Determination of the Bioavailable Fraction of Cu and Zn in Soils Amended with Biosolids as Determined by Diffusive Gradients in Thin Films (DGT), BCR Sequential Extraction, and Ryegrass Plant

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Abstract This study assessed the effect of biosolids applied at rates, 0, 30, 45, and 60 Mg ha^{-1} on the chemical associations and bioavailability of Cu and Zn in soils from an important agricultural zone of the Metropolitan Region in Central Chile. Three methods were used to determine the bioavailability of Cu and Zn in soils: ryegrass (*Lolium perenne*) plants, diffusive gradients in thin films (DGT) technique, and Community Bureau of Reference (BCR) sequential extraction. The DGT effective concentration (C_E) and sequential extract acid soluble fraction of the BCR extraction (most labile fraction of the soils, normally associated with bioavailability) were compared with total metal concentration in ryegrass plants as a means to compare the chemical and biological measures of bioavailability. Total Zn was higher in comparison to Cu for all treatments. Concentrations were within the limits set by the Chilean regulations for land-applied biosolids. Metals in the control soil were primarily found in the residual fraction of soils. Biosolids application generally decreased this fraction, with a subsequent increase observed mainly in the acid soluble fraction. The contents of Cu and Zn in ryegrass plants increased with increasing rates of biosolids. Comparison of the Cu and Zn content in ryegrass plants with C_E, showed a good correlation for Zn. However, the C_E for soil Cu was only related to plant Cu for some of the soils studied. Correlation between Zn in ryegrass plants and the labile fraction of Zn as measured by the sequential extraction was excellent, with correlation coefficients >0.9, while for Cu, correlation coefficients were lower.

Keywords Biosolid · Heavy metals bioavailability · DGT · Sequential extraction

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1 Introduction

Agricultural use of biosolids is supported by several decades of research throughout the world. A potential benefit associated with the use of biosolids in agriculture is the addition of soil micronutrients

which can be deficient in soils. An outstanding aspect of the presence of micronutrients in biosolids is that, as they are complexed with the substrate organic components, they are only slowly released and may provide a long-term nutrient source for plants. Thus, many soils of low agricultural value will regain quality and improve productivity. However, because of concerns of adding potentially phytotoxic concentrations of trace elements to soils with biosolids application, regulations regarding biosolids quality have been developed in Chile as in other countries. In Chile, these regulations consider characteristics of the receiving soil in addition to characteristics of the amendment.

Even though the existing regulations are based primarily on total metal content in soil, it is widely recognized that total content of these metals includes fractions that are not available to plants, microorganisms, or soil biota (Zhang et al. 2001). Determination of the bioavaliable fraction is important in risk assessment. This fraction is often predicted from the concentration of the free metal ion in soil or through reagents validated in single-extraction procedures (Nowack et al. 2004; Jamali et al. 2007). In addition to plant specific factors, trace metal uptake by plants is affected by soil physical and chemical factors (Cattani et al. 2006).

In the search for new, faster and more efficient methods to determine trace metal bioavailability in soil, the diffusive gradients in thin-films technique (DGT) has been developed to measure supply fluxes of metals in sediments (Harper et al. 1999). Many studies have shown it to be a good tool to assess the bioavailability of metals to plants (Nolan et al. 2005; Lehto et al. 2006; Zhao et al. 2006). However, others authors did not observe good correlations between plant metal concentrations and DGT extractable metals. They have indicated that further an evaluation of DGT use to determine Zn phytoavailability in soil systems is warranted (Degryse et al. 2003; Sonmez and Pierzynski 2005). DGT gradually lowers the metal concentration in the soil solution, thus causing resupply by diffusion from the bulk solution and desorption of the solid phase (Davison et al. 2000). In this way, the DGT device is designed to mimic metal availability in the rhizosphere.

The DGT device consists of a plastic assembly containing a layer of resin imbedded in gel, coated with a layer of diffusing gel and a protecting filter

through which ions can diffuse freely. These devices are deployed in the soil for a period of time during which metal ions are concentrated in the resin. The concentration of metal in the resin is used to calculate the rate of metal resupply through diffusion in a particular soil (Zhang et al. 2001). To relate the element concentration in resin to bioavailability, a numerical model is used which considers flux and reactions occurring between the device and deployment medium. To this end, the application of the DGT-induced fluxes in sediments and soils (DIFS) computer program has been developed. The program allows quantification of the DGT response in relation to diffusion characteristics of the soil and the supply rate from the solid phase to the solution (Sochaczewski et al. 2007). The DGT technique was first developed for use in aquatic systems. Theoretically, the resin layer binds the solutes that have diffused through the diffusion layer, thus eliminating them from the solution, effectively creating a concentration gradient. This results in a constant flow of ions towards the resin, which facilitates calculation of the solute in the soil solution. This concentration estimation requires that the concentration of the solute adjacent to the device remains constant and effectively equals the one in the bulk system beyond the influence of the DGT device. This holds very well for aquatic systems. For soil systems, however, as a result of a lack of homogeneity, it must be assumed that, in general, concentrations in the pore water adjacent to the device become smaller with time. If there is no mechanism for a new supply of solutes outside diffusion, the zone of depletion becomes progressively larger with time. The application of DIFS uses this assumption to carry out calculations leading to effective concentration, C_E, which has been related to the bioavailable concentration in plants as well as in the soil pore water (DGT Research Ltd 2003). In general, in recent investigations carried out with this technique, a good correlation has been shown between effective concentration and concentrations observed in plants (Zhang et al. 2001; Almas et al. 2006). It is important to point out that these correlations would likely not hold up in cases of phytotoxic metal concentrations (Almas et al. 2006) and that DGT measures metal concentration in a space of a few millimeters, while the plant integrates a much larger volume of soil solution (Nowack et al. 2004).

