

The application of diffusive gradient technique (DGT) for assessment of changes in Cd, Pb, and Zn mobility in rhizosphere

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ABSTRACT

Diffusive Gradient in Thin Films (DGT) technique was applied to determine Cd, Pb and Zn concentrations in rhizosphere of four different plant species: hyperaccumulator Penny-cress (*Thlaspi caerulescens* J. et C. Presl), trees with a good accumulation capacity willow (*Salix dasyclados* Vimm.) and poplar (*Populus nigra* L.) and crop maize (*Zea mays* L.). Moreover, the effect of two chelating agents (ethylenediaminetetraacetic acid – EDTA, and S,S-ethylenediaminedisuccinic acid – EDDS) on element mobility in rhizosphere like soil was investigated. DGT was able to register even small changes in heavy metal concentrations in rhizosphere according to individual crop and/or soil treatments. A particularly significant effect of EDTA application to enhance solubility of metals bound to solid phase into the soil solution was observed resulting in increased concentrations of mobile metals in rhizosphere. The suitability of DGT technique for evaluation of heavy metal behavior in the environment was demonstrated with reasonable results

Keywords: DGT; soil solution; element mobility; rhizosphere; cadmium; lead; zinc

Toxicity or bioaccumulation of heavy metals is usually predicted from correlations of their content in plant with element concentration in soil solution, with presence of free metal ions in soil solution or with some operationally defined extractable fractions. Reliable measurements of trace species are essential for studies of pollution or trace element cycling but they are difficult, partly because the distribution of chemical species often changes during sampling and storage. These measurements do not account for the buffer capacity of the soil (Nowack et al. 2004). The kinetics of this resupply determines the availability of the metals to the plant root (Nowack et al. 2004). Most of methods for heavy metal measurement use equilibrium procedures, such as ion-selective electrodes or dialysis. Other speciation procedures (for example stripping voltammetry) are kinetically controlled and so the measured species depend on the chemical reaction involved and the rate of mass transport of ions from the bulk solution to the reaction site (Davison and Zhang 1994).

Davison and Zhang (1994) also described a simple technique for measuring trace metal concentrations. The technique uses Diffusive Gradient in

Thin Films (DGT) and incorporates an ion-exchange resin separated from the solution by an ion-permeable gel membrane. Mass transport through the gel is a controlled diffusion and thus well defined, making it possible to obtain quantitative data on concentration and speciation over relatively short time periods.

Variation in solution speciation affects bio-availability, element mobility, and the partition through adsorption/desorption and dissolution/precipitation with the heterogeneous solid phase. Resin in the form of bags, capsules and membranes are used to simulate metal and nutrient movement to plant roots in a more realistic and sensitive manner. The resin continuously removes species from solution and thus provides an *in situ* measure of their availability to the resin (Zhang et al. 1998). It is important to know the thickness of gel between the solution and the reactive medium. Transport through the gel is restricted to diffusion and, by selection of an appropriate gel thickness, it controls the overall rate of mass transport irrespective of the hydrodynamics in the bulk solution. This method is simple, inexpensive and uses relatively available equipment (Davison and Zhang 1994).

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DGT technique may predict heavy metal concentration in rhizosphere and plant metal uptake better than the metal concentration in soil solution or pH (Nowack et al. 2004). DGT locally decreases the metal concentration of the soil solution and thereby triggers a resupply by diffusion from the bulk solution and desorption from solid phase.

