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INVESTIGATIONS ON ARSENIC MOBILITY CHANGES IN RIZOSPHERE OF TWO FERNS SPECIES USING DGT TECHNIQUE

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Abstract. The aim of this study was to investigate the effects of *Dryopteris filix-mas L.*, one of the indigenous fern species from Romania, and of a known As-hyperaccumulator, *Peris cretica L.*, on As bioavailability at the roots-soil interface. The ferns were grown in pot experiments on two composite soils collected from two localities from north-western Romania, containing 108 mg kg^{-1} dw and respectively 230 mg kg^{-1} dw total As. The Diffusive Gradient in Thin-films (DGT) technique was used to study the root-available As in soils, by measuring DGT-labile As fraction before and after plants grown. The *R* ratios between labile As concentration measured by DGT and As in soil solution were calculated and two different behaviors were observed for the two ferns: in rizosphere of *Pteris cretica L.*, *R* increased after plants grown, which indicate a greater proportion of As in labile form, probably due to the As solubilisation by fern roots, while contrary, in the rizosphere of *Dryopteris filix-mas L.* this ratio decreased. Also, was observed that higher organic carbon (in its dissolved form) and phosphorous in soil enhanced As biovailability. *Pteris cretica L.* accumulated higher concentrations of As in the aboveground part, the translocation factor being about 7.5, but for *Dryopteris filix-mas L.* this factor was <1, thus this cannot be considered As-hyperaccumulator. This study has also confirmed that DGT technique is a sensitive tool for the evaluation of As mobility changes in the rizosphere of plants.

Key words: arsenic, Diffusive Gradients in Thin-films, *Dryopteris filix-mas L.*, *Pteris cretica L.*, rizosphere, phytoremediation

1. INTRODUCTION

Arsenic (As) is a widespread metalloid, which in high concentration represents a serious environmental and human health problem. Exposure to As grows the risk of cancer, neurological maladies or Type 2 Diabetes (Anderson et al., 2011) and due to its high toxicity, As is ranked on the first position in the U.S. Department of Health and Human Services list of priority substances (ATSDR). In uncontaminated soils, the levels of arsenic concentration are regularly bellow 10 mg kg^{-1} (Adriano, 2001), but different pollution sources increased seriously arsenic or other toxic metals concentrations in soil in many sites in the world (Stefanescu et al., 2011). As a consequence, great efforts have been made in the last years to remediate the polluted sites, and numerous studies regarding the development of new efficient remediation techniques have been initiated (Lacatusu et al., 2012).

Phytoremediation is commonly based on the use of terrestrial plants for remediation of polluted soils by phytoextraction or by phytostabilisation and represents good alternative to the classical remediation technologies which are expensive, time-consuming and can produce new waste (Fitz et al., 2003). Hyperaccumulators plants, by definition, should be tolerant to the high metal concentrations and should accumulate high amounts of metals in their aboveground part (McGrath et al., 2001). Generally, these plants accumulate two to three orders of magnitude more of an inorganic element than "normal" plants (Wei & Chen, 2006). The mechanisms that lead to the hyperaccumulator behaviour of these plants are still not enough explored and not well understood. Plants, by their roots, actively modify conditions in the rhizosphere soil by releasing root exudates, such as low molecular weight organic acids with influence on pH and thus on metals mobility (Oburger et al., 2009). Understanding As accumulation in plants by monitoring its mobility in rizosphere is necessary in order to progress sustainable As phytotechnologies and to find appropriate candidates for phytoremediation (Bergqvist & Greger, 2012).

Normally, As uptake by normal plants is low, but several As-hyperaccumulator fern species of the family *Pteridaceae* have been discovered, among them *Pteris vittata L.* and *Pteris cretica L.*, and theirs capability to accumulate As has been evaluated in several studies in the last decade (Luongo & Ma, 2005; Silva Gonzaga et al., 2006; Fayiga et al., 2007; Cattani et al., 2009; Shelmerdine et al., 2009; Mandal et al., 2012). However, it is also very important to explore the potential of native plant species for phytoremediation processes because these plants are already adapted to the local environment and theirs chances for survival under extremes environmental are higher than plants hosted from other climates (Mahmud et al., 2008).

