RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2008; **22**: 1230–1234
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.3463

RCM

A new rapid micro-method for the molecular-chemical characterization of rhizodeposits by field-ionization mass spectrometry

Peter Leinweber[1]*, Kai-Uwe Eckhardt[1], Holger Fischer[2,3] and Yakov Kuzyakov[3]

[1]Institute for Land Use, University of Rostock, Justus-von-Liebig-Weg 6, 18051 Rostock, Germany
[2]Institute of Soil Science and Land Evaluation, University of Hohenheim, Emil-Wolff-Str. 27, 70593 Stuttgart, Germany
[3]Department of Agroecosystem Research, University of Bayreuth, 95440 Bayreuth, Germany

Received 8 December 2007; Revised 18 January 2008; Accepted 31 January 2008

Time-consuming investigation of rhizodeposit composition by leaching, freeze-drying of leachate, and pyrolysis-field ionization mass spectrometry (Py-FIMS) of solid samples was replaced by direct Py-FIMS of a 5 μL liquid rhizodeposit sample which was evaporated overnight in the quartz tube of a mass spectrometer inlet system. Application of this new rapid technique to a set of 14 liquid rhizodeposit samples from maize (*Zea mays* L.), leached twice with a time lag of 80 min, unequivocally showed the effect of soil texture on the chemical composition of the rhizodeposits. Irrespective of leaching time, a partial least-squares analysis separated the Py-FI mass spectra of the maize rhizodeposits leached from a soil from those leached from a soil + quartz sand-mixture (prepared by addition of 50% w/w quartz sand to the original soil). The signals which had the strongest discrimination power and were significantly enriched in leachates from the soil + quartz sand were assigned to sugars, peptides and polyamines. Mass signals of putrescine and cadaverine, *a priori* not expected in the rhizodeposits, were indicators of modified root environment and rhizosphere processes in the soil + quartz sand. In conclusion, the new rapid mass spectrometric profiling method is suitable for rhizosphere research because it requires very small sample volumes, is fast and highly sensitive to detect and quantify a wide range of *a priori* expected and unexpected organic substances.

Rhizodeposition is determined by plant growth, nutrient supply and soil texture[1–4] and it is enhanced by low nutrient supply, especially with N and K.[1,2] Pulse-labeling of maize with ^{14}C showed more recently assimilated C in treatment with a low than with a high nutrient supply.[3] Increased soil clay content also led to increased rhizodeposition because of more intensive mechanical impedance and microbial activity which stimulate rhizodeposition.[4]

Rhizodeposits consist of more than 200 organic compounds among which low molecular weight organic substances such as amino acids, sugars and organic acids, lipids and phenols are the most abundant.[1,5] In most previous investigations these substances were extracted from nutrient solutions, quartz sand or soil in batch experiments, subsequently divided into amino acids, carboxylic acids and carbohydrates by ion-exchange columns, and then analyzed by high-performance liquid chromatography (HPLC).[5,6] The major disadvantage of this approach is that only *a priori* expected substances will be detected while others such as lipids, fatty acids, peptides, and aromatic substances will remain undiscovered. Furthermore, the sample preparation for HPLC analysis includes many purification and concentration steps, leading to losses of individual substances or substance classes.[7]

Pyrolysis–field-ionization mass spectrometry (Py-FIMS) has been applied successfully for the characterization of rhizodeposits from maize,[8,9] and even slight differences in rhizodeposition between transgenic and non-transgenic potato lines have been detected by this approach.[10] These investigations were based on the Py-FIMS of leached and freeze-dried rhizodeposits, a procedure that (1) requires a large sample volume (about 500 mL), (2) is time-consuming, and (3) perhaps can alter the composition of the rhizodeposits. Therefore, the objective of the present study was to develop and test a new pretreatment-free, rapid micro-method for Py-FIMS that is based on sample volumes as small as 5 μL. We hypothesize that ^{14}C pulse labeling of maize, which is a well-established test system,[3,7–9] shows possible responses of plants to an artificially modified texture and nutrient supply. If the new rapid micro-method for Py-FIMS is sufficiently sensitive, modifications in the chemical composition of rhizodeposits should be detected.

