (Jacob and Otte 2003).

Table 6 Estimation of the Cu and Zn bioavailable fractions through the determination of the C_E obtained using the DGT technique

Soil	Biosolid Mg ha ⁻¹	Cu				Zn			
		C _E μg L ⁻¹	SD	C _{DGT} μg L ⁻¹	R	C _E μg L ⁻¹	SD	C _{DGT} μg L ⁻¹	R
Taqueral	0	13	15	5.449	0.222	136	8.44	5.38	1.489
	30	51	2.0	2.007	0.203	149	26.1	5.40	2.072
	90	46	3.2	2.114	0.065	324	12.4	13.2	0.360
	200	71	2.9	3.000	0.104	490	5.99	20.0	0.492
Polpaico	0	77	0.2	3.330	0.034	69	0.86	2.90	0.358
	30	72	8.9	3.283	0.045	102	3.66	4.36	0.364
	90	56	4.3	3.208	0.035	275	14.1	11.4	0.641
	200	94	0.2	3.985	0.038	518	36.3	22.2	0.261
Codigua	0	575	57	21.23	0.242	157	18.9	7.54	2.286
	30	155	52	9.485	0.149	227	17.5	12.0	0.840
	90	295	18	9.564	0.152	501	77.1	24.7	0.526
	200	141	0.5	7.620	0.038	1,033	49.5	44.3	0.172
Pelvín	0	531	12	23.85	0.290	224	10.8	8.24	1.659
	30	312	146	17.92	0.116	352	39.8	13.0	0.450
	90	351	8.1	17.39	0.080	730	61.0	31.1	0.448
	200	511	58	17.66	0.028	1,548	17.8	66.8	0.233
Lampa	0	340	24	14.04	0.260	23.4	7.13	0.978	0.261
	30	300	20	12.52	0.165	147	34.2	7.53	0.329
	90	236	29	9.940	0.048	454	30.0	21.5	0.377
	200	338	41	14.37	0.034	2,452	28.4	105.4	0.218

C_E C_{DGT}/R_{diff}

C_{DGT} Concentration accumulated by DGT

R Ratio Cu_{DGT}/Cu_{sol}

SD Standard deviation

3.4 Determination of C_E through the DGT technique

Tables 6 and 7 present the results from the DGT extraction. The DGT technique mimics plants, causing a localized reduction in soil solution concentration, limited by the rate of resupply from the solid phase. These two factors are accounted for in the determination of C_E using the DIFS computer mathematical model (Zhang et al. 2001). The results from the DGT extraction mirrored the plant results with the C_{DGT} values in all soils amended with biosolid, following the order $Zn > Cu > Ni > Cr$. In general, an increase in C_E was observed with increased biosolid application rates for Zn, Cr, and Ni. An irregular pattern was observed in the case of Cu. For the Taqueral, Polpaico, and Lampa soils, C_E decreased at the 90 $Mg\ ha^{-1}$ application rate and then increased. In contrast, with the Codigua soil, there was a sharp decrease in C_E for soils amended with 30 $Mg\ ha^{-1}$ biosolids, followed by a slight increase and subsequent decrease with increasing biosolid application rates. The observed decrease may be related to the presence of organically complexed and colloidal Cu species in the

Table 8 Relationship between the C_E values and total metal content in plants using the simple linear correlation (R) test

	Cu		Cr	
	R^a		R	
Codigua	0.374	NSS	NC	
Lampa	NC		0.303	NSS
Pelvín	NC		NC	
Polpaico	0.234	NSS	0.242	NSS
Taqueral	0.474	NSS	0.879	***
		Ni	Zn	
Codigua	NC		NC	
Lampa	0.228	NSS	0.634	NSS
Pelvín	NC		NC	
Polpaico	0.346	NSS	0.972	***
Taqueral	0.878	***	0.948	***

NC No correlation found, NSS No statistically significant relationship

^a Correlation coefficient

*** Statistically significant relationship at 99 %

Table 7 Estimation of the Cr and Ni bioavailable fractions through the determination of the C_E obtained using the DGT technique