To study the metals mobility, and to account the root-available fraction of metals in soil, sequential or single chemical extraction procedures for the measurement are commonly applied. Different chemical extractants are regularly used: chelating agents such as EDTA (ethylene-diamine-tetraacetic acid) or DTPA diethylene-triamine-pentaacetic acid), solutions of strong or weak acids (dilute HCl, CH_3COOH, etc.), neutral salts ($CaCl_2$, $NaNO_3$, NH_4NO_3, etc.), water, each of them extracting metals from different fractions of soil (Madrid et al., 2007). Nevertheless, chemical extractions are not very good predictors of metals accumulation in plant tissues, because they can determine the metals pool potentially accessible to plants, but not account for the reduction of metals concentration at the root-soil interface and the resupply from the soil solid phase (Muhammad et al., 2012). The newly developed Diffusive Gradient in Thin-films (DGT) is a sampling technique that makes possible the measurement of the fluxes of labile metal species in soil pore-water. DGT technique uses a simple device that contains a layer of resin impregnated in a hydrogel which accumulate the metallic ions, overlaid by a diffusive layer of hydrogel through which metallic ions passed according to their coefficients of diffusion. Because only the dissolved metal species can pass through the diffusive gel, only these species can be accumulated in the resin gel. The diffusion of metals ions through the diffusive layer is based on Fick's first law of diffusion. Thus, by measuring the metal mass accumulated in resin gel, the concentration of free metals species in water or in soil solution can be calculated (Davison & Zhang, 1994, Senila et al, 2008).

For DGT measurements in soil, this is mixed with a known amount of water to obtain slurry, then DGT device is deployed for a known time and the mass of metal on the resin layer is measured after elution with nitric acid by a spectrometric method (e.g. ICP-AES, ICP-MS) (Muhammad et al., 2012).

Coupling DGT measurements with a numerical program 2D DIFS (2D DGT induced fluxes in sediments and soils), created by Sochaczewski et al., (2007), allows the measuring of effective concentration (C_E) of a metal in soil, which account both the depletion at the root-soil interface and the resupply from the solid-phase reservoir and thus mimics the processes from the root-soil interface (Tandy et al., 2011). Several authors reported good correlations between effective concentration measured by DGT and metals accumulation by plants (Zhang et al., 2001; Mason et al., 2008), rendering it a promising instrument for evaluation of phytoavailability.

The aim of this study was to compare As mobility changes in the rizosphere of two fern species, one of them known as hyperaccumulator, Pteris cretica L., and an indigenous fern from Romania, *Dryopteris filix-mas L.*, (Tanase et al., 2009) grown in pot experiments on As-contaminated soils. Soil samples were collected from two localities from Baia Mare area, northwestern Romania, known for the metal pollution problems (Damian et al., 2008; Lăcătuşu & Lăcătuşu, 2008; Senila et al., 2011; Big et al., 2012; Senila et al., 2012a). The effect of plants roots on As mobility in soils was studied using Diffusive Gradient in Thin-films (DGT) technique. Also, the accumulation of arsenic in roots and shoots of the two ferns was determined in order to assess the potential use for phytoremediation of As-contaminated soil.

2. EXPERIMENTAL

2.1. Pot experiments

Top soil samples (0-20 cm) were collected from two localities from Romania, Maramures County: Sasar village (SA) and Ferneziu district of Baia Mare city (FE), located between 23˚29'- 23˚39' Est longitude and 47˚38'- 47˚44' North latitude, as presented in figure 1. The industry developed in this area was based mostly on mining and processing of non-ferrous minerals, and although the extracting and smelting activities in Baia Mare area have generally

