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The use of differential pulse anodic stripping voltammetry and diffusive gradient in thin films for heavy metals speciation in soil solution

Research article

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Abstract: In the soil solutions obtained in situ with suction cups from soils (Cambisol and Fluvisol) of pot experiment with *Salix smithiana* Smith*, Lolium perenne* L. and *Thlaspi caerulescens* J. & C. Presl heavy metals species (Cd, Pb and Cu) were assayed by differential pulse anodic stripping voltammetry and diffusive gradient in thin films. Prediction of accumulation performed best at free metal ion concentrations in unchanged pH (in 10^{-3} mol L^{-1} $NaClO_4$ base electrolyte). The speciation provided by differential pulse anodic stripping voltammetry according to pH can provide a detailed description of the soil solution matrix. The concentration of free metals in unchanged pH represents a small part of the total content and varied from 0.04 to 0.75% with two exceptions found for accumulating plants (the content of Cd^{2+} in the soil solution from *T. caerulescens* was about 6% and the content of Cu^{2+} in the soil solution from *S. smithiana* was about 30%). The available concentration as determined by diffusive gradient in thin films was not in correlation with the heavy metals concentration in plant biomass.

Keywords: *Heavy metals • Soil solution • Speciation • DP-ASV • DGT*

1. Introduction

Environmental monitoring and speciation of heavy metals (HMs) in soil solution are very important for ecological assessments as well as for understanding of the relationship between plant and soil [1]. Exposure of plants to metals occurs primarily through the aqueous phase of soil, soil solution. Several plant and soil properties affect the composition of soil solution. In this case, composition of soil solution including concentration of potentially toxic metals in the solution and speciation of these elements, i.e. distribution of the elements among their various physical and chemical forms, and possible oxidation states (free ions, complexes, ion pairs, and chelates in solution) may influence the reactivity, mobility, and bioavailability of the elements [2]. The soil solution contains HMs in different forms, such as free metal ions, simple inorganic complexes, and complexes with organic ligands [3,4]. Organic matter in a soil solution is mostly represented by low molecular weight organic acids (LMWOAs) [5]. Relations between acids and their adducts are strongly dependent on pH.

In aquatic systems, consideration of free metal

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ion activities in solution has improved predictions of bioaccumulation and toxicity, and there is some evidence that solution speciation is also important in soil systems [4,6]. For trace metals speciation in natural waters, numerous analytical techniques have been used ranging from complexation studies with simple ligands to utrafiltration studies of the influence of organic matter and colloidal particles [7,8]. A special importance was given into two methods – anodic stripping voltammetry and diffusive gradients in thin films (DGT) [9,10].

Differential pulse anodic stripping voltammetry (DP-ASV) techniques have been used for more than 30 years for laboratory trace metal measurements in waters, and routine instrumentations have been developed [11-14]. Predominantly, the detection of total metal contents, after physical and chemical pre-treatments of samples (usually after sample acidification and UV irradiation, or speciation), was made by these conventional approaches [15]. Another important analytical application of voltammetry is the determination of complexation properties (stability constants and total ligand concentrations) with complexants in natural waters [4,16,17] and sediments [18]. The voltammetric measurement of several metal ion contents has been successfully applied to potassium nitrate or ammonium nitrate soil extracts [19,20].

DGT (diffusive gradient in thin films) is a very powerful technique for assay of the available portion of heavy metals in natural waters, sediments, and in soils [21-25]. This technique can be used like a preconcentration method for trace metals determination. Metals are accumulated on the resin layer (Chelex® 100) after passing through a diffusive hydrogel. After accumulation, metal ions from the resin layer are eluted using a known volume of HNO_3 solution.

As the DGT technique is expected to measure free ions and labile complexes similar to DP-ASV [21], no voltammetry on *in situ* soil solution samples has been performed up to now. Our study aims were:

- to examine the possibilities of DP-ASV in analysis of soil solutions taken from different soils occupied by three plant species,
- to compare ionic portions in soil solution of selected metals obtained from DP-ASV with available portions in soil solution detected by DGT and with total HM content in soil solution as determined by optical emission spectrometry or atomic absorption spectrometry,
- to characterize the impact of detected HM species on bioaccumulation in plants.

2. Experimental

2.1. Pot experiment

The hyperaccumulating plant *Thlaspi caerulescens* J. & C. Presl with a high ability to accumulate Cd and Zn [26], the fast growing tree *Salix smithiana* Smith with a high metal accumulation ability [27] and the grass *Lolium perenne* L. representing common pasture plants were cultivated in pot experiments in two soils from The Czech Republic (Fluvisol – location Píšťany and Cambisol – location Příbram) differing in total content of risk elements (Table 1), physical properties characterized with soil composition [28] (Table 2), and physical-chemical characteristics (Table 3). Plants were cultivated in 6 litres plastic pots with 5 kg of air-dried soil with four replicates for each treatment. The experimental soils were passed through a 5-mm plastic sieve. Soil moisture was regularly controlled and kept at 60% maximum water holding capacity. After plant harvest (5 months growth), the above-ground biomass was gently washed with deionized water, checked for fresh and dry biomass, ground up, and analyzed (Table 4).

The soil solution was sampled (Fig. 1) at the same time as the harvest (after 5 months of plant growth

Table 1. Total content of heavy metals (mg kg^{-1}) for investigated metals in both soils, n=4.