**Correspondence to*: P. Leinweber, Institute for Land Use, University of Rostock, Justus-von-Liebig-Weg 6, 18051 Rostock, Germany.
E-mail: peter.leinweber@uni-rostock.de
Contract/grant sponsor: German Research Council (DFG); contract/grant number: LE 903/4,1-2.

EXPERIMENTAL

Setup of the labeling and rhizodeposit leaching experiment, ^{14}C-determination

Samples from the top 10 cm of a silty loamy Haplic Luvisol ('soil') from Heidfeldhof near the University of Hohenheim, Stuttgart, Germany, were air-dried and sieved <2 mm. For half of the treatments the soil was mixed with an equal weight of pure quartz sand, changing the texture of the original soil from 22.6% clay, 62.9% silt, 14.5% sand to a sandy loam (11.3% clay, 31.4% silt, 57.3% sand) (''soil + quartz sand''). Tubes of 1-mL reaction vials (Eppendorf, Hamburg, Germany), whose lids and bottoms had been removed, were inserted into the lids of 55-mL centrifugation tubes, (VWR, Bruchsal, Germany) and fixed with hot glue. The centrifugation tubes were filled with either 58 g (soil) or 69 g (soil + quartz sand). Two-day-old maize seedlings (*Zea mays* L. cv. 'Amadeo') were placed into each reaction vial. For the growth experiment the soil moisture was kept at 21% w/w. Plants were watered daily and grown for 21 days in a day/night rhythm 24°C/18°C, 50% relative humidity, photoperiod of 12 h, and light intensity of 19 kLux. On the labeling day, eight equally developed plants (four on soil, four on soil + quartz sand) with five leaves and an average height of about 180 cm (range 170–195 cm) were selected.

For labeling, the holes of the reaction vials were sealed air-tight above the seeds with non-phytotoxic silicone rubber paste (NG 3170; Thauer & Co., Dresden, Germany). The pots were put into an air-tight Plexiglass chamber (0.5 m × 0.5 m × 0.6 m) and the labeling was achieved by dissolution of $Na_2^{14}CO_3$ with 5 M H_2SO_4 and evolution of ^{14}C-CO_2 (124 kBq per plant), which was uniformly distributed in the labeling chamber by a fan.[11] After 2 h of $^{14}CO_2$ assimilation, the air remaining in the chamber was pumped out for 45 min by two membrane pumps. The CO_2, including unassimilated $^{14}CO_2$ of the labeling, was trapped in a NaOH-containing flask to evaluate assimilation efficiency.

The soil-root pots were connected to the leaching apparatus with tubes that penetrated lid and cone. An air-water mixture was pumped with a membrane pump (air) and a peristaltic pump (water) into the soil-root pot through the lid (50 mL water h^{-1}). The gas-soil solution mixture was transferred via the tube at the lower end to a leachate collection flask. This flask was placed on ice during the leaching process to inhibit microbial decomposition of organics in the leachates.

The leaching of rhizodeposits started 3 h after the start of the labeling. The flasks for leachate and NaOH were replaced simultaneously 30 min and 110 min after the start of the leaching. Finally, 0.05% v/v $CHCl_3$ was added to the sample for sterilization.

The ^{14}C activities of leachate and trapped CO_2 were measured by liquid scintillation counting using the scintillation cocktail EcoPlus (Roth Company, Karlsruhe, Germany). The leachate (10 mL) was filtered via a glass fiber filter (GF/D, Whatman, Brentford, UK) and subsequently via a 0.4 μm pore size polycarbonate filter (type 230; Sartorius, Göttingen, Germany). Afterwards the samples were concentrated from 10 mL to 1 mL using a Speedvac vacuum centrifuge (RVC 2-25; Christ GmbH, Osterode, Germany). These concentrated leachates were stored at −18°C in the dark until Py-FIMS analyses. More experimental details, including a detailed description of the soil properties and a graphical presentation of the setup, were described previously.[7]

