

## Extractability of Copper and Application of Diffusive Gradients in Thin Films: Metal Availability in Contaminated Soil by Biosolids

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O objetivo deste estudo foi utilizar a técnica de difusão em filmes finos por gradiente de concentração (DGT) para a avaliação da disponibilidade de cobre em solos com elevados níveis do metal, que foi alterado com biossólidos, além de comparar esta técnica com procedimentos de extração simples e sequenciais, considerando a extractabilidade do metal. A ordem de importância das frações de cobre no solo foi: residual > reduzida > oxidável > solúvel. A aplicação do biossólido diminuiu as frações solúvel e reduzida, bem como aumentou a quantidade de metal associada à fração oxidada. A quantidade de cobre extraída por DGT da solução de solo foi 13% do metal total em solução. No caso do solo, esse valor foi inferior a 0,3% do total do metal. Também relacionou-se diretamente o metal extraído por DGT com o metal disponível com CaCl<sub>2</sub> ou o metal ligado à matéria orgânica do solo.

The aim of this study was to use the technique of diffusion gradients in thin films (DGT) to evaluate the availability of copper in soil with high presence of this metal treated with biosolids, and to compare this technique, in terms of metal extractability, with simple and sequential extraction procedures. The order of importance of the copper fractions in the soil was: residual > oxidizable > reducible > soluble. Biosolid application decreased of both soluble and reducible fractions and increased the amount of metal attached to the oxidizable fraction. Copper amount extracted by DGT from the soil solution was 13% of total metal in solution. In the case of soil, this amount was less than 0.3% of total metal. Furthermore, it was possible to directly relate the metal extracted by DGT with the available metal with CaCl<sub>2</sub> or the metal bound to organic matter.

**Keywords:** copper availability, DGT, sequential extraction, biosolid, metallic species

### Introduction

The phytotoxicity of metallic species is not necessarily related to the total metal content since the metals can be found in various forms, such as interchangeable, associated with organic matter, or forming complexes, which together with soil properties like pH, ionic strength and cation exchange capacity determine the magnitude of processes such as adsorption, mobility across the soil profile and possible risk to humans and the environment presented by metallic species.<sup>1,2</sup>

The development of methods for the determination of the bioavailable fraction of different elements in different environmental scenarios has become very important. In that sense, biomimetic methods have been developed, such as the diffusion gradients in thin films (DGT) technique,

whose advantage lies in the simultaneous quantification of different metals, describing the kinetics of the processes involved and making *in situ* measurements.<sup>3</sup>

DGT devices have had various applications, among them the determination of metals in estuarine waters,<sup>4</sup> in seawater<sup>5</sup> or their use in the determination of metallic species in soil,<sup>6,7</sup> measuring approximately the bioavailability of metals by the continuous removal of the species either from the soil or from soil solutions. Another application is the determination of metallic species in the rhizosphere and the correlation with their content in plants,<sup>8</sup> as well as the determination of other elements like phosphorus in water used in aquaculture.<sup>9</sup> The influence of metal-humic substance interactions in the soil or in soil solution on the available amount of metal estimated by the method is an aspect that is little studied of the DGT method. The comparison of complexes with ligands of different sizes, including humic substances, shows that

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the diffusion of the metal as a complex decreases as the size of the ligand increases.<sup>10</sup> Therefore, the application of DGT to soils with high metal content that are also being subjected to various agronomic practices requires careful result interpretation.

The application of biosolids to soils as a way of disposing them is a practice that has been established for several decades at the international level,<sup>11</sup> with regulations that consider the physicochemical characteristics of the soils to which these materials are applied. Under these conditions, the bioavailability of the elements in the soil can be altered because biosolids contribute to the organic matter that can bind metals and form coordination complexes, implying in decrease of the element bioavailable to the plants or in mobilization of the element to deeper layers of the soil.<sup>12,13</sup> In this context, one of the strategies used for the recovery of soils contaminated with metallic species is the *in situ* application of high amounts of biosolids to increase the retention of the metals in the soil and favor the development of a compost layer.<sup>12,14</sup> The repetitive application of biosolids to soils may involve a gradual increase in metallic species content, as well as significant changes in the physicochemical properties, a fact that over time can pose risks to crops, environment and humans, depending of the interactions established between a metal and soil components.

Soils with high copper content have been described in Chile, in some cases with over 3000 mg kg<sup>-1</sup>,<sup>14,15</sup> and many of these soils are used for agricultural production activities. Together with this, most of the sewage treatment plants use activated sludge with an anaerobic digestion process that affects the level of degradation of the organic matter, so in the sludge from the anaerobic digestion ponds there was a higher content of total organic matter and soluble organic matter.

