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In situ sampling of small volumes of soil solution using modified micro-suction cups

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Abstract Two modified designs of micro-pore-water samplers were tested for their capacity to collect unbiased soil solution samples containing zinc and citrate. The samplers had either ceramic or polyethersulfone (PES) suction cups. Laboratory tests of the micro-samplers were conducted using (a) standard solutions with zinc and citrate concentrations that can be found in the rhizosphere and (b) two soils with contrasting texture. The results showed that both suction cups were inert with respect to citrate and zinc: they did not affect the concentrations of the two ions in freshly prepared and directly sampled standard solutions. Both micro-suction cups removed most microorganisms by filtration, though the PES cups were more effective in this respect. This filtering by the suction cups allowed for relatively accurate analysis of citrate in solutions containing microorganisms. These capacities, together with their small size and small dead volume, make the two micro-pore-water samplers highly suitable for sampling of rhizosphere soil solutions although further testing with other organic acids and trace metals may be needed.

Keywords Micro-suction cup · Citrate · Zinc · Rhizosphere · Soil solution · Pore water sampler

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Introduction

The rhizosphere is an extremely interesting soil compartment. Plant roots are capable of modifying this soil compartment chemically, physically and biologically, with profound effects on, among others, nutrient availability. The rhizosphere soil solution is of even more importance because this is the phase from which plants take up nutrients. Plant roots release exudates into the rhizosphere. Particularly low molecular weight organic anions (LMWOAs) such as citrate, malate and oxalate can play an important role in nutrient mobilization and metal detoxification (Hinsinger 2001; Jones 1998; Ryan et al. 2001). Recently it was suggested that citrate can be involved in mobilization of the micronutrient metal cations zinc (Hoffland et al. 2006) and manganese (Gherardi and Rengel 2004) from soil with low levels of these micronutrients. Characterizing the composition of rhizosphere soil solution with respect to LMWOAs and trace metal cations is therefore of utmost importance for a better understanding of plant functioning in adverse soil conditions.

Studying the rhizosphere has always been a methodological challenge (Hinsinger et al. 2005). Many methods were developed to collect root exudates, but most of them were either not quantitative or did not allow for root growth in a soil (Engels et al. 2000; Neumann and Römheld 2001). To overcome these problems, rhizotrons or rhizoboxes were developed enabling nondestructive sampling of the rhizosphere soil solution (Göttlein et al. 1996; Wenzel et al. 2001). The soil solution in the rhizotrons designed by Göttlein et al. (1996) was sampled with micro-pore-water samplers. These samplers have a high spatial and temporal resolution and can sample small volumes of soil solution in situ with minimal disturbance. These rhizotrons in combination with micro-pore-water samplers allow for studying spatial and temporal heterogeneity of rhizosphere processes along roots with an increasingly smaller spatial resolution (Puschenreiter et al. 2005).

A major drawback of micro-pore-water samplers can be biasing of samples due to adsorption or release of interfering compounds by their suction cups and their connecting tubes. In addition, organic substances in soil solution samples may change in concentration and composition due to biodegradation during and after sampling.

Previous studies on ion adsorption by suction cup materials were conducted using metal ion concentrations that may occur in the soil solution of contaminated soils (Grossmann and Udluft 1991). Wenzel and Wieshammer (1995) found that nylon membrane cups adsorbed less Cd, Cu, and Pb than porous ceramic materials using concentrations of metal ions similar to those found in soil solution. Wenzel et al. (1997) demonstrated that larger samplers with ceramic cups removed almost all Zn from the sampled solution. The sorption of Zn by ceramic micro-suction cups produced from pure aluminium oxide was negligible at low pH of 4.5, but increased under alkaline conditions at pH 8.0 (Rais et al. 2006). Larger samplers with nylon membrane cups adsorbed little Zn, however, regardless of pH.

