

Use of Diffusive Gradients in Thin Films (DGT) in Undisturbed Field Soils

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The technique of diffusive gradients in thin films (DGT) has been shown to be a promising tool to assess metal bioavailability in soils under laboratory conditions. In this study we used DGT to investigate the resupply kinetics of Cu and Zn under in-situ conditions in a polluted lysimeter soil and compared the results with laboratory measurements using undisturbed soil cores at defined water contents as well as homogenized soil samples. Results differed considerably between these treatments, although the same soil material was used in all experiments. A small pool of rapidly available Zn was found in the field but not in the homogenized soil. Soil solution pH and dissolved metal concentrations also varied significantly between the soil treatments. In addition, we compared the DGT-measured effective concentration with the uptake of Cu and Zn into the shoots of *Lolium perenne* (Ryegrass) under the same three types of conditions, i.e., field, soil cores, and homogenized soil. A close relationship was found which was not linear but could be described by a saturation-type behavior. *L. perenne* is a metal excluder plant, and thus, metal accumulation is limited by translocation of metals from roots to shoots. DGT predicted plant metal uptake much better than the soil solution concentration or pH. The results of this study suggest that DGT may be successfully used under field conditions to study the kinetics of metal resupply. Plant metal concentrations were not well predicted in all cases by the effective concentration C_E under field conditions. Some plants took up considerably more metals than estimated by C_E . Variations in metal uptake independent of their bioavailability can be caused by local variations in microsite conditions, e.g. light, temperature, water, and nutrients. To some degree, such indetermination has to be expected as an inherent feature of the system and the concept of bioavailability.

Introduction

The determination of heavy metal bioavailability is a crucial step in risk assessment for metal-polluted soils. Toxicity or bioaccumulation of heavy metals is usually predicted from correlations with soil solution concentration, free metal ion in soil solution, or some operationally defined extractable fraction (1, 2). These measurements do not account for the buffer capacity of the soil, i.e., its ability to replenish the

solution following extraction of the metal. To account for the dynamics of the soil/solute system, the technique of diffusive gradients in thin films (DGT) has been proposed (3). DGT has been developed to measure supply fluxes of metals in sediments (4), and it has proven to be a very promising tool to assess both the kinetics of resupply and the bioavailability of metals to plants (5, 6). Like plant roots, DGT locally lowers the metal concentration of the soil solution and thereby triggers a resupply by diffusion from the bulk solution and desorption from the solid phase. The kinetics of this resupply determines the availability of the metals to the plant root. Zhang et al. (5) grew the metal indicator plant *Lepidium heterophyllum* in soils containing 19–8645 mg kg⁻¹ Cu and found that plant Cu concentration closely correlated with the effective concentration C_E measured by 24-h deployment of DGT ($r=0.95$). This was the closest correlation between a measured soil property and plant concentration ever found for a large variety of different soils and a wide concentration range.

Current methods to assess metal bioavailability use homogenized and sieved soil. Often the soil is also dried prior to the analysis, although this has a significant influence on the fractionation of heavy metals (8, 9). The exchangeable Zn fraction in sediments, for example, was found to be reduced by air-drying by as much as 26–86% compared to a fresh sample (8). Other fractions (e.g., the carbonate-bound metal fraction) may increase with drying (9). Also, in soils, air-drying has been found to increase the exchangeable fraction considerably (10). We can therefore expect that the bioavailable concentration determined from sieved and dried soils is generally not representative for in-situ conditions of undisturbed field soils.

Prior studies have applied DGT in the laboratory to samples of sieved and homogenized soils. Ernstberger et al. (11), for example, used separate DGT devices to measure the resupply kinetics over different time intervals. With undisturbed samples and even more in the field, the inherent heterogeneity of soils becomes an important issue. Due to larger scatter in the resulting measurements, the approach proposed by Ernstberger et al. (11) is not feasible under heterogeneous conditions usually encountered in field soils. It is therefore necessary to adapt this method for field soils.

The aim of this work was to test and adapt the DGT method under field conditions and to compare field measurements to results obtained in laboratory tests with homogenized soil samples. DGT measurements were performed over a range of deployment times to determine the resupply kinetics of the different soils. In addition, we compared the uptake of Cu and Zn by the grass *Lolium perenne* and correlated the uptake to the effective concentration C_E measured by 24-h deployment of DGT in the different soil samples.