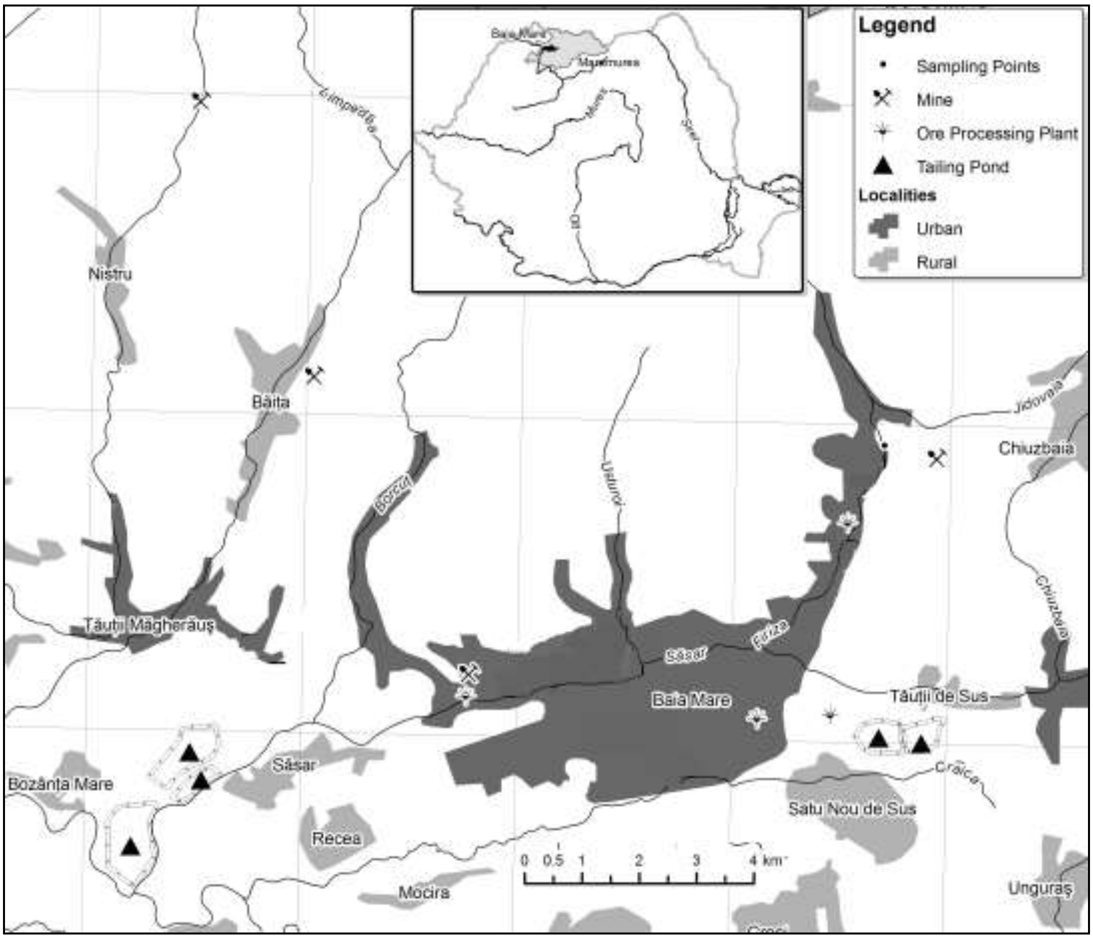


Figure 1. Map of Baia Mare and Săsar localities, NW Romania

ceased, the metals (including As) concentrations in soil are above the maximum allowable limits. For the laboratory experiment, composite soil samples were collected from each of the two localities, from cultivated fields. Fresh soil was passed through a 2 mm mesh sieve, homogenized and then the soil characteristics (including total and mobile As of soil) were determined before the experiments. For each composite soil, six parallel pot experiments were carried out, by growing three specimens of *Pteris cretica L.* and three specimens of *Dryopteris filix-mas L.*

A known mass (3.5 kg) of composite soil was placed in each plastic pot of 20 cm in height and 15 cm in diameter in which was introduced a plastic border covered with nylon mesh cloth (45 microns), with the dimensions of 16 cm in height and 8 cm in diameter. Thus, two compartments were created in each pot: rizosphere soil compartment, in which were introduced the roots of the fern and external compartment (bulk soil). *Pteris cretica L.* specimens, previously grown on As-uncontaminated soil, were obtained from Cluj-Napoca Botanic Garden. Specimens of *Dryopteris filix-mas L.*, of similar size, were collected from a forest from Cluj County, Romania, where the As content in soil was below 5.5 mg kg^{-1}. After sampling, five specimens of *Dryopteris filix-mas L.* were analysed, according to the procedure described below, and averages of the total As concentration were 1.25 mg kg^{-1} dw in roots and 0.88 mg kg^{-1} dw in shoots. These values were used as blank for the calculation of As accumulation in *Dryopteris filix-mas L.* after pot experiments. The transplanted ferns were grown for three months. The plants were watered during the study with distilled water and kept at an average temperature night/day of 15/30˚C. In total, twelve pot experiments were generated: three pots containing soil from Sasar, cultivated with *Pteris cretica L.* (SA-PT1-3), three pots containing soil from Sasar, cultivated with *Dryopteris filix-mas L.* (SA-DF1-3), three pots containing soil from Ferneziu, cultivated with *Pteris cretica L.* (FE-PT1-3), and three pots containing soil from Ferneziu, cultivated with *Dryopteris filix-mas L.* (FE-DF1-3). At the end of experiments, the roots and shoots of plants were separately harvested for the analysis of accumulated As. Soil from rizosphere compartment (adhered to the roots) and bulk soil were sampled and analysed for total As, mobile As and pH.

2.2. Reagents and instrumentation

Acids used for samples digestion were of high purity (analytical grade): 37% (m/m) HCl and 65% (m/m) HNO_3 (Merck, Germany). Calibration standards were prepared using a 1000 mg L^{-1} stock solution of As (Merck, Germany). Ultrapure water was obtained by a Millipore Milli Q system. The DGT units for As determination were purchased from DGT Research Ltd. (Lanchester, UK). Each DGT unit consists of a plastic piston covered by a layer of polyacrylamide gel containing Fe-oxide resin, a diffusive layer with a thickness of 0.76 mm and a 0.45 mm pore size filter membrane with a thickness of 0.13 mm, pressed by a plastic cap with a window 2 cm in diameter. A soil certified reference material, SRM 2709 San Joaquin Soil (NIST, New York, USA) was used for the quality control of total As determination.