For that reason, it is necessary to develop tools that can allow a more accurate *in situ* estimation of the metal fraction available to the crops. In that sense, DGT devices are highly promising tools that must be evaluated for the copper availability in Chilean soils with high concentration of this metal, which in turn have been treated with biosolids.

Considering on the one hand that in some studies the bioavailable copper in soil solution or in natural water is estimated by DGT,<sup>16</sup> and on the other hand that its direct use in the soil has been described,<sup>6,7</sup> the objective of this work is to estimate the available copper by means of DGT devices in soil solution and in soil treated with biosolids and incubated for different times. The copper estimated by DGT would be related with the available metal obtained by simple extraction with different extractants and with metal forms through a sequential extraction procedure.

## Experimental

### Calculations involved in the use of DGT

The diffusion gradients in thin films technique uses an ion exchange resin (Chelex 100) embedded in a hydrogel that is covered by another hydrogel permeable to the ions, with a thickness called  $\Delta g$ . These gels are assembled in such a way that the diffusive gel is exposed only to the solution or the soil. The element diffuses through the hydrogel, and then is immobilized onto the resin gel, provided that the resin is not saturated. If the concentration remains constant over time, the flow of the metal ( $F$ ) can be calculated by Fick's first law of diffusion.

Another definition of flow is  $M/A \times t$ , where  $M$  is the mass of metal that diffuses through an area ( $A$ ) of the permeable gel in contact time ( $t$ ). The value of  $M$  is obtained from the concentration of analyte in the eluate, after the analytical procedure, through equation 1:

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

where  $C_e$ : concentration of analyte in the eluate from the resin;  $V_a$ : volume of acid added for the elution;  $V_{gel}$ : volume of resin gel; and  $f_e$ : elution factor.

Thus, the *in situ* concentration of the element can be calculated from equation 2.

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

This equation allows the determination of the actual concentration of the element in the soil solution, provided that the concentration gradient in the diffusion gel remains constant.<sup>17</sup>

### Preparation and validation of DGT

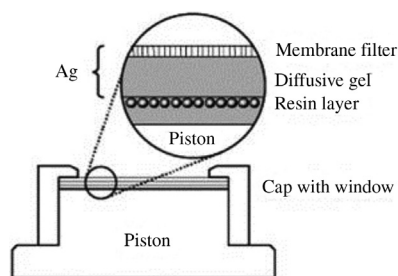
The gels were prepared according to the procedure described by Zhang and Davison.<sup>18</sup> Briefly, the hydrogel solution was prepared from 15 mL of cross-linker derived from agarose,<sup>19</sup> 47.5 mL of Milli-Q water and 37.5 mL of acrylamide (40%). To prepare the diffusive gel, 10  $\mu$ L of *N,N,N',N'*-tetramethylethylenediamine (TEMED, 99%) and 30  $\mu$ L of 10% ammonium persulfate, which act as initiators and retardants, respectively, of polymer formation, were added to 5 mL of the hydrogel solution. The preparation of the resin gel considered the use of 2 g of Chelex-100, which was added to 5 mL of hydrogel solution, followed by the addition of 10  $\mu$ L of TEMED and 30  $\mu$ L of 10% ammonium persulfate. All materials were previously decontaminated with 0.01 mol L<sup>-1</sup> HNO<sub>3</sub>, rinsed with Milli-Q water and dried in an oven.<sup>18</sup>

The formation of both gels was made on glass plates with 0.3 mm thick spacers on which the diffusive gel or the resin gel suspension was poured, heating them in an oven at 43 °C for 1 h. The gels were then removed and transferred to beakers for their hydration with Milli-Q water for 24 h, replacing the water approximately every 6 h. Once both diffusive and resin gels were hydrated, reaching a thickness of 0.73 mm, they were cut into 2.5 cm diameter circles that were stored in 0.01 mol L<sup>-1</sup> NaNO<sub>3</sub> at 4 °C.<sup>19</sup>

To ensure uniform distribution of the Chelex-100 resin particles for a correct operation of the model, each gel was observed under a stereomicroscope, discarding those batches in which the distribution was not uniform or which presented sections with more than one layer of particles. Similarly, the integrity of the diffusing gel was verified, discarding that which showed air microbubbles.