Experimental Section

DGT. Diffusive and Chelex gels were prepared according to Zhang and Davison (12) from acrylamide (Fluka, Switzerland) with cross-linker (DGT Research Ltd). The diffusive gels were washed in distilled water and stored in 0.01 M NaNO₃ at room temperature. The Chelex gel was stored in water at 4 °C. The thickness of the diffusive gel was 0.064 cm. For the preparation of Chelex gels, 0.12 g of dried Chelex 100 (Na⁺, 200–400 mesh, Fluka) was added to 2 mL of acrylamide solution. The gels were mounted on a modified Sartorius filtration unit (for 25 mm filters) with a cellulose acetate filter (0.45 µm, Sartorius) on top to protect the gel. The cap of this filter unit was cut off to expose an area of 3.8 cm² of the filter. The inlet into the filtration unit was sealed. Metals were eluted

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from the gel with 1 M HNO₃. Eluted Zn was measured by flame atomic absorption spectroscopy (VarianAA400) and Cu by graphite-furnace atomic absorption spectroscopy (Varian AA300). Even at the longest deployment time and in the soils with the highest pollution, the amount of accumulated metals (Cu and Zn) was mostly below 10% of the capacity of the gel. The DGT devices were tested according to the procedure outlined in ref 3. They were immersed for 4 h in a 2-L PE bottle with 68.5 µg/L Cu at pH 6.0 in 0.01 M NaNO₃, which is comparable to the concentration found in our soils. The average concentration measured by DGT was 58.5 µg/L.

The soils used in this study were taken from a lysimeter experiment in which the influence of metal pollution of the topsoil on a forest ecosystem was studied (13). The soil was polluted artificially by mixing filter dust from a brass smelter (a known source of pollution, e.g., around metal processing industries) into the upper 15 cm of the topsoil layer. Average metal concentrations were 2721 mg kg⁻¹ Zn, 667 mg kg⁻¹ Cu, 115 mg kg⁻¹ Pb, and 10 mg kg⁻¹ Cd at the beginning of the experiment in the year 2000 (13). Mixed stands of spruce, willows, poplar, and birch were planted together with natural understorey plants in lysimeters with contamination and with uncontaminated reference soils. The DGT measurements were carried out under natural rainfall conditions. Undisturbed soil cores were taken with a cylindrical sampler with a diameter of 5 cm and a length of 10 cm (Humax sampler). Further topsoil samples were collected, dried at 40 °C, and sieved to 2 mm. Soil properties were 1.5% organic carbon content, pH(CaCl₂) 6.45, carbonate content < 0.1%, 36% sand, 49% silt, and 15% clay (13). Unpolluted soil was also freshly mixed with different quantities of filter dust and equilibrated for 1 month. The resulting concentrations of Cu and Zn were (in mg kg⁻¹) 21/76 for the reference soil and 213/1018, 401/1944, and 548/2760 for the contaminated soils.

Resupply Kinetics. To determine the resupply kinetics we employed DGT repeatedly at the same location using the following procedure: after a certain exposure time, the DGT device was removed from the soil and the Chelex gel taken out and replaced by a new one at exactly the same spot. Gels were changed after 4, 8, 24, 72, and 168 h. In the field, the experiment was run in four replicates and in the laboratory in three. In the experiments with homogenized soil we used pots with 250 g of soil to which 100 g of water was added and equilibrated for 15 days. In the experiments with undisturbed soil cores, soil water potential was kept at -100 hPa. Water loss by evaporation was replaced daily. The field experiments were carried out under cool weather conditions after heavy rain with intermittent rain during the exposure time. The DGT units were placed into 5-cm deep holes that were then covered with plastic wrap to minimize desiccation. At each sampling, soil temperature was measured close to the DGT device. Soil solution was sampled using Soil Moisture Samplers from Rhizosphere Research Products (The Netherlands). The samplers were washed with 0.1 M HNO₃ and rinsed with distilled water before use. In the field, the samplers were buried about 5-cm away from the DGT units at the same depth. In the experiments with undisturbed soil samples, a separate core was used for soil solution monitoring. In the homogenized soils, the samplers were buried about 1.5 cm away from the DGT units. At each DGT sampling point soil solution was also sampled.