Another approach to study metal availability to plants is sequential extraction procedures that, through use of different reagents attempt to define operational speciation or metal fractionation. The majority of papers define the bioavailable/mobile pool as the most easily extracted fraction or labile fraction (Bacon and Davison 2008). The exchangeable and carbonate metal forms corresponding to the first step of BCR procedure are considered readily mobile and available to plants, while metals incorporated in clay crystalline lattices are relatively inert (Walter et al. 2006; Ahumada et al. 2009). A good correlation has been observed between the labile fraction determined by sequential extraction and Cu and Zn content in plant of Ryegrass and subterranean clover (*Trifolium subterraneum*; Ahumada et al. 2009). However, Torri and Lavado (2009) concluded that the most available fractions obtained by soil sequential extraction did not provide the best indicator of Cu and Zn availability to *Lolium perenne*.

The purpose of this study was to assess the application of two measures of bioavailability; DGT and the first fraction of BCR sequential extraction, to determine the influence of incorporating biosolids on Cu and Zn bioavailability to ryegrass.

2 Materials and Methods

2.1 Soils and Biosolid

Six composite soil samples of surface soil (0–20 cm) were collected from an agricultural zone in central Chile where biosolids are likely to be applied. The soils were three Mollisols, an Inceptisol, an Alfisol, and a Vertisol, referred to as Chada (CHD), Colina (CNA), Lonquén (LON), Linderos (LIN), Los Morros (MRR), and Chicureo (CHC), respectively. Each soil sample was air-dried and sieved through a 2-mm-mesh polyethylene sieve. 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, soil, and soil–biosolids mixtures were characterized for pH using a 1:2.5 soil-to-water ratio. Organic matter was determined by the spectrophotometric method and cation exchange capacity (CEC) by the acetate procedure at pH 7

(Sadzawka et al. 2006). Total metal concentration was determined after acid digestion (4 mL HNO_3, 4 mL HF, and 2 mL 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. Metals were determined by flame atomic absorption spectroscopy (F-AAS). A certified reference material (Montana Soil, NIST 2710) was included in the analysis to verify accuracy of the extractions. The values determined were not significantly different from the certified one ($p<0.05$).

2.2 Greenhouse Experiment

Biosolids were mixed thoroughly into the soils at rates equivalent of 0, 30, 45, and 60 Mg ha^{-1}. Pots containing the equivalent to 500 g of each treatment with three replicates were irrigated to field capacity and allowed to stand for 15 days before sowing with ryegrass. Five grams of seeds per pot were sowed. The experiment was conducted under controlled conditions with 14/10 h light/dark periods at $20\pm5^{\circ}$ C. Irrigation quantities were determined by weight at 2/3 of the water holding capacity (33 kPa) of each soil. Seedlings were watered daily with distilled water to maintain soils at field capacity conditions for 30 days from seed sowing.

At day 30, the ryegrass was harvested. Plant samples (root and shoot tissue combined) were washed with distilled water and dried at 30°C. Plant samples were ground and homogenized for each treatment. Ryegrass *L. perenne*, an excluder like most plants, will generally accumulate metals in roots, with only a small fraction translocated into shoots (Nowack et al. 2004). For this reason, root and shoot tissue were combined for analysis.

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

The greenhouse experiment was carried out using a completely randomized block design with four rates of biosolids application added to six soil types. There were three replications for each treatment.

2.3 Soil Sequential Extraction

Soil samples were subjected to a sequential extraction using the BCR scheme (Ure et al. 1993), with specific reagents used to obtain the following operationally defined fractions for Cu and Zn in soils: acetic acid-soluble (20 mL of 0.11 M HOAc for 16 h), soluble in reducing condition (20 mL of 0.50 M NH$_2$OH·HCl adjusted to pH 2 with HNO$_3$ for 16 h), soluble in oxidizing condition (10 mL of 30% H$_2$O$_2$ digestion and extraction with 25 mL of 1 M NH$_4$OAc pH 5 for 16 h), and residual fraction (10 mL aqua regia). Depending on concentration, metal determination in the extracts was performed either through flame AAE or ICP-MS. About 0.5 g of each soil, biosolids, and soil–biosolids mixture was weighed into a 50-mL centrifuge tube. Each treatment was analyzed in triplicate. The reagent corresponding to the step was added, with shaking and centrifugation. The supernatant was separated, filtered, and finally stored in polyethylene bottles at 4°C until AAS or ICP-MS analysis. The residues were washed with 10 mL distilled water, and the rinsate was discarded. The centrifuge tubes were weighed for volume correction after each step. For the oxidizable fraction (step 3), the residue from step 2 was oxidized with 10 mL hydrogen peroxide (30%) in cold for 1 h and digestion was allowed to proceed to near dryness at 85°C by heating the uncovered tube in a water bath. Forty milliliters of 0.1 M ammonium acetate (adjusted to pH 2 with nitric acid) were added to the cooled residue, which was then shaken for 16 h at room temperature. In order to determine the residual fraction, the recommended extraction protocol with aqua regia (ISO11466) was adapted for microwave oven use. Thus, the residue oxidizable fraction was digested with a concentrated mixture of HNO$_3$ and HCl (1:3 v/v), using a Milestone/mls 1,200 Mega microwave oven. The sequential extraction procedure method was validated by use of certified reference material CRM 483, the experimental results of which were statistically compared with the results reported by BCR