Pyrolysis-field ionization mass spectrometry

Immediately prior to series of measurements, 5-μL aliquots of the concentrated leachates were injected into the quartz crucible (volume = 6 μL) by means of a syringe and dried over silica gel in a desiccator for 6 h. The quartz crucible with dried sample was inserted into the ion source of a Finnigan MAT 731 (Finnigan, Bremen, Germany) modified (AMD Intectra GmbH, Harpstedt, Germany) high-performance 8 kV accelerating voltage mass spectrometer. For temperature-resolved Py-FIMS the sample was heated under high vacuum (10^{-4} Pa) from 50°C to 700°C in steps of 10°C per magnetic scan. The gas evolved was subjected to soft ionization at the carbon needles of the field ionization (FI) emitter at a potential difference of 14 kV. About 60 scans were acquired in the range *m/z* 15–900 (single spectra) in 18 min. For every single spectrum the relative abundances of ten important compound classes of organic matter were calculated on the basis of indicator ion signal intensities.[12] The instrument and operating conditions were the same as in previous studies.[13] More details of the Py-FIMS methodology and of the statistical evaluations of sample weight and residue, volatilized matter and total ion signal intensities (TIIs) of solid samples have been published previously.[13,14] The single-scan spectra were integrated over the whole temperature range to obtain one summed spectrum. These summed spectra were evaluated for differences between the two soil texture treatments and the two leaching times by partial least-squares discriminant analysis (PLS-DA) using the relative abundance of the ions in the range *m/z* 55–450 in % TII. Differences in the relative abundance of the most discriminant *m/z* were tested by the t-test.

RESULTS AND DISCUSSION

Recovery of ^{14}C assimilates in leached rhizodeposits

About 0.7% of the total applied ^{14}C remained in the atmosphere of the labeling chamber and 99.3% (equal to 123.2 kBq per plant) was assimilated via photosynthesis. On average 162.6 Bq (= 0.13%) of this amount were found in the leachate from one plant within the first 110 min after the start of leaching. The mean ^{14}C leachate activities were slightly higher for the soil + quartz sand (5.4 ± 2.1 and 3.9 ± 0.3 Bq mL^{-1}) than for the soil (2.6 ± 1.0 and 3.1 ± 0.8 Bq mL^{-1}, first and second leaching, respectively) (Table 1). The release of recently assimilated ^{14}C (during the labeling) to the rhizosphere continued at least until the second sampling after 110 min as the ^{14}C concentration in leachate did not significantly decrease (paired samples t-test). This indicates that the transfer of $^{14}CO_2$–C assimilated to rhizodeposits continued on the same level in the first and second leaching. The ^{14}C activities of the two leachings were slightly but insignificantly higher for the soil + quartz sand mixture than for the soil (Table 1). This agrees with a similar study that reported an increased ^{14}C recovery in the leachate after

DOI: 10.1002/rcm

RCM

Table 1. Treatments, ^{14}C activity (blank corrected) and total ion intensity of Py-FIMS of two consecutively leached rhizodeposit samples; means, standard errors and number of replicates in parentheses

Sample	Leachate	^{14}C activity ($Bq\,mL^{-1}$)	Total ion intensity (10^6 counts mL^{-1})
Soil	1	2.6 (1.0, n = 3)	10.0 (3.6, n = 15)
	2	3.1 (0.8, n = 3)	10.3 (2.5, n = 17)
Soil + quartz sand	1	5.4 (2.1, n = 3)	7.2 (3.2, n = 18)
	2	3.9 (0.3, n = 3)	4.6 (0.8, n = 15)

5 days.[3] The lack of significant differences in ^{14}C activity between soil and soil + quartz sand in Table 1 is explained by the short time period of 110 min between pulse labeling and leaching.

