

WATER EXTRACTABILITY OF TRACE METALS FROM SOILS: SOME PITFALLS

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Abstract. Water soluble and/or dissolved metals represent the most ecotoxicologically relevant fraction of metals in the environment. However, water extractions may be prone to errors. This study aims to evaluate the performance of 5 filters as well as Rhizon soil moisture samplers, with respect to metal adsorption and/or release by the filter. In addition, the effect of equilibration time on water extractions of different types of soils was evaluated (silty loam, silty clay loam, loamy sand). Filtrations of synthetic solutions containing $40 \mu\text{g l}^{-1}$ Zn, $20 \mu\text{g l}^{-1}$ Cu, Ni and Pb, $10 \mu\text{g l}^{-1}$ Cr and $2 \mu\text{g l}^{-1}$ Cd were conducted using the different filters. The synthetic solutions either contained (i) no other competitive cations (Ca, Mg, Na, K, Fe, Mn, Al) (A_1), (ii) competitive cations at concentrations similar to those observed in soil solutions (A_2), (iii) competitive cations at 10 times lower concentrations than those in the synthetic soil solution (A_3). Whiteband filters were observed to retain considerable amounts of trace metals (except Cr), both in the presence and absence of other competitive cations. Millipore filters did not exhibit metal retention. Rhizon soil moisture samplers did not retain trace metals from the synthetic soil solution (A_2), whereas at lower concentrations of competitive cations (A_1 , A_3) retention of Cu and Pb was observed. Whiteband filters without a predefined pore diameter allowed colloidal material of unknown particle size to pass into the filtrate, making interpretation of results very difficult and comparison between studies using different filters impossible. Millipore filters with a predefined pore diameter are to be recommended for this purpose. However, particular attention must be paid to potential constitutive Zn release by the sintered glass filter holders, the effect of which can be reduced by rigorous acid washing prior to and following every use. Rhizon samplers were also considered to be useful tools with well-defined and sufficiently small pore diameters to withhold colloidal material. However, in the absence or at reduced concentrations of competitive cations (A_1 , A_3) retention of trace metals by the Rhizons, particularly Cu and Pb, was observed. Finally, short equilibration times may be insufficient for full assessment of the water extractable pool of trace metals in the soil.

Keywords: filtration, soil, sediment, trace metals, heavy metals, water extractable

1. Introduction

Current legislation concerning allowable metal contents in soils is mainly based on total metal concentrations rather than on actual exposure concentrations. Considering the extreme variation in soil physicochemical properties, total concentrations

do not represent biological availabilities and toxicities of metals in different soils (Tack Verloo, 1995; Boyd and Williams, 2003). Water soluble metal ions can however easily be mobilised, and may be considered as highly available (Seguin *et al.*, 2004). To assess the readily available metal fractions under field conditions, collection and analysis of pore water has therefore become an important aspect of many environmental monitoring programs. Chapman *et al.* (2002) concluded that pore water testing and analyses can be effective tools provided their limitations are well understood by researchers and managers. Concentrations and chemical species of trace metals in soil solutions as affected by soil properties can become a powerful tool in environmental risk assessment of trace metals (Meers *et al.*, 2005). Carr and Nipper (2001) also concluded from a SETAC (Society of Environmental Toxicology and Chemistry) Technical Workshop on sediment pore water toxicity testing: "Determination of chemical concentrations in pore waters is recommended, in addition to the regular contaminant measurements conducted in the whole sediment, as a means of providing information on routes and levels of exposure, aiding in the interpretation of test results, and identifying sources of toxicity. It is however nearly impossible to avoid artefacts and chemical changes when removing pore water from sediment. Sampling, extraction, and storage techniques are critically important for achieving the most field-representative samples of pore water." (Carr and Nipper, 2001, cited in: Förstner, 2004). Bufflap and Allen (1995a) distinguished four means of extracting sediment pore water. Two of the methods, squeezing and centrifugation are *ex situ* and require the removal of soil or sediment from the natural system and the other two, vacuum filtration and dialysis, have the advantage that they can be performed *in situ*. Knight *et al.* (1998) also listed some additional disadvantages of centrifugation: it is time consuming and the collected sample often requires further filtration or centrifugation. In addition to each method having its own advantages and disadvantages, several general sources of error can alter pore water chemical concentrations: oxidation, sediment sampling, metal contamination, temperature artefacts and filtration (Bufflap and Allen, 1995b). If soils and sediments are too dry and pore water cannot be collected, water can be used as an extractant to estimate the mobile and potentially bioavailable metal fraction. In that case, different liquid to solid ratios (L:S) and equilibration times can be applied, which can make comparison of data between different studies very difficult (Tack *et al.*, 2002). Moreover, filtration is often applied during pore water sampling and in procedures to determine water soluble metal fractions, but it can be a source of error. If samples are not filtered during or after extraction, the residual particles can cause errors in two ways: interferences during the analytical procedure and sorption of metals to the particles altering the metal concentrations in the pore water (Bufflap and Allen, 1995b). Also, sample acidification for temporary storage would result in solubilization of metals which were previously sorbed to colloidal particles. However, if filters are used, these filters can also be a source of error as they can sorb or release metals. For example, ceramic suction cups for vacuum filtration are known to suffer from adsorption of charged groups, particularly trace metals,

