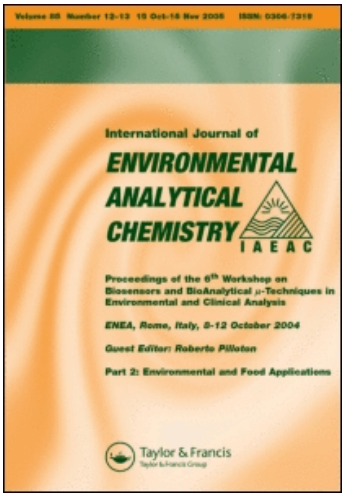

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Oxalate distribution in soils under rhubarb (*Rheum rhaponticum*)

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To cite this Article Strobel, Bjarne W. , Kristensen, Flemming and Hansen, Hans Christian B.(2004) 'Oxalate distribution in soils under rhubarb (*Rheum rhaponticum*)', International Journal of Environmental Analytical Chemistry, 84: 12, 909 — 917

To link to this Article: DOI: 10.1080/0306731042000268134

URL: http://dx.doi.org/10.1080/0306731042000268134

Intern. J. Environ. Anal. Chem.
Vol. 84, No. 12, pp. 909–917, 15 October 2004

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OXALATE DISTRIBUTION IN SOILS UNDER RHUBARB (*RHEUM RHAPONTICUM*)

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(Received 11 June 2003; In final form 12 April 2004)

Oxalate in soils may enhance phosphate availability, promote mineral dissolution, and increase the mobility of aluminium and heavy metal cations by complexation. Rhubarb (*Rheum rhaponticum* L.) has very high content of oxalate in leaves and petioles, and therefore the topsoil under rhubarb might have elevated contents of oxalate. Soil samples were collected at depths of 0–2.5 and 2.5–5 cm from 10 cm sections along 100 cm transects from rhubarb plants at four locations in Denmark, and from seven layers in a soil profile to 80 cm depth at one location. Oxalate was extracted from the soil with 0.2 M phosphate at pH 2 by reciprocal shaking for 24 h and then determined by a new fast capillary zone electrophoresis method with 300 mM KH_2PO_4 and 0.30 mM TTAB electrolyte adjusted to pH 7, developed and tested to analyse high-ionic-strength soil extracts. Rhubarb increases the oxalate content in soil under the leaves slightly. The average content of oxalate in the upper 0–5 cm soil was 444 μmol/kg at the Kaldred site, and 111–333 μmol/kg at the three other locations. In the soil profile, the content of oxalate decreased from 500 μmol/kg in 0–5 cm depth to 110 μmol/kg at 75–80 cm depth. No significant seasonal changes in oxalate contents were observed, while an annual variation of 100 μmol/kg could be observed at 0–2.5 cm depth. During plant decay in autumn, a slight increase in oxalate content was observed at 30 cm soil depth. In conclusion, the role of oxalate in weathering and metal transport appears to be limited in soils under rhubarb. Oxalate might stimulate microbiological growth and phosphate mobilisation in the rhizosphere, but concentrations observed are too low to impose any toxic effects to organisms.

Keywords: Capillary zone electrophoresis; Carboxylic acids; Ionic strength; Organic acids; Oxalic acid; Soil

INTRODUCTION

Oxalate is a common solute in plants and root exudates [1,2], which is often detected in soil and soil solution [3,4]. Oxalate is primarily formed in the petioles and leaves by a cleavage between C2 and C3 in ascorbic acid [5]. Oxalate is part of the pH regulation in plant cells and accumulates in some plants to toxic levels for potential herbivores, i.e. >5% of dry mass (dm) [1]. Spinach, sugar beet and rhubarb have high contents of oxalate; in rhubarb oxalate concentrations have been reported to be in the range 3.2–9.5% of dm, depending on the specific cultivar [6,7]. Oxalate is toxic

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ISSN 0306-7319 print: ISSN 1029-0397 online © 2004 Taylor & Francis Ltd
DOI: 10.1080/0306731042000268134

to humans with lethal doses in the range 111–167 mmol per person [8], and to rats LD_{50} 4.2–5.2 mmol/kg (oral) [9].