2.4 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 disk on the support, followed by a diffusive gel disk (DGT Research Ltd., Lancaster, UK), and last by a membrane filter. The upper cover, with a window exposed to the sample, was then fixed. The DGT were placed in the saturated paste for 24 h at 25°C in an incubator (Zhang and Davison 1995; Zhang et al. 2001). Then, the DGT 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 it was placed in test tubes with 5 mL 1 M HNO$_3$ 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 the pore water.

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

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

Where:

C_{elu} concentration of metals in 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:

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

Where:

Δg diffusion layer thickness (diffusive gel+ membrane filter)
D diffusion coefficient of the metal in the resin gel
t deployment time
A area of exposure window

Conversion of C_{DGT} to C_E, the effective available concentration of metal from the soil solution, was done using the following relation:

$$C_E = C_{DGT} / R_{diff}$$

R_{diff} is the ratio of concentration at the DGT interface to the concentration in the bulk soil solution, with only the diffusive case being considered. This parameter was calculated by using the computer numerical model of the DGT-soil, DIFS (DGT-induced fluxes in sediments) system. In order to calculate R_{diff}, it is necessary to know particle concentration (Pc) and porosity (φ).

2.5 Statistical Analysis

The results were analyzed using an ANOVA procedure. When significant F values were observed, differences between means were tested using Tukey HSD test for data from the sequential extraction. Linear correlation tests were used to assess the relationship between plant metal concentrations and effective concentration (C_E) from DGT analysis and with first fraction of BCR fractionation procedure. Both tests were carried out using Statgraphic 5.0 software.

3 Results and Discussion

General properties of the biosolids and unamended (control) soils are shown in Table 1. The biosolids had a high organic matter (35%) and near-neutral pH (6.8). The soils had pH values ranging between 7.1 and 7.8. Vertisol soil (LIN) had the highest pH value. The organic matter content in the soils ranged from 1.4% to 3.3%. The highest CEC was seen in the Inceptisol (CHC) with the lowest seen in the MRR soil, 43 and 18 cmol kg^{-1} cmol, respectively. The biosolids had higher concentrations of heavy metals than the control soils with total Zn, Cu, Cr, Ni, and