From the accumulated mass of metal the concentration C_{DGT} was calculated. C_{DGT} is the accumulated mass M multiplied with the diffusion layer thickness (diffusion gel + filter) (Δg) and divided by the exposed gel area (A), the diffusion coefficient (D) of each metal in the diffusive gel (14), and the deployment time (T)

$$C_{DGT} = (M\Delta g/ADT) \quad (1)$$

C_{DGT} is the time-averaged concentration at the DGT interface for the diffusion-only case. We can then calculate the ratio R of the mean concentration at the DGT surface and the bulk soil solution concentration, i.e.

$$R = (C_{DGT}/C_{solution}) \quad (2)$$

The dependence of R on metal resupply from the solid into the solution phase can be modeled using the DIFS model (DGT Induced Fluxes in Soils) (15). The adsorption/desorption kinetics are characterized by the equilibrium distribution coefficient K_d and the response time T_c

$$T_c = (1/k_1 + k_{-1}) \quad (3)$$

where k_1 and k_{-1} are the first-order adsorption and desorption rate constants.

Plant Experiments. English ryegrass (*Lolium perenne*) was sown in the field, in the undisturbed soil cores, in homogenized and sieved (<2 mm) field soil, and in soil freshly mixed with filter dust. The field soils received 200 mg of seeds within an area of 10 × 10 cm with three replicates. The undisturbed soil cores received only 50 mg of seeds according to their smaller surface area. Plant experiments with homogenized soil were performed with samples of 150 g of soil filled into the same polyethylene tubes (5 cm diameter and 9 cm length) used for undisturbed sampling. To each tube 50 mg of seeds were applied as in the case of undisturbed cores. The experiments with freshly mixed soil were carried out with 1 kg of soil in pots 12 cm in diameter. Each pot received 200 mg of seeds in an area of 10 × 10 cm. Pots and soil cores were placed in a growth chamber with 16 h of daylight at 23 °C and a night temperature of 16 °C. The gravimetric water content was held constant at 60% of the field capacity. The irrigation water contained (in µmol) 3.2 PO₄, 4.3 Na, 7.7 K, 5 Ca, 1.2 Mg, 28 Cl, 3.2 SO₄, 71.4 NH₄NO₃. In the field, plants received natural precipitation and during longer periods of dry weather they were watered artificially. After 5 weeks (field 8 weeks) the plants were cut 1 cm above the soil surface and air-dried at 40 °C for 24 h. Heavy metal contents were determined by X-ray fluorescence (X-Lab 2000, Spectro Germany). Small samples which did not provide enough material for XRF were digested with 5 mL of HNO₃ (65%), 2 mL of H₂O₂, and 2 mL of H₂O and analyzed by flame or graphite tube AAS for Zn and Cu. A comparison of a subset of samples has shown no significant difference between the two methods. After plant harvest, pots and soil cores were saturated with water and equilibrated for 1 day. DGT devices were placed for 24 h on the surface of the soil. With the soil cores, three subsequent DGT measurements were performed. After each measurement the top 3 cm of the soil was removed to expose fresh soil. From the accumulated mass of metal the effective concentration C_E was calculated according to ref 5 as

$$C_E = (C_{DGT}/R_{diff}) \quad (4)$$

R_{diff} represents the depleted concentration at the interface of the device and the soil for the diffusion-only case and was calculated using the numerical model DIFS (DGT Induced Fluxes in Soils) (15). Input parameters are the particle concentration P_c

$$P_c = (m/V) \quad (5)$$

where m is the total mass of soil particles, V the pore water volume, and the soil porosity ϕ