Oxalate is a strong ligand forming metal complexes in solution and at mineral surfaces, and as such contributes to several soil processes [10,11]. The following reactions of oxalate in the soil environment have been documented: (1) transport of metal ions, (2) weathering of soil minerals, (3) increased mobility and uptake of phosphate, (4) complexation of phytotoxic metals in soil solution, and (5) photodegradation of organic compounds [11–16].

A high release of oxalate from rhubarb due to root exudation or leaching from dead plant material may increase weathering rates of minerals, extract phosphate from soil sorbents and increase metal mobility. Water-extractable oxalate at near-neutral pH comprises mostly soil solution oxalate [3]. Adsorbed oxalate can be extracted from soil samples with (1) acidic solutions, e.g. HCl that reduce affinity to soil minerals by reducing surface complexation through oxalate protonation [17], or (2) solutions of strong ligands, e.g. ammonium phosphate at pH 2 that replace oxalate at the mineral surface by combined acidification and competitive adsorption [3].

Soil solutions from mineral soils typically contain very little oxalate, i.e. concentrations are below limit of detection, but for coniferous forest soils, concentrations up to 10 μM oxalate have been reported [4,18,19]. The content of oxalate extractable from mineral soil is usually in the range 30–180 μmol/kg as reviewed by Strobel [20]. However, some soils have even higher contents of oxalate; Certini *et al.* [21] found concentrations of oxalate up to 2400 μmol/kg in some Italian forest soils, and with oxalate detected at depths below 250 cm (44 μmol/kg).

The decomposition rate of oxalate is an important factor controlling the content of oxalate in soils and hence influencing the impact of oxalate on soil processes. Van Hees *et al.* [22] concluded that oxalate is decomposed very quickly (<6 h) in the topsoil. Jones *et al.* [23] concluded that the addition of aluminium ions to an oxalate solution in soil did not slow down the rate of oxalate decomposition. The microbial degradation of oxalate is lower in the subsoil compared with the topsoil [22,24], and in combination with increased adsorption of oxalate that may retard the microbiological oxalate degradation, the residence time of oxalate is expected to be longer in the subsoil. Ström *et al.* [24] examined the decomposition of oxalate in a calcareous soil, and they found that only 5–6% of the oxalate was decomposed after 24 h. These results indicate that oxalate degradation rates are under great influence of calcium, because of the formation of strong calcium oxalate complexes and precipitates that are difficult to be decompose [24]. The content of oxalate in soils under rhubarb might bein a steady state with a high influx due to root exudation, transfer from decaying rhubarb, and production of oxalate by microorganisms, and fast effluxes caused by rapid decomposition [22].

Capillary electrophoresis (CE) is a useful technique for the analysis of carboxylic acids in soil solutions and aqueous extracts [2,4], whereas HPLC or GC is usually preferred for soil extracts with high salt concentrations [3,25]. The advantage with CE is that the amount of sample necessary to analyse is very small (100 μL) and that it has a very short analysing time (<10 min). The disadvantage of CE is that if the high ionic strength of the sample is too high, the so-called stacking process is impaired, causing broad peaks, poor resolution and high detection limits. When applying CE to the analysis of solution with high salt concentrations, it is important to overcome this major drawback by using a running electrolyte with a high ionic strength combined

with a very low voltage and current, that maintains sufficient temperature control in the capillary centre.

The objectives of the present study was to develop a combined extraction and CE method for determination of oxalate in soils, and to map in different soils the spatial and temporal variation in oxalate contents around single plants of rhubarb, in order to evaluate the possible environmental implications of high-oxalate producing species.