Instrumentation used for sample preparation included Memmert oven with adjustable temperature (Memmert GmbH & Co. KG, Germany), Berghof high-pressure microwave digestion system (Berghof, Germany) and Hettich Universal 320 centrifuge (Hettich, Germany). An inductively coupled plasma atomic emission spectrometer, ICP-OES, Optima 5300 DV (Perkin-Elmer, Canada) and an inductively coupled plasma mass spectrometer, ICP-MS, ELAN DRC II (SCIEX Perkin-Elmer, SUA) were used for the determination of metals. A JENWAY 3340 pH-meter was used for soil pH determinations, while organic (OC) was determined using the Multi N/C 2100S Analyser (Analytic Jena, Germany).

2.3. Analytical methods

Aqua regia (HCl:NHO_3 = 3:1 (v/v)) extraction method was used for the determination of the total As, Fe, Mn, Al, P and K concentrations in soil, according to ISO 11466:1995. A single chemical extraction in neutral salt solution (1 M NH_4Cl) was used for the estimation of potentially available contents of As in soil, according to the procedure described by Kashem (2007). The fern samples were digested by heating 0.5 g dried sample with a mixture of 2 mL of H_2O_2 and 6 mL HNO_3 in microwave oven. The concentrations of metals in soil and vegetable extracts were measured by ICP-OES or by ICP-MS. Total concentration of N in soil was determined by Kjeldahl digestion. Soil pH was measured in 1:5 (w/v) soil/water ratio, organic carbon (OC) was determined using an automated TOC analyser, while cation exchange capacity (CEC) was determined according to ISO 23470:2007 by measurement of exchangeable major cations by ICP-OES determinations.

For As-DGT measurements, the DGT devices were introduced in soil slurries prepared by mixing 50 g soil and appropriate amount of ultrapure water to obtain 100% of water holding capacity (WHC) in a plastic container and kept for 24 hours for hydration and equilibration (Senila et al., 2012b). The DGT devices were deployed directly on the soil samples in triplicate for 24 hours at temperature of 22 ± 2°C. The containers were covered with plastic films to avoid water evaporation from the soil during the DGT deployment. After 24 h, the devices were recovered, cleaned from the adhering soil by washing carefully with ultrapure water. Arsenic was eluted from the chelating resins by introducing them in 1 mL 1M HNO_3 for 24 h, then the eluents were diluted 5 times with ultrapure water before As determination by ICP-MS.

To determine the mass (M) of As accumulated in the resin, was used the Equation (1):

$$M = C\ F\ (V_{acid} + V_{gel}) / f_e \qquad (1)$$

where C is the As concentration in eluent, measured by ICP-MS, F is the dilution factor (5), V_{acid} is the volume of HNO_3 added to the resin (1 mL), V_{gel} is the volume of resin gel (0.15 mL), f_e is the elution factor (0.8). The time averaged concentration of metal (C_{DGT}) was calculated by Equation 2:

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

where Δg is the thickness of the diffusive gel (0.078 cm) + membrane filter (0.014 cm), D is the diffusion coefficient of As in the resin gel, t is the deployment time (86400 sec), and A is the area of the sampling window of the DGT device (3.14 cm^2).

To measure the As concentration in soil solution (C_{sol}), a portion of the soil slurry prepared for the DGT measurements was introduced in 25-mL polyethylene tubes and centrifuged at 5000 rpm for 25 minutes. The supernatant was filtered by 0.45-µm pore size filter and the As concentration was measured by ICP-MS. To measure the resupply from solid phase, the ratio R between C_{DGT} and C_{soln} can be calculated.

The measured flux of As in the soil solution can be linked to effective concentration (C_E) that includes both soil solution concentration and its supplementation from the soil solid phase, and can be calculated using Equation 3:

$$C_E = C_{DGT} / R_{diff} \qquad (3)$$

where R_{diff} is calculated using the computer numerical model 2D DIFS. The input parameters to calculate R_{diff} were: particle concentration (P_c), As diffusion coefficients in water, in soil and in diffusive gels (D_0, D_s, D_d), diffusion layer thickness (Δg = 0.092 cm), deployment time (t = 24 h). For particle density (P_s), was used the typical value for mineral soils of 2.65 g cm^{-3} (Mihalik et al., 2012).

$$P_c = m / V \quad (4)$$

where m is the mass of soil used and V is the volume of water added to obtain the 100% WHC for DGT deployments.

2.4. Quality assurance

Blank samples, replicate analyses and certified reference material (CRMs) of soil (SRM 2709 San Joaquin Soil, New York, USA) were used for the QA/QC. Percent recovery (%) of As in soil CRM was calculated using the average of five replicates and the relative standard deviation at a 0.05 significance level and was 96.5 ± 9.4%.