The principle of DGT technique is based on Fick's first law of diffusion (eq. 1). An ion-exchange resin layer is separated from bulk solution by an ion-permeable hydrogel membrane of thickness Δg (Figure 1). Simple metal ions diffuse through the gel membrane and are rapidly bound with the resin. It is possible to calculate the flux of ions diffusing through the gel layer (F) and the concentration in rhizosphere C_{DGT} (eq. 2) using these equations:

$$F = DC_{DGT}/\Delta g \quad (1)$$

$$C_{DGT} = (M\Delta g)/(DtA) \quad (2)$$

where: D is the diffusion coefficient of a given metal ion, A is the area of gel membrane exposed to the bulk solution, t is deployment time, and M is the mass of metal accumulated in the resin layer (Zhang et al. 1998). M can be determined by usual analytical methods and calculated like

$$M = C_e(V_{HNO_3} + V_{gel})/f_e \quad (3)$$

where: C_e is the concentration of metals in the elution solution ($\mu\text{g/l}$), V_{HNO_3} is the volume of HNO_3 added to the resin gel, V_{gel} is the volume of the resin gel, typically 0.15 ml, and f_e is the elution factor for the metals, typically 0.8 (Zhang et al. 1998). The recovery of trace metal ions from the resin was de-

termined by Zhang and Davison (1995) as follows: $80.3 \pm 5.5\%$ Zn, $83.9 \pm 2.7\%$ Cd, $79.3 \pm 6.4\%$ Cu, $81.4 \pm 2.2\%$ Mn, and $69.7 \pm 5.0\%$ Fe. Except Fe^{2+} , all metal ions had a recovery higher than 80% regardless of the amount of metal bound. An elution factor of 0.8 was adopted for the calculation of trace metal concentrations in bulk solution.

When ions are removed from solution by DGT probe, they may be resupplied from the solid phase of the soil to the solution. There will be a net flux from soil to solution induced by the DGT device. This flux could be calculated like

$$F_{DGT} = M/(tA) \quad (4)$$

When this technique is used in solution (like water or sediment available fraction), the time necessary for transport of ions through the diffusive layer is typically minutes or hours. When DGT is applied to soils, it also measures labile species and resupplied species; a longer time is required for successful ion transport (Zhang et al. 1998).

For transport through layers in DGT device, there is a theoretically important diffusive boundary layer (DBL) between diffusive gels with filter and bulk solution, where transport is caused solely by molecular diffusion. In soils there is a domination of the gel thickness to control mass transport and DBL is negligibly small (Davison and Zhang 1994, Zhang and Davison 1995). Davison and Zhang (1994) compared concentrations of metals measured by DGT technique with test solutions (known content of metals) and recognized 97–100% recovery effect of equation prediction.

The hydrogels used for DGT technique contain 95% of water when fully hydrated. All of the water is effectively available for diffusion, there being no bound water. The diffusion of large molecules is likely to be affected by the hydrogel in the DGT devices; simple metal ions have been shown to diffuse freely with an effective diffusion coefficient indistinguishable from that for an ion in water (Zhang and Davison 1995). Humic and fulvic substances have much lower diffusion coefficients in water than simple metal ions (Zhang and Davison 1999). DGT technique only measures labile species and therefore will exclude kinetically inert organic species, large colloids and strong organic-metal complexes (Davison and Zhang 1994, Zhang et al. 1995, Harper et al. 1998).

Resin gel layer Chelex-100 is used for most of metals. Chelex-100 is polyacrylamide hydrogel comprising 15 vol % acrylamide and 0.3% patented agarose-derived cross linker (Zhang et al. 1998). Measurement is independent of pH in the range 5.0–8.3 and the ionic strength. The temperature also plays an important role, because diffusion coefficient and viscosity are temperature depend-

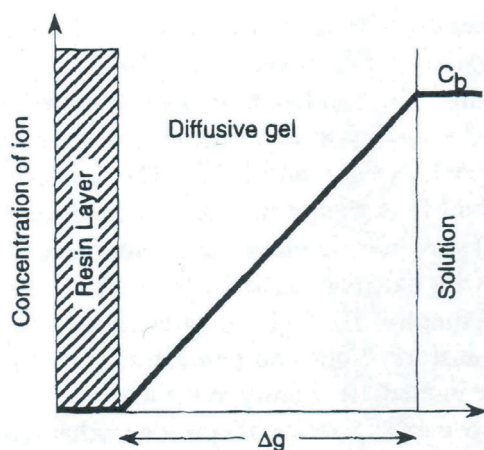


Figure 1. Schematic representation of the concentration of an ionic component through the gel and resin layer (Zhang et al. 1995); in this figure C_b is the concentration close to the DGT device (in text called C_{DGT}), Δg is the thickness of diffusive gel layer