Soil	Biosolid $Mg\ ha^{-1}$	Cr				Ni			
		C_E $\mu g\ L^{-1}$	SD	C_{DGT} $\mu g\ L^{-1}$	R	C_E $\mu g\ L^{-1}$	SD	C_{DGT} $\mu g\ L^{-1}$	R
Taqueral	0	5.49	0.57	0.239	0.063	23.4	1.02	0.953	0.394
	30	11.6	0.36	0.510	0.067	64.8	1.71	2.67	0.366
	90	25.6	0.50	1.137	0.018	257	10.0	10.7	0.065
	200	41.4	0.98	1.846	0.022	571	12.6	23.9	0.098
Polpaico	0	4.06	0.88	0.188	0.017	49.7	13.3	1.39	0.075
	30	5.54	0.34	0.258	0.019	72.8	1.06	3.18	0.097
	90	10.5	0.27	0.490	0.019	199	3.58	8.72	0.091
	200	18.6	1.07	0.892	0.017	330	4.72	14.5	0.080
Codigua	0	10.2	2.86	0.458	0.722	75.6	9.30	2.89	0.249
	30	28.8	0.18	1.159	0.033	127	11.1	6.23	0.105
	90	75.0	4.80	3.793	0.031	348	25.1	17.6	0.106
	200	134	26.5	8.115	0.028	827	5.32	34.3	0.090
Pelvín	0	17.1	10.1	0.777	0.742	68.6	12.1	2.52	0.339
	30	25.1	4.08	1.160	0.034	175	1.68	6.45	0.127
	90	52.9	4.88	2.772	0.027	273	40.1	11.9	0.082
	200	117	17.8	5.530	0.022	605	46.3	26.5	0.083
Lampa	0	nd	–	–	–	63.6	9.46	2.73	0.160
	30	18.3	3.18	0.848	0.016	236	11.2	9.28	0.088
	90	51.9	0.15	2.210	0.042	526	32.7	23.0	0.130
	200	115	11.3	5.442	0.012	892	2.00	41.6	0.057

C_E C_{DGT}/R_{diff}

C_{DGT} Concentration accumulated by DGT

R Ratio Cu_{DGT}/Cu_{sol}

SD Standard deviation

soil solutions. Some studies have demonstrated that DGT excludes these metal forms (Zhang and Davison 1995; Zhang et al. 1998, 2001; Ruello et al. 2008; Kovarikova et al. 2007).

To prove the efficacy of this tool for the soil/biosolid/plant system in the present study, C_E values were related to the concentrations of the metals in sunflower tissue using a simple linear correlation (Table 8). For Cu, positive linear correlations were found in the Codigua, Polpaico, and Taqueral soils with correlation coefficients of 0.4–0.5, but these values were not statistically significant. Similar results were found by Cattani et al. (2006), who demonstrated that DGT can be used to predict some effects of the cultivation of polluted soil but cannot be used to determine Cu bioavailability in maize plants. For Cr, a positive linear correlation was found for the Lampa, Polpaico, and Taqueral soils but was only significant for the Taqueral soil. Nowack et al. (2004) compared the DGT C_E as measured with Cu and Zn uptake in the shoot of *Lolium perenne* under three different conditions, i.e., in the field, soil cores, and homogenized soil, and a significant nonlinear relationship was found between the Cu and Zn concentrations in the plant and C_E values.

4 Conclusions

The results from this study indicate that the metals in the soil occur mainly in the inert form, with only a small proportion available for plant uptake. Biosolid application significantly increased the labile fraction of metals in most of the soils, especially at the highest biosolid application rate. However, biosolid application did not alter the plant uptake of Cu in the Taqueral, Polpaico, and Lampa soils compared with the control soil. Copper and Zn were the most phytoavailable metals for sunflower plants. Plant metal uptake generally followed the order, Zn>Cu>Cr>Ni. Biosolid application primarily increased the bioavailable fraction of Zn in all soils treated at the 90 Mg ha⁻¹ rate. Soils treated with the highest biosolid rate displayed a decrease in the plant Zn concentrations.