Pb concentrations of 1,681, 650, 381, 88, and 61.3 mg kg^{-1}, respectively. These concentrations were lower than the recommended limit values Chilean regulations (DS-4 2009). Zinc concentrations in the soils ranged from 90 mg kg^{-1} in the MRR soil to 136 mg kg^{-1} in the CHD soil. In the LIN soil, Cu was higher than other soils (142 mg kg^{-1}), in the other soils, ranging with values from 60.9 to 81.3 mg kg^{-1}.
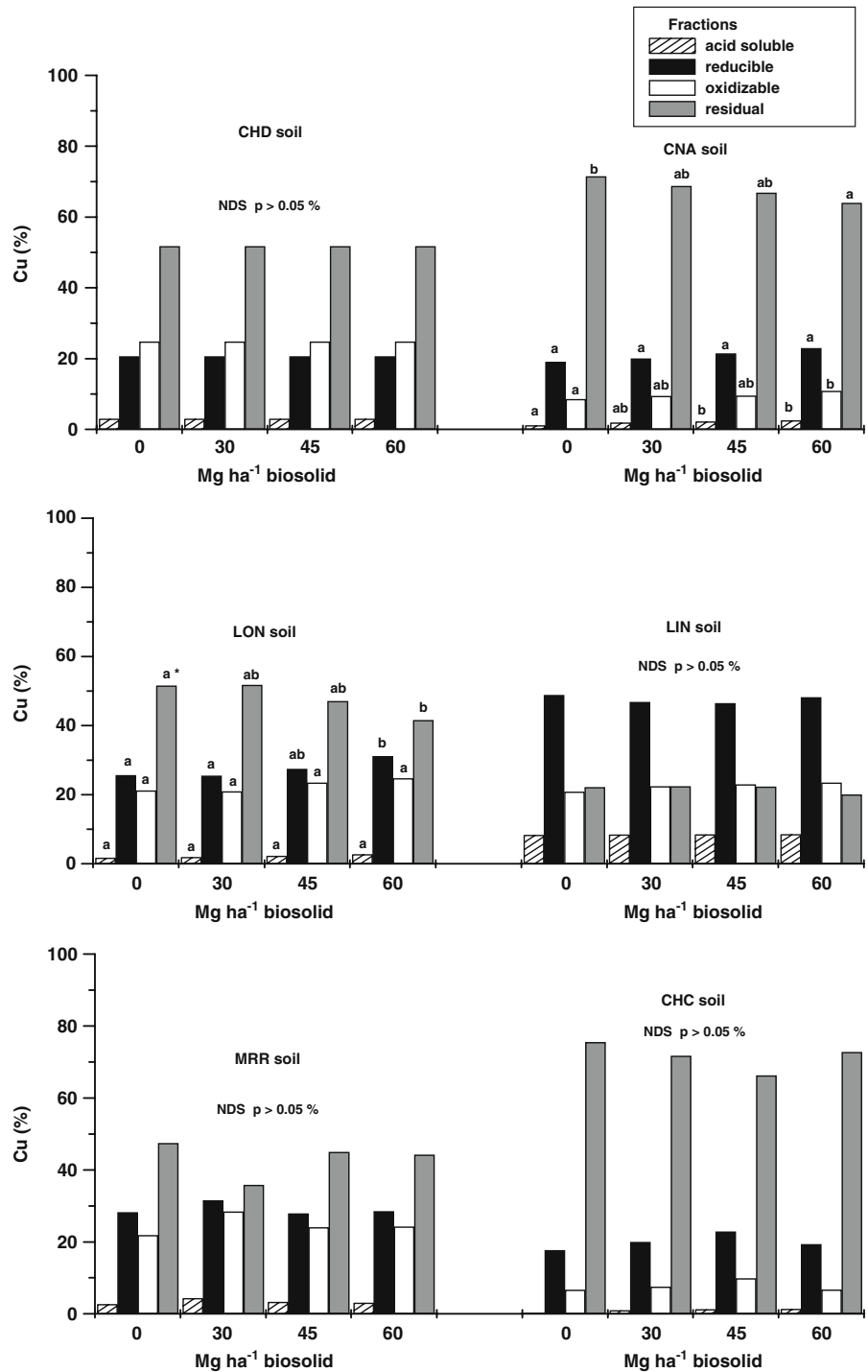
3.1 Sequential Extraction of Cu and Zn

Copper Figure 1 shows the distribution of the different Cu chemical fractions in the soils under study. Except for LIN soil which had the highest total Cu, the majority of Cu was associated with the residual fraction across all the soils. Distribution of the different Cu fractions varied by soil type, with the smallest portion of Cu found in the acid-soluble fraction. This fraction is considered to represent the most labile Cu form, available for uptake by plants. Across all soils, excluding the LIN, about 20% Cu was associated with Fe and Mn oxides. In LIN soil, this fraction was predominant; accounting for 50% of total Cu. Fifteen percent to 25% of the Cu was associated with organic matter (oxidizable fraction) for most of the soils. CNA and CHC soils showed a lower amount of Cu associated to organic matter, corresponding to 8% and 6% of total Cu. Changes were observed in the distribution of Cu following biosolids application for the CNA and LON soils. These soils showed a significant increase in the labile and organic matter associated Cu fractions, with a decrease in the residual fraction.

Table 1 Some properties of biosolids and soils under study

		Biosolid	CHD	CHC	CNA	LIN	LON	MRR
OM	(%)	35.0	2.7	2.6	1.4	1.7	3.3	3.1
pH	H$_2$O 1:2.5	6.8	7.2	7.6	7.1	7.8	7.5	7.5
CEC	cmol kg^{-1}	31.0	23.2	43.0	26.8	21.2	20.1	17.5
Total metal								
Cr	mg kg^{-1}	381	29.8	36.7	22.6	30.7	27.0	28.2
Cu	mg kg^{-1}	650	81.3	81.0	76.4	142	60.9	70.8
Ni	mg kg^{-1}	88	41.3	29.4	28.1	49.2	28.2	30.1
Pb	mg kg^{-1}	61.3	37.8	39.6	25.9	46.0	34.3	53.7
Zn	mg kg^{-1}	1,681	136	67.3	117	109	130	90.0