Sampling for LMWOA analysis in soil solution has been subject to other problems. The major problem is the rapid biodegradation of LMWOAs during sampling (Neumann and Römheld 2001). Thiele et al. (2005) showed that only polypropylene micro-suction cups could stop bacteria, whereas five other materials did not. They, however, did not demonstrate recovery of LMWOAs from a soil solution. Dessureault-Rompré et al. (2006) added formaldehyde to prevent microbial degradation. This probably caused contamination of the samples, affected sample pH and cannot prevent microbial degradation during sampling. The effect of this addition on presence of microorganisms in the samples was not examined in their study. Puschenreiter et al. (2005) related low recovery of LMWOAs to adsorption on the suction cups.

In the present study, we tested the extraction characteristics of two modified designs of micro-pore-water samplers. We compared samplers with either widely used ceramic micro-suction cups or with polyethersulfone (PES) cups for sampling of both citrate and Zn, using soil Zn and citrate levels that are likely to be found in the rhizosphere of low Zn soils.

Materials and methods

Micro-pore-water samplers

Two types of micro-pore-water samplers were tested. One type (Rhizocera) had ceramic suction cups; the other type (Microrhizon) had polyethersulfone (PES) suction cups. The ceramic cups were made of pure Al_2O_3 capillary tube; the PES cups of hollow fibre. Both cups had an outer diameter (o.d.) of 1.1 mm and a length of 8 mm (Fig. 1). Pore sizes were <0.7 µm and <0.2 µm for ceramic and PES, respectively. The porous material was sealed at the tip with hotmelt glue and mounted with PVC glue on a green 1.6 × 0.8 mm (o.d. × i.d) PEEK (polyetheretherketon) tube (length 50 mm) to facilitate handling. This 1.6 mm PEEK tube fitted exactly inside the sampling holes of the rhizotron we developed, which is highly similar to a rhizotron introduced previously (Göttlein et al. 1996) that is now widely used (Vetterlein and Jahn 2004; Sandnes et al. 2005; Dessureault-Rompré et al. 2006).

The PES cups (Fig. 1A) had a 100 mm long 0.6 × 0.4 mm PEEK tube inside the PES fibre and the 1.6 mm PEEK tube to increase mechanical

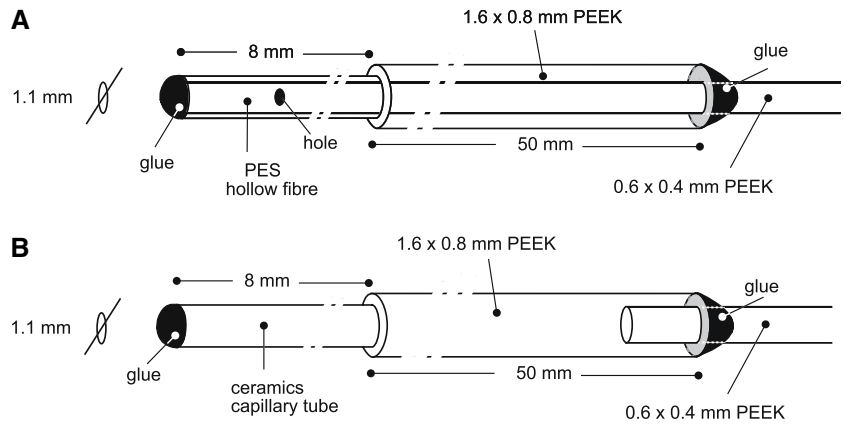

Fig. 1 The modified micro-pore-water samplers used, with PES (**A**) or ceramic (**B**) suction cups. Only the tips with the micro-suction cups are shown. The PEEK tubing at the right hand side was connected to two-part 5 ml-syringes to create lower pressure (around −90 kPa)

resistance. This 0.6 mm PEEK tube inside the PES fibre had a 0.1 mm hole in the centre to connect the PES lumen with the 1.6 mm PEEK tube lumen. The ceramic samplers (Fig. 1B) had 50 mm 0.6 mm PEEK glued in the backside of the 50 mm long 1.6 mm PEEK.

The cups were extended with 500 mm 0.6 × 0.4 mm PEEK tube with silicone connectors (inner diameter 0.5 mm). Care was taken that samples did not contact the silicone tubes because they had shown in preliminary experiments to release Zn. The dead volume of both samplers was less than 100 µl. Suction cups, connectors and tubing were supplied by Rhizosphere Research Products, Wageningen, The Netherlands.