$$\phi = (d_p/P_c + d_p) \quad (6)$$

where d_p is the density of soil particles, which is normally

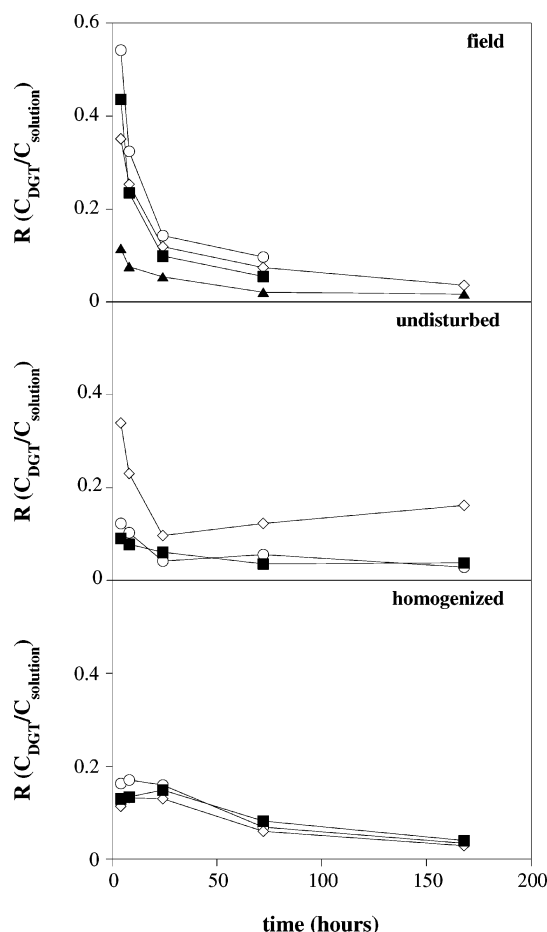


FIGURE 1. Resupply kinetics of Zn measured in undisturbed field soil (four replicates), in undisturbed soil cores in the laboratory at -100 hPa, and in homogenized soils (three replicates each). Samples have been taken after 4, 8, 24, 72, and 168 h. The different symbols represent three replicates (four for the field).

assumed to be 2.65 g cm^{-3} , and the diffusion coefficient of the metal in soil, D_s

$$D_s = (D_0/1 - \ln \phi^2) \quad (7)$$

where D_0 is the diffusion coefficient of each metal in water.

Results and Discussion

Resupply Kinetics. The adaptation of the method by Ernstberger et al. (11) employing a sequential placement of DGT devices at the same spot allowed us to determine resupply kinetics in soils where we cannot assume an equal distribution of metal concentration. To get an idea of the heterogeneity of the sampling site, we deployed up to four DGT units at the site within an area of about 10×10 cm. Figures 1 and 2 show that the ratio R between the mean concentration at the DGT surface and the bulk soil solution concentration varied much more in the field and between the undisturbed samples than between the pots with homogenized soil. By repeated deployment of DGT at the same location, we observed a continuous decrease of R with time in all experiments, indicating the depletion of a mobile pool of metals. This pool was found to be smaller in the homogenized samples than in the experiments with undisturbed soil structure, in particular for Zn. The long-term resupply of Zn was very similar in all samples. The average concentration at the DGT surface was only 5% of the soil solution composition after 168 h.

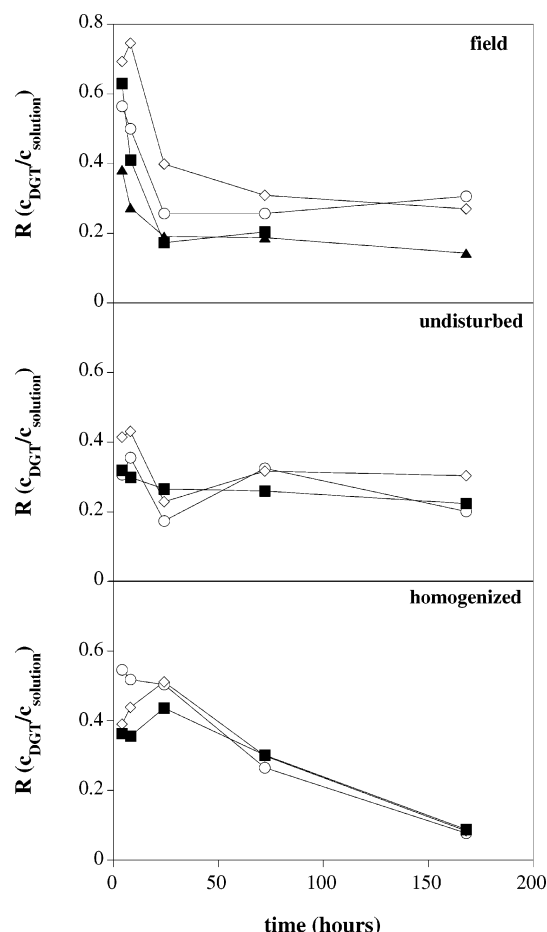


FIGURE 2. Resupply kinetics of Cu measured in undisturbed field soil (four replicates), in undisturbed soil cores in the laboratory at -100 hPa, and in homogenized soils (three replicates each). Samples have been taken after 4, 8, 24, 72, and 168 h. The different symbols represent three replicates (four for the field).