Zinc Figure 2 shows the percent distribution of Zn fractions in the soils. The residual fraction predominated across all of the soils, with 60% to 88% of total Zn associated with this fraction. As with Cu, the smallest fraction corresponded to exchangeable Zn as well as with Zn associated to carbonates, which would correspond to the Zn labile form. In most of the soils, a significant increase was observed in the
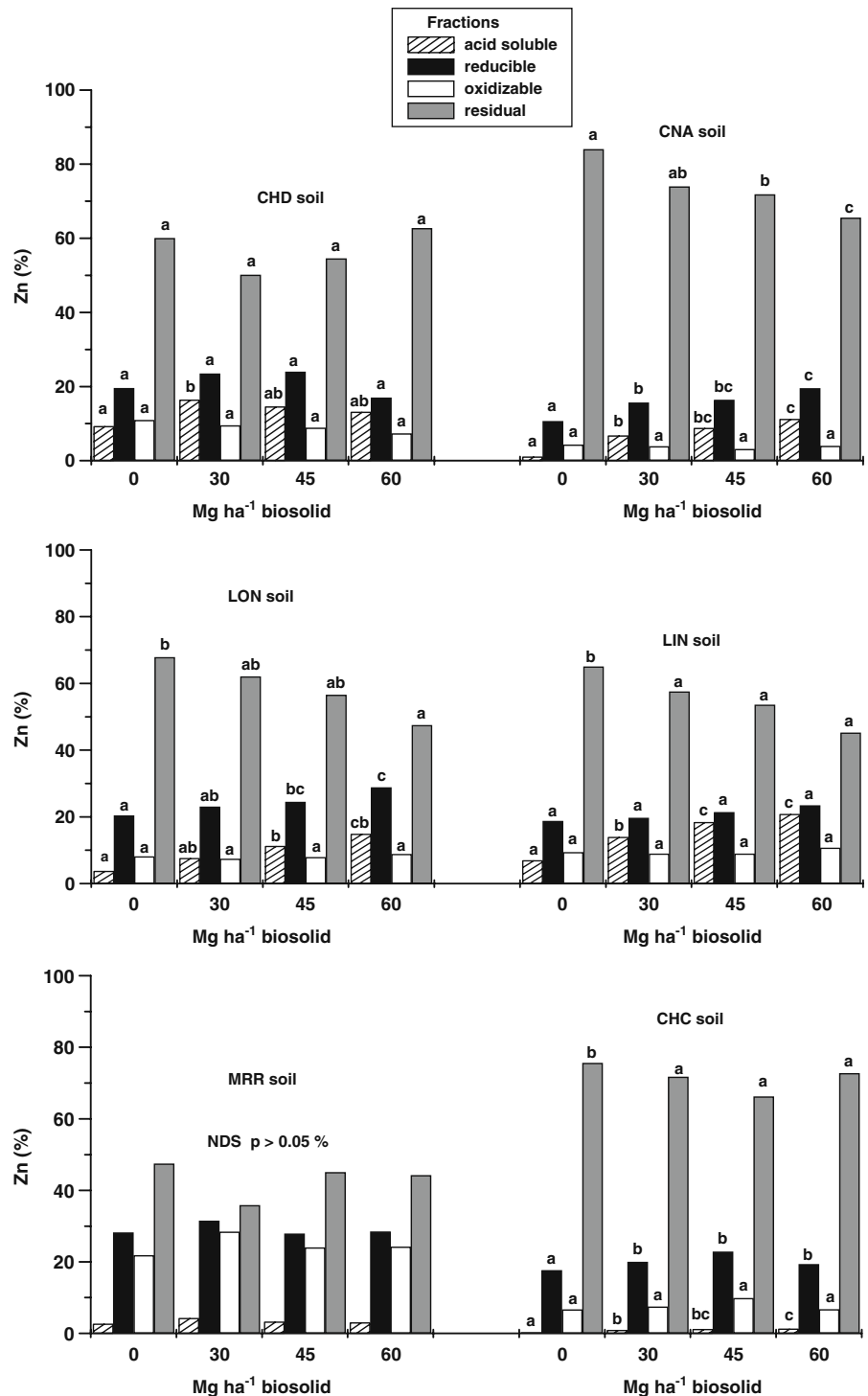
Fig. 2 Fractionation of Zn in CHD, CNA, LON, LIN, MRR, and CHC soils treated with biosolid at rates 0, 30,45, and 60 Mg ha^{-1}. *For each metal fraction, values followed by the same letter show no significant differences for p<0.05, according to Tukey HSD test

labile Zn fraction with increasing biosolids application rates. In the CHD soil, however, this increase was only observed at the 30 Mg ha^{-1} biosolids application rate. An increase in biosolids application rate caused a

significant increase in the reducible fraction in the LON and CNA soils. Increasing biosolids application resulted in a significant decrease in the residual fraction for most of the soils in consideration.

3.2 Cu and Zn Bioavailability

Table 2 shows total Cu and Zn concentration in ryegrass plants grown in the soils treated across all rates of biosolids addition. Zinc was found at higher concentrations than Cu, which would be related to a higher zinc concentration in biosolids and soils. For Cu, a gradual increase in plant Cu was observed with increased biosolids application rates for most soils. The magnitude of this increase varied across the different soils. At the highest biosolids application rate, Cu in plant tissue increased by 30% in comparison to the control in the CHD and LON soils. This increase was 50% in the CNA soil and 100% in the CHC soil. In the LIN and MRR soils, plant Cu remained similar across all rates of biosolids addition.