Locality	Cd	Pb	Cu
Příbram	3.21 ± 0.64	240.0 ± 9.6	32.0 ± 3.5
Píšťany	1.70 ± 0.78	65.4 ± 29.4	42.0 ± 2.6

Table 3. Soil properties (pH, CEC (Cation Exchange Capacity) and C_{ox} (percentage content of oxidizable carbon)) of investigated soils, n=4.

Locality	pH (H_2O)	pH (KCl)	CEC (mekv/kg)	C_{ox} (%)
Příbram	6.59 ± 0.05	5.77 ± 0.03	134 ± 11	1.90 ± 0.01
Píšťany	4.82 ± 0.02	3.85 ± 0.02	113 ± 6	5.11 ± 0.26

Table 2. Textural classes of investigated soils.

			Textural class (%)				
			Clay	I. Class	II. Class	III. Class	IV. Class
Locality	Soil type	Soil class	<0.002mm	<0.01mm	0.01-0.05mm	0.05-0.1mm	0.1-2.0mm
Píšťany	Fluvisol	Loamy soil	9.64	29.96	23.87	8.56	37.59
Příbram	Cambisol	Sandy loam	11.63	21.7	12.86	8.92	56.51

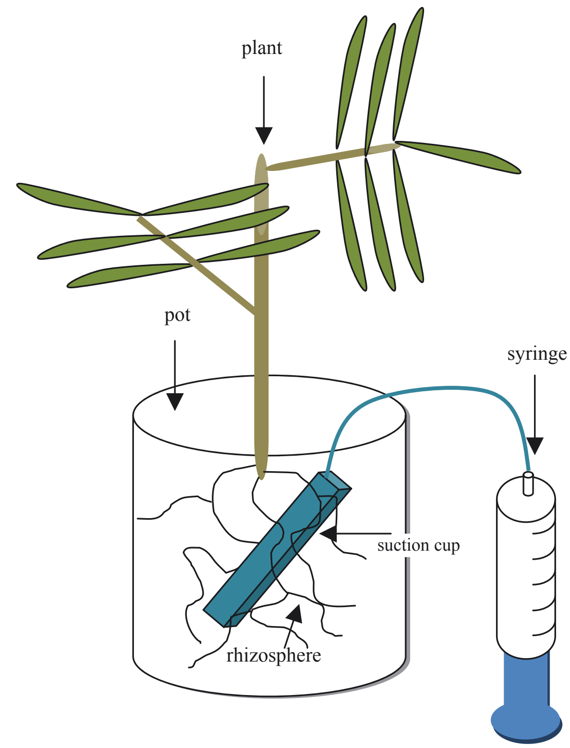

Figure 1. Design of the pot experiment for obtaining the soil solution.

using nylon suction cups (DI Gottfried Wieshammer, Wien, Austria)) and stored at 4°C until analysis (within 7 days). The soil solution (from 20 to 30 mL) was sampled after 24 hours of soil saturation with deionized water [29]. For sampling, storage, and analysis were used very clean, sterile containers and other glass oven-ware.

2.2. Chemicals and reagents

For DP-ASV analysis, purified water on Chelex® 100 (Fluka, Sigma-Aldrich Co.) was used. For base electrolytes preparation, $NaClO_4$ (p.a., Merck) and HNO_3 (Suprapur®, Merck) were used. Deionized and redistilled water (18 MΩ.cm^{-1}) (PCS Sybron Barnstead) was used for all analyses. For soil decomposition, HNO_3 (electronic grade purity, Analytika, Czech Republic) and HF (electronic grade purity, Analytika, Czech Republic) were used. Standard solutions ASTASOL Cd (1000 mg L^{-1}), Pb (1000 mg L^{-1}), Cu (1000 mg L^{-1}) and Zn (1000 mg L^{-1}) in 2% HNO_3 were purchased from Analytika, Czech Republic.

2.3. Instrumentation and analysis

The total contents of the focused metals in soil samples and in plant biomass were determinated by ICP-OES (Inductively coupled plasma optical emission spectroscopy) after decomposition by a dry ashing procedure as follows: An aliquot (~1 g) of the dried and powdered soil was weighed to 1 mg into a borosilicate glass test-tube and decomposed in a mixture of oxidizing gases ($O_2+O_3+NO_x$) at 400°C for 14 hours in a Dry Mode Mineralizer Apion (Tessek, Czech Republic) [30]. The ash obtained from plant biomass was dissolved in 20 mL of 1.5% HNO_3 and kept in glass tubes until analysis. The ash obtained from soil was decomposed in a mixture of HNO_3 and HF, evaporated to dryness at 160°C, and dissolved in *aqua regia* [31]. The oxidizable organic carbon (C_{ox}, see Table 3) was determined spectrophotometrically (Zeiss Spekol 221, Germany) after the oxidation of organic matter by $K_2Cr_2O_7$ (p.a. Merck, Czech Republic) [32].

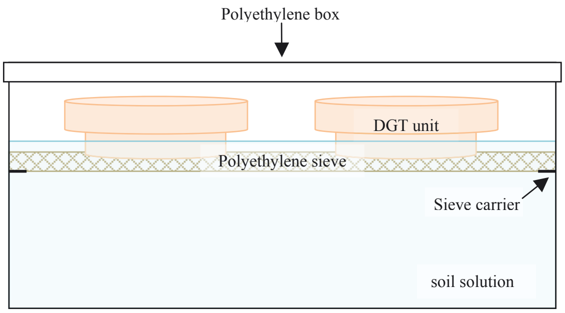

Figure 2. Design of the DGT units exposure to soil solution.