Once the DGT devices had been prepared, they had to be validated by applying a solution with a single cation or a mixture of cations under the pH and ionic strength conditions at which the cations are in their labile form, evaluating the amount of metal present in the device by elution with an acid medium. In the present case, a Cu(NO<sub>3</sub>)<sub>2</sub> set of solutions with concentrations between 0.1 and 0.3 mg kg<sup>-1</sup> of Cu<sup>2+</sup>, in 0.01 mol L<sup>-1</sup> NaNO<sub>3</sub>, at pH 5 was used.

Both diffusive and resin gels were used to assemble the device as shown in Figure 1, placing a resin gel together with a diffusive gel, and over the latter a 0.2 μm cellulose nitrate membrane filter, in a modified plastic filter holder, so that the surface of the filter exposed to the soil or solution corresponds to a 2 cm diameter circle.



**Figure 1.** DGT device assembly (with copyright permission from reference 5).

Once the stirring time was over, the devices were disassembled, separating the resin gel, which was washed with Milli-Q water, to be transferred to a previously decontaminated glass tube, to carry out the elution with 1 mol L<sup>-1</sup> HNO<sub>3</sub> for 12 h. Copper was quantified in the eluate ( $C_e$ ), in the initial ( $C_{\text{Initial}}$ ) and final ( $C_{\text{Final}}$ ) solutions by flame atomic absorption spectroscopy (FAAS). The

determination of  $C_{\text{DGT}}$  was made using equation 2, considering the used temperature and the elution volume. To obtain a representative result, it was suggested that the devices should not differ by more than 10% between  $C_{\text{DGT}}$  and  $C_{\text{initial}}$ .<sup>19</sup>

#### Preparation of soil and soil treated with biosolid

Agricultural soils near to Rancagua city in the Sixth Region of Chile was used for the study. The soil was collected from a zone with high copper levels<sup>15</sup> due to permanent irrigation with water contaminated by mining activities in the area. Field moisture was determined by the procedure described by Blakemore *et al.*,<sup>20</sup> weighing 30 g of moist soil, adding water until a homogeneous moist paste that crumbled readily was formed, and the amount of added water was determined by weight difference.<sup>21</sup> The biosolid was obtained from a domestic sewage treatment plant in the city of Santiago (Chile). The soil and the biosolid were physically and chemically characterized by standardized methods.<sup>20,22</sup>

Extraction of the soil solution was done using two soil:water ratios, 1:2 and 1:10, called  $S_2$  and  $S_{10}$ , respectively, in order to cover a wider range of Cu concentrations in solution for DGT test. In both solutions, total copper was quantified by FAAS, and then these solutions were used for deploying DGT.

The soil was treated with 200 t ha<sup>-1</sup> of biosolid and together with the untreated soil, it was moisturized until it reached the field moisture. One part of both substrates was allowed to dry in air, and this was substrate time 0 (not incubated). The remaining substrates were incubated for 130 days in darkness in half open containers at 25 °C. At the end of that period, the samples were dried in air for the general and DGT analyses.

#### Determination of free Cu by ISE in soil solution

The concentration of free Cu in soil solution was potentiometrically determined with a Cu (II) ion selective electrode (ISE), using a method of standard additions. This methodology has been used in several studies with complex matrices,<sup>23</sup> such as soil solution, to minimize the effect of ionic strength of the sample on the electrode response. The linear response range of the electrode was established between 0.01 and 9 mg kg<sup>-1</sup>. The initial reading was made on 50 mL of each extract ( $S_2$  and  $S_{10}$ ), to which were added successive 500 μL aliquots of a 4 mg kg<sup>-1</sup> Cu solution. After each addition and an equilibration time, the voltage associated with the free Cu present in the solution was recorded. A linear correlation was established between log

added Cu and the reading in mV. The concentration of free Cu in the soil solution was then determined by extrapolation.

#### Procedure for simple and sequential extraction, and determination of total Cu in soil

Available copper in the soil, treated and untreated, was estimated by means of four simple extraction procedures, namely 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub>,<sup>24</sup> citric acid, tartaric acid<sup>25</sup> and DTPA (diethylenetriaminepentaacetic acid),<sup>26</sup> while the metal forms were estimated by the European Community Bureau of Reference (BCR) sequential extraction procedure.<sup>27</sup> All the procedures were applied as described by the authors, without any modification.

