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Six out of the 33 posters displayed at the GeoSpec2010 meeting, which took place at Lancaster University in September 2010, were expanded by their authors into papers for the ECG *Bulletin*. Extended extracts from these six papers appear in the printed and web edition, respectively, of the January 2011 issue of the ECG *Bulletin*. The complete (partly edited) papers are published on these ECG web pages of the RSC web site (www.rsc.org/ecg and links).

Paper 1 Lessons from a large scale deployment of DGT in the Seine basin

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Introduction

Since the link between chemical speciation of metals in water and their toxicity has been established [1], analytical chemists have proposed a number of analytical techniques to assess the different fractions of metals in the water column in order to obtain measurements that give information about the potential risk of metals for ecosystems. Among them, the Diffusive Gradients in Thin-films technique (DGT), developed by Zhang and Davison [2], is now commonly used in the scientific literature. DGT allows the estimation of the time-weighted average concentration of labile metals in situ. First, the DGT is an in situ speciation method: it samples a labile fraction of dissolved metals in water which is composed of free inorganic metals and weak labile organic complexes. Tusseau-Vuillemin *et al.* [3, 4], and Ferreira *et al.* [5], have shown that this fraction is close to the bioavailable fraction for *Daphnia magna* and aquatic mosses. Second, DGT is a passive sampling technique, which integrates the metallic contamination during a time deployment of some hours to several weeks. Thus, DGT provides an interesting alternative to measurements from spot sampling that is much more sensible to concentration fluctuations.

For these reasons, passive sampling techniques, and particularly DGT for metals are getting gaining interest for water agencies in charge of regulatory monitoring programs. The main objective of the project presented here

was to evaluate the potentiality of DGT as a monitoring tool at a river basin scale, when scientific applications of DGTs usually take place in a limited number of sites in a same water course or in neighbouring sites. We present here the first results of a large scale deployment of DGTs, taking place in the Seine river basin in France. The whole set of measurements constitutes a rich dataset including large and small rivers, and reference as well as impacted sites.

1. Methods

Study sites and field methods

The sampling area was the Seine-Normandy basin (**Figure 1**). It is 500 km long and 200 km wide, regroups 16 millions of inhabitants and 2500 wastewater treatment plants. The water courses are under urban, industrial and agricultural pressures. The total sampling period lasted over the whole 2009 year. 45 sites have been investigated on 30 locations in the entire basin. Due to loss of some materials, 43 sites over the 45 sampled have been analysed.

At each site, six DGT devices were deployed. The DGT elements, Chelex resin and restricted gels of 0.78 mm, were purchased from DGT research. DGTs had been assembled in ultra-clean conditions the day before deployment. The first set of 3 DGT was deployed one week, and the other ones two weeks. Acid and DGT assembly blank were analysed. Labile Cd, Cr, Co, Cu, Mn, Ni, Pb and Zn were measured.

During DGT deployment, raw and 0.45 µm filtered water samples were collected once a week to measure total and dissolved metals (three spot samples were taken for each site). Water was filtered in situ with a filter syringe of PolyEther Sulfone of 0.45 µm diameter. General physico-chemical parameters were also measured weekly: pH, conductivity, anions, cations, Dissolved Organic Carbon (DOC), Total Suspended Solid (TSS). Temperature was continuously recorded by a Hoboware data logger.
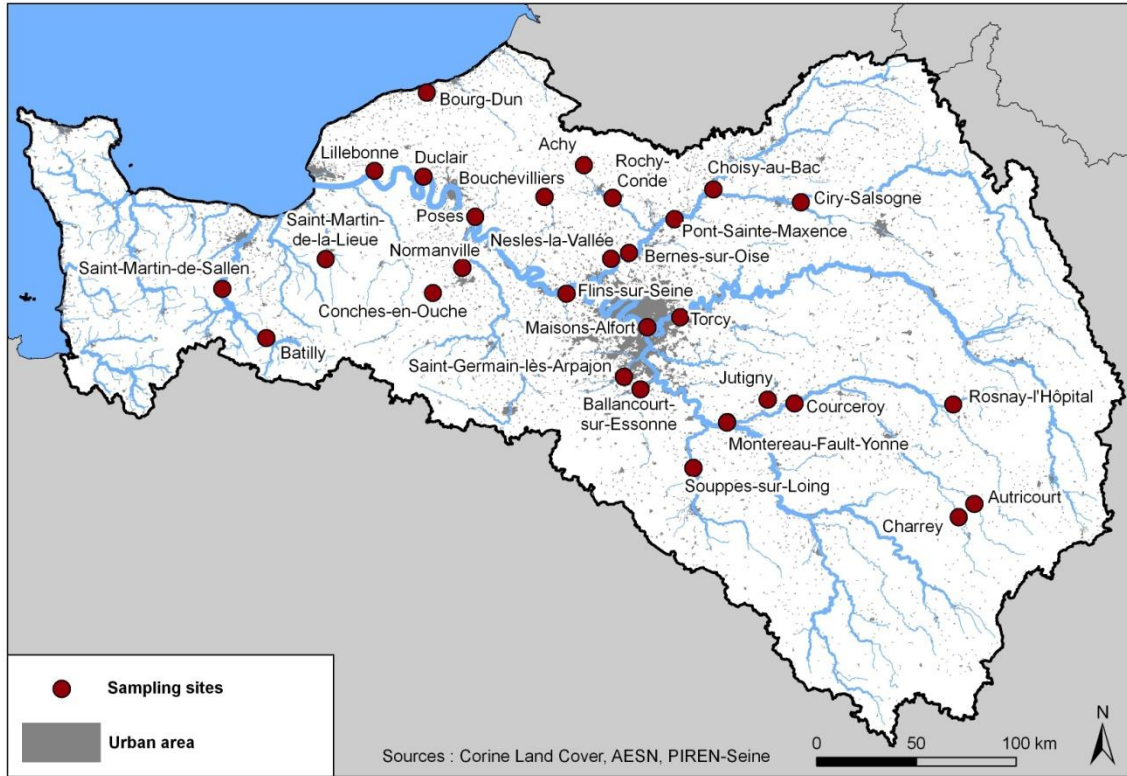

Figure 1a: *Location of the sampling area*

Figure 1b: *Location of the sampling sites*

DGT method

Metals were eluted from Chelex-resin by soaking in 1 mL HNO_3 1.2 mol.L^{-1} during a minimum time of 24h. The resulting extracts were diluted with ultra-pure water, spiked with internal standards and analysed by ICP-MS (X series 2 Thermo fisher scientific). The accumulated mass of metal on the resin M (in ng) was deduced from metal concentration in resin eluate C_e (in µg/L).

$$M = \frac{C_e(V + Vr)}{f} \quad (1)$$

V: eluate volume in mL
Vr: resin volume = 0.16 mL
f: elution factor = 0.8

From the amount of metal accumulated in the resin, the labile metal concentration in water was estimated following the equation, adapted from Davison and Zhang [2]:

$$C_{labile} = \frac{M}{tA}\left(\frac{\Delta g}{D_g} + \frac{\Delta_f}{D_f}\right) \quad (2)$$

D_g: diffusion coefficient in the gel in cm².s^{-1}. D_g (restricted pores) = 0.7x D_g (open pores) (given in DGT user guide [6]).
D_f: diffusion coefficient in the membrane filter in cm².s^{-1}.$D_f = D_g$ (open pores) [6, 7]
A: exposure area = 3.14 cm²
t: deployment time in s
Δg, Δf: thickness of the gel (0.78 mm), of the filter membrane (0.14 mm)

When two times deployment are available, mass could be plotted versus time (equation (3)).The labile metal concentration C_{labile} in water is calculated from the slope.

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$$M = \frac{C_{labile} \, A}{\dfrac{\Delta g}{D_g} + \dfrac{\Delta f}{D_f}} \cdot t \qquad (3)$$

Metals analysis method

Water was acidified in the laboratory with concentrated HNO_3 (Merck, suprapur). Raw water was acidified, and treated with HNO_3 and HCl on a hotblock (Digiprep, SCP Science) following an adaptation of the US EPA 3010A method [8]. The samples prepared in this way were then treated as DGT eluate and analysed by ICP-MS.

2. Results and discussion

Contamination of the basin

Figure 2 presents the profile of the contamination in labile, total dissolved and total concentrations of Cd, Cr, Co, Cu, Mn, Ni, Pb and Zn. Detection limits by ICP-MS have been plotted for each concentration; labile metal detection limits have been calculated for two weeks exposure at 25 °C. Although the total dissolved concentration of Cr or Cd is below the detection limit for some samples, all metals are detectable in the DGT extracts. This highlights the interest of using DGT that pre-concentrate contaminants in situ and allow to estimate even very low metal concentrations.
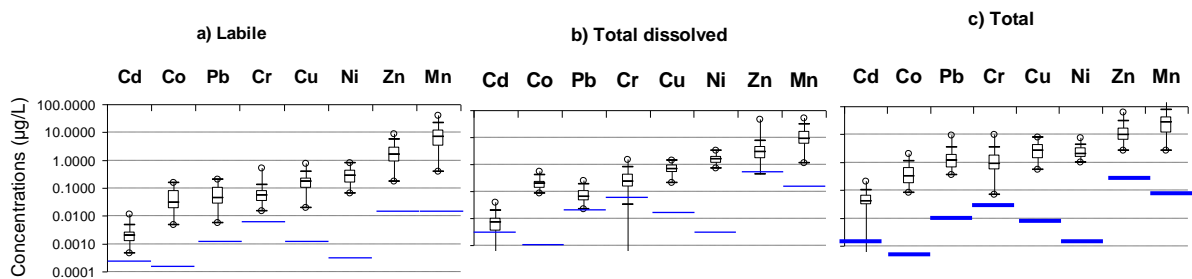

Figure 2: Ranges of concentrations in µg.L^{-1} observed on the 43 sampling sites. Rectangle: 50% of the total size, bars: min and max surrounding 99% of the total size, circles: min and max values. Horizontal bar: detection limit ICP-MS

Cd has the weakest concentrations (order of 10 ng.L^{-1}), then Co, Pb and Cr (order of 10 to 100 ng.L^{-1}). The third group is Cu-Ni (order of 100 ng/L), and finally the greatest concentrations for Zn and Mn (order of 10 µg.L^{-1}). These values are in the same range, than world average values of dissolved metal found in major no impacted rivers given by Gaillardet *et al*. [9] (**Table 1**), except for Zn which is much higher in the Seine river basin.

Cd	Co	Pb	Cr	Cu	Ni	Zn	Mn
0.080	0.148	0.079	0.70	1.48	0.80	0.60	34.0

Table 1: World average concentrations in ng.L^{-1} for "natural" river systems [9]

Results allow to draw a map of metal contamination. As illustrated in **Figure 3** for Ni and Pb, the behaviour of metal strongly varies according to the metal: while Ni is relatively homogeneous in the basin, with a coefficient of variation of 60%, Pb has more heterogeneous values (CV = 80%). The most contaminated samples are not necessarily the same for the two metals, although they are located downstream in sites under urban pressure (**Figure 1b**). The spatial distribution of the contamination is consistent with urban impact.
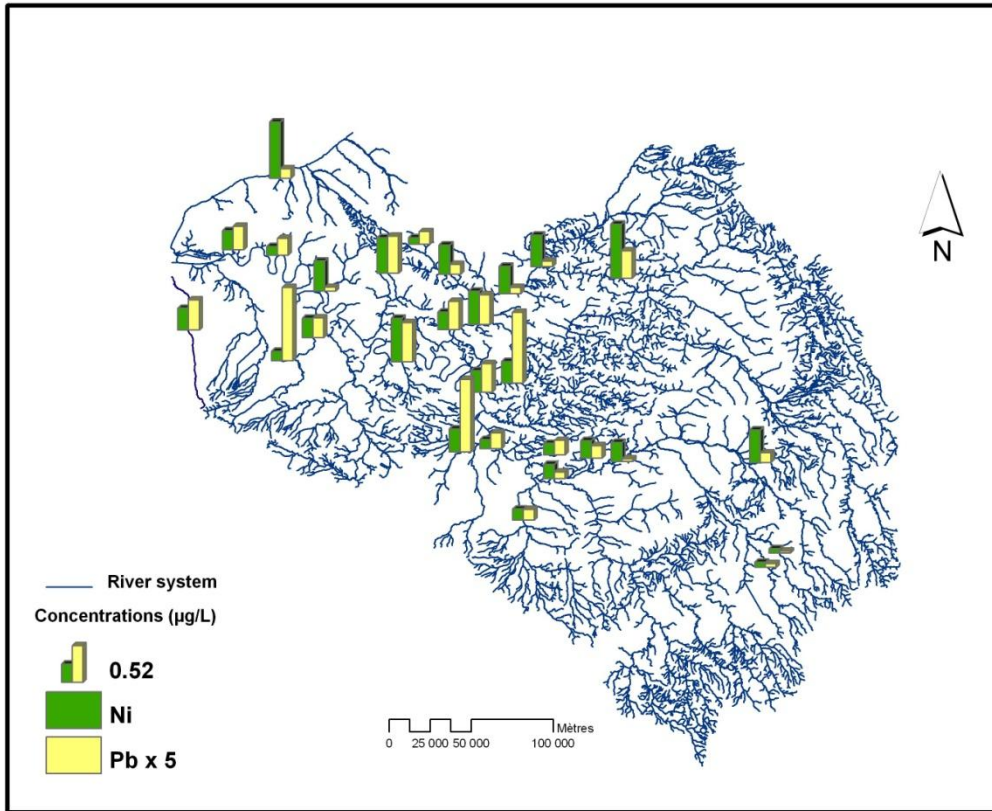
Figure 3: *Map of the contamination in Ni and Pb labile concentrations (µg.L^{-1})*

Lability of dissolved metals

The DGT samples a fraction of the dissolved metals called the labile metals, composed of free and weakly bound to organic matter metals (**Figure 4**). Lability is operationally defined, i.e. it depends on the thickness and the pore size of the hydrogel. In this study, the use of gel with restricted pores minimizes the sampling of large metal complexes (like with FA and HA). Merely only the inorganic metal are sampled by the DGT used in this study [10].
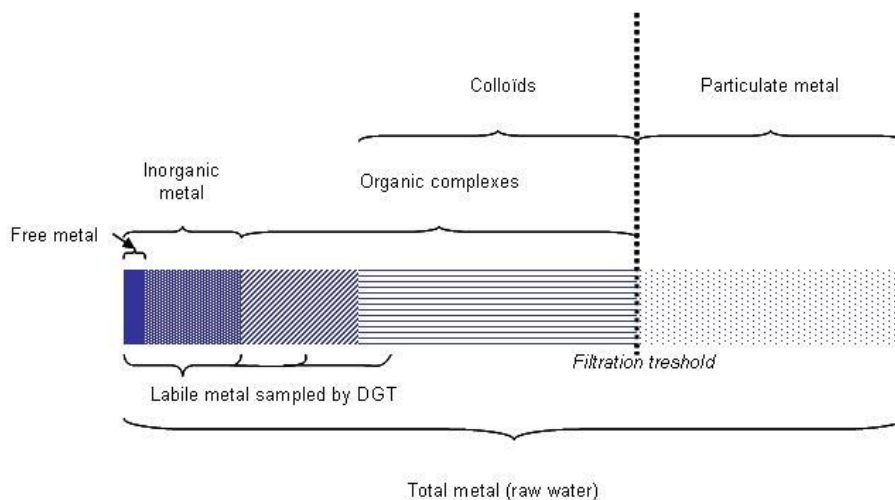

Figure 4: *Speciation of the metal in the water column*

The metal lability is estimated by the percentage of labile metals in the dissolved phase:

$$\% \, lab = \frac{C_{labile}}{C_{dissolved}}$$

Where C_{labile} is the concentration in water estimated by DGT, and $C_{dissolved}$ is the mean concentration of the 3 spot samples. The higher the percentage is, the more labile and then weakly complexed by organic matter the metal is.