The total contents of focused elements in soil (Table 1) and in plant digests (Table 4) were determined by the ICP-OES (VARIAN VistaPro, Varian, Australia). Total contents of HMs in soil solutions (Table 5) as well as in DGT eluates (Table 6) were determined by flameless atomic absorption spectrometry (ET-AAS) using Zeeman atomic absorption spectrometer AA280Z with GTA 120 graphite tube atomizer and programmable sample dispenser PSD 120 (Varian, Australia). The pyrolytically coated tubes were used for all measurements and a forked platform was applied for Cd and Pb determination.

The ionic contents of elements in the soil solution were determined by the DP-ASV (Table 5) in different types of base electrolyte; in 10^{-3} mol L^{-1} $NaClO_4$ (unmodified pH, ~ 7), in 10^{-3} mol L^{-1} HNO_3 (~ pH 3) and in 10^{-2} mol L^{-1} HNO_3 (~ pH 2). The measurement was carried out in three electrodes connected with an HMDE (hanging mercury drop electrode) as a working electrode (the reference electrode was a saturated silver chloride electrode and the auxiliary electrode was a platinum electrode), on a PC-controlled Eco-Tribo Polarograph voltammetric analyzer (Polaro-Sensors, Prague, Czech Republic) with Polar 4 software. The accumulation time was 360 s and the accumulation potential was -1200 mV for Cd and Pb and -800 mV for Cu. The pH was measured on a pH 301i/SET (WTW, Germany).

Table 4. Content of heavy metals in plant biomass (mg kg^{-1}).

			Cd	Pb	Cu
plant	locality	part	mg kg^{-1}		
L. perenne	Příbram		0.43 ± 0.03	0.86 ± 0.22	3.83 ± 0.12
	Píšťany		0.21 ± 0.03	0.64 ± 0.07	3.75 ± 0.39
T. caerulescens	Příbram		112.22 ± 23.73	2.53 ± 1.00	85.51 ± 1.37
	Píšťany		53.88 ± 2.32	1.33 ± 0.58	64.77 ± 8.39
S. smithiana	Příbram	branches	5.79 ± 0.23	0.85 ± 0.02	16.63 ± 2.01
		leaves	14.17 ±1.33	3.49 ± 0.40	21.03 ± 2.48
	Píšťany	branches	3.09 ± 0.43	0.74 ± 0.03	18.65 ± 0.87
		leaves	5.75 ± 0.86	1.90 ± 0.15	20.06 ± 2.12

The DGT technique was used for determination of available HM species. DGT units (DGT REsearch Ltd., Lancaster, GB) consist of the Diffusive Gel Strip (0.8 mm) in 0.1 mol L^{-1} NaCl, the Chelex Gel Strip (0.4 mm) in redistilled water and rectified by Milipore® system and Tuffryn® Membrane Filter (25 mm, 0.45 µm, Pall Corporation). The DGT units were exposed for 24 hours (at 4°C) to the soil solution (50 mL of the soil solution collected from 4 pots). Polyethylene boxes were used to expose the soil solution to the DGT units (Figure 2). The DGT units were placed in polyethylene sieves and their exposure windows were immersed in soil solution. After exposure, the DGT units were disassembled and the chelex gel discs (resins) were leached for 24 hours (at 4°C) in 1.5mL vials containing 1 mL 2 mol L^{-1} HNO_3.

The total metal content in eluates was determined by ET-AAS. The available concentration of elements (ions) in the bulk solution C_b [µg L^{-1}] is calculated by [22]:

$$C_b = \frac{M \Delta g}{DtA} \qquad (1),$$

where M [µg] is the element mass adsorbed on area A [3.14 cm^2] within the time t [s], Δg [cm] is the thickness of diffusive layer (0.0935 cm), and D [cm s $^{-1}$] is the diffusive coefficient [23,24] for Cd, Pb and Cu (D_{Cd} = 3.13x10^{-6} cm^2 s^{-1}, D_{Pb} = 4.19x10^{-6} cm^2 s^{-1}, D_{Cu} = 3.25x10^{-6} cm^2 s^{-1}). M is calculated by [22]:

$$M = C_e \frac{V_g + V_e}{f_e} \qquad (2),$$

where C_e is the measured element concentration (by ET-AAS) in an eluate solution of 2 mol L^{-1} HNO_3, V_g [L] is the volume of resin, V_e [L] is the volume of elution solution, and f_e is the elution factor for elution reagent (for 2 mol L^{-1} HNO_3 is f_e = 0.8 [22]). Other symbols are stated before.

For comparison of C_b with C_{ion}, the C_b has to be corrected for the accumulation time used in the stripping analysis:

$$C_b - corr = \frac{C_b}{240} \qquad (3).$$

C_b-corr is C_b corrected for different accumulation times compare to the accumulation time used in stripping analysis.

3. Results and Discussion

3.1. Speciation of HMs in soil solution using DP-ASV

The total contents of HMs in soil (Table 1) did not have any correlation to the abundance of HM species in *in situ* sampled soil solution nor to the total content of HMs in plant biomass (Table 4).

The total contents of HMs in soil solutions showed more importance than the total contents of HMs in soil. The focused soils had different textures (Table 2) and different physical-chemical properties (Table 3) caused different amounts of HMs bound with soil matrixes [33,34]. The total soil solution contents of Cd, Pb and Cu (Table 5) were determined by ET-AAS and DP-ASV in 10^{-2} mol L^{-1} HNO_3 as base electrolyte (BE). The total contents determined by these two methods were in a very good agreement.