EXPERIMENTAL

Field Sites and Soil Sampling

Soil samples were collected at four sites planted with rhubarb (*Rheum rhaponticum* L.) in Zealand in Denmark. The main site, a sandy soil in Kaldred near Kalundborg, was sampled in spring (1 May), summer (28 June), autumn (6 October) in 2000, and in summer (13 July) 2001. Three additional sites were sampled in summer (28–30 June) 2000: sandy loam at Klippinge near Køge, sandy loam at Saltvig near Maribo and loamy sand at Tåstrup near Roskilde (Table I). From the four sites, soil samples were excavated from 0–2.5 cm and 2.5–5 cm depth at seven different lateral sections each of 10 cm from the plant base, i.e. 0–10, 10–20, 20–30, 30–40, 40–50, 65–75, 90–100 cm. None of the sites showed indications of podzolisation, i.e. the downward transport of iron and aluminium mediated by organic ligands and humic substances.

At Kaldred, additional samples were collected on 13 July 2001 to monitor the vertical distribution of oxalate in the soil profile. Samples were taken at seven depths (0–2.5, 2.5–5, 5–10, 15–20, 35–40, 55–60 and 75–80 cm) and at two distances of 0–10 and 30–40 cm from the plant base. Finally, the Kaldred soil was sampled at four different times during the autumn 2001 in order to trace the transfer of oxalate from wilting or decaying rhubarb plants to soil. During this sampling, samples were taken from depths of 0–2.5, 2.5–5, 5–7.5, 7.5–10 cm, and at three distances of 0–10, 10–20 and 20–30 cm from the plant base and an additional sample 3 m depth. All soil samples were air-dried and sieved <2 mm before extraction.

The rhubarb cultivar was 'Elmsfeuer' in Klippinge and 'Victoria' in Tåstrup. The rhubarbs in Kaldred and Saltvig were old and morphologically similar to the cultivar 'Vinrabarber,' but the exact cultivars were unknown. All cultivars have high contents of oxalic acid, i.e. 'Elmsfeuer' (6.2–7.5% of dm), 'Victoria' (6.0–7.1% of dm) and 'Vinrabarber' (5.9–9.2% of dm) [6,7].

TABLE I Soil pH, organic carbon, clay and average oxalate content at a soil depth of 0–5 cm and 0–10 and 65–75 cm at the four field sites in summer 2000

Site	*Oxalate* 0–10 cm µmol/kg	*Oxalate* 65–75 cm µmol/kg	*pH*	*Organic carbon* %	*Clay* %
Kaldred	525	494	6.9	3.7	5
Tåstrup	373	252	7.4	2.2	9
Saltvig	173	177	7.5	3.0	11
Klippinge	514	165	7.1	3.7	14

Extraction

Phosphate sorbs strongly to mineral surfaces making it useful as an extraction agent for adsorbed oxalate. In earlier experiments, ammonium phosphate has been used to extract oxalate and other aliphatic acids from soil [3], and HCl has been used to extract oxalate [21]. In preliminary experiments, 5.0 g soil spiked with 200 μM oxalate was extracted with 10 or 20 mL of 0.10 and 0.20 M ammonium phosphate solutions (pH 2.0, 3.0 and 4.0) for periods ranging from 30 min to 24 h. Preliminary extractions with 0.2 M ammonium phosphate for 24 h showed clearly improved recovery from spiked soil samples with decreasing pH with recoveries of 67% at pH 2.0, 6% at pH 3.0 and no recovery at pH 4.0. Five grams of soil weighed to a 40 mL polycarbonate centrifuge tube was treated for 24 h with 20 mL of 0.2 M ammonium phosphate (pH 2) while agitated on a reciprocal shaker (150 strokes/min). Extracts were centrifuged at 10 000 g for 10 min, and filtered through a 0.45 μm Millipore filter. Before analysis 200 μL of the supernatant were mixed with 40 μL 20 mM Na_4EDTA, 80 μL of 1.0 M NaOH and 120 μL of water. EDTA was added in order to eliminate cation interferences in the CE method, which was tested and confirmed by adding different concentrations of $AlCl_3$ to a 200 μM oxalate standard with EDTA [26]. With this method, soils spiked with oxalate showed recoveries of 67%. None of the following results have been adjusted for the recovery.