phosphorus and ammonium groups (Wenzel and Wieshammer, 1995; Grossman and Udluft, 1991). Such effects may be particularly important when filtering weak extractants with low overall ionic strength, compared to stronger salt solutions for which the filters are usually designed. Wenzel and Wieshammer (1995) concluded that many reported experiments on sorption of trace metals by various materials are not relevant because of the unrealistic high metal concentrations of the test solutions. They also concluded that alternate plastic materials should be designed and tested sufficiently in the field. As a result, “Rhizon” type MOM *in situ* porous plastic samplers have been recently designed for measuring low metal contents in soil solutions during field experiments. These samplers may allow simultaneous and sequential sampling of soil pore water at different depths of the soil profile and provide an *in situ* non-destructive sampling method (Luo *et al.*, 2003). Knight *et al.* (1998) also stated that these samplers would have no cation exchange capacity.

All kinds of soil moisture sampling techniques have already been used to study metal mobility and availability in pore waters of specific soils and sediments (e.g. Rhizon samplers by Knight *et al.* (1998); centrifugation by Arnold *et al.* (2003) and Ma and Dong (2004); water extraction by Svete *et al.* (2000)). However, few studies have been conducted to compare metal extractability using these different techniques on the same soils or sediments (e.g. Tiensing *et al.*, 2001; Ludwig *et al.*, 1999). Moreover, little attention is given up to now to the performance of filter materials with respect to trace metal adsorption and desorption when filtering soil solutions with low ionic strength. Weltje *et al.* (2003) described the adsorption of five metals to eight types of 0.2 μm membrane filters used for sterilizing a plant (*Lemna minor* L.) culture medium. Results showed that metals had the lowest affinity for polycarbonate and nylon filters and the highest affinity for cellulose- and polyester-type filters. These authors suggested the use of polycarbonate or nylon filters to sterilize nutrient solutions, especially when dealing with low volumes, a high pH and low metal concentrations. Our study aimed to evaluate the performance of five filters as well as the “Rhizon” type MOM *in situ* porous plastic soil moisture sampler, with respect to trace metal adsorption and release of metals by the filter to soil solutions with low ionic strength. In addition, the effect of equilibration time on metal extractability by water was evaluated.

2. Material and Methods

2.1. FILTER COMPARISON

Table I presents the various filters used in the experiments. Filter A-D are common whiteband filter papers, E is an *in situ* porous plastic sampler under vacuum which is supposed to exhibit no cation exchange capacity (Knight *et al.*, 1998) and F is used for *ex situ* vacuum filtration. The filters originated from Schleicher & Schuell, Dassel, Germany (filter A), Machery-Nagel, Düren, Germany

TABLE I

Filters used in the experiments: A-D Whiteband filters, E Rhizon soil moisture sampler, F Millipore filter

A	S&S 589 ² white ribbon ashless filter paper	Schleicher and Schuell, Dassel, GE
B	MN 640 m white box ashless filter paper	Machery-Nagel, Düren, GE
C	Whatman 2 ^v folded filter papers	Whatman, Maidstone, UK
D	MN 280 ¹ / ₄ folded filters	Machery-Nagel, Düren, GE
E	Rhizon MOM-type soil moisture samplers (0.1 μm ore)	Eijkelkamp Agrisearch Equipment, Giesbeek, NL
F	MN porafil CM membrane filter (0.45 μm pore)	Machery-Nagel, Düren, GE

TABLE II

Synthetic solutions used for the filter comparison experiments (dissolved in deionised water)

Trace metal solution (A₁)

40 $\mu\text{g l}^{-1}$ Zn
20 $\mu\text{g l}^{-1}$ Cu, Ni, Pb
10 $\mu\text{g l}^{-1}$ Cr
2 $\mu\text{g l}^{-1}$ Cd

Synthetic soil solution (A₂)

40 $\mu\text{g l}^{-1}$ Zn	400 mg l^{-1} Ca
20 $\mu\text{g l}^{-1}$ Cu, Ni, Pb	40 mg l^{-1} Mg
10 $\mu\text{g l}^{-1}$ Cr	20 mg l^{-1} Na, K
2 $\mu\text{g l}^{-1}$ Cd	200 $\mu\text{g l}^{-1}$ Fe, Mn, Al

1/10 strength synthetic soil solution (A₃)

40 $\mu\text{g l}^{-1}$ Zn	40 mg l^{-1} Ca
20 $\mu\text{g l}^{-1}$ Cu, Ni, Pb	4 mg l^{-1} Mg
10 $\mu\text{g l}^{-1}$ Cr	2 mg l^{-1} Na, K
2 $\mu\text{g l}^{-1}$ Cd	20 $\mu\text{g l}^{-1}$ Fe, Mn, Al

(filters B, D, F), Whatman, Maidstone, United Kingdom (filter C) and Eijkelkamp Agrisearch Equipment, Giesbeek, Netherlands (filter E).

