Accumulation of Arsenic and Zinc in the Rhizosphere of Wetland Plants

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Oxidation of the rhizosphere by wetland plants leads to precipitation of iron oxyhydroxides in the rhizosphere and on the root surface of the plants. These precipitates are also known as ironplaque. Arsenic and zinc have a high binding affinity for iron oxyhydroxides (Padmanabham 1983; Belzile and Tessier 1990; Gerth et al. 1993) and were found to accumulate in ironplaque on roots of *Aster tripolium* L. (Otte et al. 1989; 1991). It was argued that rhizosphere oxidation and formation of an ironplaque would favor the accumulation of iron, arsenic and zinc in the rhizosphere (Otte et al. 1991; Otte and Ernst 1994). Oxidation of ferrous iron to its ferric form would lead to precipitation of iron oxyhydroxides in the rhizosphere, which in turn would lead to a decreasing concentration gradient of dissolved iron towards the plant roots. The iron oxyhydroxides in turn would bind arsenic and zinc, again creating a decreasing concentration gradient of both elements towards the roots. These gradients would lead to the diffusion of iron, arsenic and zinc in the direction of the roots. Assuming that uptake of the elements by the roots is slower than supply through diffusion, an increase in concentrations of all three elements would be expected to occur in the solid phase of the rhizosphere. Similar processes, but induced by changes in pH rather than Eh, occur in the rhizosphere of upland plants like soybean (*Glycine max* L.) and barley (*Hordeum vulgare* L.) as described by Youssef and Chino (1991). Already, Otte et al. (1991) found indications that arsenic accumulated in soil immediately adjacent to the roots ('rhizosphere soil').

These plant-induced processes in wetland soils may not only affect availability of zinc and arsenic to the plants, but may also play an important role in the sink function of wetlands for metals and metalloids. Knowledge of these processes is important for our understanding of the functioning of wetland ecosystems, as well as for the utilization of wetlands as so-called helophyte filters (Gersberg et al. 1986; Brix and Schierup 1989; Mickle 1993).

A research project was started to study the impact of rhizosphere oxidation and ironplaque formation on the distribution of arsenic and zinc in salt marsh soils. Here we report on the results of a pilot field study which compared concentrations of the metals in ironplaque, rhizosphere soil and bulk soil under *Spartina anglica* Hubbard and *Halimione portulacoides* (L.) Aellen. It was hypothesized that iron, zinc, and arsenic concentrations in the soil would increase from the bulk soil towards the roots.

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MATERIALS AND METHODS

The study site was at a saltmarsh located in the Rogerstown Estuary (53° 30'N, 6° 7' W) on the east coast of Ireland in north co. Dublin. Sampling took place on 21 January 1994. Lumps of soil containing *Spartina anglica* Hubbard and *Halimione portulacoides* (L.) Aellen (eight replications of each species) were collected using stainless steel spades, placed in plastic bags, transported to the laboratory and stored at 4 °C until further analysis. Bulk and rhizosphere soil were separated following Otte et al. (1991). Soil that could easily be removed from the lumps by gently shaking and that contained no roots was regarded as bulk soil. This soil was dried before analysis at 80 °C. Soil remaining on the roots after removal of most of the soil by hand (a layer of no more than a few mm thickness) was regarded as rhizosphere soil. Rhizosphere soil was washed off the roots with approximately 50 mL deionized water in 250-mL glass beakers. The suspension obtained was then dried at 80 °C and the soil material collected for further analysis.

Bulk density of soil under *H. portulacoides* stands was determined by weighing dried soil cores of known volume which were collected using PVC-tubes of 4 cm diameter and 20 cm length (two per plant species). Because it was not possible to collect cores in a similar fashion from under *S. anglica* stands, bulk density was in that case determined by taking intact lumps of soil. These were then wrapped in cling-film, submerged in water in a graduated cylinder to determine volume, and dried at 80 °C. Using the same sampling procedure as above, soil was collected for determination of root density (two per plant species). All the roots in a known volume of soil were washed out and weighed (fresh, and dry after drying at 80 °C). Loss on ignition (LOI) of three bulk and rhizosphere soil samples for each plant species was determined by ashing a known amount of sample (0.5 - 4 g) in a furnace at 500 °C for six hours.