Py-FIMS analyses of the chemical composition of rhizodeposits

All samples yielded Py-FI mass spectra with high ion intensity, in the range of $\approx 1.5 \times 10^6$ to 32.5×10^6 counts mL^{-1}. The mean TIIs were higher for the leachates from soil than from soil + quartz sand. This result is in disagreement with the ^{14}C activities, but this disagreement can be explained by the use of different methods. The ^{14}C activity is a measure of the assimilate whereas the TII of Py-FIMS also reflects organic compounds which were mobilized and co-leached by the interaction of rhizodeposits with solid organic-mineral surfaces. The standard errors of the TIIs originate from differences among the rhizodeposition of individual plants as confirmed by the good correlation of the relative standard errors of the two methods, especially the smallest standard errors for the second leaching from the soil + quartz sand, obtained by ^{14}C activity measurement and Py-FIMS (Table 1). Generally, the TIIs shown in Table 1 were smaller than in a recent Py-FIMS study on potato rhizodeposits[16] in which, however, freeze-dried rhizodeposits containing much more organic matter were measured.

The summed and averaged Py-FI mass spectra for the soil and soil + quartz sand and the two sampling times (Figs. 1(a)–1(d)) showed high intensities of the indicator ions for carbohydrates (e.g. *m/z* 60, 114, 126, 144, 162) and for phenols and lignin monomers (e.g. *m/z* 164, 166, 168, 178, 180, 182, 192, 194, 196, 208 and 210). The TII-thermograms of the second leaching were clearly reduced in intensity without there being any remarkable modifications in thermal behavior.

The mass spectral patterns showed no obvious differences between the samples differing in texture, i.e. no obvious effect of soil dilution by 50% w/w of quartz sand addition on the molecular composition of leachates. The proportions of the compound classes (%TII, averaged for the two sampl-