Determination of R depends on an accurate measurement of total Cu and Zn concentrations in soil solution. In previous DGT applications, soil solution samples were usually obtained by extraction with Rhizon Soil Moisture Samplers (5). The samplers have to be placed some distance away from the DGT device to avoid depletion of the soil water content in the vicinity of the gel. The measured soil solution concentration may therefore be different from the actual soil solution concentration close to the DGT device. With homogenized soils, this does not pose particular problems. Replicate measurements of soil solution concentration from three homogenized pots gave Cu concentrations of 98 ± 12 , 100 ± 6 , and $87 \pm 13 \mu\text{g L}^{-1}$ (six measurements over 6 days). However, three samplers in the vicinity of the DGT devices in the field gave Cu concentrations of 262 ± 21 , 212 ± 12 , and $162 \pm 32 \mu\text{g L}^{-1}$ (five measurements each). Zn concentrations in the same samplers in the field varied even more with 4163 ± 397 , 10713 ± 1700 , and $2446 \pm 625 \mu\text{g L}^{-1}$. For all calculations of R shown in Figures 1 and 2, the average value of the soil solution concentration in the vicinity of the DGT devices was used. Differences in the total metal concentration and therefore soil solution concentration in the vicinity of the DGT device result in different accumulation rates of metals by the DGT. The differences in R between undisturbed samples thus can partially be attributed to differences between the average soil solution concentration and the actual soil solution concentration close to the DGT at the beginning of the exposure. An approach to overcome this problem would be to sample soil solution

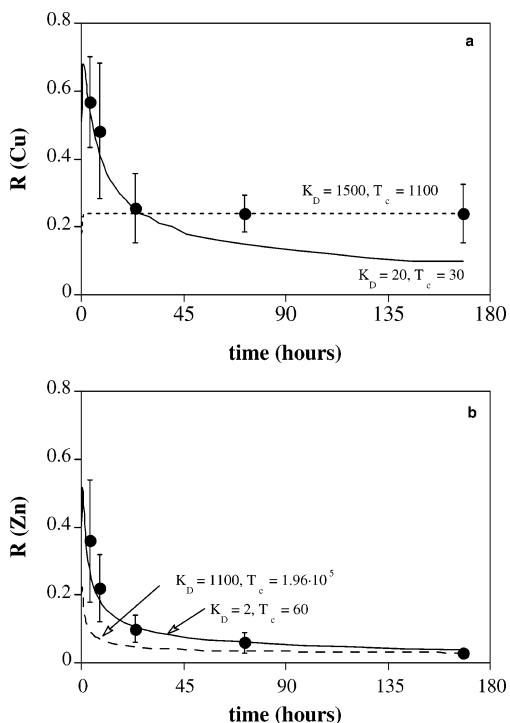


FIGURE 3. DIFS model fits of the experimental measurements of R for Cu (top) and Zn (bottom) in the field soil as a function of time. Model fits are given for two K_D/T_c ratios.

TABLE 1. Average Metal Concentration ($\mu\text{g L}^{-1}$) and pH of the Soil Solution in the Kinetic Experiments

sample	Cu	Zn	pH
field	262 ± 21	4160 ± 397	6.62 ± 0.06
soil cores	144 ± 13	5815 ± 996	6.38 ± 0.05
homogenized	219 ± 31	2034 ± 353	7.41 ± 0.2

by microsuction cups directly under the DGT device immediately before the deployment (after the deployment the soil solution concentration at the surface of the DGT decreases rapidly). The suction cups we used had a length of 10 cm and thus averaged soil solution over a much larger volume than the DGT device. A sample of 5 mL of soil solution usually represents more than 10 cm^3 of soil, while DGT lowers the soil solution concentration only over a few millimeters of soil adjacent to the filter (11); thus, the depleted zone represents not more than $1\text{--}2 \text{ cm}^3$. However, sampling of soil solution by microsuction cups in the field is not straightforward. Averaging R for the four replicates as shown in Figure 3 is a more appropriate way to deal with the heterogeneity. For further modeling of the resupply kinetics (see section DIFS modeling), we used this average value of R .