Ironplaque was removed from the roots using a dithionite-citrate-bicarbonate (DCB) extraction as described in Otte et al. (1991). Prior to analysis for Fe, Zn, and As, DCB-extracts were acid-digested to oxidize sulfur compounds remaining in the extracts that might interfere with the analysis, as follows. To 1 mL of DCB-extract 3 mL of acid-digestion mixture (HNO_3:HCl = 4:1) was added in a Teflon pressure bomb and heated at 140 °C for eight hours. After the digest had cooled down it was made up to 10 mL with deionized water. Extracts were analyzed for Fe and Zn using a Pye Unicam SP9 flame atomic absorption spectrophotometer (FAAS). Arsenic was determined using the hydride generation technique with the same equipment.

Soil samples (0.2 - 0.5 g) were digested by adding 2 mL of acid digestion mixture (HNO_3:HCl = 4:1) in a Teflon pressure bomb and heating at 140 °C for eight hours. Digests were made up to 10 mL with deionized water and analyzed for Fe, Zn and As as described above for DCB-extracts. Inter-laboratory standard reference samples were also analyzed and values on average deviated 7.5 (Fe), 14 (Zn) and 13 (As) % from certified average values. Detection limits were 0.5 µmol Fe g^{-1}, 0.03 µmol Zn g^{-1} and 0.1 nmol As g^{-1}.
Statistical analysis was carried out following Sokal and Rohlf (1981) on log-transformed data using Statview and Excel for Macintosh as indicated in the text.

RESULTS AND DISCUSSION

Iron concentrations in soil samples did not vary significantly between plant species, but were significantly lower in rhizosphere soil than in bulk soil under both species (Tables 1 and 2). The significant interaction indicates that the difference in iron concentrations between rhizosphere and bulk soil were more pronounced under *H. portulacoides* than under *S. anglica*. Differences in zinc concentrations in soil did not differ between plant species, but concentrations in rhizosphere soil were higher than in bulk soil under both plant species. Arsenic concentrations were higher under *H. portulacoides* than under *S. anglica*, and

Table 1. Loss on ignition (LOI) and element concentrations (mean±standard deviation, number of replications between parentheses) in bulk and rhizosphere soil, and element concentrations in ironplaque associated with *H. portulacoides* or *S. anglica*. The analysis for Zn in soil samples was repeated, because the initial analysis was deemed unreliable. Lack of material for some samples resulted in lower numbers of replication.

Soil samples	LOI	Element concentrations		
		Fe	Zn	As
H. portulacoides				
Bulk	41±35 (3)	766±60 (8)	1.25±0.15 (7)	0.26±0.05 (8)
Rhizosphere	41±23 (3)	633±61 (8)	1.35±0.28 (5)	0.28±0.05 (8)
S. anglica				
Bulk soil	83±1 (3)	686±29 (8)	1.06±0.13 (8)	0.20±0.03 (8)
Rhizosphere	78±8 (3)	661±51 (8)	1.22±0.28 (5)	0.24±0.04 (8)
Fe plaque				
H. portulacoides		504±62 (8)	0.40±0.19 (7)	0.26±0.28 (7)
S. anglica		486±181 (8)	0.36±0.13 (8)	0.63±0.33 (8)

LOI in g 100 g^{-1}, concentrations in soil samples in $\mu\text{mol g}^{-1}$ dry soil, concentrations in iron plaque in $\mu\text{mol g}^{-1}$ dry root.

Table 2. Significance (Probability) of differences in element concentrations between plant species, and between bulk and rhizosphere soil as determined by two-factor nested (soil samples within plant species) analysis of variance, and in element concentrations in ironplaque as determined by one-way analysis of variance.