Prior to using, the micro-suction cups were cleaned by forcing 5 ml 0.2 M HNO_3 through the cups. After that the cups remained for 2 h in 0.2 M HNO_3 before they were rinsed with 10 ml Ultra pure water. Finally, the cups were rinsed with 5 ml 1 mM $Ca(NO_3)_2$ and stored overnight in 1 mM $Ca(NO_3)_2$ as background electrolyte. The first two drops of each sample were discarded to equilibrate the cups' material and reduce the dilution effect by the $Ca(NO_3)_2$ solution retained by the cups. For the experiments on citrate recovery the suction cups, connecting tubes and syringes were autoclaved prior to use (20 min, 120°C).

To collect a sample solution the pressure inside the micro-pore-water samplers was reduced to around −90 kPa with a 5 ml two-part syringe (Terumo) connected to the 0.6 mm PEEK extension tube. Three-part syringes could not be used because the black rubber gasket released relatively large amounts of Zn in preliminary experiments.

Soil characteristics

Two types of soil were used: a sandy soil (pH-KCl 5.1; organic matter 3.3 %; water holding capacity (WHC) 270 ml kg^{-1}) and a clay soil (pH-KCl 5.1; organic matter 7%; WHC 450 ml kg^{-1}). Both soils were collected near Wageningen, The Netherlands. The sandy soil was adjusted to a moisture content of 75% of WHC, and the clay soil to 80% of WHC.

Citrate recovery

To check for adsorption of citrate on micro-pore-water sampler material citrate solutions with concentrations 3, 7, 77 or 275 µM were sampled immediately after preparation (control) and after passing through the samplers with ceramic or PES micro-suction cups.

A proxy of a soil solution containing citrate was prepared by mixing a soil suspension with a citrate solution. The soil suspension was prepared with demineralized water (2 ml g^{-1} soil). After thorough mixing and 2 h of subsequent sedimentation the extract was filtered (S & S 589[3]). Two millilitre 1.0 mM citrate solution was added to 98 ml of this soil suspension. Part of the suspension was analysed for citrate directly ($t = 0$) and after various periods of storage at room temperature (control). Another part of the suspension was sampled by either of the two micro-suction cups at $t = 0$. Sampling took about 1.3 h for PES

cups and about 4 h for ceramic cups. These samples that had passed the micro-samplers were also stored at room temperature and analysed for citrate in time. Three replicates were sampled per treatment each time.

To check recovery from a soil, citrate was added to sandy and clay soils and mixed thoroughly. A volume of 40 ml 1.0 mM citrate solution was added to 1 kg soil. The sandy soil was adjusted to a moisture content of 75% of WHC, and the clay soil to 80% of WHC. The mixture of citrate and soil was allowed to equilibrate for 1 h, and then subsamples of the mixtures were packed into Delrin/acrylic rhizotrons similar to the ones described by Göttlein et al. (1996) until dry bulk density of 1,500 kg m^{-3} for sandy soil and 1,100 kg m^{-3} for clay soil. Soil solution was sampled immediately after filling of the rhizotrons with the two types of micro-samplers. For comparison ("control"), soil solutions were collected from other subsamples of the mixtures by centrifugation for 15 min at 7,500*g*, 4°C in two-compartment containers, containing the solid phase in the upper part. The upper and lower parts were separated by a filter holder and filter membrane (0.45 μm). Centrifugation was done simultaneously to the filling of the rhizotrons and was followed by citrate analysis immediately. There were three replicates for each treatment of soil solution extracted by centrifugation, ceramic cups and PES cups. This experiment was done twice yielding similar results.

All samples were analysed immediately, to prevent further biodegradation during storage.

Zinc recovery

Standard Zn solutions (0.2, 1.5, and 15 μM $Zn(NO_3)_2$) were prepared in 1 mM $Ca(NO_3)_2$, and extracted with the two types of micro-porewater samplers. There were three replicates for each standard solution.