3. RESULTS AND DISCUSSION

3.1 Physical and chemical soil properties

Bioavailable fraction of As in soil (fraction that can be taken up by terrestrial plants) can be influenced by several soil characteristics such as pH, organic carbon (OC), total N, total K, total P, cation exchange capacity (CEC), oxides of Al, Fe and Mn (Cattani et al., 2009). The main soil characteristics, analysed before pot experiments, are presented in table 1. The soil pH was found to be neutral to slightly acidic (7.12 and 6.52) in composite soil samples from Sasar and in soil from Ferneziu, respectively. The cation exchange capacities were 23.1 cmol kg^{-1} in SA, and 29.6 cmol kg^{-1} in FE, while the organic carbon contents were 39.4 mg kg^{-1} dw in BE and 22.3 dw in FE. The contents of nutrients in soil from Sasar were higher than in soil from Ferneziu. Thus, averages of nitrogen, potassium and phosphorous were 3.25 g kg^{-1} dw, 520 mg kg^{-1} dw and 24.8 mg kg^{-1} dw in soil from Sasar, while in soil from Ferneziu the average values of these parameters were 1.21 g kg^{-1} dw, 280 mg kg^{-1} dw and 10.2 mg kg^{-1} dw, respectively.

Relatively high concentrations of *aqua regia* extractable As (considered in the study as total concentrations) were found, which overhead in both areas the intervention level of 25 mg kg^{-1} dw, established by Romanian legislation for soils from residential and agricultural areas (MO 956:1997). However, the concentrations of As extractible in 1M NH_4Cl were much lower: 4.56 mg kg^{-1} dw in SA and 5.15 mg kg^{-1} dw in FE, which represent only 4.34% and 2.24%, respectively, from the total As content.

The concentrations of total Al, Mn and Fe in composite soil from FE were 19,200 mg kg^{-1} dw, 1,100 mg kg^{-1} dw and 25,400 mg kg^{-1} dw, while in the second area (SA) the averages of total Al, Mn, and Fe were lower: 15,500 mg kg^{-1} dw, 886 mg kg^{-1} dw and 16,800 mg kg^{-1} dw, respectively.

3.2. Arsenic accumulation in ferns

In table 2 are presented the concentrations of As accumulated in the roots and in the shoots of the two fern species, grown on the two As-contaminated soils. Among the two ferns, *Pteris cretica L.* was much more effective for accumulating As, the concentrations of As both in roots and in shoots being one order of magnitude higher than in *Dryopteris filix-mas L.*

One of the required characteristics of metal hyperaccumulators is their capability to transfer the higher amount of metal from roots to shoots (Luongo & Ma, 2005). Different approaches are used in order to express the behavior of metals accumulation in plants (Garcia-Salgado et al., 2012). In this study, three ratios were calculated: $[As]_{root}/[As]_{soil}$, so-called bioavailability factor (BF); $[As]_{shoot}/[As]_{root}$ or translocation factor (TF), which provides information about the As transfer from root to shoot; and $[As]_{shoot}/[As]_{soil}$ or accumulation factor (AF). For both BF and AF calculations, total concentration of As in soil was used.

Arsenic concentrations in the shoots and roots of the two ferns were higher for those plants grown on soil with higher concentration of total As (FE). BFs for *Pteris cretica L.* were 0.33 and 0.21, while for *Dryopteris filix-mas L.* were 0.08 and 0.04 in SA and in FE, respectively. TFs, which indicate the capacity of plant to translocate metals in the abovegroud part, were 7.50 and 7.52 for *Pteris cretica L.*, whereas for *Dryopteris filix-mas L.* were only 0.79 and 0.70, respectively. The values of AF, were 2.50 and 1.57 for *Pteris cretica L.* in SA and in FE, respectively, which confirmed the hyperaccumulator character of this fern, also indicated in several previous papers (Meharg, 2003; Luongo & Ma, 2005; Wei & Chen, 2006). For *Dryopteris filix-mas L.* AFs were 0.06 and 0.03 in SA and in FE, respectively. This behaviour of accumulating more metal content in roots is characteristic for non-hyperaccumulator plants, since they cannot translocate it from roots to the aerial parts.