The contrasting environments investigated within the Seine river basin, including headwater streams and impacted river, allow us to observe the variability of the lability and to study its environmental influencing factors.

The distribution of the lability in the whole basin is shown on **Figure 5**. The range of lability observed in this study corresponds to a large number of samples with different environmental characteristics. Two different behaviours can be distinguished: Cd, Cr, Pb, Zn, Mn have a great variability of lability, while Co, Cu, Ni show an homogeneous percentage on the whole basin. For the 3 last metals, the linear regression of C_{labile} vs $C_{dissolved}$ let us to estimate with good accuracy a mean labile percentage on the basin (**Figure 5**): the mean lability is 25.5 ± 0.9 %, 15.8 ± 1.4 % and 27.4 ± 0.7 % for Ni, Co and Cu respectively.

In a previous study in the Seine river, Tusseau-Vuillemin *et al*. [11] calculated a mean labile percentage for two sites for Ni (52 and 42%), Co (20 and 29%), Cu (25%) and Cd (37 and 29%). These values are consistent with the range of lability found in the present study for Cu and Cd and tend to be higher for Co and Ni. Let us notice that the highest values for Co lability are obtained for sites located downstream in the basin, as it were the sampling sites n the previous study cited above [11].
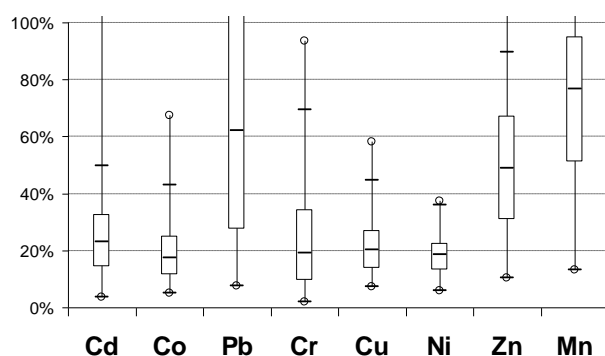

Figure 5: *Distribution of the labile percentages on the 43 sampling sites*

The lability of metals is strongly related to environmental physical and chemical parameters and especially the presence and the nature of organic matter. The contrasted sites sampled in this study offer a large type of organic matter, from natural matter mainly composed humic substances in forested and upstream areas to allochtonous anthropogenic matter in urban areas. The impact on lability is likely to differ depending on the environmental parameters.

In the following, we investigate whether physicochemical parameters can explain the variability of the lability observed on the **Figure 5**. The significant correlations between metal labilities and pH, DOC, conductivity and TSS have been analyzed by the use of Principle Component Analysis. The correlations table obtained (**Table 2**) shows the significant coefficients (alpha = 0.05) between labile percentage of metals and physicochemical parameters. The correlations of labile percentage of metals to each other are also examined.

Variables	Cd	Co	Pb	Cr	Cu	Ni	Zn	Mn
pH	-	-	-	-	-	-	-	-
DOC	-	-	-	-	-	0.304	-0.374	-
Conductivity	-	-	-	-	-	0.395	-	-
TSS	-	0.451	-	-	-	-	-	0.395
Cd	1	-	-	-	0.821	-	0.574	-
Co	-	1	-	0.722	0.354	-	-	0.463
Pb	-	-	1	-	-	-	-	-

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Cr	-	0.722	-	1	0.374	-	-	-
Cu	0.821	0.354	-	0.374	1	-	-	-
Ni	-	-	-	-	-	1	-	-
Zn	0.574	-	-	-	-	-	1	-
Mn	-	0.463	-	-	-	-	-	1

Table 2: *Significant correlations coefficients between labile percentage and physicochemical parameters*

A few correlations are revealed: as expected, Zn lability is negatively correlated to DOC. The higher the DOC is, the more complexed to organic matter Zn is. Nevertheless, the same correlation is not observed for other metals and surprisingly, Ni is positively correlated to DOC. The nature of organic matter is probably a more relevant factor than the concentration of organic matter alone to explain the variability in labilities. Co and Mn labilities have a significant correlation only with TSS. They are also correlated together. This result is consistent with conclusions of Lienemann *et al.* [12] who highlighted the association of Co and Mn in aquatic systems and in particles, and the similarity of their speciation in water. The authors attribute the link between the two metals in the particles to the sorption of cobalt to manganese hydrous oxides, and observed a similar profile in the water column.

We can identify from the **Table 2** some group of metals who have a correlated lability: Co-Cr-Cu, Co-Mn, Cd-Cu, Cd-Zn. Two metals in a same group are expected to show similarities in their lability, and thus in their chemistry in the water column. Ni and Pb, which are not correlated to any other metal, would have a specific chemistry.

Obviously, the physicochemical parameters chosen here are not sufficient to explain the variability of metal labilities observed at the basin scale. More precise indicators like the type of ligand (weak or strong) would play a role in the lability [4]. However, the results presented show a spectrum of the possible values of lability in different environmental conditions.

3. Conclusions

The large scale deployment of DGT in the Seine river basin was successful: all the samples have been interpreted and are exploitable, whereas the dissolved metal samples are sometimes under the detection limit. We have then built a representative data set on the water contamination in labile metal of an urban impacted basin. The results are also interpreted as labile percentage, showing the operationally possible values of lability in contrasted environmental conditions.

A first approach of the factors influencing the lability highlights the significance of the nature of the organic matter to interpret the lability of the metals.

References

1. Batley, G.E., S.C. Apte, and J.L. Stauber, Speciation and bioavailability of trace metals in water: Progress since 1982. *Australian Journal of Chemistry*, 2004. **57**(10), 903-919.

2. Zhang, H. and W. Davison, Performance-Characteristics of Diffusion Gradients in Thin-Films for the in-Situ Measurement of Trace-Metals in Aqueous-Solution. *Analytical Chemistry*, 1995. **67**(19), 3391-3400.

3. Tusseau-Vuillemin, M.H., *et al.*, Performance of diffusion gradient in thin films to evaluate the toxic fraction of copper to Daphnia magna. *Environmental Toxicology and Chemistry*, 2004. **23**(9), 2154-2161.

4. Buzier, R., M.H. Tusseau-Vuillemin, and J.M. Mouchel, Evaluation of DGT as a metal speciation tool in wastewater. *Science of the Total Environment*, 2006. **358**(1-3), 277-285.

5. Ferreira, D., *et al.*, More than inorganic copper is bioavailable to aquatic mosses at environmentally relevant concentrations. *Environmental Toxicology and Chemistry*, 2008. **27**(10), 2108-2116.

6. DGTresearch. *DGT User Guide*. 2003.

7. Scally, S., W. Davison, and H. Zhang, Diffusion coefficients of metals and metal complexes in hydrogels used in diffusive gradients in thin films. *Analytica Chimica Acta*, 2006. **558**(1-2), 222-229.

8. *Acid Digestion of Aqueous Samples and Extracts for Total Metals for Analysis by FLAA or ICP Spectroscopy*. 1992, US EPA 3010A.

9. Gaillardet, J., In *Treatise on Geochemistry*. 2004.

10. Zhang, H. and W. Davison, Direct in situ measurements of labile inorganic and organically bound metal species in synthetic solutions and natural waters using diffusive gradients in thin films. *Analytical Chemistry*, 2000. **72**(18), 4447-4457.

11. Tusseau-Vuillemin, M.H., *et al.*, Dissolved and bioavailable contaminants in the Seine river basin. *Science of the Total Environment*, 2007. **375**(1-3), 244-256.

12. Lienemann, C.P., *et al.*, Association of cobalt and manganese in aquatic systems: Chemical and microscopic evidence. *Geochimica et Cosmochimica Acta*, 1997. **61**(7), 1437-1446.

Paper 2   On boron in humic and inorganic components of soil: a geochemical *versus* a chemical approach

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"In the Biosphere, the migration of chemical elements occurs with direct participation of living matter or in the environment which geochemical patterns are governed by living matter."
Vernadsky's law formulated by Prof. Aleksandr I. Perelman (1972)

Introduction

The first observation on the essentiality of boron in higher plants was reported by Agulhon (1910), who showed that boron stimulates the growth of several vascular plants in water culture and that boron is abundant in lignified tissues. This was followed by several similar studies that confirmed boron essentiality (Mazé, 1914; Warington, 1923). Maluga (1964) noticed that the boron contents in plant ash were much higher than those in soils and the lithosphere (400, 10, and 12 mg kg^{-1}, respectively), indicating boron accumulation in the biosphere. The accumulation of boron greatly varies in different organisms (Bowen, 1966) (**Figure 1**). It seems that boron is not biomagnified in aquatic food chains, whereas in terrestrial food chains, boron accumulates in plants but not in animals (Underwood, 1977).
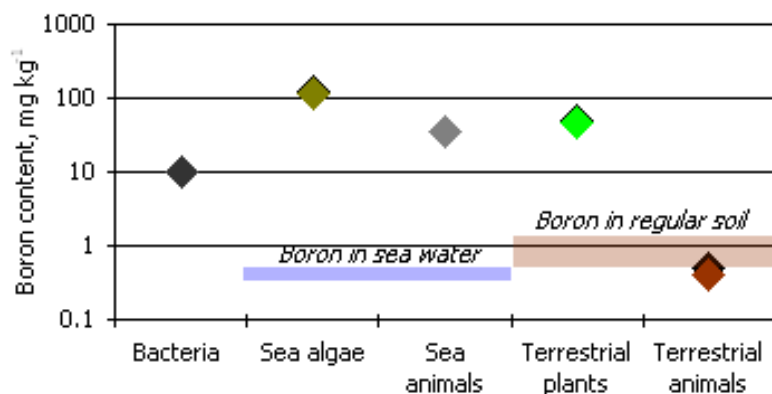

Figure 1: Boron contents in living organisms (by Bowen, 1966)

Other circumstantial evidences for the essentiality of boron in animals continued to appear in the 1980s (Woods, 1994; Nielsen, 1998). Thus, it is logical to assume that boron, when being involved in biochemical cycle, must interact with organic compounds forming complexes within living matter and its derivatives – of more or less stable nature. The formation of boron complexes in plants and – to a less extent – in animal tissues were described elsewhere (*see review* Kot, 2009). The current predictions of boron dynamics and fate in aquatic and terrestrial environments are solely based on boron associations with inorganic species whereas organoboron complexes are most often ignored. In this paper, we present some preliminary findings on boron in soil that demonstrate the organophilic character of boron and the necessity to considering its organic forms when investigating boron in soils.

According to Kovda (1973), boron is involved in the major soil processes: 1) the local soil biological cycle (litter – fresh and partly decomposed), 2) the synthesis of humus, 3) the formation of clays and the synthesis of colloids, 4) illuviation, and 5) hydrogenic accumulation (meadow soils, solonchaks). Decomposed and humified plants residues are a major source of organic matter to soil. Organic matter is an extremely reactive soil component that is hereditary to plant remnants and bacteria excretion, which are transformed by decomposition, synthesis and complexation reactions followed by aggregation processes (Schnitzer & Khan, 1978; Ponomareva & Plotnikova, 1980; Stevenson, 1994). Yermiyahu *et al*. (2001) found that the sorption capacity of composted organic matter for boron – on a weight basis – was at least four times greater than that of clay and soils. This may indicates that the soil and nutrient cycle involves boron.

Reviewing the literature on boron soil chemistry indicates that boron is associated with various fractions including soil solution, adsorbed, and occluded in mineral phases (e.g. clays and iron oxyhyroxides). However, this approach seems rather arguable. Surprisingly, only a few works have considered the necessity of including boron-organic matter forms in their schemes (*see review in* Kot, 2007, 2009). This probably is due to the difficulty in extracting soil organic matter without causing losses or chemical changes in the organically-bound boron fraction. For example, the procedure by Swift (1996), recommended by The International Humic Substances Society (IHSS), which is a modification of the procedure by Oden (1919), as well as procedures based on pyrophosphate, only allows humates and fulvates bound to solid soil particles through divalent (Ca^{2+}, Mg^{2+}) cation bridges to be extracted. These procedures affect neither humates bound to Fe/Al amorphous and crystalline hydrous oxides and clay minerals nor the fraction of humin most refractory to microbial attack (Tyurin, 1937; Russell, 1961; Kononova, 1966; Greenland, 1971; Schnitzer & Khan, 1978; Ponomareva & Plotnikova, 1980; Stevenson, 1994). The two latter fractions are rarely included in humus fractionation schemes, even though they may account for a substantial or even greater portion of soil/sediment organic matter. Treatment with sodium hypochlorite or hydrogen peroxide may be more effective (Anderson, 1963), although these reagents also affect amorphous and crystalline soil minerals, and they make the picture of fractionation rather more vague. Moreover, these reagents intensively oxidise humus/organic components and do not allow their characterisation.

It should be noted that most of the observations on boron accumulation by plants were from laboratory experiments based on solutions of inorganic boric acid and borates. Moreover, conventional analytical methods such as spectrophotometry do not distinguish the presumable forms of boron in natural waters. On the other hand, colorimetric and fluorometric methods prescribe harsh (e.g. highly acidic) procedures for sample preparation. In general, these methods suffer from numerous interferences and have low sensitivity and precision (see review in Sah & Brown, 1997). Among the modern methods, the inductively-coupled plasma-mass spectrometry (ICP-MS) technique seems most likely to be able to overcome most of the drawbacks if an adequate sample preparation could be applied. Below, we present some preliminary results on soil boron fractionation and potential mobility/bioavailability, with, as a special reference to soil humus components, examples of two irrigated soils.

Objects and methods

The objects were two Mediterranean soils – Hamra (red sandy) soil, irrigated with fresh and reclaimed effluent waters for between 5 and 7 years, and Grumosol (dark clay carbonaceous), irrigated with fresh and reclaimed effluent waters for 4 years.

The major soil humus and inorganic components were extracted from the samples. The extraction scheme (**Table 1**) was worked out in accordance with the classic scheme of Tessier *et al*. (1979), elaborated for the detailed fractionation of humus according to Waksman (1936), Turin (1937) and Ponomareva & Plotnikova

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(1980). For the extraction of humin, the procedure of Preston *et al.* (1994) was utilised, for it is relatively gentle and does not affect the organic matter much when compared to the harsh treatment of crystalline mineral components and clays by the concentrated hydrofluoric-hydrochloric acid mixture. The levels of boron in the extracts were determined by the ICP-AES technique.