A Zn solution (0.2 or 15 μM $Zn(NO_3)_2$ in 1 mM $Ca(NO_3)_2$) was added to dried sandy soil (200 ml kg^{-1}) and mixed thoroughly. The sandy soil was adjusted to a moisture content of 75% of WHC. The mixtures were packed into Delrin/acrylic rhizotrons similar to the ones described by Göttlein et al. (1996) until dry bulk density of 1500 kg m^{-3}. After overnight equilibration soil solution was collected from the rhizotrons with the two types of micro-suction cups, and from another subsample by centrifugation (see above). Samples were stored in polystyrene ICP-MS sample tubes. There were three replicates for each treatment of soil solution extraction by centrifugation, ceramic cups and PES cups.

Analyses

Citrate concentration in solutions was measured spectrophotometrically using an enzymatic method. Usually a sample volume of 100 μl was sufficient. In a cuvette containing the sample and a glycylglycine buffer (pH 7.8) citrate lyase was added to convert citrate to oxaloacetate. Oxaloacetate and its decarboxylation product pyruvate were reduced by NADH to malate and lactate by addition of malate dehydrogenase and lactate dehydrogenase, respectively. The amount of NADH oxidized in these reactions is stoichiometric with the amount of citrate in the sample. The decrease in NADH in the cuvette was determined by means of its absorption at 340 nm. The detection limit for this assay is 0.5 μM. All chemicals were from Roche Diagnostics GmbH, Mannheim, Germany.

The number of bacteria in samples was determined by plating dilutions onto King's medium B agar (King et al. 1954). Four replicates per treatment were used. After 2 days incubation at room temperature the number of colony-forming units (cfu) per plate was determined. The detection level was 3 cfu ml^{-1}.

The Zn concentration in the solutions was measured by ICP-MS, with a determination limit of 0.07 μM. Prior to analyses samples were adjusted to a final concentration of 0.14 M HNO_3. Soil solution collected by four samplers was bulked to obtain enough sample volume.

Statistical analysis

One way analysis of variance was conducted with SAS statistical software. Differences between treatments were evaluated for significance by LSD multiple range tests ($P \leq 0.05$).

Table 1 Concentrations of citrate in four standard solutions measured directly (control) or after passing through micro-pore-water samplers with ceramic or PES suction cups

Treatment	Citrate concentration (μM)			
Control	3.1 a	7.1 a	77.5 a	276 a
Ceramic cups	2.9 a	6.5 a	82.3 a	284 a
PES cups	3.2 a	6.7 a	73.2 a	291 a

Values represent means of three replicates and those followed by the same letter in each column are not significantly different among treatments ($P \leq 0.05$)

Results

Citrate recovery

Neither the ceramic nor the PES cups affected the citrate concentration in freshly prepared standard solutions within the range of 3–275 μM citrate (Table 1). There was no significant difference in citrate concentration between the directly analysed standard solution on the one hand and the samples extracted by either of the two types of samplers on the other hand. The recovery of citrate was >97% although it took about 4 min to collect 2–3 ml of the standard solutions with the samplers.

In a proxy of a soil solution containing citrate, the citrate concentration rapidly decreased with time. Within 5 h after preparation it decreased from 25 μM to 5 μM or less (Fig. 2). Collection of enough sample volume (1.5 ml for analysis) from this solution using the samplers with PES cups required 1.2 h for the sandy soils and 1.4 h for the clay soil. With the ceramic cups it took 4.2 and 3.6 h, respectively. The citrate concentration in the sample collected by the PES cups remained stable around 22 μM for a longer period than in the control solution. In the sandy soil samples it started to decline after 23 h, and in the clay soil samples after 5 h. The citrate concentration in the sample collected by the ceramic cups was substantially decreased already in the first sample analysed. Sampling by ceramic cups took about 4 h, during which microbial degradation of citrate had obviously proceeded with a similar rate as in the control solution.