Table 1. Selected characteristics of the two composite soils used in pot experiments (average ± standard deviation (SD), n = 6 parallel samples)

Parameter	Composite soil			
	Sasar		Ferneziu	
	Average	SD	Average	SD
pH (1:5 soil/water ratio)	7.12	0.10	6.52	0.11
CEC (cmol kg^{-1} dw)	23.1	2.05	29.6	2.67
OC (g kg^{-1} dw)	39.4	3.55	22.3	2.48
KjN (g kg^{-1} dw)	3.25	0.92	1.21	0.63
K (mg kg^{-1} dw)	520	50.2	280	14.9
P (mg kg^{-1} dw)	24.8	0.16	10.2	0.12
Total As (mg kg^{-1} dw)	108	11.2	230	15.5
As extractible in 1M NH_4Cl (mg kg^{-1} dw)	4.56	0.28	5.15	0.55
Al (mg kg^{-1} dw)	15500	785	19200	860
Mn (mg kg^{-1} dw)	886	85.0	1100	88.7
Fe (mg kg^{-1} dw)	16800	1200	25400	1450
Soil type*	Luvosol		Eutricambosol	

* According to Romanian Soil Taxonomy (Big et al., 2012)

Table 2. Arsenic accumulation in roots and shoots of ferns (average ± standard deviation (SD), n = 3 parallel samples)

Fern species	SA		FE	
	Roots	Shoots	Roots	Shoots
Pteris cretica L. (mg kg^{-1} dw)	36±3.5	270±14	48±3.9	361±15
Dryopteris filix-mas L. (mg kg^{-1} dw)	8.5±0.5	6.7±0.6	9.8±0.6	6.9±0.5

Our results consist with those reported by Larios et al., (2012) from a study regarding metals accumulation by plants from two polluted mining areas from Spain, which showed that accumulation factor for *Dryopteris filix-mas L.* were generally below 0.14. In a screening of As accumulation in plants grown on As-contaminated soils from Bangladesh (Mahmud et al., 2008) it was found that the arsenic bioaccumulation factor was higher than 1. In our case, the AF is greater than 1 only if it is considered the potentially available As in soils (As extractible in 1M NH_4Cl). It is also remarked that for the ferns grown on soils from SA, the bioavailability and accumulation factors were higher than for ferns grown on soil from FE, explained probably by the higher content of organic carbon, which in its dissolved form can increase As mobility in soil (Cattani et al., 2009) and by higher content of phosphorous which is also positively correlated with As uptake by plants (Silva Gonzaga et al., 2012).

3.3. Changes of arsenic root-availability in rizosphere assessed by DGT

Plant uptake is related to root-available concentration of metal in soil. In our study, the influence of the two hyperaccumulator and non-hyperaccumulator ferns grown for three months in pot experiments on As availability in two contaminated soils was studied using DGT technique. In table 3 are presented the As concentration in soil solution (C_{sol}), DGT-labile As in soil (C_{DGT}) and effective concentration (C_E) of As, calculated using 2D DIFS program, before plants grown. Also there are shown the main input parameters to calculate R_{diff}. A large value for soil response time (T_c) and a small value for distribution ratio (K_d) were used to calculate R_{diff}. Knowing R_{diff}, C_E can be calculated using the Equation 3.

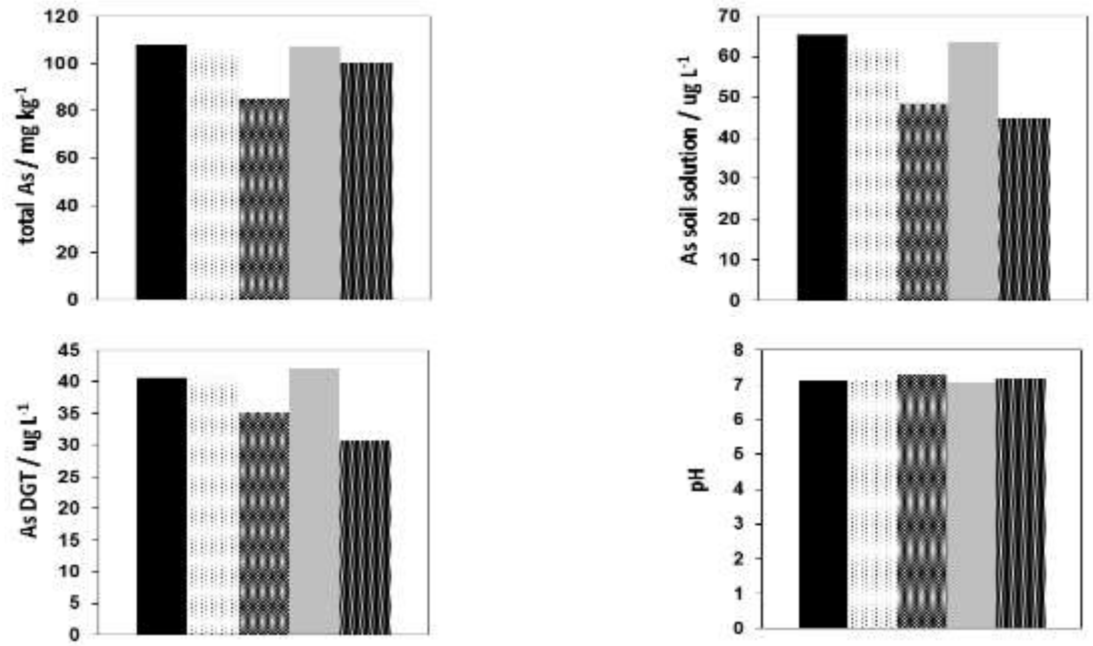
The As concentrations in soil solutions extracted by centrifugation from the two composite soils from SA and FE were 65.3 µg L^{-1} and 98.5 µg L^{-1}, while the labile-DGT concentrations of As were 40.6 µg L^{-1} and 55.2 µg L^{-1}, respectively. The *R* ratio between C_{DGT} and C_{sol} ($0 < R < 1$) indicates the capacity of the soil solid phase to resupply the soil solution with As. When $R > 0.95$, As is present as mobile species in the solid phase and the capacity of the solid phase to resupply the pore water is high. An R value approaching 0 suggests very limited or no As resupply from the solid phase. The *R* ratios for As in SA and FE soil were 0.62 and 0.56 indicating an intermediate case for As resupply from the solid phase. Also, *R* value from SA indicates that As resupply rate is higher in this soil, which explain also the greater bioavailability and accumulation factors for ferns grown on this soil. The average effective concentrations of As in soils SA and FE were 840 μg L^{-1} and 1130 μg L^{-1}, respectively, which show that despite the fact that total As concentration in FE was two time higher, the available fraction is higher with only 26% than in SA. The higher degree of bioavailability in soil from SA can be explained by higher content of OC (in its dissolved form) and phosphates one the one hand, and also by lower contents of Al, Fe and Mn in soil, since arsenic has a high affinity for the oxides of these elements, which reduce its mobility in soil (Fayiga et al., 2007).