The equilibrium among HMs and their organic ligands is strongly depended on the pH [34] and the addition of mineral acid into soil solutions changes the balance among LWMOAs complexes and HM ions, as determined by our DP-ASV measurement at different pH values for the system. The ionic content of HMs increased with decreasing pH (after adding mineral acid). The percentage of ionic portions (PIP=C_{ion}/C_{total}x100) (Fig. 3-5) increased when the pH of the soil solution decreased. The determination of real ionic contents (C_{ion}) was provided in the original pH of the system (about 7) in 10^{-3} mol L^{-1} $NaClO_4$, a good BE for this analysis. The real ionic contents of tested elements were very low (with the exception of two

Table 5. The comparison among HM total contents (C_{total}) measured by ET-AAS and DP-ASV at pH 2 (in 10^{-2} mol L^{-1} HNO_3) and HM ionic contents (C_{ion}) measured by DP-ASV at original pH (in 10^{-3} mol L^{-1} $NaClO_4$, which varied from 6.39 to 7.40) and the ionic content of HMs measured by DP-ASV at pH 3 (in 10^{-3} mol L^{-1} HNO_3) in all focused soil solutions (μg L^{-1}).

	Salix smithiana Smith				*Thlaspi caerulescens Presl*				*Lolium perenne L.*			
Method	ET-AAS	ASV (HNO_3)	ASV (HNO_3)	ASV ($NaClO_4$)	ET-AAS	ASV (HNO_3)	ASV (HNO_3)	ASV ($NaClO_4$)	ET-AAS	ASV (HNO_3)	ASV (HNO_3)	ASV ($NaClO_4$)
Locality		0.01 mol L^{-1}	0.001 mol L^{-1}	0.001 mol L^{-1}		0.01 mol L^{-1}	0.001 mol L^{-1}	0.001 mol L^{-1}		0.01 mol L^{-1}	0.001 mol L^{-1}	0.001 mol L^{-1}
	Cadmium											
Příbram	0.84	0.80	0.45	0.001	0.90	0.84	0.28	0.061	0.86	0.85	0.30	0.001
	± 0.07	± 0.07	± 0.02	LOD	± 0.07	± 0.07	± 0.01	± 0.001	± 0.40	± 0.34	± 0.06	LOD
Píšťany	1.50	1.53	1.20	0.001	1.80	1.74	0.81	0.105	0.85	0.85	0.27	0.001
	± 0.10	± 0.12	± 0.05	LOD	± 0.12	± 0.08	± 0.08	± 0.060	± 0.02	± 0.02	± 0.01	LOD
	Lead											
Příbram	13.32	14.50	7.06	0.010	2.93	2.94	0.37	0.008	9.67	9.77	2.39	0.012
	± 1.31	± 1.43	± 0.68	± 0.001	± 0.25	± 0.19	± 0.01	± 0.001	± 0.40	± 0.34	± 0.06	± 0.001
Píšťany	1.15	1.23	0.35	0.008	0.94	1.10	0.78	0.007	1.26	1.40	0.75	0.008
	± 0.09	± 0.05	± 0.02	± 0.001	± 0.03	± 0.09	± 0.08	± 0.001	± 0.10	± 0.09	± 0.04	± 0.001
	Copper											
Příbram	45.68	41.97	40.00	13.33	50.31	48.39	43.60	0.023	38.83	30.01	12.34	0.022
	± 2.11	± 2.02	± 1.83	± 1.12	± 2.30	± 2.80	± 2.10	± 0.001	± 1.50	± 1.02	± 0.71	± 0.003
Píšťany	41.20	35.51	26.34	12.77	54.92	38.89	8.30	0.021	43.48	36.59	16.64	0.019
	± 1.91	± 2.04	± 1.03	± 1.11	± 3.00	± 1.90	± 0.90	± 0.002	± 1.40	± 0.98	± 0.65	± 0.002

cases, discussed further) compared to the total content of these elements in the soil solution (Table 5, Fig. 3-5). The ionic portions in pH 3 (in 10^{-3} mol L^{-1} HNO_3) were dissimilar and varied from 15% (Pb in soil solution from *T. caerulescens* (Příbram)) to 84% (Cu in soil solution from *S. smithiana* (Příbram)). There were many differences observed in the behavior of HMs during pH change. Each metal reacts to pH change in specific ways; the ionic release depends on the soil solution matrix. It is known [35] that plants can change the pH of the system by exudation and the methodology, which allows the observation of changes in HM mobility, is good for assessment of plant impact to the soil system.

The ionic portions of the focused HMs in all analyzed soil solutions were small, contrary to the levels observed in model aqueous solutions [21] or natural waters [8]. That is why the soil solution sampled *in situ* compared to less complex water systems is more complicated and contains many organic complexants compared to natural water systems mentioned [36,5,24].