To compare filter performance, various synthetic solutions, varying in composition (Table II), were allowed to pass. In addition, the overall performance of the filters was also assessed by water extraction of air-dry soil samples. General characteristics of the soil (S₁) used for the filter comparison are presented in Table III. Protocols for soil analysis are presented below. Water extraction was performed by equilibrating 10 g of air-dried, ground and sieved soil for 24 h in 50 mL of deionised water while shaking. Shaking was performed on planar shaker. Subsequently, the mixture was filtered using the various filters under investigation. All experiments were performed in quadruplicate.

TABLE III

General properties of the soils under study: electrical conductivity (EC), soil actual acidity (pH-H₂O), carbonate content (CaCO₃), organic matter (OM), soil texture, cation exchange capacity (CEC), texture class, total metal content; intervals denote standard deviations ($n = 3$)

		Soil		
		S ₁	S ₂	S ₃
EC	$\mu\text{S cm}^{-1}$	52 ± 1	1963 ± 20	45 ± 3
pH-H ₂ O		5.9	7.6	5.2
CaCO ₃	%	0.0 ± 0.2	3.8 ± 0.6	0.0 ± 0.1
OM	%	3.5 ± 0.1	8.5 ± 0.6	4.9 ± 0.1
Clay	%	15	51	6
Silt	%	60	47	14
Sand	%	24	2	80
CEC	cmol (+) kg^{-1}	5.6 ± 0.2	39.2 ± 0.5	3.7 ± 0.8
USDA		Silt loam	Silty clay	Loamy sand
Cd	mg kg^{-1}	0.19	0.92	0.26
Cr	mg kg^{-1}	12	65	6.7
Cu	mg kg^{-1}	12	54	0.02
Ni	mg kg^{-1}	3.5	34	1.7
Pb	mg kg^{-1}	20	49	6.4
Zn	mg kg^{-1}	21	188	18

To examine potential metal retention (or release) by the filters, 50 mL of the solutions were applied to the filters. Subsequently analyses results of filtrates were compared with those of unfiltered control samples. For the Rhizon MOM soil moisture samplers (filter E), vacuettes each with a total volume capacity of 9 mL were applied instead of 50 mL solutions. To examine the Rhizon performance, a series of 20 vacuettes was subsequently connected to each Rhizon sampler. From the cumulative difference between the concentrations of metals recovered in the filtrate and those in the original trace metal solution, a theoretical cation exchange capacity for the Rhizons was calculated. All experiments were performed in quadruplicate.

Stock solutions used for the comparison of the various filters are presented in Table II. The first solution is a trace metal stock solution (Cd, Cr, Cu, Ni, Pb, Zn), without any other elements added. In addition to trace metals, the second solution also contains other cations (Ca, Mg, K, Na, Fe, Mn, Al) at concentrations typical for a soil solution. Finally, the third solution contains the other cations at concentrations 10 times below those in the synthetic soil solution to mimic effects in a single extraction with deionised water as extractant. The metals Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn were pipetted from Merck standard solutions (Merck, Darmstadt,

Germany). The exchangeable bases Ca, Mg, K and Na were applied in the form of nitrates (laboratory grade; Merck, Darmstadt, Germany). As a result of using the acidic (0.5 M HNO₃) Merck metal standard solutions to prepare the synthetic solutions, the pH of the synthetic solutions is also slightly acidic (4.3). This is an unavoidable disadvantage which does not represent realistic field conditions, but it will be taken into account in the discussion.

Metal analysis was performed using Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES; Varian Vista MPX, Varian, Palo Alto, CA, USA). Concentrations below the detection limit of the ICP-OES instrument were determined by Graphite Furnace – Atomic Absorption Spectrometry (GF-AAS) with Zeeman correction (SpectrAA 800, Varian, Palo Alto, CA, USA). The detection limits were 2 µg l⁻¹ Cd, 10 µg l⁻¹ Cu, 5 µg l⁻¹ Cr, 10 µg l⁻¹ Ni, 20 µg l⁻¹ Pb and 35 µg l⁻¹ Zn for ICPOES and 0.1 µg l⁻¹ Cd, 2 µg l⁻¹ Cu, 0.5 µg l⁻¹ Cr, 2 µg l⁻¹ Ni and 2 µg l⁻¹ Pb for GF-AAS.

2.2. EQUILIBRATION TIME

Three soils were used in the experiments: a silty soil (S₁), a clayey soil (S₂) and a sandy soil (S₃). Properties of these soils are presented in Table III. Protocols for soil analysis are presented below. To assess the importance of equilibration time in water extractions, a short equilibration time (2.5 h) was compared with a long equilibration period (48 h). In each case, 50 mL of deionised water was added to 10 g of air-dry soil and allowed to shake on a planar shaker. Finally, the mixtures were filtered over Millipore filter (CM-type, Machery-Nagel, Düren, Germany) and analysed using ICPOES (Varian Vista MPX, Varian, Palo Alto, CA, USA) or GF-AAS with Zeeman correction (SpectrAA 800, Varian, Palo Alto, CA, USA). Extractions were performed in triplicate.