ent. Fully quantitative interpretation is only possible if temperature is relatively constant ($\pm 2^\circ\text{C}$). Deployment time is from 1 h to typically 3 months till the resin becomes saturated in selected environment (water, sediment, soil). Maximum deployment time in natural waters is likely to be limited by microbial attack and biofouling (Zhang and Davison 1995).

For measurement in waters or sediments requiring only a few hours of deployment, a measurement *in situ* is possible, but for its application in soils is necessary to keep a long deployment time. In this case it is impossible to provide stable temperature conditions, which is very important for metal diffusion through a temperature sensitive diffusion coefficient for metals in solution. The variation of the temperature of several degrees during *in situ* 24 hrs depletion time can lead to a significant change in diffusion of metals through diffusion layers resulting in impossible calculation of some average diffusion coefficients. The *ex situ* measurement with DGT technique in laboratory under control conditions is then probably the only acceptable method at present.

The DGT device is continuously supplied by a diffusive flux from the pore water that depletes the pore water concentrations near the DGT device. This depletion effect is counteracted by a resupply from the metal bound to the solid phase and decreases with distance from the device (Harper et al. 1998). The interfacial concentration will be therefore less than the bulk concentration. When the depletion is insignificant, DGT calculated concentration will approach the bulk concentration (Harper et al. 1999). On the other hand it is possible to compare DGT concentrations of heavy metals with concentrations from bulk soil solution. The measured soil solution concentrations may be different from the actual soil solution concentration close to the DGT device. Suction cups that are usually used for investigation of element concentrations in soil solution have a length of 10 cm and thus average the soil solution over a much larger volume than the DGT device. A sample of 5 ml of soil solution usually represents more than 10 cm³ of soil, whereas DGT lowers the soil solution concentration only over a few millimeters of soil adjacent to the filter; thus, the depleted zone does not represent more than 1–2 cm³ (Nowack et al. 2004), which is possible to consider as the rhizosphere.

The aim of this work was to demonstrate the suitability of DGT technique for the assessment of the changes of heavy metal mobility in rhizosphere of four plant species with different accumulation capacity cultivated in pot experiments. Additionally, we have tested application of two different organic chelating agents and their effect on metal availability in rhizosphere.

MATERIAL AND METHODS

Pot experiment

DGT technique was used to simulate processes of heavy metals (cadmium, lead and zinc) release in the rhizosphere of selected plant species grown in the pot experiment involving four species with different heavy metal accumulation capacity. Hyperaccumulator Penny-cress (*Thlaspi caerulescens* J. et C. Presl), trees with a good accumulation capacity, willow (*Salix dasyclados* Vimm.) and poplar (*Populus nigra* L.), and crop maize (*Zea mays* L. hybrid Etendard) were tested in this experiment. Willow plants were cultivated in three treatments to investigate the effect of multiple application of chelating agents – untreated control, application of 1 mmol/l EDTA (ethylenediaminetetraacetic acid), and application of 1 mmol/l EDDS (S,S-ethylenediaminedisuccinic acid). Chelating agents were added three times during the vegetation.

Moderately contaminated Cambisol containing 5.46 mg Cd/kg, 956 mg Pb/kg and 279 mg Zn/kg was used for the experiment where 5 kg of air-dried soil was weighed in each pot. Plants were fertilized with NPK at the beginning and with a complex of macro- and micro- nutrients solution during the vegetation to optimize their growth. All pots were watered with demineralized water. Soil samples for DGT experiment were taken from individual pots after vegetation period, air-dried at 20°C, ground in a mortar and passed through a 2-mm plastic sieve.