3.2. The HM bioavailability

The knowledge of heavy metal behavior during pH change is essential in the evaluation of HM bioavailability and the ability to perform phytoextraction. The soil with lower pH affords greater phytoextraction abilities to HMs [37]. Surprisingly, the pH of the *in situ* sampled soil solution is higher (~ 7) than the soil itself (Table 3). What is more the differences between pH (H_2O) of the focused soils were almost 2 pH units (Příbram 6.59±0.05 and Píšťany 4.82±0.02), however the soil solution pH values were very similar (*S. smithiana* 7.40 (Příbram) and 6.70 (Píšťany), *T. caerulescens* 6.76 (Příbram) and 6.39 (Píšťany) and *L. perenne* 7.10 (Příbram) and 6.64 (Píšťany)). This fact appears to have a big impact of plant to soil solution pH values. This is in accordance with the opinion that root activity is the driving force in HM uptake and phytoextraction [38]. The plant efficiency of HM extraction (the HM´s bioavailability) is also affected by soil sorption parameters, cation exchange capacity (CEC) [39], and organic matter content [40,41]. The soil with high CEC and oxidizable carbon content is predestinated to fix HMs more than the soil with low CEC and low content of oxidizable carbon. The soil from Příbram has higher CEC (134 mekv kg^{-1}) and lower content of oxidizable carbon (1.90 ± 0.01%) than the soil from Přišťany (CEC = 113 mekv kg^{-1} and oxidable carbon 5.11 ± 0.26%). These two parameters have only predicative value, whereas the bioavailability strongly depends on the quality of organic matter. A more important bioavailability factor is an abundance of HM species in the soil solution according to [42]. If we compare the total HM content taken up into plant biomass with the ionic content of HMs in soil solution, we found that

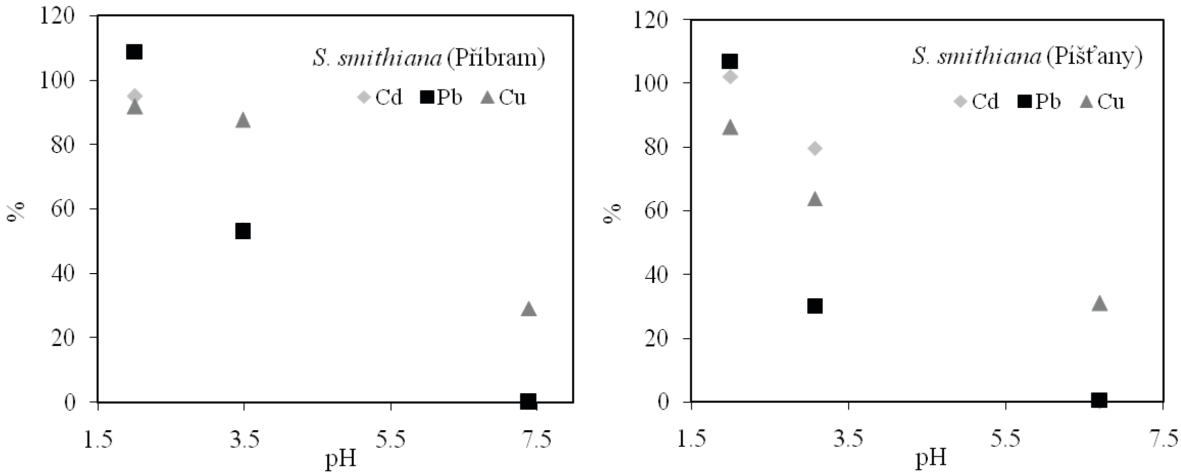

Figure 3. Dependence of ionic portion (in %) measured by DP-ASV on pH of Salix smithiana Smith soil solution grown at two localities. The total contents of metals were measured by ET-AAS and amount to 100%.

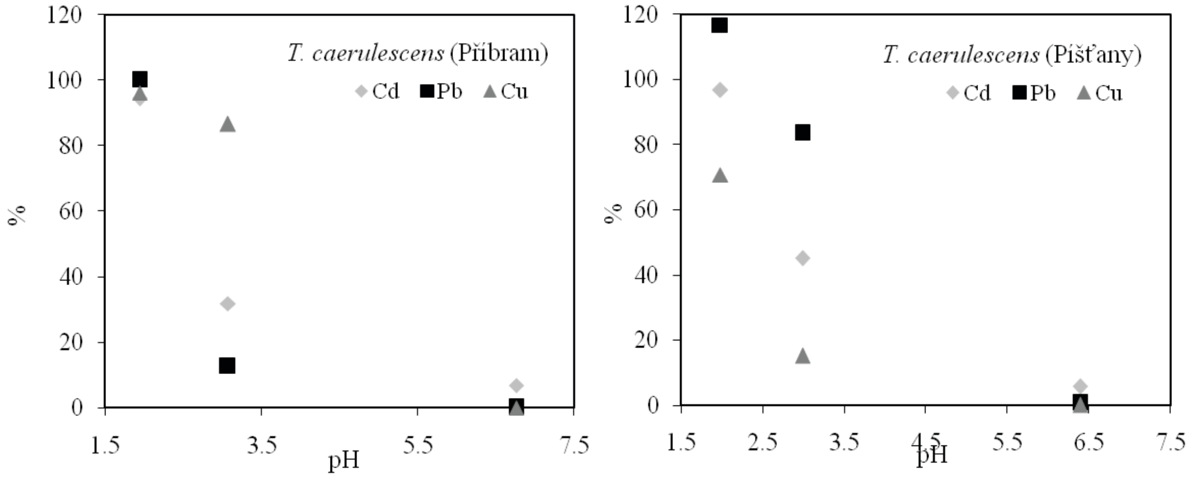

Figure 4. Dependence of ionic portion (in %) measured by DP-ASV on pH of *Thlaspi caerulescens* J. & C. Presl soil solution grown at two localities. The total contents of metals were measured by ET-AAS and amount to 100%.