2.3. SOIL PROPERTIES

All analyses for soil characterisation and total metal content assessment were performed in triplicate. Soil conductivity was measured with a WTW LF 537 electrode (Wissenschaftlich-Technischen Werkstätten, Weilheim, Germany) after equilibration for 30 min. in deionised water at a 5:1 liquid:solid ratio and subsequent filtering (white ribbon; Schleicher & Schuell, Germany). To determine pH (H₂O), 10 g of air-dry soil was allowed to equilibrate in 50 mL of deionised water for 24 h. For determination of potential soil pH, 50 mL of 1 M KCl was added to 10 g of air-dried soil and allowed to equilibrate for 10 min. The pH of the supernatant was then measured using a pH glass electrode (Model 520A, Orion, Boston, MA, USA). The total carbonate content present in the soil was determined by adding a known excess quantity of sulphuric acid and back titrating the excess with sodium hydroxide (Van Ranst *et al.*, 1999). Organic matter was determined using the method described by

Walkley & Black (Allison, 1965). The grain size distribution of the soil samples was determined using laser diffractometry (Coulter LS200, Miami, FL, USA) with the clay fraction defined as the 0–6 μm fraction (Vandecasteele *et al.*, 2002). This fraction was found to correspond with the 0–2 μm fraction using the conventional pipette method. Likewise, 6–63 μm was used as the silt fraction and 63–2000 μm as the sand fraction. The Cation Exchange Capacity (CEC) of the sediment was determined by first saturating the soil matrix with NH_4^+ , then desorbing the NH_4^+ by K^+ and measuring the quantity of the NH_4^+ in the leachate (Van Ranst *et al.*, 1999). Soil metal content was determined after *aqua regia* digestion (Van Ranst *et al.*, 1999). Analysis was subsequently performed, using ICP-OES (Varian Vista MPX, Varian, Palo Alto, CA, USA).

2.4. STATISTICAL ANALYSIS

Descriptive statistics and significance analysis (compare means *t*-test; Dunnett's multiple comparison *t*-test) were performed using the Excel 9.0 (Microsoft Inc.) and SPSS 11.0 software packages (SPSS Inc.).

3. Results and Discussion

3.1. FILTER COMPARISON

3.1.1. Trace Metal Stock Solution (A_1)

Figure 1 presents the observed concentrations of trace metals in the filtrate after filtration of a stock solution containing 40 $\mu\text{g l}^{-1}$ Zn, 20 $\mu\text{g l}^{-1}$ Cu, Pb and Ni, 10 $\mu\text{g l}^{-1}$ Cr and 2 $\mu\text{g l}^{-1}$ Cd. Cr was not retained by any of the filters, attributed to its presence in CrO_4 anionic form. Zn, Cd, Ni and Cu were largely retained by all filters except the Millipore filter (F). All filters also retained Pb to a high degree, with the Millipore filter adsorbing the lowest quantities as indicated by concentrations recovered in the filtrate. Moreover, it should be noted that the pH of this stock solution was slightly acidic (4.3) as it was prepared from acidic standard solutions. At higher pH under more realistic field conditions, retention is expected to be even higher. Trace concentrations of Zn appeared to be released in the Millipore filter system (F). Follow-up experiments however, revealed that the source of the constitutive Zn release in this system originates from the sintered glass filter holders used in the experiments. When Millipore membranes were acid washed in 50 mL 1% HNO_3 the concentrations of Zn recovered were $<5 \mu\text{g l}^{-1}$ (below detection limit). These results suggest that when working at low concentrations of trace metals, such as are observed in water samples or water extracts of soils, one should keep in mind the possible retention by the filter. The lowest interactions were observed with the Millipore filter, indicating that this type is most suitable for low concentrations of trace metals.