Analyses

Oxalate was determined with a Beckman P/ACE 5510 instrument equipped with a diode array detector (75 μm i.d. and 20-/27-cm-long capillary), with detection at 190–200 nm, an aperture of 100 × 200 μm, temperature set to 25°C and an applied voltage of −2 kV.

The running electrolyte consisted of 300 mM KH_2PO_4, and 0.3 mM tetradecyltrimethylammonium bromide (TTAB) at pH 7.0. In preliminary experiments, different concentrations of TTAB (0.1–0.9 mM) were tested to achieve the best separation of nitrate and oxalate at pH 6 and 7. The ionic strength of the running electrolyte should be at least twice as high as that in the samples to obtain effective narrowing of peaks by stacking, and hence to improve the resolution and sensitivity of the method. For soil extracts with 0.2 M ammonium phosphate at pH 2 the ionic strength is approximately 0.16, and in the running electrolyte with 300 mM potassium phosphate at pH 7, it is 0.38. A more acidic ammonium phosphate extractant would make the ionic strength in the neutralised extract too high to obtain the stacking effect. In preliminary experiments, all the above parameters were optimised for the best separation and to keep analysis time short.

The sample introduction time was 7 s, equivalent to a sample volume of 87 nL. The limit of detection (LOD) and limit of quantification (LOQ) was calculated as three and ten times the standard deviation of the oxalate concentration in a 20 μM standard solution analysed 15 times. The LOD was 5.7 μM and LOQ was 19 μM for the extracts, i.e. LOD 0.5 μmol/kg and LOQ 1.7 μmol/kg for soils.

All chemicals were pro analysis or similar purity and the reagents used were prepared with triple deionised water, passed through a 0.45 μm RC membrane filter (Sartorius, Goettingen), and finally degassed.

The final analysis procedure is a rapid method with sufficient sensitivity and resolution to separate and determine oxalate in 0.20 M ammonium phosphate extracts at

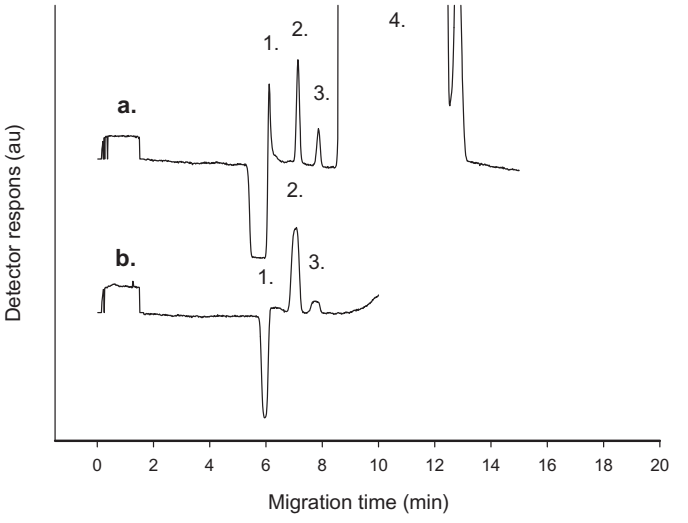

FIGURE 1 Electroferograms of: (a) standard solution with (1) EOF (electro-osmotic flow) and 200 μM chloride, (2) 200 μM nitrate, (3) 200 μM oxalate, (4) 0.2 M phosphate and 2 mM EDTA, and (b) a 0.2 M ammonium phosphate soil extract.

pH 2.0 (Fig. 1). Oxalate is well separated from the common inorganic anions in soil extracts.