Table 2 Total Cu and Zn content in ryegrass (*Lolium perenne* L.) plant grown in soils CHD, CNA, LON, LIN, MRR, and CHC soils treated with biosolids at rates 0, 30, 45, and 60 Mg ha^{-1}

Soil	Biosolid, mgha^{-1}	Cu, mgkg^{-1}	RSD	Zn	RSD
CHD	0	27.0	6.7	54.3	7.7
	30	28.0	5.7	60.0	2.0
	45	28.7	5.9	68.7	4.2
	60	35.2	9.9	75.6	12
CNA	0	24.5	7.5	57.4	9.0
	30	29.7	16	68.3	11
	45	30.9	11	77.0	1.4
	60	35.6	6.2	82.8	5.1
LON	0	26.2	9.1	57.3	4.7
	30	34.6	13	71.8	12
	45	30.8	11	86.9	2.4
	60	35.0	12	81.8	12
LIN	0	74.3	0.3	49.4	13
	30	76.8	11	74.9	1.5
	45	77.0	4.0	82.4	18
	60	72.3	7.8	82.7	12
MRR	0	32.1	6.7	46.8	7.2
	30	32.8	5.6	50.9	1.2
	45	33.5	12	58.6	6.6
	60	29.8	2.3	53.6	9.7
CHC	0	19.2	7.0	29.6	7.2
	30	35.7	2.8	46.9	1.2
	45	26.5	6.3	45.9	6.6
	60	37.8	4.3	48.7	9.7

It should be noted that LIN control soil showed the highest acid extractable Cu content and highest total Cu concentration of all soils. Plant Cu concentration for plants grown in this soil was also significantly higher than plants grown in all other soils. In addition, biosolids incorporation produced no significant changes in metal availability for this soil (Fig. 1). Observations in this soil and similarly in MRR soil, where no change in bioavailability was observed, were coincident with the findings in the chemical fractionation. For both soils, biosolid incorporation had no significant effect on distribution of Cu in the BCR extraction. The changes in plant Zn concentration in relation to increased biosolids application rate were similar to those for Cu. However, increases in plant Zn concentration with increased biosolids application rates were consistent across all soils. The increase in plant Zn in comparison to the control was about 40% for CHD, CNA, and LON soils, from 60% to 70% for LIN and CHC soils, and only 10% for MRR. These results were compared with those obtained for the available fraction in the sequential extraction. The significant increase of in the available fraction of the extract agrees with the findings in the plant assay. It should be noted that the only soil where no important variation was observed in the extraction also showed the smallest increase (10%) in plant Zn at the highest biosolids rate.

3.3 Determination of C_E by DGT

Table 3 shows C_E values obtained by the use of DGT. In general, an increase in C_E was observed with increasing biosolids application rates for both Cu and Zn. However, in the case of Cu, this increase was only observed in the LIN, LON, and CHC soils. In the CNA and CHD soils, the C_E decreased at biosolids application rates greater than 30 and 45 Mg ha^{-1}, respectively. In the MRR soil, C_E decreased with all biosolids application rates. This decrease could be due to the existence of Cu organically complexed and colloidal species in soil solution. Some studies have shown that DGT sampling excludes these metal forms (Zhang and Davison 1995, 1998; 2001, Ruello et al. 2008). In the case of Zn, the effective concentration increased in all of the soils with increasing biosolids application rates, with the exception of the CNA soil at the highest application biosolids rate. The DGT technique, like

Table 3 Estimation of Cu and Zn bioavailable fraction through determination of effective concentration (C_E) obtained using diffusive gradient in thin films technique (DGT)

Soil	Cu					Zn			
	Biosolid, mg ha^{-1}	C_E, µg L^{-1}	SD	C_{DGT} µg L^{-1}	R values	C_E, µg L^{-1}	SD	C_{DGT}, µg L^{-1}	R values
CHD	0	190	2.3	8.12	0.214	632	1.3	27.6	1.22
	30	404	8.0	17.2	0.187	984	2.0	43.0	1.48
	45	369	5.5	15.7	0.244	775	2.8	33.7	0.91
	60	426	7.3	18.2	0.318	964	5.2	42.1	1.04
CNA	0	132	3.2	5.73	0.165	160	0.9	5.37	0.278
	30	203	2.2	8.84	0.131	148	1.5	4.95	0.128
	45	290	1.1	12.6	0.232	193	6.6	6.48	0.132
	60	213	9.3	9.26	0.181	271	3.0	9.08	0.226
LON	0	112	2.5	4.97	0.145	115	1.4	3.88	0.179
	30	104	3.4	4.55	0.103	125	1.4	4.21	0.142
	45	112	1.8	4.97	0.145	171	0.7	5.76	0.205
	60	225	0.9	9.85	0.380	246	1.6	8.31	0.271
LIN	0	400	2.7	16.6	0.214	763	0.8	32.4	1.66
	30	853	8.6	35.2	0.120	943	2.8	39.9	1.46
	45	1,197	6.5	49.5	0.109	870	1.2	36.8	1.25
	60	1,405	21	57.9	0.120	918	6.0	38.7	1.00
MRR	0	441	11	17.1	0.291	176	49	6.90	0.102
	30	376	59	14.6	0.136	347	7.1	13.6	0.272
	45	227	15	8.82	0.0744	345	19	13.6	0.154
	60	195	3.7	7.56	0.0716	358	10	14.0	0.152
CHC	0	217	20	8.54	0.276	1,228	21	48,9	1.66
	30	283	72	9.64	0.302	10,456	35	416	6.97
	45	290	31	11.4	0.353	11,795	15	470	7.55
	60	321	44	12.6	0.218	14,925	20	596	9.89