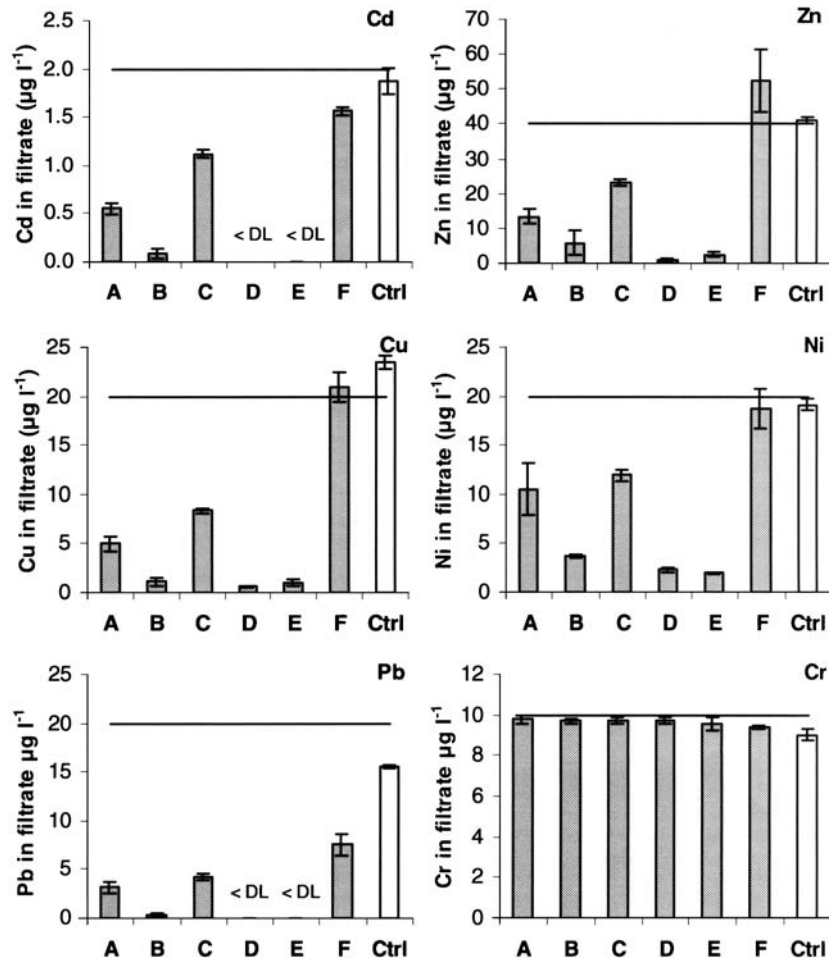


Figure 1. Observed metal concentrations in filtrates of a trace metal stock solution (A_1) using different filters: A-D: White band filters, E: Rhizon soil moisture sampler, F: Millipore filter, Ctrl: unfiltered control; line indicates theoretical stock solution concentration; intervals depict standard deviation ($n = 4$).

Figure 2 presents the metal concentrations observed in subsequent filtrations by Rhizon soil moisture samplers, placed in the A_1 stock solution. Each single filtration event (X-axis) corresponds with a vacuette sampling tube, each with a capacity of 9 mL. The Y-axis presents the observed filtrate concentration after passage through the Rhizon's porous filter material. The difference observed between the filtrate concentration and the stock solution concentrations are considered to be retained by the filter material. Cr and Cd have been omitted from the figure for clarity purposes. Cr concentrations in the filtrate were already at $10 \mu\text{g l}^{-1}$ in the first filtration event, indicating there was no retention for this

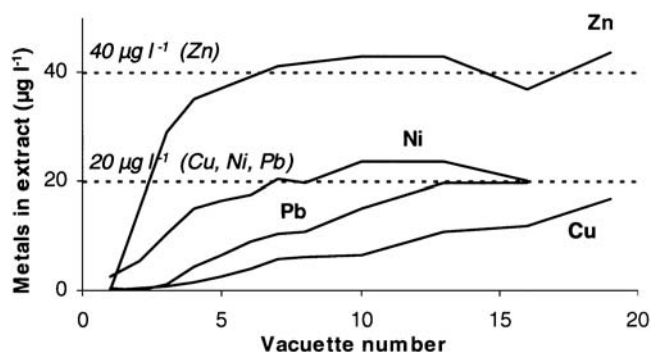


Figure 2. Observed metal concentrations in various filtrates, subsequently collected in vacuettes (9 ml volume each; each presented result is an average of 4 replicates) when filtering a trace metal stock solution (A_1) using Rhizon soil moisture samplers, dashed lines indicate actual concentrations in the original stock solution.

metal. Cd concentrations in the filtrates reached the stock solution concentrations within three filtration events and remained constant thereafter. We can estimate the general order of magnitude of the Cation Exchange Capacity (CEC) of the Rhizon's porous filter material by calculating the cumulative difference between metal concentrations in the original stock solution (A_1) and those observed in the Rhizon filtrates. The estimated CEC calculated in this manner, corresponds with $0.11 \mu\text{MOL (+)}$ per rhizon. Taking into account Rhizon dimensions (radius 0.7 mm, length 9.5 cm), this corresponds with $\pm 263 \mu\text{MOL m}^{-2}$ porous filter material. Cu appeared to be retained more than the other trace metals, while Cd and Zn were the least retained. This initial adsorption may be of some importance when Rhizon soil moisture samplers are being used for water research purposes, with low concentrations of dissolved ions. Sufficient equilibration times with the (soil) solution before sampling seem to be required to avoid net sorption of cations during sampling.

3.1.2. Synthetic Soil Solution (A_2) and 1/10 Strength Solution (A_3)

The previous experiment was repeated in the presence of competitive cations at concentrations indicative for a soil solution (A_2), as the presence of some competitive cations is far more relevant for environmental samples. In addition, to mimic a single extraction with a more diluted concentration of competitive cations, a solution containing 10 times lower concentrations of these competitive elements was also evaluated (A_3). Results are presented in Figure 3.