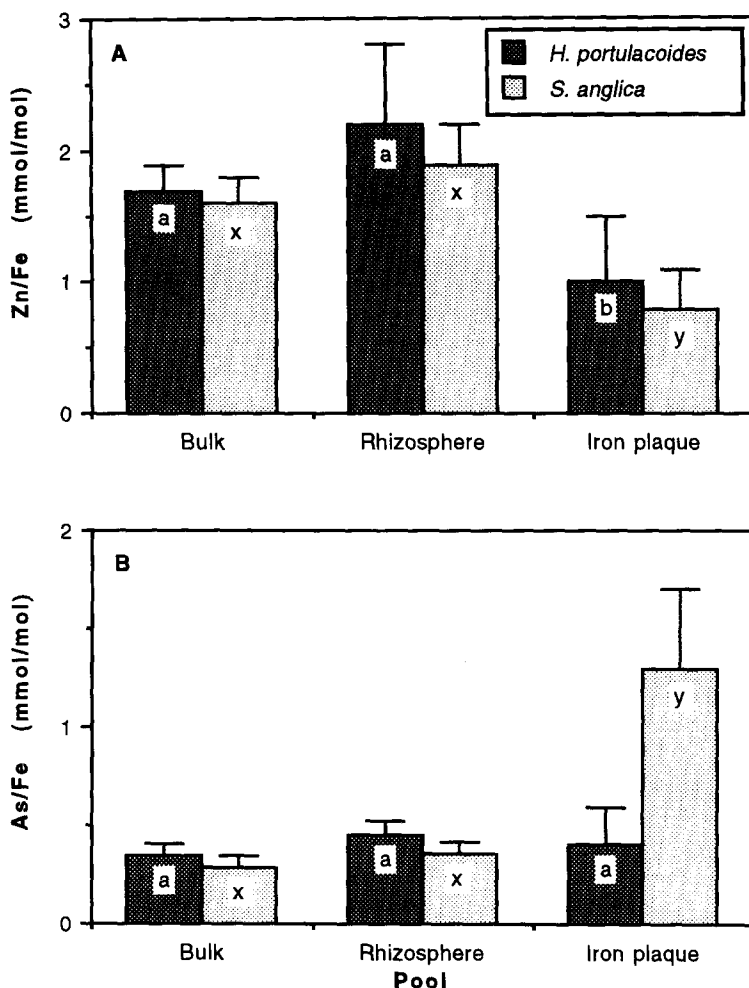|		Probability	
Source of variation	Fe	Zn	As
Soil			
A. Plant species	0.2268	0.0775	0.0045
B. Bulk/rhizosphere soil			
(within plant species)	0.0018	0.0397	0.0365
Interaction (A x B)	0.0225	0.2724	0.4348
Ironplaque			
Plant species	0.6879	0.6738	0.0168

Figure 1. Mean Zn/Fe (A) and As/Fe (B) ratios (mmol mol^{-1}) in bulk and rhizosphere soil, and ironplaque associated with *H. portulacoides* or *S. anglica*. Bars indicate standard deviations. Different letters within data of one species indicate significant differences at $P < 0.05$ (one-way analysis of variance followed by Scheffé 's test for comparison of means).

were significantly higher in rhizosphere soil than in bulk soil under both plant species.

Loss on ignition (LOI) was highly variable and tended to be lower in soil under *H. portulacoides* than under *S. anglica* (Table 1). No differences occurred in LOI between rhizosphere and bulk soils.

Concentrations of the elements in ironplaque can not be directly compared with concentrations in soil because they are based on different units. However, a

comparison of these concentrations can be made between plant species (Tables 1 and 2). Neither iron nor zinc concentrations in ironplaque varied between plant species, but arsenic concentrations were significantly higher in ironplaque on *S. anglica* roots than on *H. portulacoides* roots. Arsenic and zinc concentrations strongly correlated with iron concentrations in ironplaque. Pearson product-moment correlation coefficients were 0.795 ($v=13$, $P<0.01$) for As-Fe and 0.567 ($v=13$, $P<0.05$) for Zn-Fe for all data pooled for both plant species.

Table 3. Distribution of Fe, Zn and As in top 20 cm soil under *H. portulacoides* and *S. anglica*.

Plant species	*H. portulacoides*	*S. anglica*

Physical parameters (means±standard deviations, number of replications between parameters)

Bulk density (g l^{-1})	350±47 (2)	430±14 (2)
Root density (g l^{-1})	11.0±1.6 (2)	8.8±1.1 (2)
g rhizosphere soil/ g roots (dry weight)	2.6±2.4 (8)	1.0±0.6 (8)

Amounts of iron, zinc and arsenic associated with bulk soil (less rhizosphere and ironplaque), rhizosphere soil or ironplaque in 1 liter of the top 20 cm soil.

Fe	mmol	% of total	mmol	% of total
Bulk	316	97	221	90
Rhizosphere	6	2	19	8
Ironplaque	4	1	5	2
Total	326	100	245	100
Zn	μmol	% of total	μmol	% of total
Bulk	516	97	331	89
Rhizosphere	12	2	35	9
Ironplaque	4	1	4	1
Total	532	100	370	100
As	μmol	% of total	μmol	% of total
Bulk	107	95	64	81
Rhizosphere	3	3	8	10
Ironplaque	2	2	7	9
Total	112	100	79	100

The As/Fe and Zn/Fe ratios in soil and ironplaque can give indications of the enrichment or depletion of As and Zn relative to iron in these pools (Fig. 1). The As/Fe ratio under *H. portulacoides* did not vary significantly between ironplaque, rhizosphere soil or bulk soil. In contrast, the As/Fe ratio was significantly higher in ironplaque on roots of *S. anglica* compared to rhizosphere and bulk soil under the plants. The Zn/Fe ratio was lower in ironplaque on roots of both species compared to rhizosphere and bulk soil.