The effectiveness of the DGT technique as a method for the estimation of the phytoavailable trace metals in the soil depends on such factors as the metal and soil type. For most of the tested metals, the C_E was found to increase as the biosolid application rate increased. Comparison of the results of the bioavailable fraction obtained through the DGT technique with the metal concentration in sunflower plants using a simple linear correlation indicated that this technique was able to predict the bioavailable fraction of Cr, Ni, and Zn in the Taqueral soil but only that of Zn in the Polpaico soil.

Acknowledgments The authors thank FONDECYT (Grant 1110115) for its financial support.

References

- Ahumada I, Escudero P, Carrasco MA, Castillo G, Ascar L, Fuentes E (2004) Use of sequential extraction to assess the influence of sewage sludge amendment on metal mobility in Chilean soils. *J Environ Monit* 5:327–334
- Ahumada I, Gudenschwager O, Carrasco MA, Castillo G, Ascar L, Richter P (2009) Copper and zinc bioavailability to ryegrass (*Lolium perenne* L.) and subterranean clover (*Trifolium subterraneum* L.) grown in biosolid treated Chilean soils. *J Environ Manage* 90:2665–2671
- Almas A, Lombnaes P, Song T, Mulder J (2006) Speciation of Cd and Zn in contaminated soils assessed by DGT-DIFS, and WHAM/Model VI in relation to uptake by spinach and ryegrass. *Chemosphere* 62: 1647–1655
- Ashworth DJ, Alloway BJ (2008) Influence of dissolved organic matter on the solubility of heavy metals in sewage-sludge-amended soils. *Commun Soil Sci Plant Anal* 39:538–550
- Black A, McLaren RG, Reichman SM, Speir TW, Condron LM (2011) Evaluation of soil metal bioavailability estimates using two plant species (*L. perenne* and *T. aestivum*) grown in a range of agricultural soils treated with biosolids and metal salts. *Environ Pollut* 159:152–1535
- Brown S, Cotton M (2011) Change in soil properties and carbon content following compost application: result of on-farm sampling. *Compost Science & Utilization* 19:88–97
- Cattani I, Fragoulis G, Boccelli R, Capri E (2006) Copper bioavailability in the rhizosphere of maize (*Zea mays* L.) grown in two Italian soils. *Chemosphere* 64:1972–1979
- Chang AC, Page AL, Foster K, Jones TE (1982) A comparison of cadmium and zinc accumulation by four cultivars of barley grown in sludge-amended soils. *J Environ Qual* 1:409–412
- Davison W, Zhang H (1994) In-situ speciation measurements of trace component in natural waters using thin-film gels. *Nature* 367:546–548
- Davison W, Hooda P, Zhang H, Edwards AC (2000) DGT measured fluxes as surrogates for metal by plants. *Adv Environ Res* 3:550–555
- DS-4 (2009) República de Chile. Reglamento para el manejo de lodos generados en plantas de tratamiento de aguas servidas.
- Evanylo G, Sherony C, Spargo J, Stamer D, Brosius M, Haering K (2008) Soil and water environmental effects of fertilizer-, manure-, and compost-based fertility practices in an organic vegetable cropping system. *Agr Ecosyst Environ* 127:50–58
- Feng MH, Shan XQ, Zhang S, Wen B (2005) A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl₂, and NaNO₃ extraction methods for prediction of bioavailability of metals in soil to barley. *Environ Pollut* 137:231–240
- Filgueiras AV, Lavilla I, Bendicho C (2002) Chemical sequential extraction for metal partitioning in environmental solid samples. *J Environ Monitor* 4:823–857
- Fuentes A, Lloréns M, Sáez J, Soler A, Aguilar MI, Ortuño JF, Meseguer VF (2004) Simple and sequential extractions of heavy metals from different sewage sludges. *Chemosphere* 54:1039–1047
- Guerra P, Ahumada I, Carrasco A (2007) Effect of biosolid incorporation to mollisol soils on Cr, Cu, Ni, Pb and Zn fractionation and relationship with their bioavailability. *Chemosphere* 68:2021–2027
- Huynh TT, Zhang H, Laidlaw WS, Singh B, Baker AJM (2010) Plant-induced changes in the bioavailability of heavy metals in soil and biosolids assessed by DGT measurements. *J Soils sediments* 10: 1131–1141
- Jacob DL, Otte ML (2003) Conflicting processes in the wetland plant rhizosphere: metal retention or mobilization? *Water Air Soil Pollut* 3:91–104
- Kennedy VH, Sanchez AL, Oughton DH, Rowland AP (1997) Use of single and sequential chemical extractants to assess radionuclide and