Step		Procedure	Fraction/Extract
1.		H_2O, filtered subsequently 1.20, 0.45, and 0.20 µm	Water-mobile colloids
2.		NH_4NO_3, 1 *M* (Han & Banin, 1995)	Exchangeable
3.		NaAc + HAc, 1 *M* (Han & Banin, 1995)	Carbonates
4.		0.1 *M* NaOH separated at pH 2 into:	
	4.1.	Acid insoluble	Humates-Ca/Mg
	4.2.	Acid soluble	Fulvates-Ca/Mg
5.		0.02 *M* NaOH at 60 ^oC separated at pH 2 into:	
	5.1.	Acid insoluble	Humates-Fe/Al
	5.2.	Acid soluble	Fulvates-Fe/Al
6.		0.1 *M* hydroxylamine hydrochloride	Amorphous Mn minerals
7.		Na dithionite-citrate buffer	Free (non-silicate) Fe
8.		HF + HCl, 5 vol. % each, 1 month	Clays, Fe-Al(-Mn-Ti) crystalline minerals
9.		0.02 *M* NaOH at 60 ^oC	Humin extractable
10.		HNO_3 + HCl at 60 ^oC for 72 h	Residue, total element contents

Table 1: *The extraction procedure scheme*

Furthermore, a water extract was subsequently filtered through 1.20 µm, 0.45 µm, and 0.20 µm filters to separate the mobile water compounds. Some major soil parameters were also determined by conventional methods, such as pH, conductivity, C_{org} contents – total and in the extracted humus fractions, as well as the major elements Na, K, Ca, Mg, Al – in the water extract filtrates and exchangeable complex. Plastic laboratory equipment was used throughout. All equipment in contact with the samples and extracts was pre-cleaned by filling with or soaking in 3% HNO_3 solution for 48 hours. The detection limit for boron, calculated as three times the standard deviation of the procedural blank, was 0.02 mg kg^{-1}.

Results and discussion

Boron in water-mobile compounds: In the sandy and slightly acidic to neutral Hamra soil, boron was found to be highly "soluble" – on average 7% of the total boron content – in <0.45 µm water filtrate, compared to Na and the other alkali and alkaline-earth metals considered in the same soil sample. Surprisingly, no boron was detected in the <0.20 µm water filtrate, indicating the binding of boron to coarser mobile colloids, probably bacterial cells ("living colloids"), as the majority of soil bacteria are known to be larger than 0.20 µm (Christensen *et al.*, 1999). In the water extract from soil irrigated by freshwater for 5 years (**Figure 2**), one can see that boron was bound to the filtrates >0.20 to <0.45 µm, whereas both the application of reclaimed effluent water and the longer irrigation period resulted in a higher boron content in the coarser >0.45 µm colloid fraction. This was probably due to the propagation of microalgae and some other bacterial species and/or the appearance of specific suspended particles from the reclaimed water. In the clayey and slightly alkaline Grumosols, water-mobile boron compounds were found, on average, in 0.4% of the "fresh" and 1.2% of the "reclaimed" surface soil, decreasing to 0.0% and 0.5% in the deeper layers, respectively. Thus, the water-mobile boron components were dependent on the duration watering and the quality of the irrigation water.
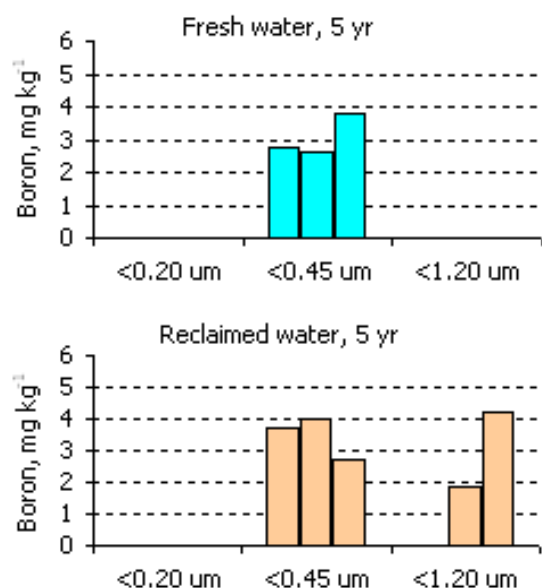
Figure 2: *Boron contents in filtrates of water extract from Hamra soil irrigated with fresh and reclaimed effluent waters*

For the <0.45 μm water filtrate, the extinction coefficients at wavelengths of $\lambda = 465$ and 665 nm (e4 and e6) gave a meaningful R^2 value for the boron content in the "reclaimed" soil, but not in the "fresh" soil, which may also indicate different origins of boron in the irrigation waters. For both kinds of soil, the spectrophotometric data showed the presence of water-mobile slightly coloured fulvic (fulvic-like) substances (e4/e6 = 8-11). Keren & Communar (2009) found that dissolved organic matter from treated effluents enhances boron transport in soil due to boron-organic matter formation. There is little knowledge about the mechanisms and pathways involved in the microbial uptake and utilisation of humus compounds. We do not know whether they are really taken up completely or if the cleavage is done extracellularly (Steinberg & Münster, 1985). At least one bacterial species (*Arthobacter*) is reported to utilise fulvic acids directly (De Haan, 1977). Boron is known to be essential for vascular plants and several other organisms, including cyanobacteria; however, in no case is its functional role well understood. Bacteria and algae do not need boron for the synthesis of lignin, like higher plants (Lewis, 1980), so we have to find another reason for the presumable accumulation of boron by the soil bacteria. Recent investigations have provided evidence of a biochemically defined function for boron in bacterial quorum sensing (Chen *et al*., 2002), although a passive boron accumulation by bacteria should not be repudiated either.

Thus, the mobile boron-water loads in the investigated soils were related to the water colloidal material of a varied, but most probably biotic, origin, and not just simply to dissolved inorganic boric acid or borates. Thus, the mobile B-water loads in the investigated soils were related to the water colloidal material of a varied, but most probably biotic, origin, and not just simply to dissolved inorganic boric acid or borates. The fact of the absence (or probably very low contents) of "truly soluble" B compounds leads to questions concerning the source of the plant-available element in the soil. Unlike most other micronutrients, plant roots require a continuous external supply of boron otherwise loss of membrane function occurs within minutes (Blaser-Grill *et al*., 1989). Like other micronutrients, the minimum solution concentrations of boron required for plant growth are too low to easily measure directly. In recent years, the concept of nutrients available in the soil in a soluble ionic form has been brought under critical re-evaluation. As long ago as eighty years, Jenny & Overstreet (1939) proposed a mechanism that includes nutrient transfer when the root makes direct contact with hydrated or dissolvable species held on colloid surfaces. Asad *et al*. (1997) suggested the following mechanism: they supposed that most of the micronutrients are complexed by the chelator and this acts as a buffer for nutrient supply. The formation constant for the metal-chelate complex, the excess of chelator in solution and the solution composition govern the activity of the micronutrient metals in solution. These systems have been able to maintain sub-micro molar concentrations of micronutrients.

Atmospheric precipitation and aerosols may be an underestimated source of plant-available boron (Boyd & Walley, 1972; Shann & Adriano, 1988; Brown & Shelp, 1997). It is interesting that fluxes of B with rainwater were found to correlate with the concentration of organic matter and the pH (Sedliský & Veselý, 2004).

Boron in major humus and inorganic fractions: In the Hamra soil, most of the boron was found bound to extractable humic compounds, including the extractable part of humin – on average 54% (28-71%) of the total boron content – as well as in fraction of free (non-silicate) Fe and Al amorphous and crystalline minerals (**Figure 3**). Among the humus components, the major boron carriers were humic-Fe/Al complexes and humin (extractable) (**Figure 4**), i.e. the most resistant formations to chemical and bacterial attack. It was estimated that the boron-Ca/Mg-humate complexes (the fraction obtained according to the IHSS procedure) only contained about 5-18% of the total boron in the Hamra soil and 4-7% in the Grumosol.
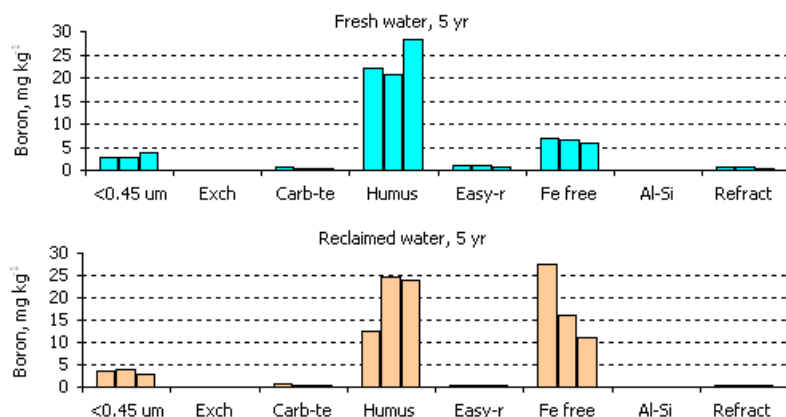

Figure 3: Boron contents in major components of Hamra soil irrigated with fresh and reclaimed effluent waters: <0.45 µm – water extract filtered through 0.45 µm filter; Exch – exchangeable complex; Carb-te – carbonates; Humus – sum of extracted humus components; Easy-r – easy-reducible manganese amorphous minerals; Fe free – non-silicate amorphous and crystalline iron/aluminium minerals; Al-Si – aluminosilicates; Refract – residual refractory fraction.
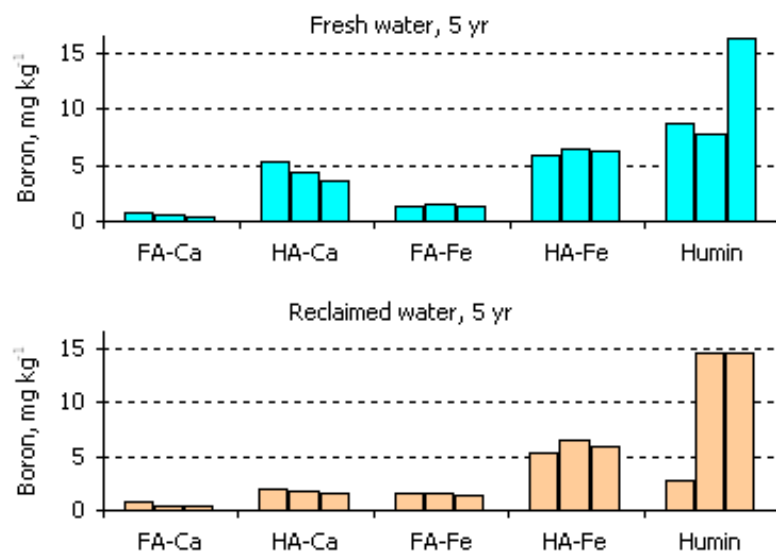

Figure 4: Boron contents in humus fractions of Hamra soil: FA-Ca, HA-Ca – Ca/Mg fulvates and humates; FA-Fe, HA-Fe – Fe/Al fulvates and humates; Humin – extractable humin.

It is known that humic and fulvic acids serve as binding agents for the cohesion of clay particles through H-bonding and coordination with polyvalent cations; the main cations are Ca^{2+}, Mg^{2+}, Fe^{3+}, and Al^{3+}. The divalent cations do not form strong coordination complexes with organic molecules and would only be effective to the extent that a bridge linkage could be formed. As suggested by Greenland (1971), organic matter bound in this manner should be rather easily displaced by a monovalent cation. In contrast, Fe^{3+} and Al^{3+} form strong coordination complexes with humic substances, in which case displacement of the bound metal is difficult and

may require extraction with a strong chelating agent (Stevenson, 1994), by alternative treatments with 0.1 M H_2SO_4 and 0.1 M NaOH (Tyurin, 1937), or by heating in alkaline solution (Ponomareva & Plotnikova, 1980). In our case, most of the organically-bound boron was found within refractory humus components that were not affected by the commonly adopted procedures, and they should not be omitted from estimates such as this.

According to the specific boron content, on the weight basis, humin was found to be the most effective boron concentrator (**Figure 5**), indicating the origin of humin from lignin of plant cell walls and membranes (Lewis, 1980; Lewis & Yamamoto, 1990). In the carbonaceous and clay-rich Grumosol, boron-humus compounds were the third largest boron pool, mostly comprised of Ca-humate and Ca-hymatomelanic complexes in the "fresh" soils and Ca-Fe-humate and hymatomelanic complexes in the "reclaimed" soils.
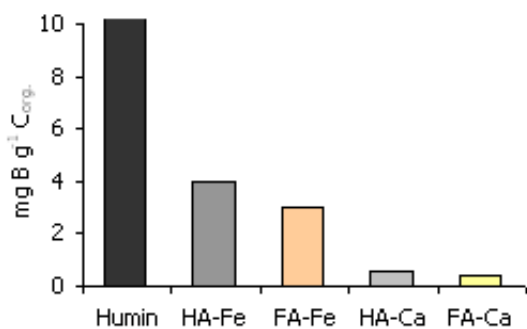

Figure 5: *Specific contents of boron in humus fractions of Hamra soil, mg B·g^{-1} C$_{org}$*

In the residual solid material left after all of the extraction procedures (Step 10 in **Table 1**), some boron was detected after harsh digestion by boiling with strong acids for 72 h – on average 1.3% (0.7-2.4%) of the total boron content – reflecting the soil components most resistant to chemical treatment, presumably: plant waxy cuticles, paraffin (insoluble humin portion), and tourmalines.

In addition to the water-mobile colloids, boron from carbonates, fulvic complexes, and easy-reducible amorphous manganese minerals should be referred to soil boron-mobile and potentially bioavailable components. As is well known, carbonates are vulnerable to environmental pH conditions, the solubility of fulvates becomes increased in the presence of alkali metals, and manganese minerals are vulnerable to redox-potential changes as well as to bacterial effects.

Conclusions

1. Boron in soil is much more organophilic than is often supposed.
2. Humus components are the principal carriers and determinants of boron in soil.
3. The mobile boron-water loads in the investigated soils were related to colloids of a varied, but probably biotic, origin, not simply to inorganic boric acid or borates, as it is often supposed.

Acknowledgements

References

Agulhon, H. (1910). 'Presence et utilité du bore chez les vegetaux', *Annales de l'Institut Pasteur (Paris)*, **24**, 321–329.

Anderson, J.U. (1963). 'An improved pretreatment for mineralogical analysis of samples containing organic matter', *Clays and Clay Minerals*, **10**, 380-388.

Asad, A.; Bell, R.W.; Dell, B.; Huang, L. (1997). 'Development of a boron buffered solution culture system for controlled studies of plant boron nutrition', *Plant and Soil*, **188**, 21–32.

Blaser-Grill, J.; Knoppik, D.; Amberger, A.; Goldbach, H. (1989). 'Influence of boron on the membrane potential in Elodea densa and Helianthus annuus roots and H^+ extrusion of suspension cultured Daucus carota cells', *Plant Physiology*, **90**, 280-284.

Bowen, H.J.M. (1966). *Trace Elements in Biogeochemistry*. Academic Press, London.

Boyd, C.E.; Walley, W.W. (1972). 'Studies of the biogeochemistry of boron. I. Concentrations in surface waters, rainfall and aquatic plants', *The American Midland Naturalist*, **88**, 1-14.

Brown, P.H.; Shelp, B.J. (1997). 'Boron mobility in plants', *Plant and Soil*, **193**, 85-101.

Chen, X.; Schauder, S.; Potier, N.; Van Dorsselaer, A.; Pelczer, I.; Bassler, B.L.; Hughson, F.M. (2002). 'Structural identification of a bacterial quorum-sensing signal containing boron', *Nature*, **415**, 545-549.

Christensen, H.; Hansen, M.; Sørensen, J. (1999). 'Counting and size classification of active soil bacteria by fluorescence in situ hybridization with an rRNA oligonucleotide probe', *Applied Environmental Microbiology*, **65**, 1753–1761.

De Haan, H. (1977). 'Effect of benzoate on microbial decomposition of fulvic acids in Tjeukemeer (The Netherlands)', *Limnology and Oceanography*, **22**, 38-44.

Greenland, D.J. (1971). 'Interactions between humic and fulvic acids and clays', *Soil Science*, **111**, 34-41.

Jenny, H.; Overstreet, R. (1939). 'Cation exchange between plant roots and soil colloids', *Soil Science*, **47**, 257-272.