C_E, C_{DGT}/R_{diff}

C_{DGT}, concentration accumulated by DGT; R ratio Cu_{DGT}/Cu_{sol}

SD standard deviation

plants, causes 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 (Table 4) (Zhang et al. 2001). To test the efficacy of this tool for the soil/biosolids/plant conditions of the present study, we related the C_E values with the Zn and Cu concentrations in ryegrass using a simple linear correlation (Table 5). For Cu, positive linear correlations were found in CHD, CNA, LON, and CHC soils, with correlation coefficients of 0.50 to 0.87. A positive correlation was found in all the soils for Zn, with R values ranging from 0.55 to 0.97. Nowack et al. (2004) compared the DGT-measured effective concentration with the uptake of Cu and Zn into the shoots of *L. perenne* (Ryegrass) under three different conditions, i.e., field, soil cores, and homogenized soil and observed a significant non-linear relationship between plant Cu and Zn and C_E. In this study, the acid soluble fraction of Zn and Cu generally showed a closer relationship with plant metal concentrations (Table 5). For Cu, R values higher than 0.5 were obtained for most of the soils (except LIN and MRR). In contrast, the R values obtained for Zn, approximately 0.9 for most soils, were better than those obtained with C_E.

Table 4 DIFS model input parameters of soils for R simulation

Soil		Cu				Zn			
	Biosolid, mg ha^{-1}	P_c, gcm^{-3}	D_0, cm^{2}seg^{-1}	D_s, cm^{2}seg^{-1}	φ	P_c, gcm^{-3}	D_0, cm^{2}seg^{-1}	D_s, cm^{2}seg^{-1}	φ
CHD	0	4.88	5.42E–06	1.75E–06	0.352	4.88	5.29E–06	1.71E–06	0.352
	30	4.97	5.42E–06	1.74E–06	0.348	4.97	5.29E–06	1.70E–06	0.348
	45	5.00	5.42E–06	1.74E–06	0.346	5.00	5.29E–06	1.70E–06	0.346
	60	4.92	5.42E–06	1.75E–06	0.350	4.92	5.29E–06	1.70E–06	0.350
CNA	0	6.24	5.42E–06	1.82E–06	0.298	6.22	6.08E–06	1.78E–06	0.299
	30	6.21	5.42E–06	1.82E–06	0.299	6.18	6.08E–06	1.78E–06	0.300
	45	6.18	5.42E–06	1.83E–06	0.300	6.18	6.08E–06	1.78E–06	0.300
	60	6.18	5.42E–06	1.83E–06	0.300	6.20	6.08E–06	1.78E–06	0.300
LON	0	5.92	5.42E–06	1.86E–06	0.309	5.92	6.08E–06	1.82E–06	0.310
	30	5.97	5.42E–06	1.86E–06	0.307	5.96	6.08E–06	1.81E–06	0.308
	45	5.92	5.42E–06	1.86E–06	0.309	5.94	6.08E–06	1.81E–06	0.308
	60	5.95	5.42E–06	1.86E–06	0.308	5.95	6.08E–06	1.81E–06	0.308
LIN	0	5.65	5.42E–06	1.65E–06	0.319	5.66	5.29E–06	1.61E–06	0.319
	30	5.75	5.42E–06	1.64E–06	0.315	5.75	5.29E–06	1.60E–06	0.316
	45	5.78	5.42E–06	1.64E–06	0.314	5.76	5.29E–06	1.60E–06	0.315
	60	5.82	5.42E–06	1.63E–06	0.313	5.82	5.29E–06	1.59E–06	0.313
MRR	0	5.91	6.23E–06	1.86E–06	0.310	5.93	6.08E–06	1.82E–06	0.309
	30	5.92	6.23E–06	1.86E–06	0.309	5.92	6.08E–06	1.82E–06	0.309
	45	5.92	6.23E–06	1.86E–06	0.309	5.90	6.08E–06	1.82E–06	0.310
	60	5.94	6.23E–06	1.86E–06	0.309	5.94	6.08E–06	1.81E–06	0.308
CHC	0	5.48	6.23E–06	1.92E–06	0.326	5.50	6.08E–06	1.87E–06	0.325
	30	5.23	6.23E–06	1.96E–06	0.336	5.50	6.08E–06	1.87E–06	0.325
	45	5.50	6.23E–06	1.92E–06	0.325	5.50	6.08E–06	1.87E–06	0.325
	60	5.48	6.23E–06	1.92E–06	0.326	5.47	6.08E–06	1.88E–06	0.326

P_c soil particle concentration, D_0 diffusion coefficient of Cu or Zn, D_s diffusion coefficient in soil, φ porosity

Δg (diffusion layer thickness), 0.094 cm

In general, we observed increasing metal extractability for both Cu and Zn with increasing biosolids application rates across all soils included in the study. However, the magnitude of this increase varied for both metals and by soil type and was not always reflected by changes in plant metal concentration. The increased C_E values for Cu in CHD, CNA, LON, MRR, and CHC soils with increased biosolids rates which generally mirrored plant Cu concentrations. However, the values of the first BCR fraction for Cu were disproportionate in CHC soil (Table 6). Kovaříková et al. 2007 also found DGT concentration of metals were lower by approximately one to two orders of magnitude in comparison to sodium nitrate extractable metals. The sodium nitrate extraction is commonly used for assessment of the bioavailable part of metals. On the other hand, for Zn, with the exception of the CHC soil, the C_E values were significantly related to increased plant Zn concentrations. The BCR extractable portion of this element generally overestimated the bioavailable fraction in comparison to plant metal uptake.