The pH was much higher in the soil solution extracted from the homogenized soil samples than from the field soil or the undisturbed soil cores (Table 1). Changes in soil solution pH after drying and rewetting have been reported (16). Higher soil pH is normally correlated with a lower dissolved Zn concentration due to stronger retention by the soil matrix (17). This is exactly what we observed for Zn. The Cu concentration in soil solution does not show a clear trend with pH, but the lowest dissolved concentration is found in the soil solution with the lowest pH, again in accordance with the known pH dependence of Cu in soils. The differences in the exchange kinetics between the different treatments are therefore most probably caused by pH changes induced by homogenization and drying.

Another important factor to consider when working in the field is the variability of the water content during the measurement. The samples measured in the laboratory were kept at approximately constant water content. In the field, such control is usually not possible, although we conducted the field measurements after rainfall during cool weather conditions with intermittent rain and covered the soil around the DGT devices to prevent evaporation. Hooda et al. (18) showed that DGT deployment in soils with different moisture content complies with theory when used at or above 80% field capacity. The metal flux into the gel is reduced at low water content, yielding lower metal accumulation rates. However, we observed very low accumulation rates after 168 h of exposure to the DGT device not only in the field soils (Figure 1a) but also in the homogenized soils (Figure 1c), which were kept at high moisture content. Moreover, we observed the same high Cu accumulation rates after 168 h in the field and the undisturbed samples that were kept at constant moisture content. Whereas our field soils had a high moisture content during the deployment time, this may be a critical factor under other weather or climatic conditions. The cool weather conditions also ensured only minimal temperature fluctuations during the deployment time. Temperature affects the DGT measurements since diffusion through the diffusive gel is temperature dependent. Measurements under varying temperature would need to use a temperature-logging device to ensure accurate DGT calculations.

DIFS Modeling. Davison and co-workers (11, 15) developed a model called DIFS which describes the dependence of R on metal resupply from the solid into the solution phase. Ernstberger et al. (11) used only one metal pool to describe their data, but the short-term resupply of Zn in their soil was not well fitted because they focused on fitting the kinetics for long exposure times. We focused on short exposure times and were not able to model the data using only one metal pool. For the undisturbed field soil, at least two copper pools were necessary to describe the observed kinetics: a slow pool with a response time of 1100 s and a K_D of $1500 \text{ cm}^3 \text{ g}^{-1}$ and a fast pool with a response time of 30 s and a K_D of $20 \text{ cm}^3 \text{ g}^{-1}$ (Figure 3a). The K_D value of $1500 \text{ cm}^3 \text{ g}^{-1}$ was imposed rather than being obtained by a best model fit. It was calculated using the regression equation given by ref 17, which accounts for pH and organic matter content. The Cu resupply kinetics from the homogenized soils could not be described well using DIFS with two Cu pools, neither with a best-fit K_D nor with the calculated K_D .

Zn resupply kinetics in the field soils was well described by assuming a single pool with a K_D of $2 \text{ cm}^3 \text{ g}^{-1}$ and a response time T_c of 60 s. Kinetics at long exposure times are also well described using a T_c of $2 \times 10^5 \text{ s}$ and a K_D value of $1100 \text{ cm}^3 \text{ g}^{-1}$, resulting from the regression equation reported in ref 17. The two model fits for Zn are also shown in Figure 3. We assume that the fast pool corresponds to the exchangeable fraction and the slow pool to the dissolution of surface precipitates or crystalline metal phases. Column experiments have shown that the release of the exchangeable pool is fast while acidic conditions were needed to achieve a significant dissolution of the more strongly bound phases (19, 20).

We found much lower R values after 168 h than Ernstberger et al. (11). For Zn, they found an R of about 0.25 after 140 h of deployment while we found a value below 0.05. Both the field and the homogenized soil gave a similar value at 168 h. The low value of R found in our soil may be due to the higher pH value of 6.3 as compared to 5.8 in ref 11 and therefore a stronger fixation of heavy metals.

The absence of the weakly bound Zn pool in the homogenized soil could therefore be explained by stronger binding of the metals at the higher pH.