Using the physical parameters bulk density, root density and amount of rhizosphere soil per amount of roots, the distribution of the elements over the three pools in a unit volume of the top 20 cm of saltmarsh soil could be calculated (Table 3). According to these calculations the soil under *H. portulacoides* contained more iron, zinc and arsenic per unit volume than soil under *S. anglica*. These data further indicate that 3 - 5% of the elements is associated with pools under the influence of roots (ironplaque plus rhizosphere) of *H. portulacoides*, while this amounts to 10 - 19% in the case of *S. anglica*.

Contrary to previous observations (Otte et al. 1991) iron concentrations were lower in the rhizosphere of both plant species than in the bulk soil. In contrast, concentrations of zinc and arsenic were higher in the rhizosphere of both species compared to the bulk soil, as was expected. The observed differences in element concentrations between bulk and rhizosphere soils may partly be due to differences in composition and concentration of organic matter in the soil, although organic matter content as indicated by LOI did not vary. Differences in redox characteristics were probably more important in determining differences in iron, zinc and arsenic concentrations between rhizosphere and bulk soils (Otte et al. 1991; 1993).

Relative to iron, arsenic appeared to be enriched in the ironplaque of *S. anglica*, but not in *H. portulacoides*. Zinc on the other hand appeared to be depleted relative to iron in the ironplaque. The concentrations of the elements in soil and ironplaque at a given point in time are the result of the dynamic processes of supply (mass flow, diffusion) and demand (uptake by the plants, leaching, etc.) (Jungk 1991). The chemical speciation of the elements plays an important role in these processes as well, as it determines the mobility of the metals, and depends on factors like pH and Eh. These in turn can be strongly affected by the physiological activity of the plants (Fischer et al. 1989; Flessa 1989; Bowen and Rovira 1991; Youssef and Chino 1991). The differences in element concentrations between bulk soil, rhizosphere soil, and ironplaque are therefore also likely to be affected by season.

The estimates of the size of the pools of the elements associated with bulk soil, rhizosphere soil, and ironplaque (Table 3) have to be treated with caution, because several sources of variation are combined in the outcome of the calculations. Although the bulk density and root density data were based on two replications only, the variation between the replications was low. A more important source of variation is probably the distinction between rhizosphere and bulk soil. The decision what is or what is not rhizosphere soil is somewhat arbitrary and operator sensitive. The thinner the layer of rhizosphere soil is taken to be the higher the chance of detecting differences between rhizosphere and bulk soil. But too thin a layer of rhizosphere soil will render the elements undetectable because of lack of material. This taken into account the method can be used to estimate at least the order of magnitude of the various pools. The calculations (Table 3) indicate that up to 10, 10 and 19% of the total amount of Fe, Zn and As, respectively, present in a unit volume of the top 20 cm of saltmarsh soil is under the influence of plant roots. Rhizosphere oxidation and ironplaque formation are seasonal processes (Crowder and Macfie 1986; Howes and Teal 1994) and since this project was carried out in January, when physiological activity of the plants was low, these can be expected to be low rather than high estimates. This indicates that the processes resulting in mobilization/immobilization in the rhizosphere of plants may significantly affect the source/sink function of wetlands

regarding iron, arsenic, and zinc. In the past decade the utilization of wetlands as so-called helophyte filters has gained enormous interest (Mitch and Gosselink 1986; Brix and Schierup 1989; Mickle 1993). What role plants play in the filter function of wetlands in relation to metals and metalloids is still a matter of debate (Gersberg et al. 1986). It has been shown that wetlands can retain large quantities of metals and it is thought that adsorption onto iron oxyhydroxides can play a key role in this process (Zedler et al. 1992). The most important effect that plants have on retention of metals by wetlands may not be through uptake of the metals, but through oxidation of the rhizosphere, thereby increasing the amount of iron oxyhydroxides in the soil. This in turn could be strongly dependent on season. We are currently investigating the effects of season on distribution of iron, zinc, and arsenic in the bulk soil, rhizosphere soil, and ironplaque of wetland plants.

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