Both ceramic and PES cups had a significant reducing effect on the number of bacteria in the

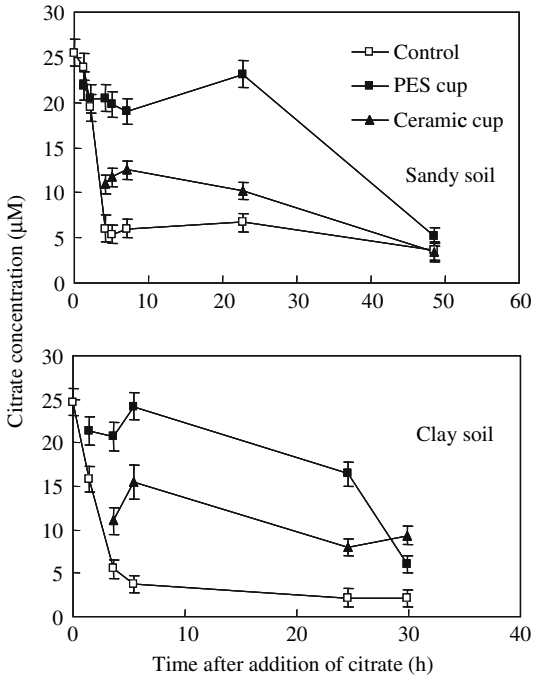

Fig. 2 Change in citrate concentration with time in a proxy of a soil solution stored as such (control), or stored after passing the PES or ceramic suction cups. At $t = 0$ h citrate was added to the soil solution. Sampling took about 1.3 h for PES cups and about 4 h for ceramic cups. Results are means of three replicates for each vertical bars represent ± SE ($n = 3$)

samples (Table 2). In the original proxy of the sandy soil and clay soil solution the number of bacteria was, on average, around 13×10^5 cfu ml^{-1}. In the solution extracted with the two types of suction cups, the number of bacteria was reduced dramatically. The PES cups did not let pass any detectable number of cfu (Fig. 3). After extraction with the ceramic cups only occasionally a few colonies were detected in undiluted samples.

Sampling of about 250 μl of soil solution from a soil packed into a rhizotron took 1.5–2.0 h for ceramic cups and about 3 h for PES cups. This sample volume originated from 0.5 and 0.8 cm^3 of soil for clay and sand, respectively. Sampling by centrifugation only took about 20 min. Citrate concentration was highest in the samples collected by centrifugation and lowest in samples collected with the samplers with PES cups (Fig. 4). The citrate concentration in the sample collected by centrifugation was about 20–30%

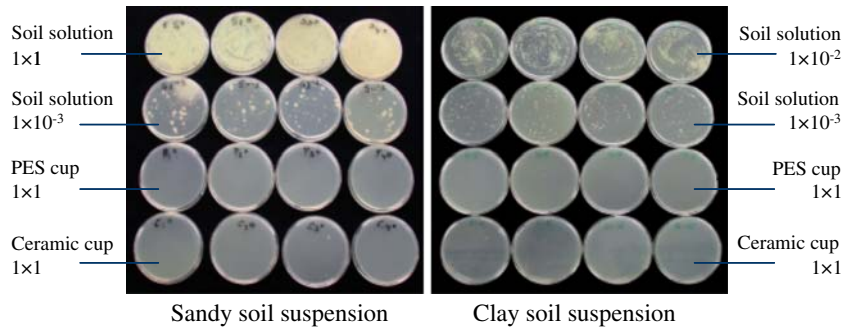

Fig. 3 Effect of micro-suction cups on filtering microorganism in collecting soil or rhizosphere solution in sandy or clay soils. The original soil solution was prepared with demineralized water (2 ml g^{-1} soil). "1 × 1" indicates results of original soil solution or not-diluted solution collected from two micro-suction cups. The symbol "1×10^{-n}" represents that the solution is diluted by 10^n times

Table 2 Number of colony forming units (cfu) on King's medium B in a proxy of a soil solution

Treatment	Number of bacteria (cfu ml^{-1})	
	Sandy soil solution	Clay soil solution
Control	$(9.5 \pm 0.5) \times 10^5$	$(16.0 \pm 0.5) \times 10^5$
Ceramic cup	27 ± 2	n.d.
PES cup	n.d.	n.d.