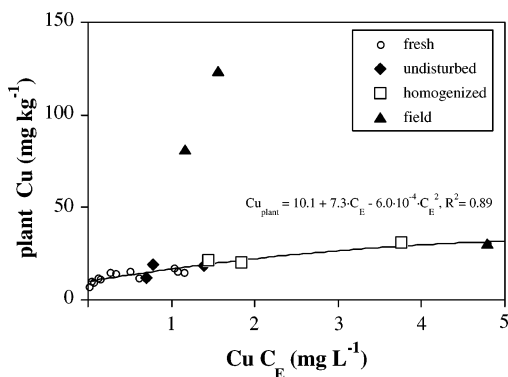


FIGURE 4. Uptake of Cu by rye grass (*Lolium perenne*) in soils freshly mixed with different amounts of filter dust (fresh), in homogenized field soils (homogenized), in undisturbed soils in the laboratory (undisturbed), and in the field (field) as a function of the effective concentration C_E determined by DGT.

Plant Uptake. Figure 4 shows Cu uptake by ryegrass as a function of the effective concentration C_E , which is the concentration that would have to be present in the soil solution to supply the same mass of metal to the DGT solely by diffusion (5). Uptake of Cu by *L. perenne* did not increase linearly with C_E but was better described by a second-order polynomial ($R^2 = 0.89$). Two of the three replicates in the field (each consisting of about 10–15 individual plants) took up significantly more Cu than predicted by C_E . They were excluded from the regression. These plants had a very low biomass and obviously suffered from competition for light, water, and nutrients with the other plants in the ecosystem (discussion see below).

An important point is that DGT measures the metal concentration within a few millimeters of the DGT while a plant integrates over a much larger volume. We tried to compensate for this by exposing DGT devices in the field at different depths at the place where the plants were grown and at different depth in the soil cores. The reported C_E represents an average value over the rooted zone.

Plant uptake was higher for homogenized soils than for undisturbed soil cores (24.23 and 16.64 mg kg⁻¹, respectively). However, also C_E was larger for the homogenized soil than for the soil cores (2344 and 954 μg L⁻¹, respectively). Soil solution pH was larger in the homogenized soils (7.41) than in the soil cores (6.38) (Table 1). The higher bioavailability of Cu in the homogenized soils was therefore reflected by a higher C_E measured by DGT. In comparison, the experiments using soil which was mixed with heavy metal containing filter dust 1 month earlier gave lower Cu uptake as well as a lower C_E .

We measured C_E after harvest of the plants, and the measurements of C_E therefore include a possible effect of the plants on metal fractionation in the soil. Plants are able to mobilize metals and make them bioavailable, and this has been included in the measurements.

Zhang et al. (5) found a linear relationship between the log C_E and the logarithm of Cu content of *Lepidium heterophyllum*. This plant is an indicator of Cu, while *L. perenne* is an excluder like most plants. A linear relationship between C_E and root uptake translates into a linear relationship between C_E and shoot uptake only if the relationship between root and shoot metal concentration is also linear. *L. perenne* accumulates Cu in the roots, and only a small fraction is translocated into the shoots (21). An increase in the total soil concentration from 10 to 650 mg kg⁻¹ increased the copper concentration in shoots from 12 to 15 mg kg⁻¹. The root content, however, increased from 13 to 380 mg kg⁻¹ in the same plants. Another study (22) found that the Cu contents of *L. perenne* grown on polluted soils varied only

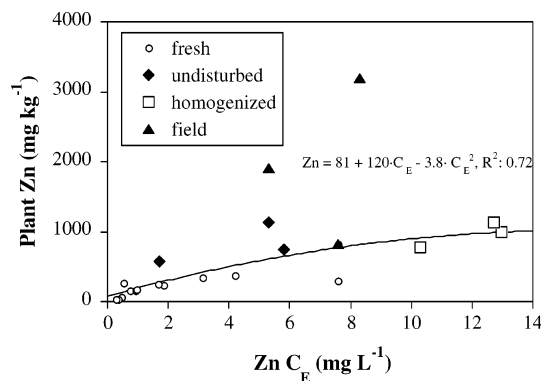


FIGURE 5. Uptake of Zn by rye grass (*Lolium perenne*) in soils freshly mixed with different amounts of filter dust (fresh), in homogenized field soils (homogenized), in undisturbed soils in the laboratory (undisturbed), and in the field (field) as a function of the effective concentration C_E determined by DGT.