The sequential extraction procedure consisted of three stages. In the first stage, the water-soluble and exchangeable fraction (HOAc-soluble fraction) was obtained; in the second stage, the forms bound to manganese and iron oxides (reducible fraction) were obtained; and in the third step, organically bound metals and sulfides were extracted (oxidizable fraction). Finally, the difference between the sum of the fractions mentioned above and the total metal was considered as metal occluded in the crystal structures of the primary and secondary minerals (residual fraction). Total metal in the soil was determined by FAAS, after digestion of the samples in a 2:1:1 mixture of HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub>:HF in a microwave oven.<sup>12,27</sup>

#### Use of DGT device in soil treated and untreated with biosolids and in the soil solution

For the application of the devices to the soil solutions, the DGT devices were placed in contact with 40 mL of each solution (S<sub>2</sub> and S<sub>10</sub>) in duplicate, for 4 h, verifying that there were no bubbles interfering with the contact between the gel and the solution, slowly stirring the system to avoid the vortex formation.<sup>19</sup>

After completing the contact time, the resin gel was removed to carry out the elution with 4 mL of 1 mol L<sup>-1</sup> HNO<sub>3</sub> for a period of 24 h. Finally, the total Cu was determined in the resulting solution.

To apply the DGT method to the substrates, a suspension was made mixing 20 g of substrate and 15 mL of water. The suspension was stirred for 24 h and the DGT devices were installed, keeping them in contact with the suspension for 24 h at 26 °C. After completing the contact time, Cu was determined as explained in the preceding paragraph. The analysis was carried out in duplicate because of the small amount of available material and because the relative standard deviations of the preliminary tests were less than 5%.

To estimate the effective concentration (C<sub>E</sub>), the procedure of Zhang *et al.*<sup>6</sup> was followed. The calculation considered the diffusion coefficient (D<sub>0</sub>), diffusion coefficient in soil (D<sub>s</sub>), soil particle concentration (P<sub>c</sub>), diffusion layer thickness (Δg) and porosity (j), which yield parameter R<sub>diff</sub> by means of the DIFS mathematical computational model.<sup>28</sup>

The amount of Cu extracted with DGT was correlated with the amount extracted with the different extractants by means of a simple correlation analysis, getting the correlation coefficient and its significance. Data of simple and sequential extraction were subjected one-way analysis of variance, and the mean values of the different treatments were compared with Duncan's multiple range test at a level of 5%. Analysis was carried out with Statgraphics statistical software, version 4.0 for Windows.

## Results and Discussion

### Characterization of soil and biosolids

Table 1 shows the general characteristics of the soil and biosolids used in this study. The soil from the Rancagua series is characterized by an almost neutral pH and a sandy loam texture, with an organic matter content of 2.2% and a total Cu content of 1227 mg kg<sup>-1</sup>. These results are similar to those reported in other studies with soils from the same zone.<sup>14,15</sup> An important part of the Cu (27%) was extracted with DTPA, a fact that is consistent with an anthropic origin of this metal due to continuous irrigation with water contaminated by mining wastes. Several recent reports indicate that, regardless of the forms of anthropogenic copper, its availability to plants is significantly higher than that of natural origin.<sup>2</sup>

Copper extracted with DTPA has been considered by several authors<sup>26,29</sup> as the fraction (bio)available to crops in uncontaminated soils. But the use of DTPA in Chilean soils with high Cu content, like those in this study, would release large amounts of the metal that would reach levels that can be toxic to plants (Table 1). However, in the field there is no evidence of this happening to the crops in this area.<sup>12,30</sup> This inconsistency between the chemical tests and the biological response would support the search for more effective indicators to predict the bioavailability of Cu. In this respect, the DGT method would be a complementary tool that should be evaluated.

The amendment used was a biosolid with an organic carbon content of 22%, pH 6.6 and a total copper content of 377 mg kg<sup>-1</sup>. These biosolids comply with Chilean regulations for the application of waste water treatment plants to soils.<sup>31</sup>

**Table 1.** Physical and chemical properties of soil and biosolid

Analysis	Rancagua soil	Biosolid
Coordinates	34°12'29.1" S 70°53'10.8" W	–
UTM	326215 E 6213163 N	–
Texture	sandy loam	–
Classification	fluventic, haploxerolls (mollisol)	–
pH, H <sub>2</sub> O	6.70 <sup>a</sup>	6.57
pH, KCl	5.60	6.40
EC / (dS m <sup>-1</sup> )	0.91	1.7
Organic carbon / %	2.2	22
Organic matter / %	3.8	–
CEC / (cmol <sup>+</sup> kg <sup>-1</sup> )	21	44
Total metal		
Cd / (mg kg <sup>-1</sup> )	2.1	4.7
Cr / (mg kg <sup>-1</sup> )	nd <sup>b</sup>	260
Cu / (mg kg <sup>-1</sup> )	1227	377
Mn / (mg kg <sup>-1</sup> )	910	442
Ni / (mg kg <sup>-1</sup> )	31	130
Pb / (mg kg <sup>-1</sup> )	nd	66
Zn / (mg kg <sup>-1</sup> )	185	1214
Ca / (g kg <sup>-1</sup> )	7.7	42
Fe / (g kg <sup>-1</sup> )	52	25
Mg / (g kg <sup>-1</sup> )	0.7	6.8
Available Cu		
DTPA / (mg kg <sup>-1</sup> )	331	–

<sup>a</sup>All results in duplicate; <sup>b</sup>nd: not detected.