Statistical Evaluation of Data

General linear models with SAS proc GLM were used for statistical processing of data [27].

RESULTS AND DISCUSSION

Horizontal Distribution of Oxalate

In preliminary experiments with water extraction of soils sampled in May, all concentrations were below the limit of detection for water samples, meaning that soil solution concentrations were below 1 μM in the mineral soil.

The horizontal distribution of soil oxalate contents was either decreasing or almost uniform in 75 cm transects starting at rhubarb plant, for both depths and all locations examined. At the Klippinge and Tåstrup sites, the content of oxalate decreased regularly from about 450 μmol/kg closest to the plant base to about 200 μmol/kg 75 cm away (Table I). The oxalate contents were almost uniform at all distances with around 500 μmol/kg in Kaldred (Fig. 2), and around 175 μmol/kg in Saltvig (Table I). No simple correlation between oxalate content and soil pH, texture or content of organic matter could be established, although the highest oxalate content was found at the most sandiest site (Kaldred).

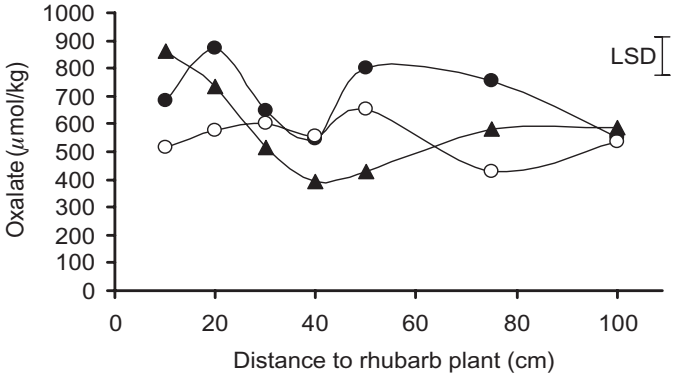

FIGURE 2 Seasonal variation in oxalate content in Kaldred at the depth of 0–2.5 cm. Spring (●), summer (○) and autumn (▲) 2000.

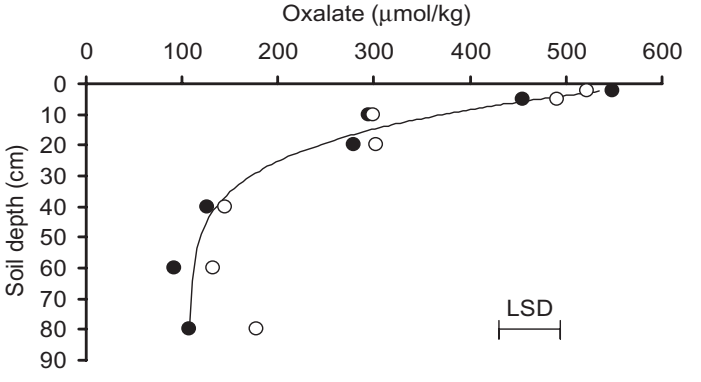

FIGURE 3 Oxalate content vs. depth in soil samples at two distances from the rhubarb plant determined at Kaldred summer 2001. 0–10 cm (●) and 30–40 cm (○) from the plant root. The solid line is drawn as a guide to the eye.

Vertical Distribution of Oxalate

In the soil materials from Kaldred sampled during the summer of 2001, the oxalate content decreases strongly with depth from about 550 μmol/kg soil in the top 2.5 cm to 300 μmol/kg at 10–20 cm depth (Fig. 3). The content of oxalate is about 133 μmol/kg in the subsoil from 40–80 cm, approximately four times lower than in the organic rich surface horizon. It is usually found that oxalate contents in the topsoils are below 100 μmol/kg as reviewed by Strobel [20], and hence the contents extracted from the 0–5 cm soil layers are clearly higher as a result of deposition from the rhubarb plants (Table I). In other investigations oxalate contents in subsoils are typically observed at a level of approximately 50 μmol/kg, which is only slightly lower than the reported values for topsoils. In our investigation, the content of oxalate in the subsoil under rhubarb at Kaldred, about 100 μmol/kg, is about twice as high as the reported value [3,19,20]. Hence, rhubarb has significantly increased the content of oxalate in both the topsoils and subsoil (Fig. 3). The rather high subsoil contents of oxalate is likely to be caused by exudation from roots and production by micro-organisms; leaching is not likely, as oxalate is strongly retained in these non-acid soils [24].