Keren, R.; Communar, G. (2009). 'Boron transport in soils as affected by dissolved organic matter in treated sewage effluent', *Soil Science Society of America Journal*, **73**, 988-1994.

Kononova, M.M. (1966). *Soil Organic Matter*. Pergamon, Elmsford, N. Y.

Kot, F.S. (2007). 'Boron speciation in soils irrigated with fresh and effluent municipal waters', *Bulletin of Environmental Contamination and Toxicology*, **79**, 259-263.

Kot, F.S. (2009). 'Boron sources, speciation and its potential impact on health', *Reviews in Environmental Science and Biotechnology*, **8**, 3-28.

Kovda, V.A. (1973). *Fundamentals of Soil Science*, Volume 2. Nauka, Moscow. (in Russian)

Lewis D.H. (1980). 'Boron, lignification and the origin of vascular plants – a unified hypothesis', *New Phytologist*, **84**, 209-229.

Lewis, N.G.; Yamamoto, E. (1990). 'Lignin: occurrence, biogenesis and biodegradation', *Annual Revue of Plant Physiology and Plant Molecular Biology*, **41**, 455-496.

Mazé, P. (1914). 'Influences respectives des éléments de la solution minérale sur le développement du maïs', *Annales de l'Institut Pasteur (Paris)*, **28**, 21–68.

Maluga, D.P. (1964). *Biogeochemical Methods of Prospecting*. Consultants Bureau Plenum Press, New York.

Nielsen, F.H. (1998). 'Ultratrace elements in nutrition: current knowledge and speculation', *Journal of Trace Elements in Experimental Medicine*, **11**, 251–274.

Oden, S. (1919). 'Die Huminsäuren', *Kolloidchemische Beihefte*, **11**, 75-260.

Perelman, A.I. (1972) *Landscape Geochemistry*. Translation No. 676, Geological Survey of Canada, Ottawa.

Ponomareva, V.V.; Plotnikova, T.A. (1980). *Humus and Soil Formation*. Nauka, Leningrad. (in Russian)

Preston, C.M., Hempfling, R.; Schulten, H.-R.; Schnitzer, M., Trofymow, J.A.; Axelson, D.E. (1994). 'Characterization of organic matter in a forest soil of coastal British Columbia by NMR and pyrolysis-field ionization mass spectrometry', *Plant and Soil*, **158**, 69-82.

Russell, E.W. (1961). *Soil Conditions and Plant Growth*. Longmans Green, London.

Sah, R.N.; Brown, P.H. (1997). 'Boron determination – a review of analytical methods', *Microchemical Journal*, **56**, 285-304.

Schnitzer M.; Khan S.U. (1978). *Soil Organic Matter*. Elsevier, New York.

Sedliský, D.; Veselý, J. (2004). 'Biogeochemical cycle of boron in watersheds of the Bohemian forest lakes', *Aktuality Šumavského Výzkumu*, **II**, 89 – 94. (in Czech)

Shann, J.R.; Adriano, D.C. (1988). 'The chronic exposure of selected crop species to boron aerosols', *Environmental and Experimental Botany*, **28**, 289-299.

Steinberg, C.; Münster, U. (1985) 'Geochemistry and ecological role of humic substances in lakewater', In *Interactions at the Soil Colloid – Soil Solution Interface*, Bolt, G. H. *et al*. (eds.), pp 105-145.

Stevenson, F.J. (1994). *Humus Chemistry, Genesis, Composition, Reactions*. John Wiley & Sons, Denver.

Swift, R.S. (1996). 'Organic matter characterization'. Ch. 35. In *Methods of Soil Analysis. Part 3: Chemical Methods*. Sparks, D.L. *et al*. (eds.). Soil Science Society of America Book Series: 5. Soil Science Society of America, Madison, WI, pp 1018-1020.

Tessier, A.; Campbell, P.G.C.; Bisson, M. (1979). 'Sequential extraction procedure for the speciation of particulate trace metals', *Analytical Chemistry*, **51**, 844-851.

Tyurin, I.V. (1937). 'Organic matter in soils and its role in pedogenesis and fertility'. In *Study of Soil Humus,* Selhozgis. (in Russian)

Underwood, E.J. (1977). *Trace Elements in Human and Animal Nutrition*. Academic Press, New York.

Waksman, S.A. (1936). *Humus: Origin, Chemical Composition, and Importance in Nature*. Williams & Wilkins, Baltimore.

Warington, K. (1923). 'The effect of boric acid and borax on the broad bean and certain other plants', *Annals of Botany (London)*, **37**, 629–672.

Woods, W.G. (1994). 'An introduction to boron: history, sources, uses, and chemistry', *Environmental Health Perspectives*, **102**, 5–11.

Yermiyahu, U.; Keren, R.; Chen, Y. (2001). 'Effect of composted organic matter on boron uptake by plants', *Soil Science Society of America Journal*, **65**, 1436-1441.

Paper 3   Impact of metals on the biodegradation of polycyclic aromatic hydrocarbons in soil

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic substances which contain two or more fused aromatic rings and are common in a wide range of petroleum products, as well as crude oil (Bamforth and Singleton, 2005; Wong *et al*., 2004). They are by-products from incomplete combustion of organic substances such as coal, oil, petrol, and wood and are ubiquitous in the soil environment (Sokhn *et al*., 2001; Maliszewska-kordybach and Smreczak, 2003). While concentrations of individual PAHs in soil produced by natural processes are estimated to be around 1-10 µg kg^{-1}, their concentrations in highly polluted soils vary from 10 mg kg^{-1} to 10 g kg^{-1} dry weight (Sokhn *et al*., 2001). PAHs are highly recalcitrant molecules that can persist in soil due to their hydrophobicity and low water solubility, and because some are known to be mutagenic, teratogenic and carcinogenic their fate in nature is therefore of environmental concern. (Sokhn *et al*., 2000; Bamforth and Singleton, 2005).

The persistence of PAHs in soil depends on a variety of factors, for example the chemical structure of the PAH, the concentration and dispersion of the PAH, and the bioavailability of the contaminant. Soil type and structure, pH, temperature, nutrients and water for the activity of the pollutant-degrading microbial community will also influence the time that PAHs persist in soil (Bamforth and Singleton, 2005). The association of PAHs with co-pollutants such as heavy metals will also prolong PAH residence time in soil. The presence of heavy metals in soil could inhibit microbial growth and hence limit the metabolism of PAHs in soil.

PAHs can be biologically degraded and removed from the natural environment and/or converted into less harmful products by the indigenous microbial community present in a contaminated environment (Bamforth and Singleton, 2005). PAH-degrading microorganisms are ubiquitously distributed in the natural environment, such as soils. These include bacteria such as the genus *Pseudomonas* and *Rhodococcus* as well as fungi such as *Chrysosporium* and *Aspergillus.* However, the presence of heavy metals in soil could adversely affect microflora populations and hence PAH biodegradation.

Metals are ubiquitous in nature and even those metals generally considered as pollutants are found in trace concentrations in the soil environment. Such metals include mercury, lead, arsenic, cadmium, chromium, manganese which are commonly associated with pollution and toxicity, but also include elements (e.g. Zn, Cu and Ni) which are essential in the metabolism of living organisms, albeit at low concentrations (Wong *et al*., 2004). Mining and ore refinement, nuclear processing, and industrial manufacture of a variety of products such as batteries, metal alloys, and fungicides are some of the sources of these metals in the soil environment. These metals in soil can exist as individual metals or more often as metal mixtures and their elevated concentrations in the soil environment have wide-ranging impacts on animal, plant, and microbial species (microbial growth is often slowed or inhibited completely in the presence of excessive amounts of metals) (Roane *et al*., 2005). Due to their toxic nature, the presence of metals in organic-contaminated sites often complicates and limits the bioremediation process.

PAHs and heavy metals are among mixed pollution of major concerns in the soil environment because of the risk they pose to soil health. Soils contaminated with PAHs often contain other pollutants such as heavy metals (HM) this is because often, these contaminants come from same sources (Maliszewska-kordybach and Smereczak, 2003). Sources of these pollutants in soil include atmospheric deposition, power and heat generation, old gasification and wood preserving plants, metallurgical industries, and fungicide application.

Fate and behaviour of PAHs and metals in soil

Diverse pathways in the soil environment influence the fate and behaviour of both PAHs and metals. PAHs in soil can volatilize, be leached, complex with soil minerals and organic matter, biodegrade to CO_2, or undergo carbon assimilation by the microbial biomass (**Figure 1**). Metals can be removed by volatilization, and when in an aqueous phase, losses can be a result of plant uptake, surface runoff, complexation or sorption to minerals and organic matter, and precipitation of the metal salt.

Figure 1: Fate and behaviour of an organic chemical (e.g. a PAH) in soil
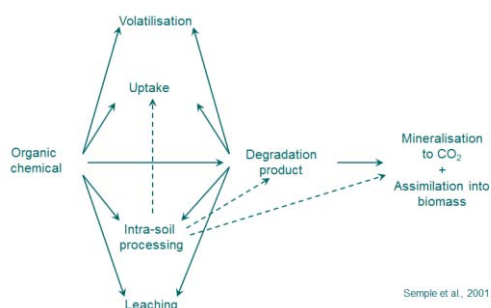
Ageing of PAHs and metals in soil is a major factor in determining their bioavailability, because of the affinity these co-contaminants have for soil organic matter and clay content. In well-aged soils, mild extractability, bioavailability and biodegradation assays for PAHs are usually low (Stokes *et al*., 2005). Ageing of metals in soils has been demonstrated to be a major factor in determining their bioavailability or lability, with bioavailability decreasing with increases in metal-soil contact times (Wendling, 2009). Several processes contribute to metal ageing in soils including incorporation into mineral structures, diffusion into pore spaces within minerals, nucleation or precipitation, mineral surface oxidation and entrainment via the formation of chemical complexes with soil solids (Wendling *et al*., 2009).

Metal toxicity to the microbial cell

Metals are essential components of microbial cells; for example, sodium (Na) and potassium (K) regulate gradients across the cell membrane, and copper, iron and manganese are required for the activity of key metalloenzymes in photosynthesis and electron transport (Roane *et al*., 2005). However, metals can also be extremely toxic to microorganisms (especially at high concentrations) impacting on microbial growth, morphology and biochemical activities as a result of specific interactions with cellular components (Giller *et al*., 1998; Wong *et al*., 2005). This toxicity of metals towards microorganisms affects the degradation of PAHs in soil.

Mechanisms of metal toxicity differ. Toxicity may occur as a result of binding of the metal to ligands containing sulfhydryl, carboxyl or phosphate groups such as proteins or nucleic acids (Roane *et al*., 2005). Mercury (Hg) and cadmium (Cd) cations readily bind sulfhydryl groups causing protein synthesis inhibition (**Figure 2**). It could also be as a result of metal-catalyzed decomposition of essential metabolites and analogue replacement of structurally important cell components. A good example is arsenic (As), which is bactericidal because it acts as an analogue of phosphate, disrupting nucleic acid structure and enzyme action (**Figure 2**) (Roane *et al*., 2005). High concentrations of lead (Pb) and nickel (Ni) retard cell division, while copper (Cu) and zinc (Zn) cause cell membrane disruption (**Figure 2**).
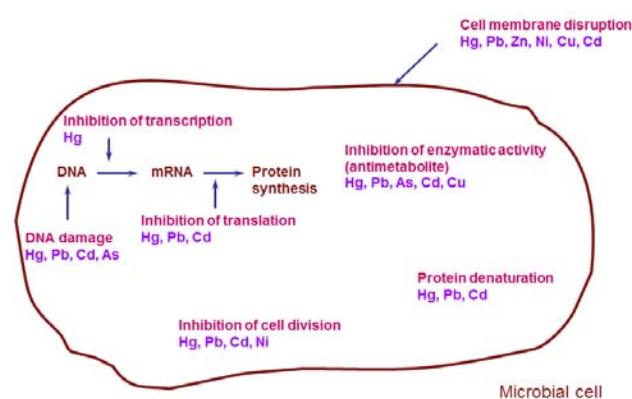

Figure 2: *Mechanisms of metal toxicity to a microbial cell*

A wide range of soil properties such as pH, organic matter content, clay content, iron oxide content and redox potential affect soil metal concentrations and hence the impact of metals on soil microbes (Giller *et al*., 1998). Of these, pH has the greatest influence because pH determines the solubility and speciation of metals in soil. The free metal ion is generally assumed to be the chemical species which is taken up by soil microbes and is toxic when present in an excess (Giller *et al*., 1998).

These negative effects of heavy metals on soil microbes and soil microbial processes can potentially limit the bioremediation of organic pollutants. Examples from the literature include:
- Decreases in phenanthrene degradation, microbial respiration and microbial numbers with increasing concentrations of Cu (Sokhn *et al.*, 2001; Obuekwe and Semple, 2011).
- Inhibition of phenanthrene biodegradation and microbial metabolic activity at high Zn concentrations (Wong *et al.*, 2005).
- Decrease in PAH biodegradation and mycelial soil colonization in Cd–PAH–contaminated soils (Baldrin *et al*., 2000).
- Lower microbial count and CO_2 evolution in soil in the presence of PAHs and cupric sulphate ($CuSO_4$). This is thought to be as a result of inhibition of microbial growth and metabolism in the presence of $CuSO_4$ (Atagana, 2006).

Methodologies for studying PAHs and metals in the laboratory

Techniques for studying soils contaminated with PAHs and metals in the laboratory examine:
(a) **PAH degradation** (b) **microbial activity** (respiration, enzyme activity *etc.*) and (c) **microbial population**. The PAH–metal(s) co-contaminant mixtures may be either artificially simulated in the laboratory or soils already contaminated with the co-contaminants in the environment are used. The co-contaminants are incubated in the

presence of indigenous microflora or introduced inocula for a period of time after which PAH degradation, microbial activity as well as bacteria count and/or fungal soil colonization are assessed.

Techniques for analysing **PAH degradation** include: solvent extraction and GC (Sokhn *et al*., 2001); solvent extraction and GC-MS (Baldrin *et al*., 2000; Wong *et al*., 2005); conversion of ^{14}C PAH into $^{14}CO_2$ using respirometry (Reid *et al*., 2001; Obuekwe and Semple, 2011). **Microbial activity** can be assessed by measuring CO_2 evolution with an infrared CO_2 analyser (Sokhn *et al*., 2001) or $^{14}CO_2$ evolution using respirometry (Reid *et al*., 2001; Obuekwe and Semple, 2011). Microbial metabolic activity can also be determined by measuring various enzyme activities (Wong *et al*., 2005; Baldrin *et al*., 2000). **Microbial populations** may be examined using a bacteria plate count technique (Sokhn *et al*., 2001; Atagana 2006; Obuekwe and Semple, 2011). Fungal soil colonization can be used to assess fungal soil growth (Baldrin *et al*., 2000).

Factors that influence the impact of metals on PAH biodegradation in soil

Soil type: Soil pH has the highest influence on metal toxicity because of its impact on metal solubility and speciation in soil. Soils with low pH usually contain metals in a more soluble form resulting in enhanced metal bioavailability and toxicity. For example, Wendling *et al.*, (2009) found an increase in isotopically exchangeable cobalt in acidic soils compared with neutral or alkaline pH. Biodegradation of PAHs in the presence of metals in soil with a low pH could therefore be impeded.

Organic matter content, clay content, and cation exchange capacity (CEC) are other soil properties that affect metal toxicity. Low lability of Cu in soils with high organic matter has been reported (Ma *et al*., 2006). Finer textured soil, which is much higher in clay content and cation exchange capacity, possesses a greater binding ability for added metals than coarse-textured soil (Kim *et al*., 2008).