In consistence with the previous experiment, Cr was not retained by any of the filters. This was observed in filtrations of both the A_2 and A_3 solutions. In addition, no significant retention was observed for Cd, Ni or Zn under these conditions. For Cu, no retention was observed for any of the filters after passage of the synthetic soil solution (A_2). However, at reduced concentrations of competing cations (A_3),

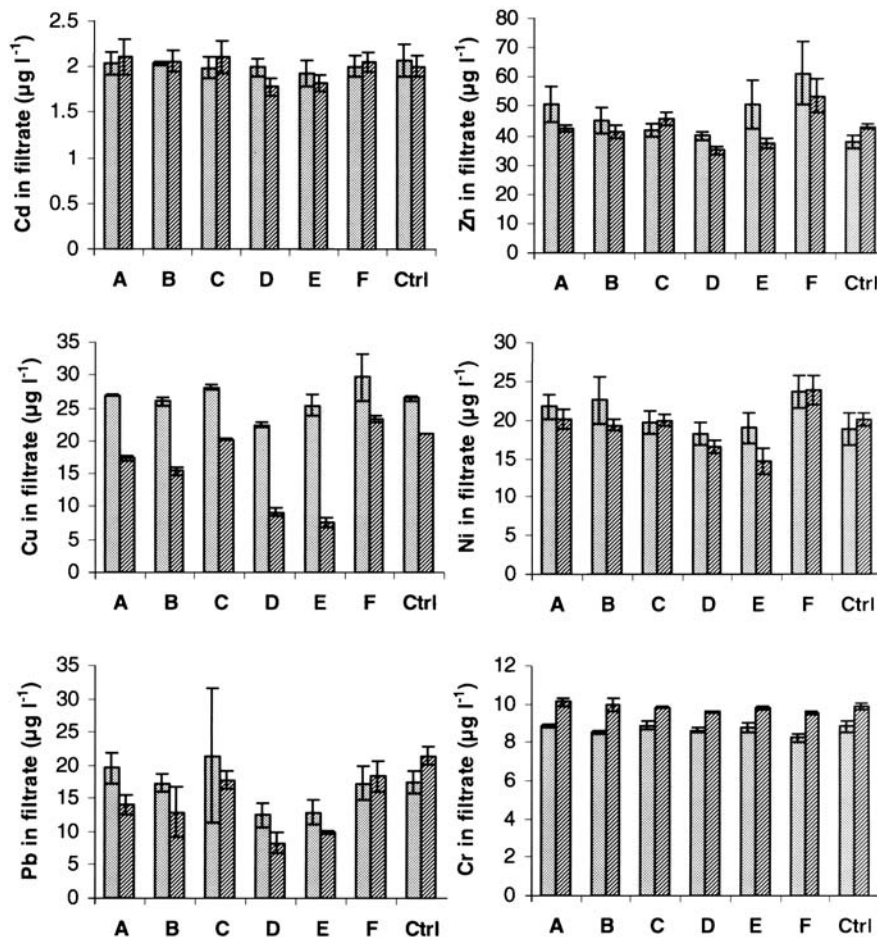


Figure 3. Observed concentrations of heavy metals in various filtrates: A-D: White band filters, E: Rhizon soil moisture sampler, F: Millipore filter, Ctrl: unfiltered control; grey bars present filtration of synthetic soil solution (A₂), striped bars present filtration of a similar solution with the same heavy metal concentrations, but containing 10 times lower concentrations of competitive cations (A₃); intervals depict standard deviation ($n = 4$).

slight retention was observed for filters A and B (resp. $3.8 \mu\text{g l}^{-1}$ and $5.8 \mu\text{g l}^{-1}$) while filter D and the Rhizon (E) removed considerably higher amounts from the solution (resp. $12.0 \mu\text{g l}^{-1}$ and $13.8 \mu\text{g l}^{-1}$). For Pb, some adsorption was observed for filters D and E after filtering of the synthetic soil solution (A₂) (resp. $4.9 \mu\text{g l}^{-1}$ and $4.5 \mu\text{g l}^{-1}$). A higher degree of adsorption was observed after passage of the A₃ solution. For filters D and E this was respectively $13.1 \mu\text{g l}^{-1}$ and $11.4 \mu\text{g l}^{-1}$ out of the $20 \mu\text{g l}^{-1}$ Pb in the stock solution. For the other filters this was somewhat lower, with observed retention of $7.2 \mu\text{g l}^{-1}$ for A, $8.4 \mu\text{g l}^{-1}$ for B, $3.6 \mu\text{g l}^{-1}$ for C and $3.0 \mu\text{g l}^{-1}$ for F.

We can therefore conclude that no problems were observed for Cr, Cd, Ni or Zn in a matrix with soil solution concentrations of competitive cations or with concentrations 10 times lower and at slightly acidic pH. Cu was not considerably retained by any of the filters after passage of the synthetic soil solution. However, at 1/10 strength (A_3) retention by filters D and E proved to be problematic. Slight retention of Pb was observed at soil solution concentrations of competitive cations (A_2). Furthermore, adsorption was found to be considerable at 1/10 strength of competitive cations (A_3) for filters D and E, and to a lesser extent A and B. However, it should again be noted that these synthetic solutions were slightly acidic as they were prepared from acidic standard solutions. At a higher pH under more realistic field conditions, retention could be somewhat higher. On the contrary, soil solutions in situ are expected to contain also chelating agents, which could neutralize part of the metal retention by the filters. Therefore, filter performance was also evaluated by comparing metal concentrations in water extracts obtained from some soils differing in texture.