#### Preparation of gels and validation of the DGT devices

The information given by Zhang *et al.*<sup>17</sup> and Zhang and Davison<sup>18</sup> led to the successful preparation of the DGT devices. The use of 0.3 mm spacers allowed

the gels to reach an average thickness of 0.8 mm after the hydration and swelling process. The devices were validated for Cu before their use to ensure a response in agreement with that described in the literature.<sup>17,18</sup> The results of the validation of the gels are presented in Table 2. Differences between the concentration determined with the device ( $C_{DGT}$ ) (equation 2) and the concentration of the initial solution ( $C_{initial}$ ) were less than 10%. Furthermore, for each point, the quotient obtained between both parameters had relative standard deviations below 10%, reflecting the reproducibility of the results obtained for the used concentration range. Likewise, a good correlation was found between both parameters with equation 3 (Table 2):

$$C_{DGT} = 0.0031 + 0.918 C_{initial} \quad (r = 0.995; p\text{-value} < 0.01) \quad (3)$$

#### Determinations in soil solution and soil treated with biosolid

The addition of biosolids to the soil increased the pH significantly, approaching a neutral value, being this effect more evident for the pH determined with KCl (Table 3). Therefore, the biosolids had the capacity to decrease the potential acidity of the soil, regardless of incubation time. Similarly, an increase of about two percentage points in the content of soil organic matter was found, an increase that was maintained until the end of the incubation period. The rise in both parameters was probably due to the high amount of organic matter and alkaline metals added to the soil with the high dose of biosolids.<sup>32</sup>

The results of the simple extraction of Cu in the incubated soils showed a clear difference between the amount extracted with CaCl<sub>2</sub> and that extracted with citric or tartaric acid or with DTPA. In the former case, an average of 3.5 mg L<sup>-1</sup> was extracted, and represents a small fraction of the exchangeable Cu present in the soils that can be leached by a solution of pH, composition, and concentration similar to that of a soil solution, such as 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub>.<sup>24</sup> In the second case, the use of complexing agents succeeds

**Table 2.** Validation of results for DGT devices prepared in the laboratory

n <sup>a</sup>	$C_{initial} / (\text{mg L}^{-1})$	$C_{DGT}^b / (\text{mg L}^{-1})$	$(C_{initial} - C_{DGT}) / \%$	$C_{DGT}/C_{Final}$		
				Value	Mean	SD (RSD)
1	0.124	0.115	7	0.93	0.936	0.024 (2%)
2	0.124	0.118	5	0.95		
3	0.215	0.196	9	0.91		
4	0.215	0.209	3	0.97		
5	0.248	0.233	6	0.94		
6	0.248	0.226	9	0.91		

<sup>a</sup>n represents each batch prepared on different days; <sup>b</sup>values in duplicate; SD: standard deviation; RSD: relative standard deviation.

**Table 3.** General characteristics and copper extracted from soil and soil-biosolid incubated for 0 and 130 days

	t = 0 day		t = 130 days	
	S	S + BS	S	S + BS
General characteristic				
pH, H <sub>2</sub> O	6.6 ± 0.1 <sup>a</sup>	7.2 ± 0.1	6.5 ± 0.1	7.3 ± 0.1
pH, KCl	5.5 ± 0.1	6.7 ± 0.1	5.5 ± 0.1	6.8 ± 0.1
Organic matter / %	4.2 ± 0.1	6.2 ± 0.1	3.9 ± 0.1	6.1 ± 0.1
Simple extraction <sup>b</sup>				
Citric acid / (mg kg <sup>-1</sup> )	466 ± 6a	425 ± 2c	466 ± 2a	449 ± 3b
Tartaric acid / (mg kg <sup>-1</sup> )	462 ± 2a	400 ± 4c	470 ± 6c	439 ± 10b
DTPA / (mg kg <sup>-1</sup> )	330 ± 4a	282 ± 4c	309 ± 2b	255 ± 4d
CaCl <sub>2</sub> / (mg kg <sup>-1</sup> )	3.2 ± 0.1a	5.4 ± 0.1a	3.8 ± 0.1b	1.9 ± 0.1d
Sequential extraction				
HOAc-Soluble / (mg kg <sup>-1</sup> )	165 ± 4b	130 ± 1d	178 ± 2a	134 ± 1c
Reducible / (mg kg <sup>-1</sup> )	321 ± 4a	255 ± 3b	325 ± 1a	247 ± 2c
Oxidizable / (mg kg <sup>-1</sup> )	387 ± 2c	457 ± 12a	356 ± 2d	427 ± 13b
Residual / (mg kg <sup>-1</sup> )	407 ± 7	373 ± 5	422 ± 1	408 ± 16
Total / (mg kg <sup>-1</sup> )	1281 ± 22	1215 ± 31	1281 ± 22	1215 ± 31