Metal ageing in soil: Ageing of metals in soils is a major factor in determining their bioavailability and toxicity, with metal bioavailability decreasing as metal-soil contact time increases. For example, a decrease in the readily soluble and weakly sorbed Co pools in soils with time has been reported (Wendling *et al*., 2009). Recent unpublished research (Obuekwe and Semple, 2011) on the impact of Zn or Cu on phenanthrene catabolism in soil showed significant increases in phenanthrene catabolism at high Cu concentration (500 mg kg^{-1}) with time [also *vide infra*]. This could be as a result of Cu diffusing into nanopores or interstices and becoming less bioavailable to prevent phenanthrene catabolism in soil.

Metal concentration in soil: Heavy metals are known to be potentially toxic to soil microorganisms at high concentrations and can hinder the biodegradation of organic contaminant in soil (Wong *et al*., 2005). Many studies have reported an increase in metal toxicity with increasing concentration; Cu at concentrations of 700 and 7000 mg kg^{-1} soil were found to reduce microbial activity and phenanthrene biodegradation (Sokhn *et al*., 2001). Raised Zn concentrations in soil (720 and 1440 mg kg^{-1}) inhibited both phenanthrene biodegradation rates and microbial metabolic activity. High Cu concentrations (500 and 1000 mg kg^{-1}) reduced microbial numbers and phenanthrene biodegradation in soil (Obuekwe and Semple, 2011) [also *vide supra*].

Metal speciation: The potential toxicity of a heavy metal in soil depends upon its speciation and availability. Heavy metals are present in various forms with different degrees of mobility and bioavailability. The toxicity of a heavy metal can be linked to its free-ion activity (Chaperon and Sauve, 2008; Kim *et al*., 2008). Kim *et al*., (2008) provided evidence that Cu^{2+} was a better surrogate for estimating the toxic effect of Cu than Cu extractable by $CaCl_2$. Free Cu^{2+} ion is the likely toxic species of Cu, reacting directly with sulfhydryl groups on the active site of the enzyme (dehydrogenase) and causing its inhibition. Metal speciation in soil is influenced by a number of physico-chemical properties including soil pH, clay and organic matter content, Fe and Mn oxide composition and content, as well as long term fixation or ageing effects (Wendling *et al*., 2009).

Conclusion

Heavy metals at elevated concentrations in the soil environment have a range of effects on microbial species. These include a decrease in microbial count and disruption of nucleic acid and enzyme function, which subsequently reduce or impede the biodegradation of PAHs in soil. Heavy metal toxicity in soil depends on factors which affect their interaction with PAHs. Due to their toxic nature, the presence of metals in PAH–contaminated land sites can often complicate and limit bioremediation processes.

References

Atagana, H. I. (2006). Biodegradation of polycyclic aromatic hydrocarbons in contaminated soil by biostimulation and bioaugmentation in the presence of copper (II) ions. *World J. Microbiol. Biotechnol.*, **22**, 1145-1153.

Baldrin, P.; Wiesche, C.; Gabriel, J.; Nerud, F.; Zadrazil, F. (2000). Influence of cadmium and mercury on activities of ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotus osreatus* in soil. *Appl. Environ. Microbiol.*, **66**, 2471-2478.

Bamforth, S. M.; Singleton L. (2005). Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *J. Chem. Technol. Biotechnol.*, **80**, 723-736.

Chaperon, S.; Sauve, S. (2008). Toxicity interactions of cadmium, copper, and lead on soil urease and dehydrogenase activity in relation to chemical speciation. *Ecotoxicol. Environ. Saf.*, **70**, 1-9.

Giller, K. E.; Witter, E.; Mcgrath, S. P. (1998). Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. *Soil Biol. Biochem.*, **30**, 1389-1414.

Kim, B.; Mcbride, M. B.; Hay, A. G. (2008). Urease activity in aged copper and zinc-spiked soils: relationship to $CaCl_2$–extractable metals and Cu^{2+} activity. *Environ. Toxicol. Chem.*, **27**, 2469-2475.

Ma, Y.; Lombi, E.; Nolan, A. L.; Mclaughlin, M. J. (2006). Short-term natural attenuation of copper in soils: effects of time, temperature and soil characteristics. *Environ. Toxicol. Chem.*, **25**, 652-658.

Maliszewka-Kordybach, B.; Smereczak, B., (2003). Habitat function of agricultural soils as affected by heavy metals and polycyclic aromatic hydrocarbons contamination. *Environ. Int.*, **28**, 719-728.

Obuekwe, I. S.; Semple, K. T., Impact of Zn or Cu-phenanthrene contamination on phenanthrene catabolism in soil. (2011). Manuscript in preparation.

Roane, T. M.; Pepper, I. L.; Miller, R. M. (2005). Microbial remediation of metals. In *Bioremediation Principles and Applications*, Crawford R.L.; Crawford D.L. (eds.), Cambridge University Press, Cambridge, pp 312-340.

Semple, K.T.; Reid, B. J.; Fermor, T. R. (2001). Impact of composting strategies on the treatment of soils contaminated with organic pollutants. *Environ. Pollut.*, **112**, 269-283.

Sokhn, J.; De Leij, F.A. A. M.; Hart, T. D.; Lynch, J. M. (2001). Effect of copper on the degradation of phenanthrene by soil microorganisms. *Lett. Appl. Microbiol.*, **33**, 164-168.

Stokes, J. D.; Paton, G. I.; Semple, K. T. (2005). Behaviour and assessment of bioavailability of organic contaminants in soil: relevance for risk assessment and remediation, soil use and management. **21**, 475-486

Wendling, L. A.; Kirby, J. K.; McLaughlin, M. J. (2009). Aging effects on cobalt availability in soils. *Environ. Toxicol. Chem.*, **28**, 1609-17.

Wong, K. W.; Toh, B. A.; Ting, Y. P.; Obbard, J. P. (2005). Biodegradation of phenanthrene by the indigenous microbial biomass in a zinc–amended soil. *Lett. Appl. Microbiol.*, **40**, 50-55.

Paper 4   Characterisation of soils containing naturally occurring radioactive material (NORM) using *in situ* and rapid laboratory techniques. Case study: South Terras, Cornwall.

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Introduction

In-situ analysis is an important field for development as it becomes an increasingly important aspect of any

contaminated land investigation and remediation. At present, portable techniques are used predominantly as scanning tools to assess the spread and magnitude of any contamination, and to perform a preliminary analysis of the collected samples in a timely manner. It has been recognised that there is a need for a better understanding regarding the nature of rapid techniques available for assessment of NORM contaminated sites, and how the information provided by each technique can be coordinated to present a more detailed picture of the assessed site. The PhD project conducted by **Anna Kutner** at the University of Reading makes an attempt to bridge this gap. The preliminary data presented in this paper are a follow-up to the poster presented at the GeoSpec2010 conference in Lancaster in September 2010.

Case study site: South Terras, Cornwall

South Terras is a historical mining site located to the west of St Austell in the valley of the River Fal, Cornwall. The area is metaliferrous in character, located directly on a tin and uranium mineral ore vein, hence there is a long history of mining activities, which can be roughly divided into two periods: iron-tin mining (1870-1890s), uranium mining (1890s – 1910s), and subsequent radium extraction works (1910s – 1930s) [1, 2]. After closure in the early 1930s the site has been left undisturbed and currently has a SSSI (Site of Special Scientific Interest) status due to the occurrence of rare uranium ore minerals. Consequently South Terras provides an ideal environment for research on the behaviour of natural radionuclides in a post-industrial environment.

Methodology

The site has been investigated in two stages, each comprising a number of techniques currently employed in the industry and academia: (1) *in-situ investigation*, and (2) *sampling and laboratory analysis* (**Table 1**).

Table 1: A range of techniques employed (until December 2010)

(1) In-situ	(2) Laboratory techniques
• Geiger counter	• Gamma spectroscopy
• Radon detector (RAD7)	• Infrared (IR) techniques
• Portable X-Ray Fluorescence (PXRF)	• X-Ray Diffraction (XRD)
• Groundhog (GH)	• X-Ray Fluorescence (XRF)
• Portable gamma spectroscopy	• ICP-OES

The techniques aim to fully characterise the location (Groundhog) and nature of NORM contamination present at the site/in the samples (gamma spectroscopy), radon in ambient air (RAD7), composition of the surface of buildings still remaining at the site (PXRF), elemental composition (XRF, ICP-OES) and mineralogy of the topsoil (XRD, IR) based on the samples collected during the first field visit in April 2010. An additional aspect of this project involves a comparison of the methods employed in terms of their accuracy and precision, duration of analysis, speed of delivery of the results and portability.

Preliminary observations and results

Radon measurements were conducted throughout the site to establish the average concentration of radon in the ambient near ground, surface air of between 100-300 Bq m^{-3}. However in the Northern corner of the site the radon measurements reached 16,000 Bq m^{-3} (RAD7). It might indicate the location of the Northern shaft, not recorded on the current OS maps (**Figure1**, Mark A). A Geiger counter proved to be a useful tool to roughly indentify "hot spots", especially in the woodlands were the GPS signal used by the Groundhog system was lost. The areas of elevated count rate from the Geiger counter correlated perfectly with the historical location of the ore processing and extracting units (data for these findings will be reported at a later date). The ruined buildings located at the site still contain some level of surface contamination. The highest readings between 550-800 cps from Geiger counter were recorded on the walls of Building A (**Figure 1**, Mark B; the background at the site ~40-60 cps). The building was considered to be an office/storage unit, however the concentration of U above 200 ppm (PXRF) indicated that the building material used for construction contained materials enriched in uranium and thorium, possibly the spoil material from the industrial processes at the site. This would have meant elevated levels of radon gas during times of occupation. Slime tanks (**Figure1**, Mark C) located behind Building A contain radium-contaminated waste which were deposited there after the closure in 1930s. It is assumed that waste slurry was deposited in the tanks and then capped with wet concrete and covered with a concrete slab for better shielding. Uranium levels on the surface of the slab were below LOD (PXRF), but a radon peak was

recorded (>270 Bq m^{-3}), which might indicate imperfections in the concrete structure as a result of corrosion or concrete heterogenity resulting in emanation of radon from the decay of radium contained in waste.
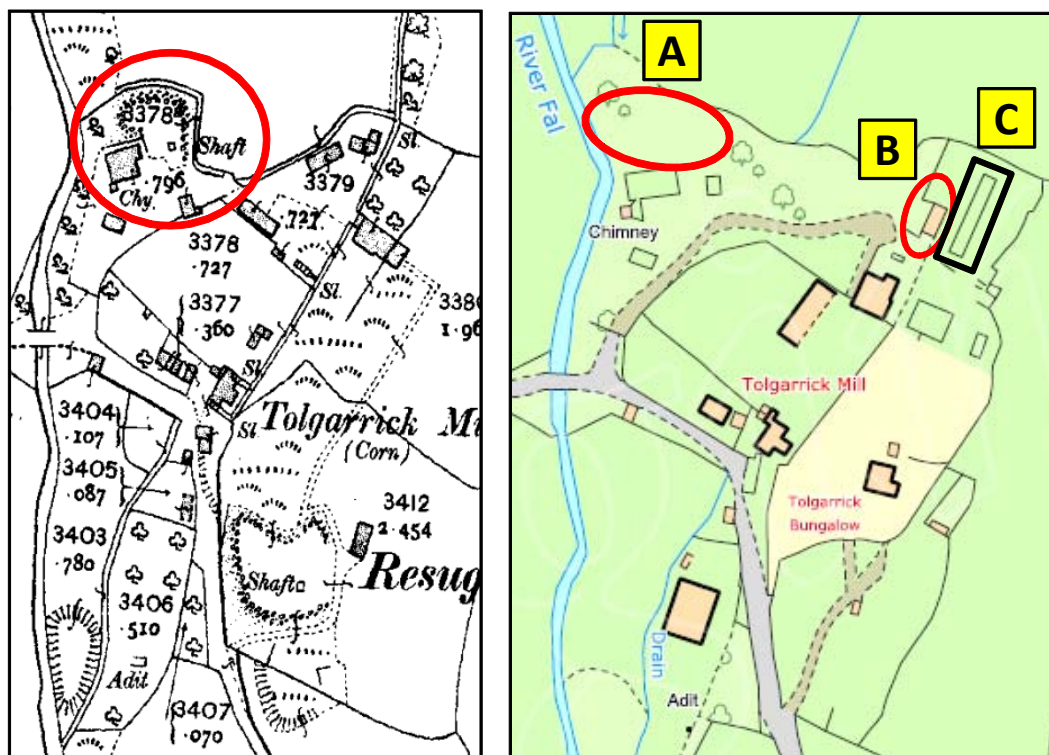

Figure 1: *Detailed maps of South Terras from 1907 (left) and 2010 (right, with marked "hot spots") [3].*

Mineralogy of soil samples from the site comprise: quartz (SiO$_2$) and hematite (Fe$_2$O$_3$), as well as calcite (CaCO$_3$) and aluminosilicate clays minerals, mostly kaolinite, the presence of which is to be expected considering the proximity of the China Clay industry located just a few miles north from South Terras. The soil samples have a high organic content which might provide a natural repository for some radionuclides due to complexing properties of humic and fulvic acids [4, 5].

Discussion

Due to the local geology, the study area is naturally enriched in U and Th and their decay products. However, due to industrial activity, the surface soils have been exposed to a high-grade uranium ore (approx. 36%) [1] comprising pitchblende and uraninite (UO$_2$-U$_3$O$_8$), as well as the products of ore extraction and processing procedures. As expected, the samples collected in the vicinity of extraction and smelting facilities were enriched in radionuclides from ^{238}U and ^{232}Th decay chains. The levels were compared to limits set by Radioactive Substances Act 1993 (RSA93). The presence of calcite (CaCO$_3$) and hematite (Fe$_2$O$_3$) at the site might indicate a potential for entrapment of the radium bivalent ion, Ra^{2+}, by substitution for calcium in calcite (due to their similar alkaline earth element affinity) or sorption to insoluble complexes with iron oxides [6]. However, uranyl ions (UO$_2^{2+}$) form highly soluble complexes with carbonates over a wide range of pH, thus there is a potential for leaching of U into groundwater or transport off-site to the nearby River Fal. However, analysis of the River Fal showed no increase in the concentration of uranium in the water, which is consistent with the chemistry of uranium which is bound to the sediments [7]. Additionally, the presence of high organic matter contents in the soils can indicate another potential for immobilisation of radionuclides due to the complexing properties of U with humic and fulvic acids.

Summary

The data presented in this short article illustrate the initial steps in data collection and processing as part of an in-situ and ex-situ study of South Terras. In the future the focus will be to finish interpreting the data and incorporating further data from various techniques to create a more detailed picture of the site. Those findings will be augmented by the historical records on the types of extraction techniques used in the mining process

extracted from the Cornwall Records Office.