3.1.3. Soil Extraction

Filter performance was also compared in soil water extracts of an unpolluted silty soil with low content of trace metals (S_1). In addition to filtration procedures, centrifugation/decantation (1660' g; 15') was performed (G). Filters A-D exhibited percolation of colloidal material, resulting in a turbid filtrate. From the turbidity, filters A and B appeared to pass relatively more colloidal material than did filters C and D. This was also reflected in lower concentrations of Cd and Pb for the last two filters. Filtrate from E and F was transparent, as expected from their defined pore diameters of 0.1 and 0.45 μm respectively. Decantated liquid from G was transparent, but floating particles could be observed both on top of as well as inside the supernatant fluid. Subsequent sample acidification before analysis may therefore result in metal release from these particles and induce a bias in the estimation of the soluble, water extractable fraction. The exclusion of colloidal material in filtrate of E and F, and their (partial) passage through filters A-D make direct comparison between results obtained by using various filters impossible. In addition, the smaller pore diameter of the porous filter material of the Rhizons (E) in comparison to the Millipore filter (F) also hamper direct comparison between E and F since colloids can exhibit intermediate dimensions between 0.1 and 0.45 μm . Indeed, higher concentrations for all trace metals were observed in F- than in E-filtered soil extractions, the difference of which may be attributed to metals associated with metal colloids with dimensions $0.1 \mu\text{m} < d < 0.45 \mu\text{m}$. Considering the pore diameters of both filter systems, retention of humic and/or inorganic colloids is expected to be higher on the surface and within the spongy matrix of the Rhizon as opposed to the membrane filter.

Generally, we conclude that filters A–D, without pre-defined pore diameters, allow the passage of more than merely the dissolved fraction. Unknown amounts of colloidal material with unknown particle sizes pass the filters as well, which

makes interpretation and comparison of results very difficult. The decantate in *G* was transparent, but exhibited floating particles which may release trace metals upon sample acidification. Only E and F filter well-defined fractions of metals from water extracts (resp. $0.1 \mu\text{m}$ and $0.45 \mu\text{m}$).

3.2. EQUILIBRATION TIME

Proposed equilibration times for water extracts of soils in literature may vary substantially. Respected equilibration time can be as short as 1–2 h in some procedures. For example, Keller and Védý (1994) proposed an adaptation of the Tessier sequential trace metal fractionation procedure (Tessier *et al.*, 1997) by adding an initial water extractable step, making use of a 1 h equilibration period. The adaptation has been adopted in other research (e.g. Ma and Rao, 1997 ; Darmawan and Wada, 1999). Crommentuijn *et al.* (1997) respected a 2 h equilibration period in bioavailability assessment of trace metals. While short equilibration times allow for the rapid processing of large amounts of samples, it can be argued that such times are insufficient to achieve full equilibration of the soil sample with the extractant fluid (*in casu* H_2O). To assess the effect of equilibration time, soils with a predominantly silty (S_1), clayey (S_2) or sandy (S_3) texture were exposed to a 5:1 ratio of deionised water for 2.5 h or 48 h. For this particular experiment, unpolluted soils were chosen to evaluate performance at low concentrations of trace metals.

Figure 4 presents the observed water extractable concentrations for Cd, Cu, Cr, Ni, Pb and Zn. Relatively high standard deviations were encountered at the low extraction concentrations, hampering detection of significant differences. For the predominantly silty and clayey soil types (S_1 and S_2), concentrations of Cu, Zn, Ni, Cr and Pb were higher in extractions after 48 h in comparison with extractions after 2.5 h. The differences were statistically significant for Cu, Pb and Cr. No significant increases were observed for the sandy soil type (S_3) after a prolonged equilibration period, except for Cr. These results suggest that for the less readily exchangeable trace metals, 2.5 h was too short to successfully extract the full water soluble fraction of Cu, Pb and Cr from silty and clayey soil types (S_1 , S_2) and the full soluble fraction of Cr from sandy soil types (S_3). Observed increases for the more readily exchangeable trace metals Zn, Cd and Ni were statistically insignificant, indicating a more rapid equilibration for these metals. However, Svete *et al.* (2000) concluded that an extraction time of 2 h is also too short for Zn and Cd, and the results must be interpreted with caution as every individual soil may behave very differently in regards to metal extraction kinetics. Also, Sinaj *et al.* (1999) reported that 72 h was required to reach a steady state for Zn extractable by ultrapure water for two unpolluted soils. In addition, the same authors observed that water extractable Zn continued to decrease after 336 h of shaking in a soil, atmospherically polluted by Zn smelter activities. The observed decrease was attributed to the presence of Zn-rich colloids, which were re-adsorbed onto soil particles over time. Based on these observations, we can state that a sufficiently long time period must be