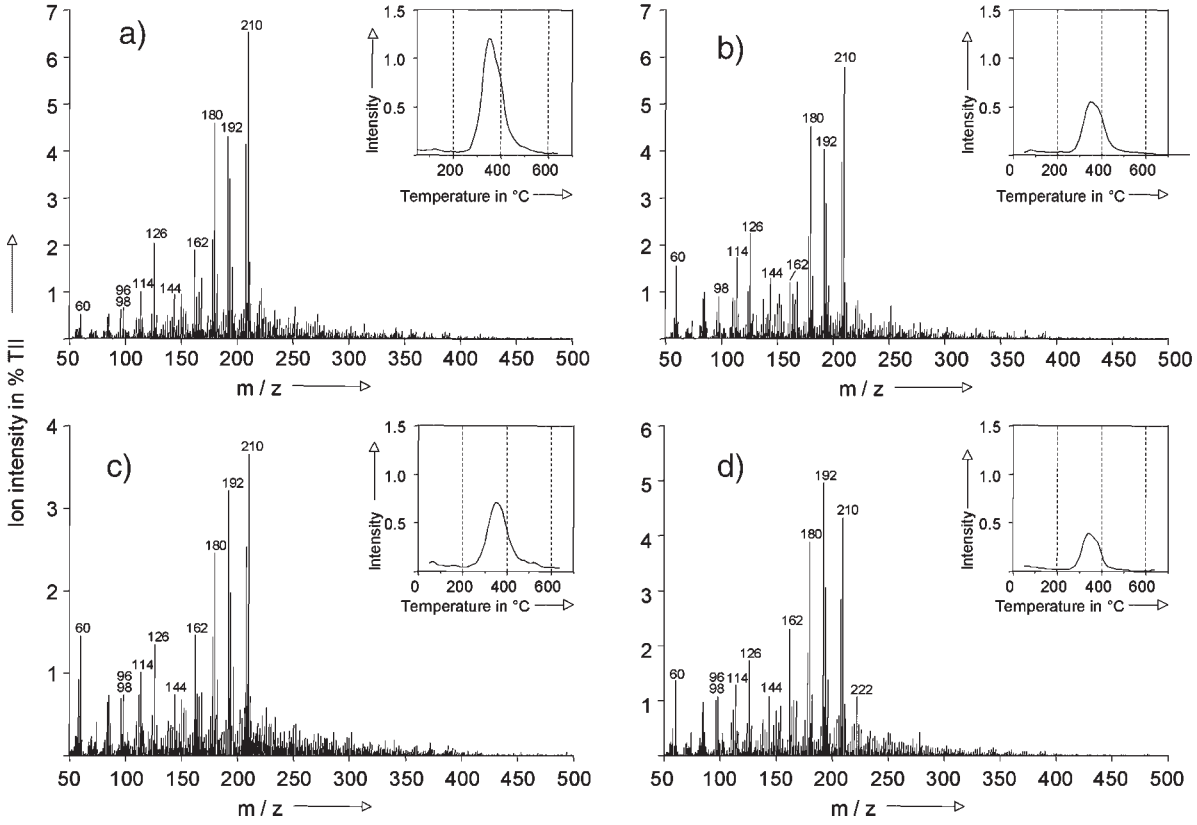

Figure 1. Thermograms of total ion intensity (inserts upper right) and summed and averaged pyrolysis–field ionization mass spectra of (a) soil, first leaching; (b) soil + quartz sand, first leaching; (c) soil, second leaching; and (d) soil + quartz sand, second leaching.

DOI: 10.1002/rcm

ing times) resulted in the following order (soil/soil + quartz sand): phenols + lignin monomers (29.7/33.7) > alkylaromatics (17.6/17.6) > carbohydrates (12.3/15.8) > peptides (4.9/5.5) > lignin dimers, lipids, N-containing compounds (3.7 to 4.7 each/2.9 to 4.3 each) > free fatty acids (1.8/1.4) > sterols (1.0/0.7) > suberin (0.1/0.1). This order was different from those found in previous analyses of maize rhizodeposits,[9] which had more N-containing compounds than alkylaromatics, phenols + lignin monomers and carbohydrates. Furthermore, rhizodeposits leached from potato crops grown under similar experimental conditions showed the order alkylaromatics > phenols + lignin monomers > N-containing compounds.[10] These disagreements in the order of compound classes indicate a strong influence of soil, crop (species and growth stadium) and leaching period on the leachate composition. In addition, the avoidance of freeze drying also may have an influence on the order of the compound classes but this has not been proven.

For a more detailed examination of the differences between the two treatments, the data sets for the first and second leaching were evaluated separately by PLS-DA. The plots of the principal components t2 vs. t1 clearly separated the two treatments. This was true for the data sets from all Py-FIMS measurements (n = 65) (not shown) as well as for the two data sets, separately for the first (Fig. 2(a)) and the second leaching (Fig. 2(b)). This provided unequivocal evidence that the textural change caused by adding 50% w/w of quartz sand altered the chemical composition of the rhizodeposits, irrespective of sampling time.

For an explanation of differences in the chemical composition of rhizodeposits between the soil and the soil + quartz sand treatment the ions at the *m/z* values with the strongest discrimination power are shown in Table 2. Ions for sugars and peptides, more abundant in the soil + quartz sand treatment than in the soil, discriminated the two treatments in the first leaching (Table 2). Similarly, polyamines were relatively enriched in the first leachate from the soil + quartz sand. The factors of enrichment were 2.9 for putrescine and 2.1 for cadaverine. The same factor of enrichment was found for cadaverine in the second leachate from the soil + quartz sand treatment. This indicates that the plant-soil-rhizosphere system reacted with modified polyamine contents on the change in texture. Polyamines have been described as important small molecules in root exudates along with carbohydrates and amino acids,[15] but their functional role in the rhizosphere remains unclear. Polyamines are formed in plant leaves, affect many cell processes and show pronounced diurnal changes,[15] comparable with diurnal changes in rhizodeposition[9] and CO_2 respiration.[11,16] Furthermore, polyamines can be