DGT device

Materials for DGT technique were purchased from DGT Research Ltd. (Lancaster, United Kingdom). For the elements investigated in this experiment resin gel Chelex-100 and diffusive gel layers were used (Zhang and Davison 1995). The resin is allocated on one side of the resin gel. For the identification of the resin side the gel was placed on clear fluent plate and looked for grainy surface. For unambiguous identification of right side of the gel, a piece of clean tissue paper was placed on a small area of the gel to take up the excess water on the surface and the roughness caused by resin particles on the resin side was then visible.

Each DGT device consists of a 25 mm diameter plastic backing cylinder and a front cap with a window of 20 mm in diameter. A layer of resin gel is placed on the base with the side containing the resin facing upward. A layer of diffusive gel is placed directly on the top of it (Figure 2). In our experiment, 0.8 mm thick diffusive gel disc and

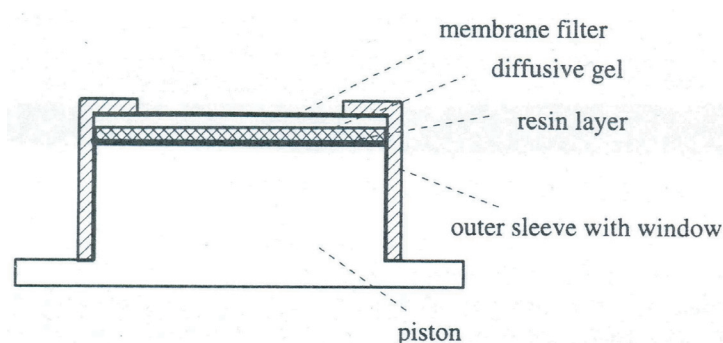


Figure 2. Schematic representation of a section through the DGT device (Zhang et al. 1998)

0.4 mm thick resin gel disc were used (Zhang et al. 1998). To prevent soil particles adhering to the gel surface 0.145 mm thick and 0.45 μm pore size hydrophilic polysulfone membrane filter (HT – 450 Tuffryn® Membrane Filter, diameter 25 mm, Pall Corporation, New York, USA) was placed on the top of the diffusive gel. Both gels and filter membrane should be moistened with a few drops of water. No bubbles between plastic base, both gels and filter membrane are acceptable and full contact of all layers is unavoidable for the best effect of DGT system. The hydrogel was wetted with demineralized water and stored in a sealed plastic bag in a refrigerator (Gimpel et al. 2001).

DGT procedure

For DGT procedure, the dry soil samples were re-humidified as follows: a portion of 10 g of dry soils was weighed from all variants to small plastic beakers in three replications and moistured with demineralized water to maximum water holding capacity and leaved 24 hrs to equilibrate in stable dark place (stable temperature, moisture, light). Dočekal et al. (2003) have recognized that metal ion concentration in the soil solution increases hyperbolically with decreasing soil moisture content. We humidified soil samples to maximum water holding

capacity to ensure a good reproducibility of tests with constant moisture. After this time, about 3.5 g of wet soil (with exactly measured weight and later specified moisture and dry weight – about 2.1 g of dry soil) was weighed on the top of signed DGT devices. Devices were placed in a small plastic box, covered with a film and stored at a dark place for 24 hrs at 24°C. We used a system to place a defined amount of soil on the top of device facing as shown on Figure 2. Next step was to open DGT device: the soil from device was washed out with a small amount of water, the plastic device was opened with a screwdriver, and membrane filter and diffusive gel layer were removed. Then, the resin gel layer was put into 1.5 ml plastic vial and 0.6 ml of 1 mol/l HNO_3 was added to elute metals from the resin gel, as described by Zhang et al. (1998). After 24 hrs 0.5 ml of eluate was diluted 6 times with demineralized water, mixed and immediately measured. Elements were determined simultaneously in diluted solutions by using an inductively coupled plasma optical emission spectrometer (ICP-OES, Varian Vista Pro, Varian, Australia).