<sup>a</sup>Error: standard deviation (n = 3); <sup>b</sup>for simple and sequential extraction, the results followed by different letters in each row refer to significant difference ( $p < 0.05$ ). S: soil. S + BS: soil-biosolid.

in extracting more than 300 mg L<sup>-1</sup> of metal which can come from exchangeable forms such as metal bonded to carbonate. The amount extracted by complexing agents is frequently associated with the amount of metal available to plants,<sup>29</sup> and those agents are widely used due to their ability to form well defined, stable and water-soluble complexes with metal cations. Considering that low molecular weight organic acids (LMWOA) can represent the process of exudation through the roots, a process that is also simulated by DTPA, it can be stated that the amounts extracted with these agents represent the metal found in a bioaccessible form<sup>33</sup> for the plant in the soil of the Rancagua series. This contains a high Cu concentration due to the use of irrigation water contaminated with metal coming from the mining operations located in that region.<sup>12,14</sup> On the other hand, the application of biosolids always caused a decrease of the Cu extracted with any of the three complexing agents, regardless of incubation time.

Sequential extraction of Cu in the soil indicated that the order of importance of the metal forms was residual > oxidizable > reducible > soluble. This order did not change with incubation time, but the application of biosolids implied a change of the order, which in this case was oxidizable > residual > reducible > soluble. Incubation time caused a slight decrease of the oxidizable form and a slight increase of the residual form of both substrates. Comparison of the distribution of Cu forms in the soil treated and untreated with biosolids, at both incubation

times, shows that the biosolids produce a decrease of soluble Cu and Cu attached to the reducible fraction, as well as an increase of the metal bound to the oxidizable fraction. Such changes can be attributed, in part, to the increased metal binding capacity produced by the large input of organic matter from the biosolids, decreasing the soluble form and increasing the oxidizable form. Likewise, the decrease of Cu bound to Fe and Mn oxides (reducible form) might be due to a greater reducing environment<sup>1</sup> generated by the large contribution of organic matter that takes place when biosolids are applied at a rate of 200 t ha<sup>-1</sup>. This environment would favor the release of Cu, which would be incorporated in the organic matter, an effect that in this study was observed immediately, with no changes detected after the incubation time.

#### Application of DGT to soil solution and to soil treated with biosolids

Regardless of the soil:water ratio used for the extraction ( $S_2$  or  $S_{10}$ ), the metal determined by DGT (Table 4) corresponds to approximately 20% of the free metal determined by specific electrode and to 13% of the total metal present in the soil solution. This result is the opposite of what would be expected if there is free diffusion of the metal toward the resin gel as well as toward the electrode surface. However, the presence of the diffusing gel and of the organic components of the soil solution sets a barrier

that modifies the diffusion processes of the ion toward the device. This would not exist in the case of measurements with specific electrodes. It must be pointed out that the total metal concentration determined in the soil solution was equal to 97% of the saturation concentration of the resin gel, so it is not possible to attribute the lower value of  $Cu_{DGT}$  compared to  $Cu_{ISE}$  to saturation of the resin.

**Table 4.** Determinations in soil solution and soil treated and untreated with biosolids and incubated

	Soil solution	
	Extract S <sub>2</sub>	Extract S <sub>10</sub>
$Cu_{DGT} / (mg L^{-1})$	$0.30 \pm 0.03^a$	$0.20 \pm 0.01$
$Cu_{ISE} / (mg L^{-1})$	$1.41 \pm 0.13$	$1.09 \pm 0.01$
$Cu_{Total}^b / (mg L^{-1})$	$2.26 \pm 0.09$	$1.60 \pm 0.04$
$R^c$	0.133	0.125
	Soil and soil-biosolid, incubated	
	S	S + BS
t = 0 day		
$Cu_{DGT} / (mg L^{-1})$	0.591	1.991
$C_E^d / (mg L^{-1})$	0.594	2.002
t = 130 days		
$Cu_{DGT} / (mg L^{-1})$	0.398	0.624
$C_E / (mg L^{-1})$	0.401	0.628

<sup>a</sup>standard deviation (n = 3); <sup>b</sup>total Cu in solution; <sup>c</sup> $R = Cu_{DGT}/Cu_{sol}$ , where  $Cu_{sol} = Cu_{Total}$ ; <sup>d</sup> $C_E = C_{DGT}/R_{diff}$ .