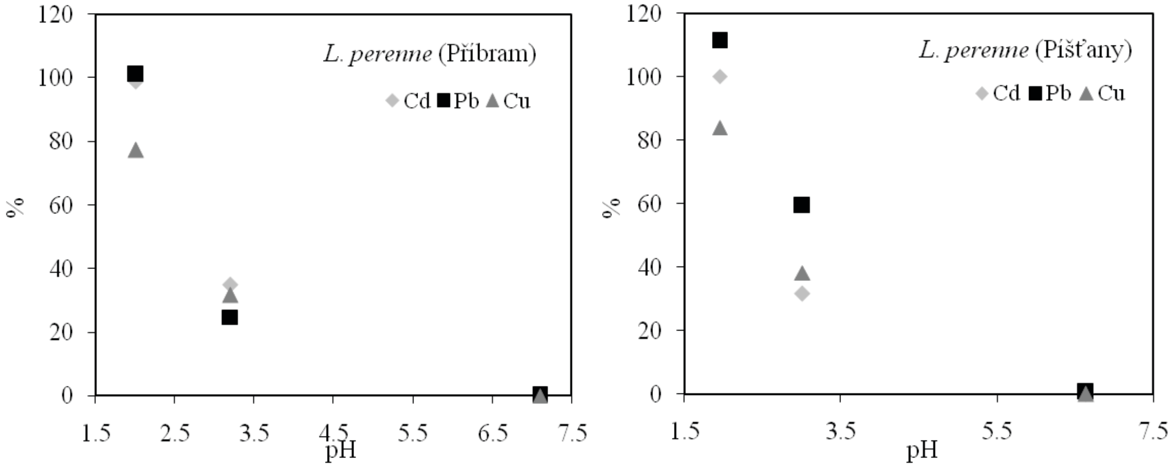

Figure 5. Dependence of ionic portion (in %) measured by DP-ASV on pH of *Lolium perenne* L. soil solution growth on both localities. The total contents of metals were measured by ET-AAS and amount to 100%.

Table 6. The comparison among C_b (available content of heavy metals determined by DGT, equation (1)), C_b-corr (Cb corrected to 360 s of accumulation according to: C_b-corr = C_b/240) (μg L^{-1}).

plant	locality	C_b			C_b-corr			C_{ion}		
		Cd	Pb	Cu	Cd	Pb	Cu	Cd	Pb	Cu
		μg L^{-1}								
S. smithiana	Příbram	0.103	3.020	7.850	0.0004	0.0126	0.0327	0.001	0.010	13.330
		± 0.029	± 0.315	± 0.961	± 0.0001	± 0.0013	± 0.0040	LOD	± 0.001	± 1.120
	Píšťany	0.230	2.220	6.040	0.0010	0.0093	0.0252	0.001	0.008	12.770
		± 0.038	± 0.274	± 0.492	± 0.0002	± 0.0011	± 0.0021	LOD	± 0.001	± 1.110
T. caerulescens	Příbram	0.096	2.400	5.570	0.0004	0.0100	0.0232	0.061	0.008	0.023
		± 0.013	± 0.566	± 1.220	± 0.0001	± 0.0024	± 0.0051	± 0.001	± 0.001	± 0.001
	Píšťany	0.201	1.920	6.500	0.0008	0.0080	0.0271	0.105	0.007	0.021
		± 0.012	± 0.188	± 0.447	± 0.0001	± 0.0008	± 0.0019	± 0.060	± 0.001	± 0.002
L. perenne	Příbram	0.235	3.310	5.800	0.0010	0.0138	0.0242	0.001	0.012	0.022
		± 0.017	± 0.678	± 0.897	± 0.0001	± 0.0028	± 0.0037	LOD	± 0.001	± 0.003
	Píšťany	0.175	2.040	4.790	0.0007	0.0085	0.0199	0.001	0.008	0.019
		± 0.022	± 0.257	± 0.392	± 0.0001	± 0.0011	± 0.0016	LOD	± 0.001	± 0.002

the ionic content of HMs in soil solution is a crucial parameter for estimation of phytoextraction range. In the soil solutions from *L. perenne* at original pH the HMs were not in ionic form (they are complexed) and they were converted into free forms after acidification to pH 3 (30%) (Fig. 4) and the phytoextracted amounts of HMs were very low (Table 4), which is in agreement with [43]. In the soil solutions from *S. smithiana* and *T. caerulescens* there were differences in uptake among the focused HMs, because *S. smithiana* and *T. caerulescens* have different growing strategies [35], and it is better to allude about each focused HM separately.

The values of the ionic portions of Pb determined in all soil solutions in unchanged pH were in the same order and negligibly small (varied from 0.08 to 0.75%). This is in a good agreement with low expected Pb availability (mobility) in soils and with low phytoextracted content in the plant biomass (in all three potted plants) (Table 4). On the other hand, the ionic portions of Cd in soil solutions from *T. caerulescens* were relatively high; ionic portions of Cd were 5.84% in Píšťany and 6.87% in Příbram, compared to ionic portions of Cd in soil solutions from *S. smithiana* and *L. perenne*, which varied from 0.07 to 0.12%. This is correlated to the high accumulation of Cd in *T. caerulescens* biomass (Table 4) and with the hyperaccumulating ability of Cd in *T. caerulescens* biomass [35]. The ionic portions of Cu in soil solutions from *S. smithiana* were relatively high, 29.17% in Příbram and 31.00% in Píšťany. In other soil solutions Cu content varied from 0.04 to 0.06%. These differences are supported by a relatively high Cu concentration in *S. smithiana* biomass (Table 4), and are also supported by [27]. On the other hand the concentration of Cd in *S. smithiana* biomass was relatively high (vary from 3.09 to 14.17 mg kg^{-1}, Table 4), however the concentration of Cd^{2+} in the soil solution (at original pH) was negligibly small (Fig. 3). This could be caused by dissimilar plant uptake strategies of Cu and Cd as mentioned by [44,45] and also by the fact that the high content of Cd in *S. smithiana* biomass can be affected by the ease of Cd release from the soil solution matrix, which was supported with measurement of Cd^{2+} at pH 3 (Table 5). The amount of Cd^{2+} at pH 3 in the soil solutions from *S. smithiana* were about 55% (Příbram) and 80% (Píšťany).