The solution was plated directly (control) or after extraction with micro-pore-water samplers with ceramic or PES cups. Values represent means ± SE ($n = 4$)

n.d. = not detectable

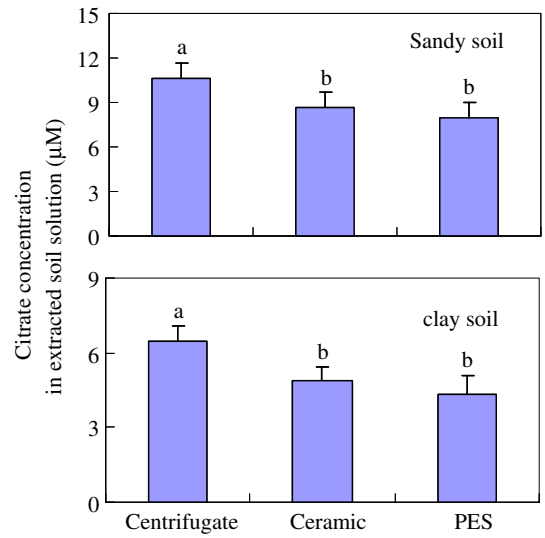

Fig. 4 Citrate concentrations in a soil solution collected by centrifugation, or by ceramic or PES micro-suction cups. Standard citrate solution was added to sandy and clay soils and mixed thoroughly. Soil solution was sampled by centrifugation immediately, or after packing in rhizotrons and subsequent sampling with PES or ceramic micro-suction cups. Results are means of three replicates for each vertical bars represent ± SE ($n = 3$). The data with the different letters show significant difference among treatments at $P \leq 0.05$

higher than in the sample collected by the two suction cups.

Zinc recovery

Zinc concentration was not affected by sampling with either of the two types of suction cups at the intermediate and high standard solution Zn concentrations (1.55 μM and 15.09 μM, Table 3). At the low standard solution Zn concentration (0.22 μM), the Zn concentration in samples extracted by ceramic or PES cups was higher than in the control (Table 3).

The Zn concentration in the sandy soil solution collected by PES cups or by centrifugation were highly similar (Fig. 5). A higher Zn concentration was found in the extracted solution by ceramic cups for the 0.2 μM Zn treatment (Fig. 5a). For the 15 μM Zn treatment, there was no significant difference in Zn concentration among all treatments (Fig. 5b). The final soil solution concentration of Zn in the two different Zn treatments did not differ.

Discussion

Our results show that neither of the two types of micro-pore-water samplers tested interfered

Table 3 Concentrations of Zn in three standard solutions measured directly (control) or after collection with micro-pore-water samplers with ceramic or PES suction cups

Treatment	Zn concentration (μM)		
Control	0.22 b	1.55 a	15.1 a
Ceramic cups	0.28 a	1.59 a	14.9 a
PES cups	0.28 a	1.59 a	14.8 a

Values represent means of three replicates and those followed by the same letter in each column are not significantly different among treatments ($P \leq 0.05$)

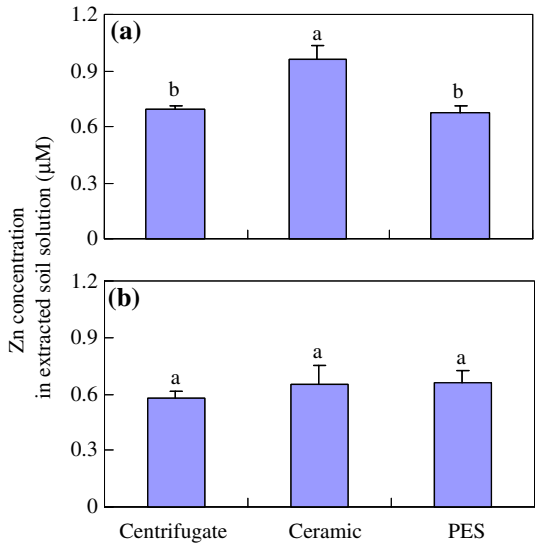

Fig. 5 Zn concentrations in a soil solution collected by centrifugation, or by micro-pore-water samplers with ceramic or PES cups. A sandy soil was mixed with a 0.2 μM (**a**) or 15 μM $Zn(NO_3)_2$ solution (**b**), and packed into rhizotrons. Results for the cups are means of three replicates. Vertical bars represent ± SE ($n = 3$). The data with the different letters show significant difference among treatments at $P \leq 0.05$

chemically with the concentrations of Zn and citrate in the sampled solutions except for the low Zn concentration treatment. Almost identical concentrations of citrate and Zn were found for directly analysed standard solutions on the one hand and samples collected by either of the two samplers on the other hand.