After plants grown, soils from rizosphere compartment and bulk soil were sampled from the each pot experiment. The samples were analysed for total, in soil solutions, DGT-labile As species and pH.

In the rizosphere compartment of *Pteris cretica L.*, C_{sol} and C_{DGT} of As decreased by about 25% and 19% from the initial concentrations. In the rizosphere of *Dryopteris filix-mas L.* the decreasing of C_{sol} and C_{DGT} were 24% and 43%, respectively. Similar to soil from SA, the percentage of decreasing for DGT-labile As was higher for rizosphere of *Dryopteris filix-mas L.* Thus, it is very interesting to observe that the *R* ratio (C_{DGT}/C_{sol}) in the rizosphere of *Pteris cretica L.* both in soil from SA and FE, increased from 0.62 and 0.56 to 0.73 and 0.61, respectively, which indicate the modification of soil solution by the greater proportion of As in labile form. Contrary, *R* values in the rizosphere of *Dryopteris filix-mas L.* declined to 0.42 and 0.46, respectively, indicating the decrease of As in available form after plants grown. Soil pH increased slowly in the rizosphere of *Pteris cretica L.* during the experiment but only with 0.08 pH units.

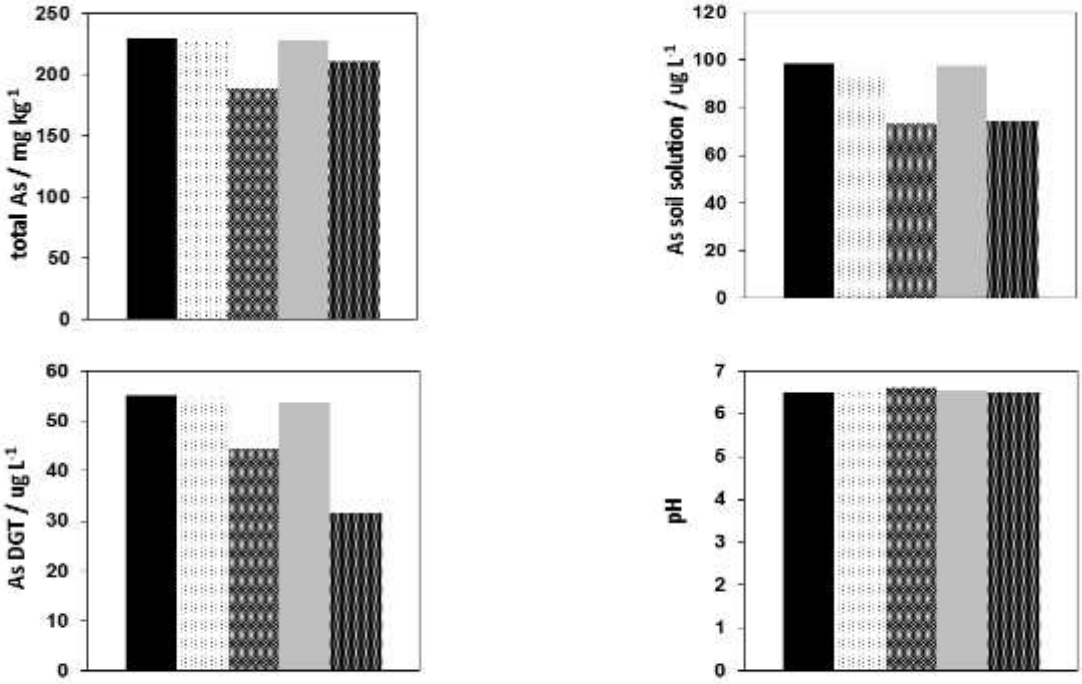
Table 3. Input parameters for 2D DIFS model (P_c – soil particle concentration, D – diffusion coefficient of As through diffusive gel), C_{sol}, C_{DGT} and C_E of arsenic (averages ± standard deviation (SD), n = 3 parallel samples) and R_{diff} ratio