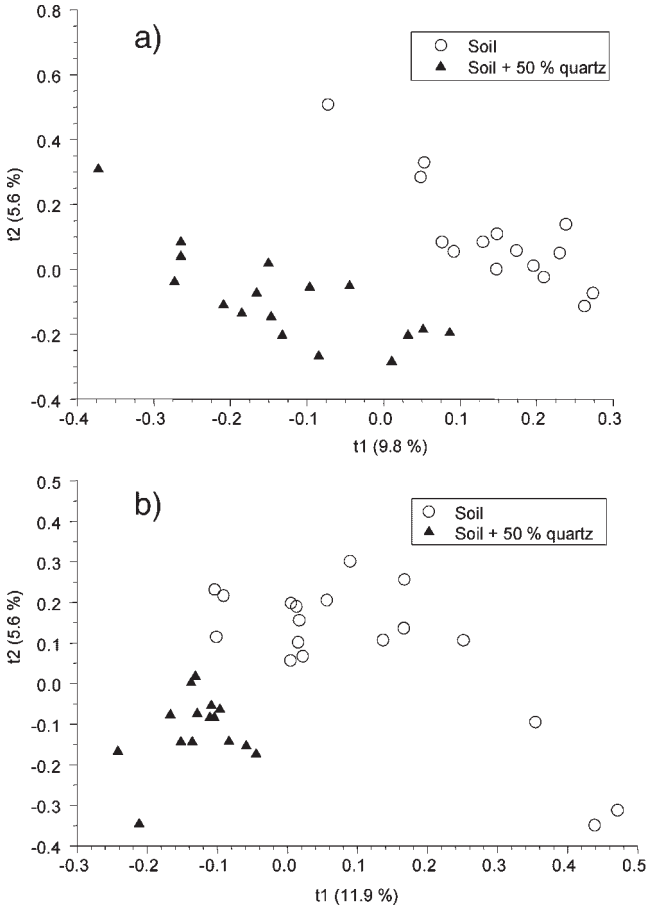

Figure 2. Plot of the principal components 1 and 2 calculated from pyrolysis–field ionization mass spectra of leachates from soil and soil + quartz sand: (a) first leaching and (b) second leaching.

Table 2. Overview on the most discriminating *m/z* values which differed significantly ($P < 0.05$) in abundance: tentative assignment and mean proportions (% total ion intensity) in leachates from soil and soil + quartz sand at two sampling dates (leachate 1 and leachate 2)

Leachate 1					Leachate 2				
			Proportion in					Proportion in	
Rank	*m/z*	Tentative assignment	soil	soil + quartz sand	Rank	*m/z*	Tentative assignment	soil	soil + quartz sand
1	58	Sugar/acetate	0.269	1.193	2	218	Alkylaromatic	0.247	0.508
2	60	Sugar	0.822	2.276	3	222	Alkylaromatic	0.664	1.036
3	88	Putrescine	0.064	0.185	11	102	Cadaverine	0.263	0.552
4	102	Cadaverine	0.176	0.376	14	124	Ligninmonomer	1.153	0.722
5	74	Peptide	0.198	0.600	23	166	Ligninmonomer	0.619	1.029
7	61	Sugar	0.072	0.299	71	160	Alanyl-alanine	0.567	0.283
8	245	Phenol, sugar	0.046	0.098	83	148	Alkylaromatic	1.344	0.670
11	220	Alkylaromatic	0.754	0.444	143	193	Unassigned	0.683	1.028
13	138	Phenol	0.809	0.469					
15	72	Sugar	0.162	0.354					

DOI: 10.1002/rcm

generated by microbial decarboxylation of amino acids.[17] In the rhizosphere, putrescine can be taken up by roots,[18] and it has been shown to be involved in inhibiting reactions in the rhizosphere.[19] Thus the putresince and cadavarine, detected in the present rhizodeposits, could originate from exudation and/or microbial decomposition of amino acids. Furthermore, the modification in polyamine content due to soil dilution by quartz sand could be a result of modified exudation, root uptake, or peptide and amino acid decomposition in the rhizosphere. The latter seems more likely since the leachates from the soil + quartz sand contained more peptides, and the larger porosity may have favored aerobic microbial decarboxylation of amino acids and peptides.

Some alkylaromatics, probably originating from lignin, were more abundant in the leachate from the soil than from the soil + quartz sand (Table 2). This could be due to the interaction of the rhizodeposits with humified soil organic matter, since lignin building blocks and alkylaromatics are considered as important building blocks of humic substances.[20] This indicates that the leaching procedure collects not only exudates and other rhizodeposits, but also their interaction products with soil organic matter in the rhizosphere.