Seasonal Change

The highest contents of oxalate were observed in spring and autumn in the top 0–2.5 cm soil layer at Kaldred with values in the range 684–872 μmol/kg at 0–20 cm soil depth. This was 24–58% higher than the oxalate 90–100 cm away (Fig. 2). Summer sampling from 0 to 2.5 cm, and for all three seasons at 2.5–5 cm, showed an almost uniform distribution vs. distance from the plants, with average values of about 500 μmol/kg. The horizontal distribution of oxalate in 0–2.5 cm varies only slightly from year to year, as shown for the summer 2000 and 2001 samples (Fig. 4), and no differences between years was observed for oxalate contents from the 2.5–5 cm layer. The annual difference in the topsoil layer might be attributed to variations in precipitation and its influence on biomass production, and organic matter turnover. Tani *et al.* [18] observed some seasonal variation with a maximum in summer and a minimum in early spring in a *Pinus densiflora* plantation.

On the first day of the 10-week period in autumn 2001, when soil samples were collected in Kaldred, the plants were upright, and the leaves were still green. As the next samples were collected in September and October, the plants rapidly wilted, and when the last soil samples were sampled, there were no plant remnants above the ground. During the decay, the content of oxalate 0–5 cm below the leaf debris increased from about 280 μmol/kg early September to 510 μmol/kg in November (Fig. 5). The observed increase was significant for 0–5 cm depth 10–30 cm from the base of the plant, whereas the contents were constant at a distance of 0–10 cm. No significant changes in oxalate contents could be observed over time for the samples from a depth of 5–10 cm; the average content of 283 μmol/kg (data not shown) was 10% higher than the average content 3 m away, 256 μmol/kg.

The Fate of Oxalate in Soil under Rhubarb

The net deposition of oxalate from rhubarb to soil has not been determined. Rhubarb dm production is at least 5 tons/ha/yr, and assuming an oxalate content of 5% in the plant dry mass, the annual oxalate production is estimated to 250 kg/ha/yr. If just 20% of this amount were transferred to the upper 5 cm soil, the soil would receive 1000 μmol/kg/yr. The observed soil oxalate contents are clearly much lower than this estimate, demonstrating that oxalate is rapidly degraded in these non-acid soils. The

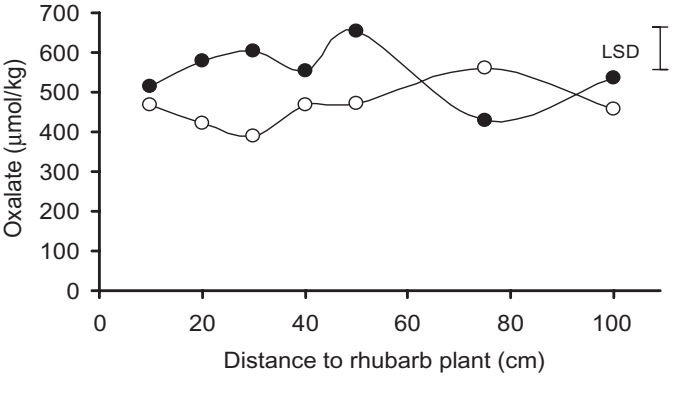

FIGURE 4 Oxalate content at a depth of 0–2.5 cm in summer 2000 (●) and summer 2001 (○) in Kaldred.