The situation is similar in the case of copper in the soil solutions from *T. caerulescens*. The concentration of Cu in *T. caerulescens* biomass was quite high (85.51 ± 1.37 μg g^{-1} (Příbram) and 64.77 ± 8.39 μg g^{-1} (Píšťany)), and the concentration of Cu^{2+} in the soil solutions (at original pH) was negligibly small, but after acidification to pH 3 the copper was present in ionic forms about 85% in Příbram and about 35% in Píšťany.

According to these facts, the ionic content and ability to release ions due to acidification can be considered as the driving forces of phytoextraction.

3.3. Comparison of ionic content and DGT-available content of HMs

We compared ionic portion detected by DP-ASV (C_{ion}) with the plant available portion (C_b) obtained by DGT (Table 6). The values obtained by DGT were not comparable with the concentration of ions (C_{ion}), contrary to similar measurements in model aqueous solutions or natural waters published by [21,8,46].

This disagreement was due to a more complicated soil solution matrix compared to model aqueous solutions and longer exposure time (in the articles mentioned the exposure time was on the order of minutes and in our study was on the order of hours). We used longer exposition times due to low HM concentrations in the soil solution. The longer exposure times allowed withdrawal of free HM ions by capturing them in the resin and was compensated by disintegration of the complexes present, according to their different stability constants. As a consequence of HM complex elimination, the C_b is orders of magnitude higher than the C_{ion} and did not reflect the real situation in the soil solution. The DGT-available concentrations are almost the same for all focused HMs in all soil solutions. No correlation with concentration of these HMs was found in the plant biomass. In addition, we suggest correcting C_b for the same accumulation time as was used in the DP-ASV measurement (360 s) according to equation (3). C_{ion} and C_b-corr then become comparable (10^{-4} µg L^{-1}) for all soil solutions from *S. smithiana* and *L. perenne.* The C_{ion} and C_b-corr of Pb were on the same order (varied from 8.02×10^{-3} to 1.26×10^{-2} µg L^{-1}) in all focused soil solutions and the C_{ion} and C_b-corr of Cu were on the same order (10^{-2} µg L^{-1}) in all soil solutions from *T. caerulescens* and *L. perenne*.

Only two discrepancies were found: for Cd in the soil solutions from *T. caerulescens* and for Cu in the soil solutions from *S. smithiana.* In both cases, higher ionic portions were found by voltammetry in the soil solutions.

According to the data mentioned, the DGT technique is less suitable in complicated matrixes (such as soil solution) where the long term accumulation (due to low concentrations) is sought as compared to DP-ASV, which gives predicative values of free HM ions content.

4. Conclusion

In this study, the methodology for HM speciation according to pH changes in soil solution was developed using DP-ASV measurements. DP-ASV measurements in 10^{-3} mol L^{-1} $NaClO_4$ can be used for determination of Cd^{2+}, Pb^{2+} and Cu^{2+} concentrations in the soil solution at original pH (C_{ion}), which is suitable for assessment of HM bioavailability in plants. The dependence of the physical-chemical balance in soil solution on pH can be studied by DP-ASV in different concentrations of HNO_3 as base electrolyte. Data from these measurements can be used in assessment of HM bioavailability in plants.

The C_b (DGT-available concentration of metals) concentration did not have predicative value in bioavailability assessments of HMs (any serious correlation among the total concentration of HM in plant biomass and C_b was not found). The C_{ion} was compared to C_b and values of these concentrations were not on the same order; C_{ion} was much lower than C_b. Alternatively, agreement in order of magnitude of DGT and DP-ASV was provided by recounting C_b for the same accumulation time (conversion to C_b-corr) as was used for voltammetric measurements. The orders of magnitude of C_{ion} and C_b-corr were in agreement among themselves, besides two exceptions (cadmium in the soil solution from *T. caerulescens* and copper in the soil solution from *S. smithiana*). Neither C_b nor C_b-corr reflected the plant accumulation specifics, compare to C_{ion}, which is a very good characteristic for HM accumulation into plant biomass.

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References

[1] B. Walna, Cent. Eur. J. Chem. 5(1), 349 (2007)

[2] A.L. Nolan, M.J. McLaughlin, S.D. Mason, Environ. Sci. Technol. 37, 90 (2003)

[3] A.M. Ure, C.M. Davidson (Eds), Chemical Speciation in the Environment (Blackie Academic Press and Professional, Glasgow, 1995)

[4] J. Buffle, M.L. Tercier-Waeber, in: J. Buffle, G. Horvai (Eds), In situ Monitoring of Aquatic Systems; Chemical Analysis and Speciation, IUPAC Ser. Anal. Physical Chem. Environ. Syst. 6 (John Wiley & Sons, Chichester, 2000)

[5] D.L. Jones, P.G. Dennis, A.G. Owen, P.A.W. van Hees, Plant Soil 248, 31 (2003)

[6] A. Kabata-Pendias, H. Pendias, Trace Elements in Soils and Plants, 3rd edition (CRC Press, Boca Raton, 2001)

[7] H. Zhang, W. Davison, Pure Appl. Chem. 73(1), 9 (2001)