- heavy metals availability from soils for root uptake. *Analyst* 122: 89–100
- Kovarikova V, Docekalova H, Docekal B, Podborska M (2007) Use of the diffusive gradients in thin films technique (DGT) with various diffusive gels for characterization of sewage sludge-contaminated soils. *Anal Bioanal Chem* 389:2303–2311
- McGrath SP, Chang AC, Page AL, Witter E (1994) Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and the United States. *Environ Rev* 2:108–118
- McLaren RG, Clucas LM (2001) Fractionation of copper, nickel, and zinc in metal-spiked sewage sludge. *J Environ Qual* 30:1968–1975
- Mundus S, Lombi E, Holm PE, Zhang H, Husted S (2012) Assessing the plant availability of manganese in soils using diffusive gradients in thin film (DGT). *Geoderma* 183–184:92–99
- Nolan AL, Zhang H, McLaughlin MJ (2005) Prediction of zinc, cadmium, lead, and copper availability to wheat in contaminated soils using chemical speciation, diffusive gradients in thin films, extraction, and isotopic dilution techniques. *J Environ Qual* 34:496–507
- Nowack B, Koehler S, Schulin R (2004) Use of diffusive gradients in thin films (DGT) in undisturbed field soils. *Environ Sci Technol* 38: 1133–1138
- Parkpian P, Leong ST, Laortanakul P, Torotoro JL (2002) Influence of salinity and acidity on bioavailability of sludge-borne heavy metals. A case study of Bangkok municipal sludge. *Water Air Soil Pollut* 139:43–60
- Remon E, Bouchardon JL, Le Guédard M, Bessoule JJ, Conord C, Faure O (2013) Are plants useful as accumulation indicators of metal bioavailability? *Environ Pollut* 175:1–7
- Ruello ML, Sileno M, Sani D, Fava G (2008) DGT use in contaminated site characterization. The importance of heavy metal site specific behaviour. *Chemosphere* 70:1135–1140
- Sadzawka A, Carrasco MA, Grez R, Mora M, Flores P, Neaman A (2006) Métodos de análisis recomendados para suelos de Chile. Instituto de Investigaciones Agropecuarias, Serie Actas INIA-N°34, Santiago
- Shuman LM (1988) Effect of organic matter on the distribution of manganese, copper, iron and zinc in soil fraction. *Soil Sci* 146: 192–198
- Solhi M, Shareatmadari H, Hajabbasi M (2005) Lead and zinc extraction potential of two common crop plants, *Helianthus annuus* and *Brassica napus*. *Water Air Soil Pollut* 167:59–71
- Ure AM, Quevauviller P, Muntau H, Griepink B (1993) Speciation of heavy metals in soils and sediments. An account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the commission of the European communities. *International J Environ Anal Chem* 51:135–151
- Zhang H, Davison W (1995) Performance-characteristics of diffusion gradients in thin-film for the in situ measurement of trace-metal in aqueous-solution. *Anal Chem* 67:3391–3400
- Zhang H, Zhao FJ, Sun B, Davison W, McGrath SP (2001) A new method to measure effective soil solution concentration predicts copper availability to plants. *Environ Sci Technol* 35:2602–2607
- Zhang H, Davison W, Knight B, McGrath SP (1998) In situ measurement of solution concentration and fluxes of trace metals in soil using DGT. *Environ Sci Technol* 35:2602–2607
- Zhang H, Lombi E, Smolders E, McGrath S (2004) Kinetics of Zn release in soils and prediction of Zn concentration in plants using diffusive gradients in thin films. *Environ Sci Technol* 35:2602–2607
- Zimmerman AJ, Weindorf D (2010) Heavy metal and trace metal analysis in soil by sequential extraction: a review of procedures. *Int J Anal Chem* Article ID 387803:1–7