Acknowledgments

References

1. Smale, C.V., *South Terras: Cornwall's premier uranium and radium mine.* Journal of the Royal Institution of Cornwall, 1993. **1**: p. 304-322.
2. Dines, H.G., *The metalliferous mining region of SW England. Volume 2: Memoirs of the Geological Survey of Great Britain (England and Wales).* 1956, London: HMSO.
3. EDINA, *South Terras.* 2010, Digimap Collection.
4. Keith-Roach, M.J., *The speciation, stability, solubility and biodegradation of organic co-contaminant radionuclide complexes: A review.* Science of the Total Environment, 2008. **396**(1): p. 1-11.
5. Carlson, L., *The role of organics on the migration of radionuclides in the geosphere. Final Report.* . 1989, Luxembourg: Nuclear Science and Technology, Commission of the European Communities.
6. Baker, A.C. and C. Toque, *A review of the potential for radium from luminising activities to migrate in the environment.* Journal of Radiological Protection 2005. **25**: p. 127-140.
7. Molimer-Martinez, Y., *et al.*, *The impact of a disused mine on uranium transport in the River Fal, South West England.* Journal of Environmental Monitoring, 2004. **6**: p. 907-913.

Paper 5   The Interest of Kinetic Considerations in Soil Heavy Metal Mobilization Assessment: An Overview

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Abstract

A general comprehensive picture of kinetic methodologies in soil heavy metal mobilization research area is presented in this paper. The author's experiences and the existing literature have been analysed and discussed briefly in terms of merits and disadvantages of each kinetic approach in mobilty and bioavailability prediction. The contribution of three kinetic methodologies including fractionation based on a single extractive scheme, Diffusive Gradients in Thin-films (DGT), and metal monitoring in Soil/Solution/Chelex batch system, in soil metal mobilization studies is discussed outlining concordances and discrepancies.

Introduction

Many agricultural and industrial activities such as sewage sludge, wastewaters, fertilizers, and smoke from factories lead to trace metal contamination in soils. This environmental problem and its effects on the health of living organisms, including human beings, is a matter of great concern to many researchers. However, it is known that the total concentration of metal in soils is not a reliable indicator of its potential ecological risks. The assessement of different physical-chemical forms of metal, usually called "speciation[1]", in soil/solution systems is subject to active environmental researches. There is an attempt to determine the part of metal in solution, which is available to biota. Chemical extractive schemes have been largely proposed for assessing the "reactive"

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[1] According to the glossary of terms used in toxicokinetics (IUPAC Recommendations 2003), speciation in chemistry is defined as the distribution of an element amongst defined chemical species in a system; source: PAC, 2004, 76, 1033.

or "labile" pools of metal in the soil solid phase (Young *et al.* 2006). Single and sequential extractive schemes were frequently used for estimating the operational mobile pool using different chemical reagent (Barona and Romero 1996, Tipping *et al.* 2003, Mocko and Waclawek 2004, Feng *et al.* 2005, Young *et al.* 2006, Manouchehri and Bermond 2009).

In most of these methods, the metal concentration is measured at equilibrium whereas natural systems are generally subject to changing conditions and are practically never at the equilibrium. Indeed, for these dynamic systems, the metal availability may be believed to be controlled by kinetic factors (Errecalde *et al.* 1998, Ma *et al.* 1999, Fortin and Campbell 2000, Hasseler and Wilkinson 2003, Slaveykova *et al.* 2003). Consequently, some rare methodologies involving the kinetic considerations like as kinetic fractionation (Bermond *et al.* 1998, Fangueiro *et al.* 2002, Bermond *et al.* 2005, Wasay *et al.* 2007) and "DGT" Diffusive Gradients in Thin-films (Zhang *et al.* 1995, Harper *et al.* 1998) have been proposed and developed. These investigations about the dynamic behaviour of diffrent metals, whether in their distribution in the soil solid phase or their concentration in pore solution, give useful informations for the improvement of soil-cleaning procedures, bioavailability prediction and risk assessment.

This paper aims to give a global picture of kinetic methodologies proposed in the literature for the evaluation of the dynamic of metal distribution in soils and related materials. The main objective is to focus on the advantages of kinetic approaches over the conventional methods and to evaluate their contribution in dynamic metal distribution assessment.

Kinetic Fractionation

The kinetic fractionation is a simple and rapid chemical speciation approach using a single extraction scheme and a mathematical model. A number of papers apply this approach to assess the concentrations of metal liable to release from the soil solid phase and the potential related environmental risk. The method consists of monitoring a leaching process *vs.* time using a chemical reagent. The fitting of leaching data (**Figure 1**) to a two-reaction model (**Equation 1**) provides the concentration of metals in two pools and the associated kinetic constants rate *i.e.* C_L or concentration of "labile" (quickly extracted) pool, C_{SL} or concentration of "slowly labile" (less quickly extracted) pool; k_L and k_{SL} the rate constants of desorption associated to these pools.

Indeed, the two reaction model, based on a pseudo-first order kinetic equation rate, represent the sum of two distinct patterns occuring simultaneously characterized by a fast-leaching phase and a second slower leaching pattern.

The labile and slowly labile pools present the operationally-defined metal concentration associated to different chemical soil constituents. The method does not involve the real soil fractions such as iron oxides or carbonates but it can be applied in mobility prediction field.

Equation 1

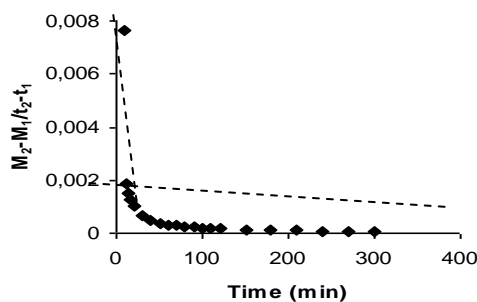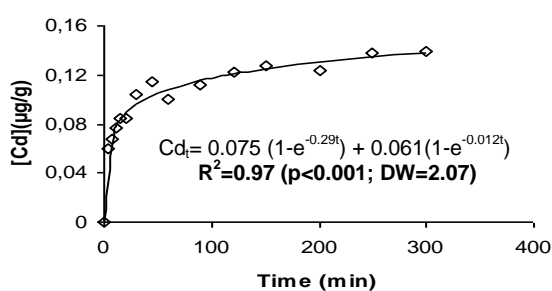$$C(t) = C_L(1 - e^{(-k_{(L)}t)}) + C_{SL}(1 - e^{(-k_{(SL)}t)})$$

Figure 1: An example of the kinetic fractionation using EDTA leaching, the case of Cd in a naturally polluted agricultural soil (a) kinetic fit of leaching data to the two reaction model; (b) distinction of two segments on the rate curve associated to two fast and slow kinetic patterns

Some organic acids such as citric acid and tartaric acid and chelating agents such as EDTA and DTPA were used for studying the desorption kinetics. EDTA has been extensively used for kinetic fractionation of heavy metals in the soil or sediment samples (Manouchehri *et al.* 2009). The feasibility of two-reaction model using EDTA was, at first, tested and validated by Ghestem and Bermond (1998) for a series of slightly polluted soil samples. Since then, several authors applied this approach for different heavy metals (Pb, Cu, Zn, Cd, Co, Ni, As and Hg) in a variety of soil and sediment matrices using EDTA, citrate and tartarate salts and confirmed the existence of two distinct (labile and less-labile) metal pools (Song and Greenway 2004, Fangueiro *et al.* 2005, Bermond *et al.* 2005, Brunori *et al.* 2005, Labanowski *et al.* 2008, Santos *et al.* 2010). A global survey of these works leads to some general remarks:

a. The kinetic fractionation is considered as an "operational" classification tool to evaluate the relative lability and mobilization rate of individual heavy metals.

b. The rate parameters, estimated by this approach, seem to be more influenced by experimental conditions than the arbitrary response of the reagent. The results obtained from the kinetic fractionation using EDTA and acetic acid showed no significant difference in terms of different metal (Cd, Zn, Cu, Pb and Hg) mobilization rate (Gismera *et al.* 2004, Wasay *et al.* 2007, Labanowski *et al.* 2008). The same order of mobility is reported in different works (**Table 1**). For example, whatever the operational or environmental conditions, a general trend is observed in terms of mobility i.e. cadmium is generally considered as the most mobile metal followed by copper and zinc. Lead represents the less mobile pattern.

c. Kinetic fractionation reveals generally concordant and complementary information with equilibrium based approaches such as simple and sequential extractive schemes and the confrontation of kinetics and equilibrium based approaches may permit to push on some conclusions in terms of metal mobilization in natural or contaminated soils or sediments.

d. The kinetic fractionation could be also considered as an efficient tool for determining the different species of some metals owning complex chemistry like as Mercury and Arsenic. It has been recently employed for studying the speciation of Hg bound to the soil organic matter (Issaro *et al.* 2010). The fit of the leaching data (in a soil-thiosulfate batch) to the two-rate reaction model resulted in two distinct segments on the rate curve suggesting the "labile" and "slowly labile" patterns. The identified pools could correspond to two different functional groups of reduced sulphur in soil organic phase.

e. The simplified assumptions made in kinetic modelling (extraction reactions are considered as pseudo first-order reactions) are sometimes inadequate for complex matrices such as soil compost. Besides, the application of pseudo-first order two-reaction model implies the use of reagent in excess with respect of the extractable metal; *e.g.* 0.05 mol.l^{-1} for EDTA; whereas, the use of EDTA at this level of concentration, as a natural ligand model, does not represent the environmental reality. Indeed, low level of EDTA concentrations were used to simulate the kinetics of Pb, Cu and Cd desorption (Manouchehri *et al.* 2006, Manouchehri *et al.* 2010). Low concentration of EDTA seems to render the kinetic simulation complicated and further assumptions are required in different operational and environmental conditions.

f. Finally, in an attempt to evaluate the reliability of this method in risk prediction fields, certain authors investigate the relationship between the labile concentrations resulted from the kinetic fractionation and the experimentally measured concentrations in soil living organisms. Bermond *et al.* (2005) demonstrated that the labile concentrations of Cd estimated by the kinetic fractionation in a series of agricultural soil-EDTA batches were correlated to the concentrations of Cd in wheat samples grown on these soils. However, the applicabiltyof labile-defined concentrations for quantitative interpretation of bioavailability is rarely investigated.

Table 1: *Kinetic rate constants reported in different works using the kinetic two-reaction model*

	k_L (min^{-1}) Pb	k_L (min^{-1}) Cd	k_L (min^{-1}) Cu	k_L (min^{-1}) Hg and As)	k_L (min^{-1}) Zn and (Ni)
Soil polluted by metallurgical fallout EDTA-leaching (Labanowski *et al.* 2008)	0.076-0.082	0.417-0.423	0.72-0.79	-	0.128-0.138
Non-polluted agricultural soil 0.002 mol.l^{-1} EDTA-leaching (Manouchehri *et al.* 2010)	0.16	0.81	0.41	-	-
Soils contaminated by agricultural processes Na-thiosulfate leaching (Issaro *et al.* 2010)	-	-	-	0.3 8.8	-
Urban compost samples (Song and Greenway 2004)	-	-	-	EDTA (0.017) citrate (0.014)	EDTA 0.025 citrate 0.016
Non-polluted agricultural soil (n=10) 0.05 mol.l^{-1} EDTA-Leaching (Bermond *et al.* 2005)	0.3	0.47	0.64	-	0.49
Naturally contaminated soils EDTA, citrate and tartarate leaching (Wasay *et al.* 2007)	EDTA 0.101 citrate 0.066 tartarate 0.071	EDTA 0.144 citrate 0.099 tartarate 0.093	EDTA 0.079 citrate 0.053 tartarate 0.074	EDTA 0.002 citrate 0.008 tartarate 0.052	EDTA 0.084 citrate 0.067 tartarate 0.074
Experimental agricultural field recieving organic amendment EDTA leaching (Santos *et al.* 2010)	0.25	-	0.30	-	0.38
Naturally contaminated soils Triton-X and anionic ligands leaching (Shin *et al.* 2006)	0.00002	0.008	0.0007		0.002
Contaminated soils from a mining site EDTA leaching (Brunori *et al.* 2005)	-	-	-	-	0.086 (0.085)

Diffusive Gradients in Thin-films (DGT)

DGT (diffusive gradients in thin-films) technique, developed by Davison and Zhang (1994 nature), permits the determination of kinetically labile species of metals and to follow their fluxes in water, soil and sediment. The DGT probe consists of a gel resin layer (Chelex for metals) as a sink of uptake the potentially labile pools. The masses of the metals trapped in the resin, after their diffusive transport through the hydrogel due to the concentration gradient, are used to calculated the flux of the metal from soil to DGT device (Zhang *et al.* 1995, Harper *et al.* 1998) providing information on the ability of the solid phase to replenish the soil solution with dissolved metal (free ion or labile complexes). The full description of the DGT procedure and modeling is given in the literature (Hooda *et al.* 1999, Harper *et al.* 2000, Ernstberger *et al.* 2002) and is not the scope of this paper. The main objective is to present this technique briefly to focus on its limits, among the rare dynamic approaches, to provide information about the potential labile fraction of metal and the related risk. Numerous recent works are reported applying this technique for measuring the fluxes of metals from the soil solid phase, modelling the kinetic exchanges of soil/plant transfer and finally studying the relationship between measured fluxes and bioavailable concentrations. Based on these works, following remarks are noteworthy:

a. Contrary to the chemical extractive schemes based on desorption/adsorption processes performed in dilute chemical solutions (chelating agent, dilute salts, etc...), DGT is an *in situ* technique and its application does not change the natural chemical environment.

b. The labile pool determined by kinetic fractionation using a chemical reagent may not adequately represent the in situ environmental distribution of metal. In some environmental conditions, the DGT-measured concentration could be considered as a better indicator of metal labile pool than the concentration in soil solution (Degryse *et al.* 2003).

c. DGT technique mimics the process of plant uptake and considers both labile concentration in soil solution (intensity[2]) and the capacity of soil resuply (capacity[3]); while in most chemical extractive approaches including the kinetic fractionation only the soil solid phase components are aimed.

d. In DGT technique, the transport through the system is supposed to be solely diffusion controlled and, contrary to extractive approaches using soil/extracant batches, the suspension is often not stirred.

e. As in kinetic fractionation, DGT device requires a model providing kinetic parameters and other divers information on the dynamic system. The DIFS model (DGT-Induced Fluxes in Soils) is developed assuming a single labile pool of metal in the soil solid phase in interaction with the solution and the kinetic of metal desorption/adsorption is studies focused on new versions of DIFS to improve the quantitative interpretation of DGT measurement (Lehto *et al.* 2006, Lehto *et al.* 2008).

f. Many authors studied the relationship between DGT-measured concentration and plant fluxes in order to test the reliability of the method to predict the bioavailability. The literature on the capacity of DGT to predict the bioavailability is relatively contradictory. Some authors consider it as a promise tool for quantitative measuring of bioavailable concentrations (Zhang *et al.* 2001, Zhang *et al.* 2004, Nolan *et al.* 2005, Black 2010, Fitz *et al.* 2003, Nolan *et al.* 2003). Others consider that DGT predict the bioavailability at non-toxic conditions (Almas *et al.* 2006) and other papers report that DGT does not provide significant advantages over conventional extractive approaches in terms of metal bioavailabilty (Duquene *et al.* 2010). In fact, the response of DGT device is affected by a range of conditions including the nature of target metal, binding properties of resin, soil physico-chemical properties (the moisture content or water retention capacity of soil, pH, oragnic matter, etc...), the level and source of contamination or the size of labile pool (Hooda *et al.* 1999, Conesa *et al.* 2010, Harper *et al.* 2000, Ernstberger *et al.* 2005, Garmo *et al.* 2006). On the other hand, the applicability of DGT in bioavailability prediction fields depends on the plant specific physiology, great diversity of route uptake mechanisms, diffusional and convective transport to the root and influence of root exudates.

g. Finally in some recent studies, the DGT technique is used (Bravin *et al.* 2010; Degryse *et al.* 2006) to identify the rate-limiting process (plant uptake kinetics or soil metal dissociation rate) in the soil-plant transfer of a given metal. For this purpose, the soil flux (measured by DGT) is assimilated to the plant metal fluxes (measured experimentally or by modelling). The identification of the rate-limiting process should greatly aid the quantitative interpretation of DGT measurements in bioavailability prediction.