[8] S. Meylan, N. Odzak, R. Behra, L. Sigg, Anal. Chim. Acta 510, 91 (2004)

[9] J. Viers, B. Dupré, M. Polvé, J. Schott, J.-L. Dandurand, J.-J. Braun, Chem. Geol. 140, 181 (1997)

[10] B. Dupré, J. Viers, J.-L. Dandurand, M. Polvé, P. Bénézeth, P. Vervier, J.-J. Braun, Chem. Geol. 160, 63 (1999)

[11] J. Buffle, M.-L. Tercier-Waeber, Trends Anal. Chem. 24, 172 (2005)

[12] J.C. Van Loon, R.R. Barefoot, Analyst 117, 563 (1992)

[13] J.F. Nolan, M.A. Gaillard in: M. Taillefert, T.F. Rozan (Eds), Environmental Electrochemistry: Analysis of Trace Element Biogeochemistry, American Chemical Society Symposium Series No. 811 (Washington, 2002)

[14] P. Ugo, L. Sperni, G.A. Mazzocchin, Ann. Chim. (Rome) 92, 301 (2002)

[15] N. Finžgar, D. Leštan, Chemosphere 63, 1736 (2006)

[16] H.B. Xue, L. Sigg, In: M. Taillefert, T.F. Rozan (Eds), Environmental Electrochemistry: Analysis of Trace Element Biogeochemistry, American Chemical Society Symposium Series No. 811 (Washington, 2002)

[17] A.M. Mota, M.M.C. dos Santos, In A. Tessier and D.R. Turner (Eds), Metal Speciation and Bioavailability in Aquatic Systems (John Wiley and Sons, New York, 1995) 205

[18] M.M.C. dos Santos, M.F. Vilhena, M.L.S. Gonçalves, Anal. Chim. Acta 441, 191 (2001)

[19] S. Sauvé, W.A. Norvell, M.B. McBride, W.H. Hendershot, Environ. Sci. Technol. 34, 291 (2000)

[20] T. Nedeltcheva, M. Atanassova, J. Dimitrov, L. Stanislavova, Anal.Chim. Acta 528, 143 (2005)

[21] H. Zhang, W. Davison, Anal. Chem. 67, 3391 (1995)

[22] H. Zhang, W. Davison, B. Knight, S. McGrath, Environ. Sci. Technol. 32, 704 (1998)

[23] K.W. Warnken, H. Zhang, W. Davison, Anal. Chem. 77, 5440 (2005)

[24] S. Scally, W. Davison, H. Zhang, Anal. Chim. Acta 558, 222 (2006)

[25] Ø.A. Garmo, O. Røyset, E. Steinnes, T.P. Flaten, Anal. Chem. 75, 3573 (2003)

[26] A.J. Pollard, K.D. Powel, F.A. Harper, J.A.C. Smith, Crit. Rev. Plant Sci. 21, 539 (2002)

[27] M. Vysloužilová, P. Tlustoš, J. Száková, Plant Soil Environ. 49, 542 (2003)

[28] Soil Survey Staff: Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys, USDA-SCS, Agric. Handb. 436 (U.S. Gov. Print. Office, Washington, 1975)

[29] J. Dytrtová, M. Jakl, I. Šestáková, P. Tlustoš, J. Balík, In: The Book of Abstracts. The Seventh European Meeting on Environmental Chemistry (EMEC7), 6.-9.12.2006, Brno (Brno, 2006) 50

[30] D. Miholová, P. Mader, J. Száková, A. Slámová, Z. Svatoš, Fresenius J. Anal. Chem. 345, 256 (1993)

[31] J. Száková, P. Tlustoš, J. Balík, D. Pavlíková, V. Vaněk, Fresenius J. Anal. Chem. 363, 594 (1999)

[32] J.R. Sims, V.A. Haby, Soil Science 112, 137 (1971)

[33] P. Tlustoš, J. Száková, A. Stárková, D. Pavlíková, Cent. Eur. J. Chem. 3(4), 830 (2005)

[34] F.D. Dakora, D.A. Phillips, Plant Soil 245, 35 (2002)

[35] M. Arienzo, In: I. Shtangeeva (Ed.): Trace and Ultratrace Elements in Plants and Soil, (WIT Press, Southampton, 2005) 69

[36] D.L. Jones, Plant Soil 205, 25 (1998)

[37] K.J. Reddy, L. Wang, S.P. Gloss, Plant Soil 171, 53 (1995)

[38] M.-H. Feng, X.Q. Shan, S.Z. Zhang, B. Wen, Chemosphere 59, 939 (2005)

[39] R. Moore, W.D. Clark, K.R. Stern, Botany (WCB Publishers, Dubuque, 1995)

[40] C. Bliefert: Umweltchemie (Wiley Europe-VCH, Weinheim, New York, 1994)

[41] Z. Li, L.M. Shuman, Soil Sci. 161, 656 (1996).

[42] I. Atanassova, Water Air Soil Pollut. 113, 115 (1999)

[43] S. Madyiwa, M.J. Chimbari, C.F. Schutte, J. Nyamangara, Phys. Chem. Earth 28, 859 (2003)

[44] R. Unterbrunner, M. Puschenreiter, P. Sommer, G. Wieshammer, P. Tlustoš, M. Zupan, W.W. Wenzel, Environ. Pollut. 148, 107 (2007)

[45] M.N. Dos Santos Utmazian, G. Wieshammer, R. Vega, W.W. Wenzel, Environ. Pollut. 148, 155 (2007).

[46] H. Zhang, W. Davison, Anal. Chem. 72(18), 4447 (2000)