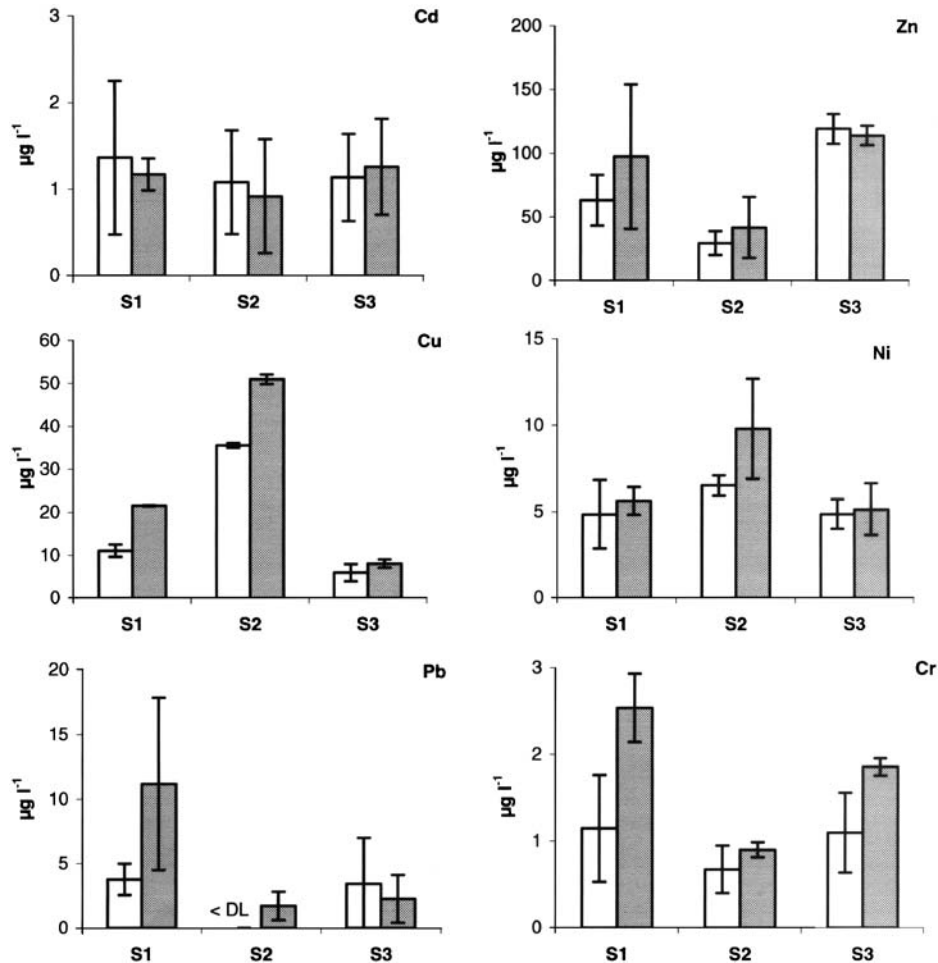


Figure 4. Extractability of heavy metals by water after 2.5 h (white bars) and 48 h (gray bars) from soils of three different texture classes (S₁ silty loam, S₂ silty clay loam and S₃ loamy sand); intervals depict standard deviations ($n = 4$).

respected for full equilibration of the soil sample. However, if the total pool of water extractable metals are to be determined, then excessively long periods are likewise to be avoided due to the occurrence of secondary effects, such as readsorption of trace metals. The selection of the equilibration time will therefore depend on the intended purpose of the extraction procedure. For assessment of the maximum pool of trace metals that can be released by water extraction, which constitutes the most labile soil fraction, secondary effects such as readsorption may not be desirable. However, for assessment of steady state equilibrium between trace metals in the soil matrix and the water extractant, then longer equilibration times may be required to reach steady state conditions.

4. Conclusion

Considerable metal retention (except Cr) was observed for all filters as well as for Rhizon soil solution samplers (E), when filtering slightly acidic aqueous solutions with metal concentrations at the $\mu\text{g l}^{-1}$ level. Zn was observed to be released into the filtrate following filtration by the Millipore filter (F). However this was attributed to constitutive release by the sintered glass filter holders, the effect of which can be reduced by rigorous acid washing and rinsing with deionised water prior to filtration. Metal retention was not significant for most metals (slight retention for Pb) in the presence of competing cations at concentrations indicative for soil solution concentrations. When reducing the concentrations of competing cations by a factor 10, such as can be observed in more dilute water extracts of soils, some problems arose for Cu and Pb, particularly for filters D and E, and to a lesser extent for A and B.

Based on filtrate turbidity, it was concluded that for soil extractions all filters except E and F allowed colloidal material to pass into the filtrate. The use of white-band filters to estimate soluble trace metals can therefore induce an overestimation of actually dissolved trace metals. Metal release after an equilibration period of 48 h proved to be significantly higher than after 2.5 h for some metals. This effect was less pronounced for a sandy soil than for clayey or silty soils. Based on these and literature observations, sufficiently long equilibration times must be respected making proposed shaking times of 1–2 h in some protocols too short.

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