Kinetic Monitoring in a soil/solution/resin batch model

Owning to the complexity of physical and chemical interactions in the soil, the modelling of TMs dynamic under natural environmental conditions seems still to be out of the capacity of the most recent methodologies. One way to try to avoid the complicated complexation, desorption and readsorption of metals is to simplify the metal desorption from the soil and its removal from the solution. For this purpose, the desorption process could be considered within a single extractive scheme using a single chemical complexing agent (as used in kinetic fractionation) and the removal process may be modelled using a sink mimicking the metal uptake by the root membrane handled by a chelating resin (as used in DGT technique). In this way, a batch experiment of soil/solution/sink is proposed (Manouchehri *et al.* 2011) as a simple model for mimicking the kinetics of soil/plant transfer and evaluating the limiting and controlling role of the metal kinetic transfer from soil to the Chelex sink. The difference with DGT technique is that both mechanisms of metal transport from the bulk–soil to plant roots (mass flow and diffusion) are considered in this approach. The kinetic regimes of Pb, Cu and Cd were monitored in this ternary batch system of soil, EDTA (the chelant) or water as natural soil solution, and Chelex (the uptake sink). The batch experiment was tested for a range of different soil matrices i.e. non-polluted agricultural soil, industrial contaminated soil and urban compost. The kinetic profiles of dissolved concentration

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[2, 3] The trace metal bioavailability and the related risk are the results of both "capacity" factor *i.e.* the soil solid phase aptitude to supply the metal to the soil solution and the "intensity" i.e. the concentration in pore solution (Barber 1995).

of Pb and Cu/Cd in soil/EDTA/Chelex or soil/water/resin mixture are assigned to P_1 and P_2, respectively. The kinetic pattern of Pb was distinctly different from those of Cd and Cu (**Figure 2**).
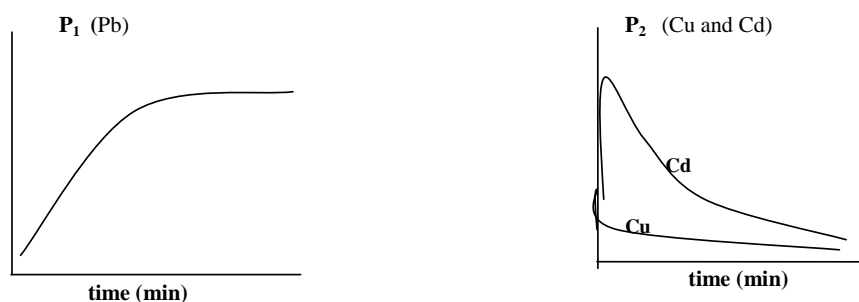

Figure 2: Typical kinetic profiles observed for TM's monitoring in soil/EDTA/Chelex mixture, P_1 for Pb and P_2 for Cu and Cd

P_1 is characterized by a slow increase of metal concentration in the solution reaching a plateau after a given time and no appreciable depletion in dissolved concentration is observed in the frame time of the reaction. Hence, Pb transfer to Chelex seems to be controlled by kinetics of desorption. The slow kinetic of desorption sustains a low level of resupply and no appreciable decline in dissolved concentration is observed over 300-min of the reaction time. The peak of the depletion may occur in the extended times where the solid phase concentration is more likely to become depleted.

Besides for P_2, the metal concentration attains rapidly (after a few minutes) a maximal value and then the dissolved concentration in solution is markedly depleted at higher reaction times. In the case of P_2, and particularly for Cd, the kinetics of resupply is presumably not limiting in the metal transfer to Chelex and the depletion in C_{soln} should be associated with the limited capacity of soil reservoir to replenish the solution. The different kinetic trends observed in the proposed batch system are not solely affected by the kinetics of resupply from the soil solid phase but depend also on the size of labile reservoir in the soil solid phase. The kinetic profiles of soil/EDTA/resin system of two agricultural soils were discussed in a recent paper (Manouchehri *et al.* 2011).

Conclusion

In all the kinetic methodologies presented in this paper, there is an effort to provide complementary information on potential metal availability to soil living organism. The kinetic fractionation is a simple extractive method to determine the concentration of metal in labile pool and desorption rate constants. It provides consensus conclusions in terms of relative mobility of different metals. DGT technique mimics the metal soil flux and measures the concentration liable to be trapped by a sink model. Applied by numerous authors, it is reported in the literature as a good surrogate of plant uptake. However, the interpretation of results in bioavailability prediction field requires considerations related to the environmental conditions. Up-to-date works report improvement in quantitative interpretation of DGT measurements in terms of modelling and field conditions. Kinetic methodologies are widely used, as bioavailability predictor, in the field of risk assessment analysis. However, numerous factors affect bioavailability predictions including soil environmental conditions, chemical speciation of metal in soil and solution, kinetics of metal transfer, diffusional and convective transport to the root, influence of root exudates, the plant specific physiology and great diversity of uptake route. Hence, a simple chemical treatment (kinetic fractionation), DGT or soil/solution/chelex monitoring could be considered as conformational tools to reduce the complexity of natural environmental systems but the obtained kinetic information give useful keys on potential mobility of trace elements and aid greatly the quantitative interpretation of bioavailability. They are particularly interesting to identify rate-limiting process in soil-plant metal transfer. This knowledge is critical for interpreting the results provided by different speciation approaches.

References

Barona A. and Romero F. (1996). "Fractionation of lead in soils and its influence on the extractive cleaning with EDTA." *Environmental Technology* **17**, 63-70.

Bermond A., Ghestem J.P. and Yousfi I. (1998). "Kinetic approach to the chemical speciation of trace metals in soils." *Analyst* **123**, 785-790.

Bermond A., Varrault G., Sappin-Didier V. and Mench M. (2005). "A kinetic approach to predict soil trace metal bioavailability: preliminary results." *Plant and Soil* **275**, 21-29.

Black A. (2010). "Bioavailability of cadmium, copper, nickel and zinc in soils treated with biosolids and metal salts." PhD Thesis, Lincoln University.

Bravin M. N., Le Merrer B., Denaix L., Schneider A. and Hinsinger Ph. (2010). "Copper uptake kinetics in hydroponically-grown durum wheat (Triticum turgidum durum L.) as compared with soil's ability to supply copper." *Plant and Soil* **331**, 91-104.

Brunori C., Cremisini C., D'annibale L., Massanisso P. and Pinto V. (2005). "A kinetic study of trace element leachability from abandoned-mine-polluted soil treated with SS-MSW compost and red mud. Comparison with results from sequential extraction." *Analytical and Bioanalytical Chemistry* **381**(7), 1347-54.

Conesa H. M., Schulin R. and Nowack B. (2010). "Suitability of using diffusive gradients in thin films (DGT) to study metal bioavailability in mine tailings: possibilities and constraints." *Environmental Science and Pollution Research* **17**, 657-667.

Degryse F., Smolders E. and Merckx R. (2006). "Labile Cd Complexes Increase Cd Availability to Plants." *Environmental Science and Technology* **40**, 830-836.

Duquene L., Vandenhove H., Tack F., Van Hees M. and Wannijn J. (2010). "Diffusive gradient in thin films (DGT) compared with soil solution and labile uranium fraction for predicting uranium bioavailability to ryegrass." *Journal of Environmental Radioactivity* **101**, 140-147.

Ernstberger H., Zhang H. and Davison W. (2002). "Determination of chromium speciation in natural systems using DGT." *Analytical and Bioanalytical Chemistry* **373**(8), 873-9.

Errecalde O., Seidl M. and Campbell P.G. (1998). "Influenc of a low molecular weight metabolite (citrat) on the toxicity of cadmium and zinc to the unicellular green alga Selenastrum Capricornutum: An exception to the free ion model." *Water Research* **32**(2), 419.

Fangueiro D., Bermond A., Santos E., Carapuça H. and Duarte A. (2002). "Heavy metal mobility assessment in sediments based on a kinetic approach of the EDTA extraction : search for optimal experimental conditions." *Analytica Chimica Acta* **459,** 245-256.

Fangueiro D., Bermond A., Santos E., Carapuça H. and Duarte A. (2005). "Kinetic approach to heavy metal mobilization assessment in sediments: choose of kinetic equations and models to achieve maximum information." *Talanta* **66,** 844-857.

Feng M. H., Shan X. Q., Zhang Sh. and Wen B. (2005). "A comparison of the rhizosphere-based method with DTPA, EDTA, $CaCl_2$ and $NaNO_3$ extraction methods for prediction of bioavailability of metals in soil to barley." *Environmental Pollution* **137**, 231-240.

Fitz W. J., Wenzel W. W., Zhang H., Nurmi J., ŠTipek K., Fischerova Z., Schweiger P., Köllensperger G., Ma L. Q. and Stingeder G. (2003). "Rhizosphere characteristics of the arsenic hyperaccumulator pteris vittata L. and monitoring of phytoremoval efficiency." *Environmental Science and Technology* **37**(21), 5008-5014.

Fortin C. and Campbell P. G. (2000). "Silver uptake by the green alga *CHLAMYDOMONAS REINHARDTII* in relation to chemical speciation : influence of chloride." *Environmental Toxicology and Chemistry* **19**, 2769-2778.

Garmo O. D., Lehto N. J., Zhang H., Davison W., Royset O. and Steinnes E. (2006). "Dynamic Aspects of DGT as Demonstrated by Experiments with Lanthanide Complexes of a Multidentate Ligand." *Environmental Science and Technology* **40**, 4754-4760.

Gismera M. J., Lacal J., Da Silva P., Garcia R., Teresa Sevilla M. and Procopio J. R. (2004). "Study of metal fractionation in river sediments. A comparison between kinetic and sequential extraction procedures." *Environmental Pollution* **127**(2), 175-82.

Harper M. P., Davison W. and Tych W. (2000). "DIFS-a Modelling and simulation tool for DGT induced trace metal remobilisation in sediments ansd soils." *Environmental Modelling and Software* **15**, 55-66.

Hasseler C. S. and Wilkinson K. J. (2003). "Failure of the biotic ligand and free-ion activity models to explain zinc bioaccumulation by Chlorella kesslerii." *Environmental Toxicolgy and Chemistry* **22**, 620.

Hooda P. S., Zhang H., Davison W. and Edwards A. C. (1999). "Measuring bioavailable trace metals by diffusive gradients in thin films (DGT): soil moisture effects on performance in soils." *European Journal of Soil Science* **50**, 285-294.

Issaro N., Besançon S. and Bermond A. (2010). "Termodynamic and kinetic study of the single extraction of mercury from soil using sodium-thiosulfate." *Talanta* **82**, 1659-1667.

Labanowski J., Monna F., Bermond A., Cambier Ph., Fernandez Ch., Lamy I. and Van Oort F. (2008). "Kinetic extractions to assess mobilization of Zn, Pb, Cu, and Cd in a metal contaminated soil: EDTA *vs.* citrate." *Environmental Pollution* **152**, 693-701.

Lehto N.J., Davison W., Zhang H. and Tych W. (2006). "Analysis of micro-nutrient behaviour in the rhizosphere using a DGT parametrised dynamic plant uptake model." *Plant and Soil* **282**, 227-238.

Lehto N.J., Sochaczewski L., Davison W.., Tych W.. and Zhang H. (2008). "Quantitative assessment of soil parameter (K_D and T_C) estimation using DGT measurements and the 2D DIFS model." *Chemosphere* **71**, 795-801.

Ma H., Kim S. D., Cha D. K. and Allen H. E. (1999). "Effect of kinetics of complexation by humic acid on toxicity of copper to *Ceriodaphina dubia*." *Environmental Toxicology and Chemistry* **18**(5), 828-837.

Manouchehri N. and Bermond A. (2009). "EDTA in soil science: A review of its application in soil trace metal investigations." *Terrestrial and Aquatic Environmental Toxicology* **3**, 1-15.

Manouchehri N., Besançon S. and Bermond A. (2006). "Major and trace metal extraction from soil by EDTA : Equilibrium and kinetic studies." *Analytica Chimica Acta* **559**, 105-112.

Manouchehri N., Besançon S. and Bermond A. (2011). "Kinetic characterizing of soil trace metal availability using soil/EDTA/chelex mixture." *Chemosphere, in press.*

Manouchehri N., Besançon S., Bispo A. and Bermond A. (2010). "Kinetic characterizing of trace metals in soil/solution/chelex-100 mixture." 20th annual meeting SETAC 2010 Seville.

Mocko A. and Waclawek W. (2004). "Three-step extraction procedure for determination of heavy metals availability to vegetables." *Analytical and Bioanalytical Chemistry* **380**(5-6), 813-7.

Nolan A. L., Lombi E. and Mclaughlin M. J. (2003). "Metal bioaccumultion and toxicity in soils: Why bother with speciation?" *Australian Journal of Chemistry* **56**, 77-91.

Nolan A. L., Zhang H. and Mclaughlin M. J. (2005). "Prediction of Zinc, Cadmium, Lead, and Copper Availability to Wheat in Contaminated Soils Using Chemical Speciation, Diffusive Gradients in Thin Films, Extraction, and Isotopic Dilution Techniques." *Journal of Environmental Quality* **34**, 496-507.

Santos S., Costa C.A. E., Duarte A., Scherer H.W., Schneider R. J., Esteves V. I. and Santos E. (2010). "Influence of different organic amendments on the potential availability of metals from soil: A study on metal fractionation and extraction kinetics by EDTA." *Chemosphere* **78**, 389-396.

Slaveykova V. I., Wilkinson K. J., Ceresa A. and Pretcsh E. (2003). "Role of fulvic acid on lead bioaccumulation by Chlorella kesslerii." *Environmental Science and Technology* **37**, 1114-1121.

Song Q. J. and Greenway G. M. (2004). "A study of the elemental leachability and retention capability of compost." *Journal of Environmental Monitoring* **6**(1), 31-7.

Tipping E., Rieuwerts J., Pan G., Ashmore M. R., Lofts S., Hill M. T. R., Farago M. O. and Thornton I. (2003). "The solid-solution partitioning of heavy metals (Cu, Zn, Cd, Pb) in upland soils of England and Wales." *Environmental Pollution* **125**, 213-225.

Wasay S.A., Barringal S.F., Tokunagal S. and Prasher S. (2007). "Kinetics of heavy metal desorption from three soils using citric acid, tartaric acid, and EDTA." *Journal of Environmental Engineering and Science* **6**, 611-622.

Young Sd, Zhang H, Tye Am, Maxted A, Thums C and Thornton I (2006). "Characterizing the availability of metals in contaminated soils. I. The solid phase: sequential extraction and isotopic dilution." *Soil Use and Management* **21**, 450-458.

Zhang H. and Davison W. (1995). "Performance Characteristics of Diffusion Gradients in Thin Films for the in Situ Measurement of Trace Metals in Aqueous Solution." *Analytical Chemistry* **67**(19), 3391-3400.

Zhang H., Lombi E., Smolders E. and Mcgrath S. (2004). "Kinetics of Zn release in soils and prediction of Zn concentration in plants using diffusive gradients in thin films." *Environmental Science and Technology* **38**(13), 3608-3613.

Zhang H., Zhao F. J., Sun B., Davison W. and Mcgrath S. P. (2001). "A new method to measure effective soil solution concentration predicts copper availability to plants." *Environmental Science and Technology* **35**, 2062-2067.