CONCLUSIONS

Despite considerable standard errors in TIIs, which, however, could be explained by differences between individual plants grown in separate containers, the newly developed Py-FIMS method was well suited for the direct characterization of plant rhizodeposits because it required minimal sample pretreatment (only drying in the quartz crucible), and it was rapid, reproducible and highly sensitive.

The sensitivity of the new method was proved by unequivocal evidence for soil texture and/or nutrient status effects on the chemical composition of rhizodeposits at the molecular level. These included relative enrichments and or depletions of compounds, either well known from rhizodeposits (e.g. peptides and sugars) or *a priori* not expected compounds (e.g. polyamines such as putrescine and cadaverine).

These successful first tests of the new Py-FIMS method call for comparisons between the Py-FIMS of freeze-dried, large-volume rhizodeposit samples and their directly analyzed liquid micro-samples. This will help to ascertain to what extent previous results were affected by sample changes upon freeze-drying. Establishment of the method described in the present paper will open the door for systematic investigations of soil texture, fertilizer, plant development and genetic modification effects on rhizodeposit quality. Furthermore, deeper insight into rhizodeposit composition and its interaction with the soil matrix and contaminants will improve understanding of the process of soil decontamination by phytoremediation.

Acknowledgements

This work was financially supported by the German Research Council (DFG; project LE 903/4,1-2). We also thank two anonymous reviewers for helpful comments.

REFERENCES

1. Kraffczyk I, Trolldenier G, Beringer H. *Soil Biol. Biochem.* 1984; **16**: 315.
2. Neumann G, Römheld V. *Plant Roots: The Hidden Half*. Marcel Dekker: New York, 2002; 617.
3. Werth M, Kuzyakov Y. *Plant Soil* 2006; **284**: 319.
4. Nguyen C. *Agronomie* 2003; **23**: 375.
5. Farrar J, Hawes M, Jones DL, Lindow S. *Ecology* 2003; **84**: 827.
6. Gransee A, Wittenmayer L. *J. Plant Nutr. Soil Sci.* 2000; **163**: 381.
7. Fischer H, Meyer A, Fischer K, Kuzyakov Y. *Soil Biol. Biochem.* 2007; **39**: 2926.
8. Kuzyakov Y, Leinweber P, Sapronov D, Eckhardt K-U. *J. Plant Nut. Soil Sci.* 2003; **166**: 719.
9. Melnitchouck A, Leinweber P, Eckhardt K-U, Beese R. *Soil Biol. Biochem* 2005; **37**: 155.
10. Melnitchouck A, Leinweber P, Broer I, Eckhardt K-U. *Environ. Biosafety Res.* 2006; **5**: 37.
11. Kuzyakov Y, Siniakina SV. *J. Plant Nutr. Soil Sci.* 2001; **164**: 511.
12. Schulten HR, Leinweber P. *Eur. J. Soil Sci.* 1999; **50**: 237.
13. Schulten HR. *Mass Spectrometry of Soils*. Marcel Dekker: New York, 1996; 373.
14. Sorge C, Müller R, Leinweber P, Schulten H-R. *Fresenius J. Anal. Chem.* 1993; **346**: 697.
15. Gemperlova L, Novakova M, Radomira V, Eder J, Cvikrova M. *J. Exp. Bot.* 2006; **57**: 1413.
16. Kuzyakov Y, Cheng W. *Soil Biol. Biochem.* 2001; **33**: 1915.
17. Simon-Sarkadi L, Holzapfel WH. *Z. Lebensm. Unters. Forsch.* 1995; **200**: 261.
18. Hart JJ, DiTomaso JM, Linscott DL, Kochian LV. *Plant Physiol.* 1992; **99**: 1400.
19. Kuiper I, Bloemberg GV, Noreen S, Thomas-Oates JE, Lugtenberg BJJ. *MPMI* 2001; **14**: 1096.
20. Schulten HR, Schnitzer M. *Soil Sci.* 1997; **162**: 115.

DOI: 10.1002/rcm