TABLE 2. Dry Weight of *Lolium perenne* normalized to an Area of 100 cm² after 5 Weeks (8 weeks in the field) of Growth. Dry Weight Was Normalized to 100 cm² Because Different Areas Were Covered in the Field, the Pots, and the Soils Cores

experiment	g dry weight/100 cm ²	
	polluted	control
field	0.09 ± 0.10	0.03 ± 0.01
soil cores	0.44 ± 0.25	0.87 ± 0.09
homogenized	0.97 ± 0.24	5.45 ± 0.16
freshly mixed ^a	4.67 ± 0.35	6.19 ± 0.66
freshly mixed ^b	3.59 ± 0.44	

^a 213 mg kg⁻¹ Cu, 1018 mg kg⁻¹ Zn. ^b 548 mg kg⁻¹ Cu, 2760 mg kg⁻¹ Zn.

from 17 to 60 mg kg⁻¹, while the total concentration in soil varied from 32 to 640 mg kg⁻¹. The shoot concentration in *L. perenne* is therefore not linearly related to the root metal concentration because translocation inside the plant is limited. The C_E concept actually predicts root uptake of metals. The translocation from roots to shoots is related to plant physiology and not to the soil chemistry. A linear relationship between C_E and shoot metal concentration is therefore only expected for an indicator plant where root and shoot metal concentration are linearly related.

Zinc uptake by ryegrass (Figure 5) exhibited more scatter than Cu uptake. As in the case of Cu, the data were described by a second-order polynomial between C_E and plant uptake. However, the correlation coefficient was only 0.72. The same two field samples as in the case of Cu exhibited a very high metal uptake despite a low C_E . Thus, they were also considered as outliers and excluded from the regression analysis. The plants grown on freshly polluted soils took up less Zn than the plants grown in soil with the aged Zn pollution, even at the same C_E . Homogenizing the soil from the soil cores increased C_E from 4 to 12 mg L⁻¹, while plant uptake increased on average from 750 to 970 mg kg⁻¹. The soil solution concentration of Zn decreased significantly and pH increased by homogenizing the soil (Table 1). On the basis of these two parameters, we would have expected a decrease in bioavailability. On the basis of DGT, however, we would have correctly predicted an increased Zn accumulation by the plant.

The plant biomass increased in the order field < soil cores < homogenized < freshly mixed (Table 2). Biomass was also considerably lower in the metal-polluted soils than in the reference soil. The biomass produced in the field was particularly low. The plants in the undisturbed soil cores,

which were grown in the laboratory with sufficient supply of light and water, produced 4 times more biomass than in the field. Homogenizing the soil doubled growth once more. The higher biomass of the plants grown in the fresh and homogenized soils may have "diluted" the accumulated metals. Metal uptake is related to the transpiration rate, which was much higher in the freshly mixed pots (23). Metal uptake in the field, however, could not be well predicted by C_E . Some plants took up considerably more metals than predicted by C_E . These plants were, however, much smaller. Metal content of plants has been found to decrease with growth (24), which might be one explanation for the lower metal content in the plants in the laboratory experiments. Variations in environmental characteristics other than soil composition (e.g., light or water) between habitats can also result in large differences in metal uptake (25). These factors are not included in the measurement of C_E but obviously can have a strong influence on plant uptake. However, we have to keep in mind that plants that showed growth comparable to the plants in the laboratory fit very well into the relationship between C_E and plant metal concentration (see Figures 4 and 5).

Implications for Assessing Bioavailability. Bioavailability of metals in soils has normally been determined in samples that were taken to the laboratory, dried, and sieved. These conditions are not representative for those prevailing in the field. Our study shows that DGT can be used to determine the bioavailable metal concentration directly in the field. The differences between the resupply kinetics of field soil and homogenized soil samples demonstrates that laboratory measurements may not give adequate prediction for resupply kinetics in the field. Plant uptake in laboratory experiments with homogenized soils showed that the effective concentration C_E could be used to predict Cu and Zn uptake by an excluder plant. However, the influence of additional parameters such as the availability of light or water that also have an influence on metal uptake but that are not captured by C_E has to be taken into account. To some degree such indetermination has to be expected as an inherent feature of the system and the concept of bioavailability. The results suggest that DGT is a promising tool for further research about the in-situ determination of the bioavailable metal fraction.

Acknowledgments

We thank Martin Keller and René Saladin for fabricating the DGT devices, Anna Grünwald for help with the metal analyses, Madeleine Günthardt-Goerg for allowing us to use the lysimeters at WSL Birmensdorf for the field experiments, and Kathrin Wenger for critical comments.

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Received for review August 5, 2003. Revised manuscript received November 7, 2003. Accepted November 28, 2003.

ES034867J