Paper 6 **Speciation and distribution of arsenic for studies of availability and mobility in mining environments**

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Introduction

Arsenic is the twentieth most abundant element in the earth's crust. It is a toxic trace element naturally present in all terrestrial and aquatic environments. High arsenic levels in water and soil endanger human health. Normal arsenic levels in soils range from 1 to 40 mg kg^{-1} [1], although some anthropogenic and volcanic activities can raise this upper limit. Various arsenic compounds are present in the environment and in biological systems [2]. Inorganic forms (arsenate and arsenite) are the predominant species, and at lower concentrations, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are found.

Arsenic toxicity depends on its speciation. Trivalent arsenic compounds are considered to be more toxic than pentavalent arsenic compounds. An approximate order of decreasing toxicity is as follows: R_3As > As(III) > $(RAsO)_n$ > As(V) > $R_2AsO(OH)$ > R_4As^+ > As(0); R = H > alkyl > aryl. Physico-chemical and environmental factors such as pH, redox potential, absorption-desorption processes and the presence of microorganisms contribute to arsenic speciation and toxicity.

The work described in this paper focuses on mercury mining areas in Asturias, a Spanish region with a historical tradition in mining and mineral processing. In particular, the extraction of mercury ores with a high arsenic content was quite widespread. As a result of these activities and related mechanical and chemical dispersion, significant amounts of arsenic have been released into the environment. Hyphenated analytical techniques are used to determine the extent and nature of arsenic pollution in the region.

Sampling area

The area we studied for this paper is located in Asturias (Northern Spain). Mieres and Pola de Lena are two districts where important mercury mines were exploited for decades and are nowadays abandoned. Three important mines belong to these areas: "La Soterraña", "La Peña-El Terronal" and "Los Rueldos" and were part of our study.

Abandoned mercury mine at Mieres, **Asturias**

Asturias was an important location for the production of mercury during the decade 1962-1972, with average annual productions of 15,000 flasks [3]. "La Peña-El Terronal" and "La Soterraña" were the most productive mines, located in Mieres and Pola de Lena districts, respectively, both in Central Asturias. The mines closed between 1973 and 1974 leaving a legacy of abandoned underground mines, extraction machinery and spoil heaps. In these Hg-mineralized areas, As is present in the form of As-rich pyrite, orpiment, realgar and occasionally arsenopyrite. Mine drainage and leachates from spoil heaps are often acidic with elevated As levels, leading to pollution of the Caudal River tributaries.

Arsenic speciation in mining waters

Arsenic is a common constituent of many metal ores and a trace element in sulphides associated with waste deposits from mining activities. Both natural and accelerated weathering of these arsenic-bearing materials results in the release of arsenic into the waters. The concentration of As in unpolluted waters typically ranges 1-10 μg L^{-1} [4]. However, arsenic levels in water in exploited sulphide mineralization areas can be extremely high. In Asturias, arsenic concentrations up to 57,000 μg L^{-1} have been found [5].

Speciation analysis [6] indicates that inorganic arsenic species, As(III) and As(V), prevail in natural waters, especially in sulphide oxidation environments [7]. Methylated compounds, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are not quantitatively important, and are only present in waters affected by industrial pollution [8].

Speciation of inorganic arsenic is a function of pH and redox potential (**Figure 1**). Under oxidising conditions at pH less than 6.9, $H_2AsO_4^-$ is dominant; at higher pH $HAsO_4^{2-}$ predominates. H_3AsO_4 and AsO_4^{3-} are present in extremely acidic or alkaline conditions, respectively. Under reducing conditions at pH less than 9.2, the uncharged arsenic species H_3AsO_3 prevails. At moderate or high redox potentials, As may be present as pentavalent oxyanions (arsenate): H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, and AsO_4^{3-}. However, under more reducing conditions (acidic and mildly alkaline) and lower redox potential, trivalent arsenic species (H_3AsO_3) are found [9, 10].
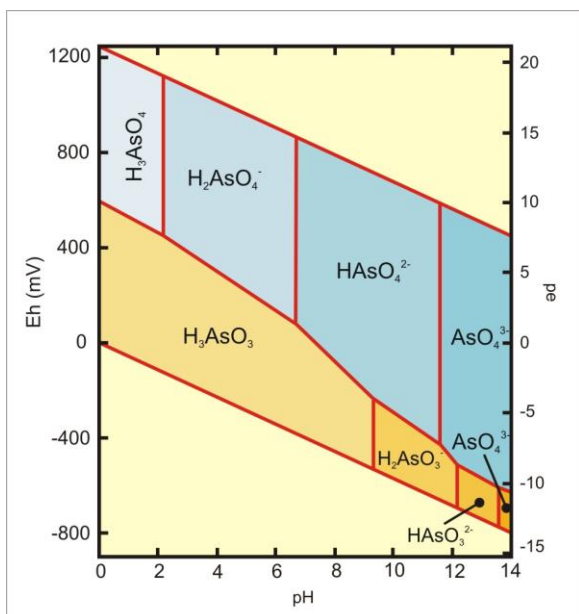
Figure 1: Eh-pH diagram for arsenic(III) and arsenic(V) oxyanions

In mining sites, acid mine drainage (AMD) is responsible for the release of relatively high levels of arsenic into surface and other waters. The high acidity generated during the oxidising processes gives rise to accelerated hydrolysis of minerals in the spoil materials, causing large quantities of the constituent elements to solubilise [11]. Arsenic minerals are associated with iron oxides or hydroxides, and a relationship between arsenic and iron speciation has been observed [11]. Correlations between the As(III)/(V) ratio and Fe(III) concentrations have been reported in waters from rivers affected by AMD. Also there is an inverse relationship between dissolved sulphate and arsenic which means that mobilisation of arsenic is not caused by oxidative dissolution of pyrite [12].

Analytical techniques for determining arsenic species

High-performance liquid chromatography is widely used for the analysis of arsenic species. Buffers (mainly phosphate buffers) are usually employed as mobile phases at different pH values ranging from 4.5 to 8.5, and they can be used both in isocratic and in gradient modes [13]. For the separation of cationic species, cationic exchange columns are used e.g. Hamilton PRP X-200 [14] and Supelcosil LC- SCX [15]. For the determination of all species, two columns of different nature can be placed in parallel [16]. A favoured option for the separation of many arsenic compound is a Dionex Ion Pac As7 column [17]; separation of As(III), MMA, DMA, As(V), AsB, TMAO, AsC, TMA can be achieved within 14 min with nitric acid as a mobile phase in gradient mode.

For detection, Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) is commonly used, since it provides a great sensibility, multi-elemental capability, and large dynamic range. And coupling with HPLC is relatively easy, because the usual flow rates for HPLC are totally compatible with the uptake flow rate of an ICP system.

Hydride generation (HG), followed by spectrometry, is also popular. This technique is based on the formation of volatile hydrides by means of chemical treatment of a sample with a reducing agent, typically sodium borohydride. The advantage of this technique is that the target arsenic species can be separated by volatilization from almost all other accompanying constituents in the sample through the HG process, so spectral and chemical interferences encountered in the detection systems are essentially eliminated. However, one drawback is that several organoarsenic compounds do not form volatile hydrides, so derivatisation methods to convert them in hydride-forming species are necessary. Microwave-assisted oxidation [18] and UV photo-oxidation [19] with potassium persulphate and sodium hydroxide have been proved successfully in this task. Among the possible spectrometry techniques, atomic fluorescence (AFS) is the most attractive one, given its lowest detection limits.

For our field work in Asturias, HPLC-HG-AFS was the technique selected for the analysis of waters at the three mining sites. We showed that the four more common arsenic species could be separated using the technique (**Figure 2**), but in the actual samples from the Asturian mining sites, only inorganic species were found (**Figure 3**).
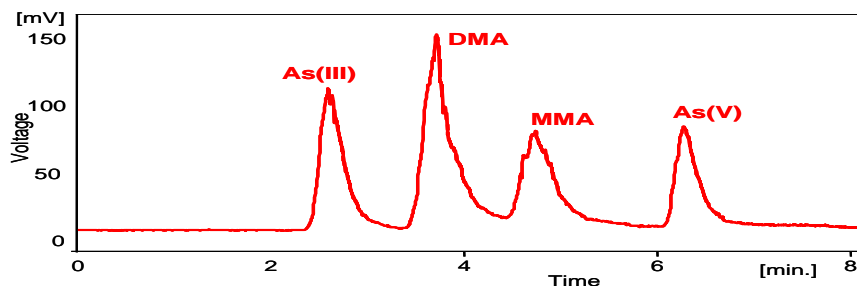
Figure 2: *Chromatogram showing the separation and identification of four arsenic species using HPLC-HG-AFS*
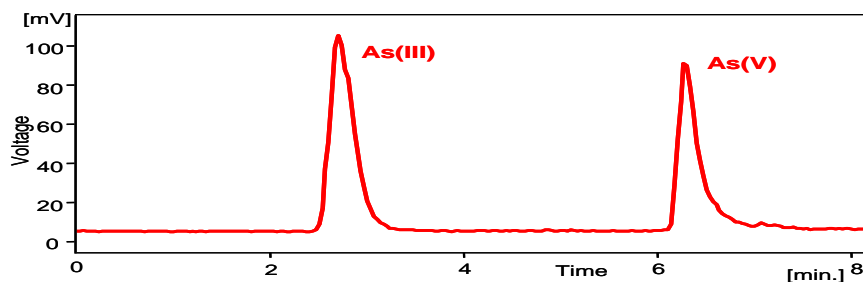

Figure 3: *Chromatogram of the arsenic species found in Asturian mining samples using HPLC-HG-AFS*

As (V) was the only species found in all the samples from the three mining sites. This is agreement with the Eh-pH diagram, given the Eh and pH value of our samples (Soterraña: Eh~200mV, pH~8; La Peña-El Terronal: Eh~200mV, pH~8; Los Rueldos: upstream, before the mine: Eh~100mV, pH~7; downstream, after the mine: Eh~600, pH~3).

The most elevated arsenic concentrations occur at La Soterraña mine (~43,000 µg L^{-1}). Arsenic concentration is lower at La Peña-El Terronal (~1600 µg L^{-1}). This mine was subject to preventive measures in 2002 when spoil heap wastes from tailings were segregated in a secure landfill site to avoid the formation of leachates. The influence of AMD is evident in the Los Rueldos mining site. Samples collected upstream had low As concentrations. Here the waters are at neutral pH and oxidative capacity and heavy metal concentrations are low. Downstream from the spoil heap the aqueous environment is altered, solubilisation of heavy metals occurs and arsenic levels are raised (~10,000 µg L^{-1}).

References

1. Ruiz-Chancho, M. J.; López-Sánchez, J. F.; Rubio, R. (2007). Analytical speciation as a tool to assess arsenic behaviour in soils polluted by mining. *Anal. Bioanal. Chem.*, **387**, 627-635.
2. [Gong, Z.; Lu, X.; Ma, M.; Watt, C.; Le, X. C. (2002). Arsenic speciation analysis. *Talanta*, **58**, 77-96.
3. SADEI, Datos y cifras de la economía asturiana; 1968-1991, Oviedo, Sociedad Asturiana de Estudios Económicos e Industriales, 1993, Oviedo, Spain.
4. Terlecka, E. (2005). Arsenic speciation analysis in water samples: A review of the hyphenated techniques. *Environ. Monit. Assess.*, **107**, 259-284.
5. Loredo, J.; Petit-Dominguez, M. D.; Ordóñez, A.; Galán, M. P.; Fernández-Martínez, R.; Alvárez, R.; Rucandio, M. I. (2010). Surface water monitoring in the mercury mining district of Asturias (Spain). *J. Hazard. Mater.*, **176**, 323-332.
6. Templeton, D. M.; Ariese, F.; Cornelis, R.; Danielsson, L. G.; Muntau, H.; Van Leeuwen, H. P.; Lobinski, R. (2000). Guidelines for terms related to chemical speciation and fractionation of elements. Definitions, structural aspects, and methodological approaches. *Pure Appl. Chem.*, **72**, 1453-1470.
7. Nordstrom, D. K.; Southam, G. (1997). Geomicrobiology of sulfide mineral oxidation. In: Banfield, J.F., Nealson, K.H. (Eds.), *Geomicrobiology: Interactions between Microbes and Minerals*, Reviews in Mineralogy, Vol. 35. Mineralogical Society America, Washington, DC, pp. 361–390.
8. Smedley, P. L.; Kinniburgh, D. G. (2002). A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochem.*, **17**, 517–568.

9. Yan, X. P.; Kerrich, R.; Hendry, M. J. (2000). Distribution of arsenic(III), arsenic(V) and total inorganic arsenic in porewaters from a thick till and clay-rich aquitard sequence, Saskatchewan, Canada. *Geochim. Cosmochim. Acta*, **64**, 2637–2648.
10. Mandal, B. K.; Kazno, T.; Suzuki, K. T. (2002). Arsenic round the world: A review. *Talanta*, **58**, 201–235.
11. Sarmiento, A. M.; Nieto, J. M.; Casiot, C.; Elbaz-Poulichet, F.; Egal, M. (2009). Inorganic arsenic speciation at river basin scales: The Tinto and Odiel Rivers in the Iberian Pyrite Belt, SW Spain. *Environ. Pollut.*, **157**, 1202-1209.
12. Harvey, F. H.; Swartz, C. H.; Badruzzaman, A. B. M.; Keon-Blute, N.; Yu W.; Ashraf Ali ,M.; Jay, J.; Beckie, R.; Niedan, V.; Brabander, D.; Oates, P. M.; Ashfaque, K. N.; Islam, S.; Hemond, H. F.; Ahmed, M. F. (2002). Arsenic mobility and groundwater extraction in Bangladesh. *Science* **298**,1602-1606.
13. Koch, I.; Wang, C. A. L.; Ollson, C. A.; Cullen, W. R.; Reimer, K. J. (2000), The predominance of inorganic arsenic species in plants from Yellowknife, Northwest territories of Canada, *Environ. Sci. Technol.*, **34**, 22-26.
14. Súñer, M. A.; Devesa, V.; Rivas, I.; Velez, D.; Montoro, R. (2000). Speciation of cationic arsenic species in seafood by coupling liquid chromatography with hydride generation atomic fluorescence detection. *J. Anal. At. Spectrom.*, **15**, 1501-1507.
15. Feldmann, J.; John, K.; Pengrepecha, P. (2000). Arsenic metabolism in seaweed-eating sheep from Northern Scotland. *Fresenius J. Anal. Chem.*, **368**, 116-121.
16. Kuehnelt ,D.; Goessler, W.; Irgolic, K. J. (1997). Arsenic compounds in terrestrial organisms: II. Arsenocholine in the mushroom amanita., *Appl. Organomet. Chem.*, **11**, 459-470.
17. Londensborough, S.; Mattusch, J.; Wennrich, R. (1999). Separation of organic and inorganic arsenic species by HPLC-ICPMS. *Fresenius J. Anal. Chem.*, **363**, 577-581.
18. Lamble, K. J.; Sperling, M.; Welz, B. (1996). Arsenic speciation in biological samples by on-line high performance liquid chromatography-microwave digestion hydride generation-atomic absorption spectrometry, *Anal. Chim. Acta* , **334**, 261-270.
19. Zhang, X.; Cornelis, R.; de Kimpe, J.; Mees, L. (1996). Arsenic speciation in serum of uraemic patients based on liquid chromatography with hydride generation atomic absorption spectrometry and on-line UV photo-oxidation digestion. *Anal. Chim. Acta*, **319**, 177-185.

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