Measured metal concentrations were tested for statistic deviation (Statgraphics Plus v. 5.0) and outliers were identified using Dixon's test. Mass of selected metals in resin gel and their concentrations in the rhizosphere were calculated and statistically evaluated by Student's *t*-test ($\alpha = 0.05$).

Table 1. Mean content of heavy metals in the selected plant species of aboveground biomass ($\bar{x}$ in mg/kg) and standard deviation (*s*), *n* = 6; contents of Cd and Pb were published and discussed in Fischerová et al. (2004)

Species	Cd		Pb		Zn	
	$\bar{x}$	<i>s</i>	$\bar{x}$	<i>s</i>	$\bar{x}$	<i>s</i>
<i>Thlaspi</i>	271	126	57.6	49.8	1501	495
<i>Salix</i> 0	57.5	15.3	5.67	1.94	900	230
<i>Salix</i> EDTA	27.9	16.6	206	43.1	371	155
<i>Salix</i> EDDS	47.6	14.7	23.3	11.9	723	244
<i>Populus</i>	21.3	3.52	14.4	5.39	502	127
<i>Zea</i>	2.67	0.355	7.59	3.18	152	22.3

RESULTS AND DISCUSSION

The element contents of Cd and Pb in plant biomass from the pot experiment were already published (Fischerová et al. 2004). On the other hand, content of Zn in plant biomass and the effect of chelating agent application on willows have not been published yet. The results are summarized in Table 1. Application of chelating agents lowered the content of cadmium (EDTA to 27.9 mg/kg; EDDS to 47.6 mg/kg) and zinc (EDTA to 371 mg/kg; EDDS to 723 mg/kg) in leaves of willows (EDTA more than EDDS) and statistically enhanced the content of lead (especially EDTA application – up to 206 mg/kg; EDDS application – 23.3 mg/kg).

The concentrations measured from the eluates were calculated by eq. 2 and eq. 3 to get metal concentrations in the resin gel and subsequently relevant content in rhizosphere (Figure 3). The results reflected the differences in the accumulation capacity of individual plant species. A relatively high variability of results is caused by the variability among the pots within one treatment. Hyperaccumulator *Thlaspi caerulescens* and crop *Zea mays* were able to take up a high amount of all the measured metals resulting in depletion of element content in rhizosphere compared to the control pots without plants, as demonstrated in Figure 3. But this depletion was statistically significant only for cadmium. In the case of both trees with a good heavy metal accumulation capacity (*Populus nigra* and *Salix dasyclados*) the element concentration in rhizosphere varied around control level for all metals with an insignificant difference. In this case, the removal of soil solution elements by plants was balanced probably by a release of metals bound to the solid phase in soil and their conversion to exchangeable and plant available forms due to the root exudates. Application of EDTA to *S. dasyclados* treatment caused a statistically significant increase of metal concentration in rhizosphere (Figure 4). On the other hand, only statistically non-significant depletion of metal concentration was registered after EDDS application compared to the variant without chelating agent application. There is an evident effect of EDTA on metal release from solid phase in soil and an enhancement of mobile Cd, Pb, and Zn concentrations in soil. However, enhanced Cd and Zn portions were not present in plant-available forms because the plant uptake of these elements did not increase at EDTA treatments. These findings are in a good agreement with the results published by Wu et al. (2004) in the case of *Brassica juncea*, and by Lai and Chen (2004) in the case of *Dianthus chinensis* and *Vetiver zizanioides*. Cui et al. (2004) reported that the application of EDTA at 8 mmol/kg increased soil mobile Pb (0.01 mol/l CaCl₂ extract-