The concentrations of citrate used cover a wide range that can be found in bulk or rhizosphere soils. Because citrate is a relatively strong tricarboxylic acid, it is one of the more reactive LMWOAs exuded by roots, with relatively high adsorption to previously tested micro-suction cups (Sandnes et al. 2005; Puschenreiter et al. 2005). We therefore expect that sampling of rhizosphere solution can be done with these samplers, though recovery experiments with other LMWOAs are advised.

The Zn soil solution concentrations we found are within the range of what is usually found in noncontaminated soils (Barak and Helmke 1993). In previous tests Zn was shown to adsorb in relatively large amounts on ceramic cups (Wenzel et al. 1997). Instead we found release of low amounts of Zn by the suction cups tested, indicating contamination with Zn released by tubes and cups. Zinc is a very difficult trace element in this respect because it is used in the production process of many types of plastics, silicon, rubber, PVC, etc. A preliminary experiment showed larger amounts of Zn released from silica tubes in comparison to PEEK or Teflon materials (data not shown). Avoidance of contact between the sampled solution and silica tubes is a necessary precautions when sampling solutions with low Zn concentrations (μM range). The use of the modified micro-suction cups in combination with PEEK tubing reduced the problem of Zn contamination to acceptable proportions, even when low Zn soils are studied. We have found no indications of sorption of Zn on cup material. This may be different at higher pH values, though. Other trace metals than zinc, however, may be adsorbed more strongly on suction cup material (Wenzel and Wieshammer 1995; Rais et al. 2006). Experiments on these metals, especially at higher pH, should be preceded by a careful test of the micro-suction cups.

The major problem in sampling for LMWOAs has always been microbial degradation. Malate and citrate in rhizosphere samples 'disappear' within hours after sampling. The seriousness of this problem is illustrated by Fig. 2: although bacterial numbers were below our detection limits in the PES samples, still biodegradation occurred after a period of time due to microorganism reproduction. Sterilization of the sample during sampling is therefore necessary, and sampling time should be as short as possible. The results of the agar plate counting test show that both types of suction cups can remove almost all microorganisms from the soil solution. PES cups seem

more reliable in this respect, which is in line with information on pore sizes provided by the manufacturers. This sterilization is a major advantage over other sampling techniques. A similar pore size nylon membrane was used to develop micro-suction cups by Puschenreiter et al. (2005) and Wenzel et al. (1997) but it was not tested on it's sterilizing capacity. The whole system from soil solution to storage vial can be sterilized and is inaccessible for microorganisms from the air. Plants do not need to be grown under axenic conditions that may interfere root exudation processes (Neumann and Römheld 2001).

Figure 2 illustrates that the samples collected by the samplers do not provide a snap shot of the soil solution citrate concentration in time. Instead, the sample represents an average soil solution concentration over the whole sampling time. Within the period of sampling, the citrate concentration in the soil solution may change due to production, microbial degradation and interaction with the solid phase. Depending on the dynamics of the sampled system, this may bias results and affect the choice for cup material. Sample collection from a solution was faster with the PES cups, but from a soil packed into rhizotrons it was faster with the ceramic cups. This is probably due to the larger pore size of the ceramic cups. These larger pores probably get obstructed when a suspension is sampled. So for soil sampling the ceramic cups do better in this respect. Preferably sampling time should be less than 2 h. This can be partially realized through lowering sample volumes if analysis procedures allow for small sample volumes. For this reason Zn analysis would ideally need downscaling as reported by Puschenreiter et al. (2005). Alternatively, samples from different samplers can be bulked.

Concludingly the modified micro-pore-water samplers in combination with previously presented rhizotrons (Göttlein et al. 1996) offer valuable opportunities for in situ collecting small volumes of soil solution and investigating dynamics of citrate and Zn in the rhizosphere although their use for sampling other organic acids and trace elements will require further testing.

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