Soil	P_c g cm^{-3}	D cm^2 s^{-1}	Δg cm	C_{sol} μg L^{-1}	C_{DGT} μg L^{-1}	R_{diff}	C_E μg L^{-1}
SA	4.11	4.7 x 10^{-6}	0.092	65.3±5.2	40.6±5.8	0.0483	840±120
FE	3.89	4.7 x 10^{-6}	0.092	98.5±6.1	55.2±7.9	0.0489	1130±162



Before phytoextraction
Bulk soil SA-PT1-3 after phytoextraction
Rizosphere SA-PT1-3 after phytoextraction
Bulk soil SA-DF1-3 after phytoextraction
Rizosphere SA-DF1-3 after phytoextraction

Figure 2. Total As, soil solution, DGT concentrations of As and soil pH (n = 3 parallel samples) in composite soil from SA before and after growth of P. cretica and D. filix-mas



Before phytoextraction
Bulk soil FE-PT1-3 after phytoextraction
Rizosphere FE-PT1-3 after phytoextraction
Bulk soil FE-DF1-3 after phytoextraction
Rizosphere FE-DF1-3 after phytoextraction

Figure 3. Total As, soil solution, DGT concentrations of As and soil pH (n = 3 parallel samples) in composite soil from FE before and after growth of P. cretica and D. filix-mas

In order to check if the effects of plants growth on the four parameters presented in figure 2 and figure 3 (total As, As in soil solution, DGT concentrations of As and soil pH) in bulk soil and rizosphere soil of the two fern species were statistically significant, one-way Analysis of Variance (one-way ANOVA) test was applied using STATISTICA software, Version 10.0. This test shows if three or more means are equal or are statistically different. According to the test, the all forms of As concentrations (total, DGT and in soil solution) differ statistically significant ($p<0.05$) as effect of plant growth, while in case of pH, p values were 0.13 for soil from SA and respectively 0.061 for soil from FE ($p>0.05$), showing that pH values did not varied significant during the tests.

The present study represents one of the first applications of DGT technique for the assessment of roots induced changes on As availability in the rizosphere of ferns grown on As-contaminated soil and is the first evaluation of influence of *Dryopteris filix-mas L.* roots on As availability using DGT technique.

4. CONCLUSIONS

The values of accumulation and transfer factors for *Pteris cretica L.* confirmed the hyperaccumulator character of this fern. For *Dryopteris filix-mas L.* the two factors were below 1, indicating that this fern is not an As-hyperaccumulator. Two composite soils collected from Sasar village and Ferneziu district of Baia Mare city, were characterized for total As, As in soil solution and DGT-labile contents. The results showed higher content of total As in soil from Ferneziu, but the percentages of As in labile forms in soil from Sasar were higher, probably due to the higher content of organic carbon and phosphorus and lower content of Fe, Mn and Al.

The two ferns had different effects on As content and fractionation in rizosphere soil. Total arsenic concentration from the rizosphere *Pteris cretica L.* decreased with about 21% and 18%, respectively, while in the rizosphere of *Dryopteris filix-mas L.* the reducing was much lower. The decreasing of labile concentrations of As from soil do not account the As accumulated by *Pteris cretica L.*, showing that As was extracted also from less available form of soil which imply that this fern was capable to solubilize As. The *R* ratio (C_{DGT}/C_{sol}) increased in the rizosphere of *Pteris cretica L.* during the experiment, indicating the greater proportion of As in labile form in solution of soil, while this ratio decreased in the rizosphere of

Dryopteris filix-mas L., indicating the lowering of As in available form from soil solution after plants grown.

Results showed that *Dryopteris filix-mas L.* is not a suitable candidate to be used for As phytoextraction, but because it decrease the labile As concentration in soil solution and accumulate As mainly in roots can be considered as being appropriate for stabilization of As-contaminated soil. However, further investigation on phytostabilization capacity on different types of soils is needed. The DGT technique showed a high sensitivity to As bioavailability determination in As-contaminated soil and was confirmed as a useful tool for identification of changes of As mobility in the rizosphere of plants.

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