able) by over 1000 mg/kg compared with 3 mg/kg, and Zn by 0.9 mg/kg compared with 0.3 mg/kg in the control. However, the Pb and Zn uptake by *Brassica juncea* shoots increased 10-fold, and 3-fold, respectively. Collins et al. (2002) observed that after the addition of 3.4 mmol/l EDTA per kg of soil 89 ± 9% of Zn in soil solution was present as Zn-EDTA complex. However, the mean concentration of Zn-EDTA in the xylem of *Hordeum vulgare* and *Solanum tuberosum* represented only 4.0–9.1% of mean concentration of Zn-EDTA measured in soil solution. Similar results were published for Pb-EDTA complex in *Triticum aestivum* (Crowdy and Tanton 1970). Therefore, the changes in element uptake by experimental plants did not reflect exactly the expectable changes in mobility of these metals in the rhizosphere soil solution. Evidently, a reasonable interpretation of the influence of EDTA

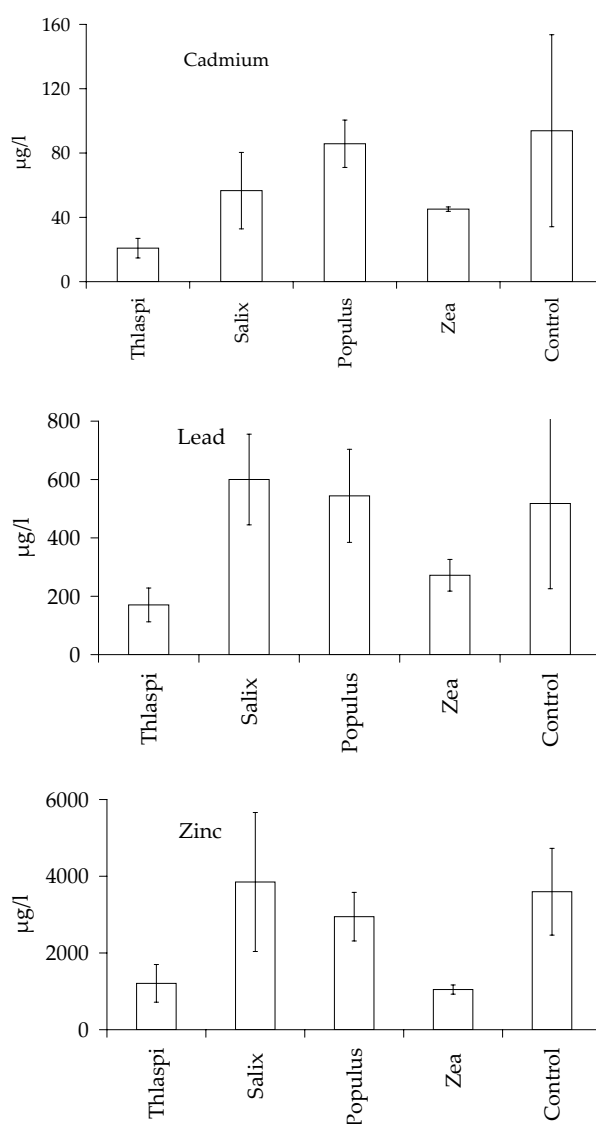


Figure 3. Content of heavy metals in the rhizosphere of different plant species (µg/l)