In this respect, it has been shown that Cu can form stronger complexes than other divalent cations, making the metal less available to be taken up by plants.<sup>34</sup> On the other hand, it has been described that biogeochemistry of Cu is controlled mainly by the interaction of the metal with natural organic matter (NOM), with the high affinity of NOM for Cu being more important than the metal abundance and polyfunctionality.<sup>35</sup> NOM is composed mostly of humic substances that can act as important chelating agents for Cu, with the formation of complexes being highly dependent on factors such as pH, ionic strength and competition with other ligands present in the medium.<sup>36</sup>

Based on the study of Scally *et al.*,<sup>37</sup> our results can be explained by the lability of the complexes formed between a metal and a ligand that is manifested in the diffusing gel. This, in our case, correspond to complexes formed by Cu and humic substances dissolved in the soil solution. The same authors<sup>37</sup> developed an expression that includes an exponential term that represents the kinetic limitation. The expression relates the amount of metal accumulated by the resin gel with the concentration, the diffusion coefficient of the free metal and the complex, and

the thickness of the diffusing gel. When the complex is partially labile, the measurement by DGT is controlled by the dissociation kinetics of the complex. Under this condition, a small thickness of the diffusion gel, like that used in our experiments, implies in insufficient time for the dissociation of the complex, and therefore its contribution to the measurement obtained by DGT is negligible. In this relation, Zhang *et al.*<sup>6</sup> indicate that the thickness of the diffusing gel is a factor that controls the path of the complexes formed with Cu (II), since as the thickness increases, the pore size varies, and it is therefore possible for the Cu complexes with the humic substances to pass through this barrier.

On the other hand, Scally *et al.*<sup>10</sup> described changes in the value of the diffusion coefficient when the size of the ligand changes, with the diffusion coefficient decreasing from 20 to 30% compared to that found for the free metal when ligands such as nitrilotriacetic acid (NTA) or diglycolic acid (DGA) were used, and between 75 and 90% when fulvic or humic acids were used. In the specific case of the formation of Cu-NTA complexes, the same authors<sup>37</sup> report that the complex diffuses 14% more slowly than Cu(II), and this can be extrapolated to divalent cations of similar size.

The above information is in agreement with the low value determined for  $Cu_{DGT}$  in our experiments with soil solution. Also, the low values of  $R$  (Table 4), as indicated by Cattani *et al.*<sup>38</sup> ( $R = Cu_{DGT}/Cu_{sol}$ ), agree with the values obtained by the same authors in soils with high Cu content (vineyards). A low value of  $R$  would be indicative of slow sorption kinetics (desorption) of copper in soils contaminated with that metal, as well as Cu replenishment from the solid phase to the soil solution.<sup>38</sup>

Calculation of  $C_E$  gave a result of the same magnitude as  $Cu_{DGT}$  since parameter  $R$  had a similar magnitude (0.995) regardless of biosolid application and incubation time. For that reason, the comparisons were made considering the  $Cu_{DGT}$  results. Therefore, this parameter in soil treated with biosolids at time 0 was about four times greater than in untreated soil (Table 4), pointing to higher bioavailability of Cu in this substrate. At time 130 days, the same trend exists, but the difference becomes smaller.

The comparison of the incubation times for each treatment shows a decrease of  $Cu_{DGT}$  with the natural aging of the soil, an effect that was more noticeable in soil treated with biosolid. At time 0, these results would be in agreement with a greater availability of labile Cu species contributed directly by the biosolid or by the solubilization of Cu from the soil due to the increased dissolved organic matter (DOM) contributed by the biosolid. After the incubation time,  $Cu_{DGT}$  drops noticeably, in agreement

with the lower availability observed with DTPA and  $\text{CaCl}_2$ , corresponding to a stabilization of the fixation of Cu on the organic matter supplied by the biosolid. In that respect, some works have described DOM provided by the biosolids as favoring both the mobility of Cu and its retention in the soil.<sup>39,40</sup>