4 Conclusions

The results obtained in this study suggest that biosolids application to soils altered the distribution

Table 5 Assessment of two methods to estimate the bio-available fraction through correlation (R) between C_E or acid soluble fraction and plant total content

	Cu			Zn		
Correlation between CE and plant total content						
	R^a	SE^b		R^a	SE^b	
CHD	0.62	2.9	NSS	0.55	7.9	NSS
CNA	0.58	3.7	NSS	0.81	6.5	d
LON	0.50	3.6	NSS	0.70	9.3	c
LIN	NC			0.84	8.6	e
CHC	0.87	4.2	e	0.97	2.0	e
MRR	0.36	1.5	NSS	0.75	3.2	d
Correlation between acid soluble fraction and plant total content						
	R^a	SE^b		R^a	SE^b	
CHD	0.78	2.3	d	0.90	4.1	e
CNA	0.94	1.6	e	0.99	1.2	e
LON	0.70	3.0	c	0.91	5.5	e
LIN	0.10	2.9	NSS	0.97	4.0	e
CHC	0.58	7.0	c	0.90	4.1	e
MRR	NC			0.68	0.37	NSS

NC no correlation found, *NSS* not statistically significant relationship

[a] Correlation coefficient

[b] Standard error

[c] Statistically significant relationship at 90%

[d] Statistically significant relationship at 95%

[e] Statistically significant relationship at 99%

p value in the ANOVA table is greater or equal to 0.10

Table 6 Availability factor (AF) corresponding to the relationship between plant concentration, effective concentration, or first fraction of sequential extraction determined in soils treated with different biosolid rates and the obtained in control soils

Soil	Biosolid mgha^{-1}	Plant AF Cu	CE AF Cu	First fraction AF Cu	Plant AF Zn	CE AF Zn	First fraction AF Zn
CHD	0	1.0	1.0	1.0	1.0	1.0	1.0
	30	1.0	2.1	1.2	1.1	1.6	1.9
	45	1.1	1.9	1.6	1.3	1.2	2.0
	60	1.3	2.2	1.7	1.4	1.5	2.2
CNA	0	1.0	1.0	1.0	1.0	1.0	1.0
	30	1.2	1.5	1.8	1.2	0.9	7.7
	45	1.3	2.2	2.3	1.3	1.2	13
	60	1.5	1.6	2.4	1.4	1.7	14
LON	0	1.0	1.0	1.0	1.0	1.0	1.0
	30	1.3	0.9	1.3	1.3	1.1	2.5
	45	1.2	1.0	1.6	1.5	1.5	4.1
	60	1.3	2.0	1.8	1.4	2.2	4.9
LIN	0	1.0	1.0	1.0	1.0	1.0	1.0
	30	1.0	2.1	1.1	1.5	1.2	2.4
	45	1.0	3.0	1.1	1.7	1.1	3.4
	60	1.0	3.5	1.1	1.7	1.2	3.9
MRR	0	1.0	1.0	1.0	1.0	1.0	1.0
	30	1.0	0.9	1.7	1.1	2.0	1.7
	45	1.0	0.5	1.4	1.3	2.0	1.5
	60	0.9	0.4	1.3	1.1	2.0	1.5
CHC	0	1.0	1.0	1.0	1.0	1.0	1.0
	30	1.9	1.3	4.0	1.6	8.5	28
	45	1.4	1.3	5.3	1.6	9.6	41
	60	2.0	1.5	6.6	1.6	12	46

of Cu and Zn. Soil type also had a significant effect on metal distribution. The residual fraction of both metals was predominant in most of the soils. For Cu, biosolids application only significantly increased the labile Cu with an associated decrease in the residual fraction for some soils. For Zn, a significant increase in the acid-soluble fraction was observed with increasing biosolids application rate for all soils included in this study. In general, Cu and Zn concentrations in ryegrass plants increased with increasing biosolid loading rates. The DGT technique effectively predicted changes in phytoavailability of Zn across all soils. However, this technique was much less effective for predicting plant Cu. Similar results were found with the first metal fraction in the sequential extraction. It should be noted that both methods provided similar results for the bioavailable fraction of Cu and Zn in most of the cases. In general, the C_E overestimated the bioavailable fraction of both Cu and Zn for some of the soils studied. Likewise, the first fraction of the sequential extraction method also overestimated the bioavailable fraction, especially for Zn in LON and CHC soils, in relation to the findings in ryegrass plants.

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