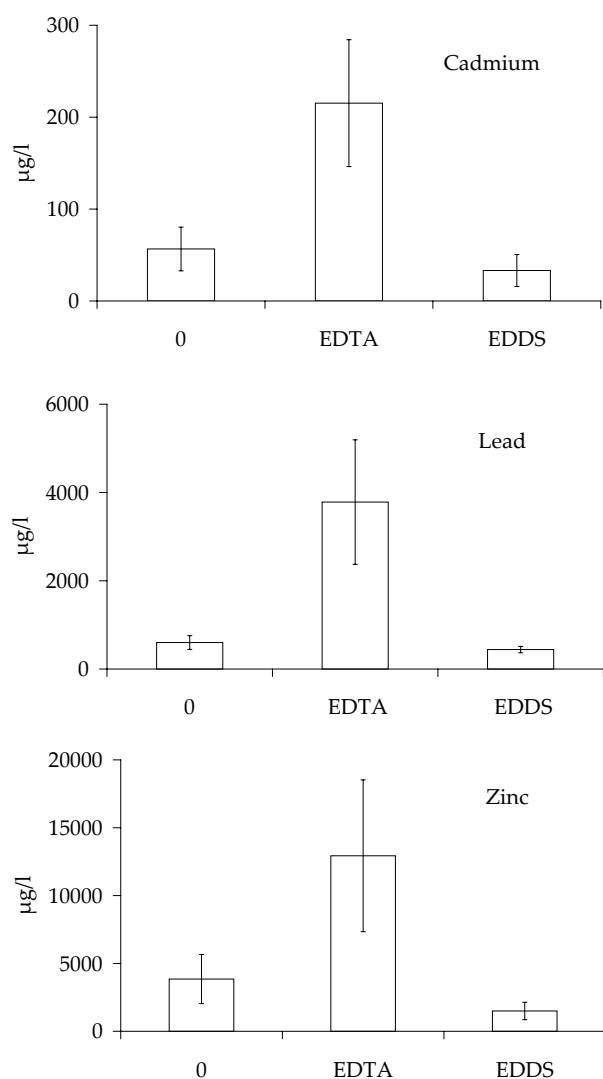


Figure 4. Content of heavy metals in the willow rhizosphere after chelate application ($\mu\text{g/l}$)

on plant-availability of the heavy metals is impossible without identification and quantification of free ions of individual metals.

The reasonable interpretation of the DGT results is essential for the assessment of rhizosphere processes. Zhang et al. (1995) tried to answer the question about the DGT interpretation. They found out in some experiments that Zn and Cd data obtained by DGT could be interpreted as concentrations, whereas the Ni, Cu, and Fe data could be interpreted as the maximum flux of metal from the solid phase to pore water. However, the differences between the behaviors of individual elements under differing physicochemical parameters of the investigated soils as well as the influence of roots and root exudates of individual plant species must be taken into account. Our results confirmed the suitability of DGT technique for the assessment of the changes of heavy metal (Cd, Pb and Zn) mobility in plant

rhizosphere. For the assessment of plant-available portion of these elements as well as for the prediction of element uptake by individual plant species a more detailed investigation of the composition of DGT eluate is necessary with regard to an element speciation in this solution.

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ABSTRAKT

Použití techniky difuzního gradientu (DGT) pro odhad změn mobility Cd, Pb a Zn v rhizosféře

V nádobovém vegetačním pokusu jsme testovali techniku DGT pro měření koncentrace Cd, Pb a Zn v rhizosféře čtyř různých druhů rostlin: hyperakumulátoru penízku (*Thlaspi caerulescens* J. et C. Presl), dřevin s dobrou akumulací kapacitou vrby (*Salix dasyclados* Vimm.) a topolu (*Populus nigra* L.) a zemědělské plodiny kukuřice (*Zea mays* L.). Dále byl zkoumán vliv dvou chelatujících látek (kyseliny ethylendiamintetraoctové – EDTA a kyseliny S,S-ethylen-diamindisukciniové – EDDS) na mobilitu prvků v rhizosféře. Pomocí DGT jsme byli schopni zaznamenat dokonce i malé změny v koncentracích těžkých kovů u jednotlivých plodin a (nebo) aplikací chelátů. Byl zjištěn významný vliv aplikace EDTA na zvýšení rozpustnosti kovů vázaných na pevnou fázi půdy, jejíž výsledkem byla zvýšená koncentrace mobilních obsahů kovů v rhizosféře. Výsledky demonstrují, že technika DGT je vhodnou metodou pro hodnocení chování kovů v systému půda – rostlina.

Klíčová slova: DGT; půdní roztok; mobilita prvků; rhizosféra; kadmium; olovo; zinek

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