The  $\text{Cu}_{\text{DGT}}$  results were used to evaluate the correlation with Cu forms obtained by simple and sequential extraction of soil (Table 5). Among the extractants,  $\text{CaCl}_2$  reflected more closely the relation between  $\text{Cu}_{\text{DGT}}$  and available Cu estimated by a chemical agent since a direct correlation between them was found ( $r = -0.77$ ). This is in agreement with the diffusion process of Cu toward DGT from the soil solution of Cu linked weakly with the solid phase of the substrate. On the contrary, citric and tartaric acids correlate inversely, and DTPA did not correlate probably because those extractants can extract Cu from different less labile forms in the solid phase.<sup>14</sup>

**Table 5.** Simple correlation between available  $\text{Cu}_{\text{DGT}}$  and Cu obtained by simple or sequential extraction

Y / ( $\text{mg L}^{-1}$ )	X / ( $\text{mg kg}^{-1}$ )	Equation	r	p-value
$\text{C}_{\text{DGT}}$	$\text{Cu}_{\text{citric}}$	$Y = 16.4 - 0.0343 X$	-0.92	< 0.01
	$\text{Cu}_{\text{tartaric}}$	$Y = 10.4 - 0.0215 X$	-0.93	< 0.01
	$\text{Cu}_{\text{DTPA}}$	$Y = 2.86 - 0.00667 X$	-0.30	0.35
	$\text{Cu}_{\text{CaCl}_2}$	$Y = -0.472 + 0.385 X$	+0.77	< 0.01
	$\text{Cu}_{\text{soluble}}$	$Y = 4.20 - 0.0217 X$	-0.70	0.01
	$\text{Cu}_{\text{reducible}}$	$Y = 3.82 - 0.0102 X$	-0.58	0.05
	$\text{Cu}_{\text{oxidizable}}$	$Y = -4.43 + 0.0131 X$	+0.81	< 0.01

As to the Cu forms estimated by sequential extraction, a direct relation was obtained only between  $\text{Cu}_{\text{DGT}}$  and Cu linked with organic matter ( $r = 0.81$ ) (Table 5). This result can be explained by the fact that Cu can interact with functional groups of the organic matter with interaction forces of variable magnitude, involving weaker or stronger attraction depending on the predominant functional group. If it is also considered that Cu easily tends to form complexes with organic matter and that the biosolid contributes a large amount of organic matter, there would be a substantial fraction of Cu bound weakly with organic matter that may control the replenishment of Cu in the soil solution.<sup>38</sup>

There are several studies linking DGT devices with the availability of copper, among these, a study by Scally *et al.*<sup>37</sup> attempts to relate the Cu determined by DGT with metal absorbed by corn plants. The authors<sup>37</sup> conclude that this technique does not allow predicting bioavailability of copper in the metal-tolerant plants since

the values determined in the aerial part do not correlate with the concentration determined by DGT. This happens mainly because of the physicochemical changes produced by plants, which interfere with the bioavailable metal content. On the other hand, it has been shown that DGT is a useful tool for the prediction of copper content in soil, where the bioavailability of copper depends on its speciation and its interaction with other existing ions.<sup>41</sup>

In the specific case of the determination of metallic species in mine tailings, Conesa *et al.*<sup>42</sup> showed that the competition between the metals present in the resin is an important factor in the estimation of available metal, but the thickness of the gel was not significant. In our study, no high levels of other elements were detected that could lead to the assumption of competition between Cu and other divalent cations in the resin of DGT.

The studies mentioned above show that the results obtained from diffusion gradient in thin films present great variability, depending on the different scenarios in which it has been applied. This raises the need to evaluate a greater number of parameters that can influence the estimation of bioavailable copper. In this sense, the results of this study are a contribution because it applies the technique to a soil with high copper content of both natural and anthropogenic origin.

## Conclusions

The bioavailability of Cu in soils with high levels of the metal, which have also been treated with biosolids, can be addressed through the application of DGT devices. In this sense, the preparation of the diffusive and resin gels in the lab as well as the assembly of a device to support the gels is possible. It can therefore be stated that the amount of copper extracted by the DGT devices applied to soil solution, for a time frequently described in the literature, corresponds to a small fraction of the total metal, in this case 13% of the total metal present in the soil solution. In the case of soil, this amount was less than 0.3%. Furthermore, it was possible to relate directly the metal extracted by DGT with the metal available with  $\text{CaCl}_2$  or the metal bound to the organic matter estimated by sequential extraction. It can therefore be concluded that DGT devices as well as of sequential extraction methods are reliable tools to evaluate the concentration of available Cu in soils contaminated with this metal.

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