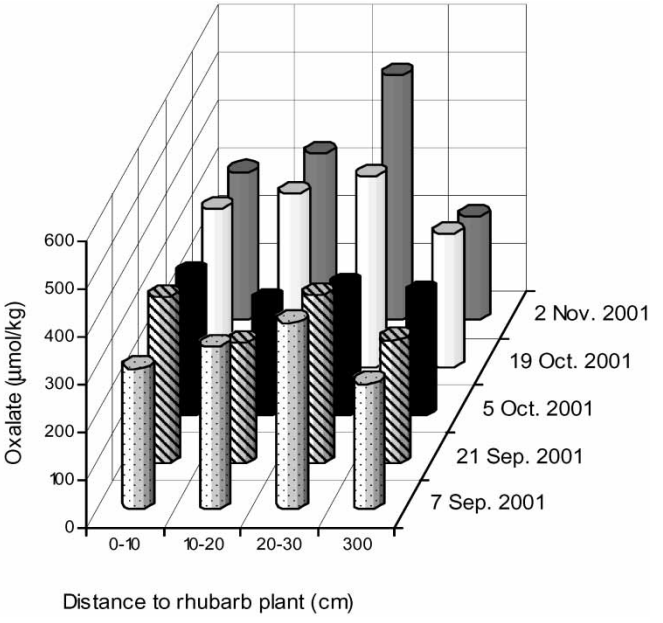

FIGURE 5 Change of oxalate contents over a 10-week period in autumn 2001 in the upper 5 cm in Kaldred. (LSD = 12.9, $n = 40$).

lack of significant correlation between oxalate contents and the distance from rhubarb plants, and the lack of significant seasonal variation, indicate furthermore that oxalate added to the soil is degraded so fast that it is not found in high concentrations in our monitoring. Despite the high degradation rates, the contents of oxalate in soil under rhubarb are two to four times higher than is usually observed for soils not covered with high-oxalate plants [20]. The increased contents may be due to either (1) high influx of oxalate causing higher steady-state concentrations, or (2) the presence of a pool of oxalate that is slowly degraded. If hypothesis (1) were true, we would expect a higher seasonal variation in oxalate contents, and therefore explanation (2) is more likely. Strong sorption of oxalate to soil minerals may retard the degradation of oxalate.

In conclusion, the role of oxalate in soil chemical processes such as metal transport and weathering appears to be limited in these soils. Oxalate might stimulate microbiological growth and phosphate mobilisation in the rhizosphere, but the concentrations observed are too low to impart any toxic effects on organisms.

CONCLUSIONS

Capillary electrophoresis was successfully used to determine oxalate in high-ionic strength extracts, such as 0.2 M phosphate, and with a satisfactory LOD of 5.7 µM for extracts and 0.5 µmol/kg for soil samples. The combined extraction and analysis methods resulted in an easy and rapid method for determining oxalate in soil samples with LOD of 71 µmol/kg. The vertical distribution of oxalate showed a regular decrease with depth from 533 µmol/kg in the top 2.5 cm to a constant subsoil content of about

133 μmol/kg at depths of 40–80 cm. The contents by depth were the same at both 0 and 35 cm from the rhubarb plants. The observed oxalate contents were two to four times higher than oxalate contents typically reported for soils. Results from Kaldred in 2000 show no signs of a seasonal change of the oxalate content; however, oxalate contents in 2000 and 2001 were statistically different. The tree soils with higher clay content contained less oxalate than the soil poorer in clay. The investigation indicates that oxalate from rhubarb deposited to the soil is rapidly degraded.

Acknowledgements

We gratefully acknowledge W. Perch-Nielsen, Aa. Andersen, K. Kristensen, I. Kristensen, K. Andersen, H. Andersen and P. Andersen, for access to rhubarb soils, and I. Skovgaard, C. Ritz and C.B. Pipper for assistance with statistical data evaluation.

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