

## Metals (Fe, Mn, Zn) in the root plaque of submerged aquatic plants collected *in situ*: Relations with metal concentrations in the adjacent sediments and in the root tissue

LOUISE ST-CYR & PETER G.C. CAMPBELL

Université du Québec, INRS-Eau, C.P. 7500, Ste-Foy, Québec, Canada G1V 4C7

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**Abstract.** We have investigated the extent of iron oxyhydroxide deposition on the roots of two common freshwater species, *Vallisneria americana* Michx. and *Heteranthera dubia* (Jacq.) MacM., collected from different sites in the St. Lawrence River, Québec, Canada, and have related metal concentrations in the root plaques both to the geochemical conditions prevailing in the host sediments (pH; metal partitioning) and to the metal concentrations within the plant root tissue. Possible effects of root plaque on sediment geochemistry are also discussed.

At those sites where the two submerged plants co-existed, the amounts of Fe deposited on their respective root surfaces were positively correlated, indicating that sediment geochemistry (pH; concentration of labile metal) exerted a more important influence on plaque formation than did inter-species differences (root physiology, morphology). Iron and Mn concentrations in the root plaque were positively correlated with each other, and with the readily extractable fractions (F1, F2) of these metals in the adjacent sediments. In contrast, Zn concentrations in the root plaque of *V. americana* were not related to Zn concentrations in the sediments – the dominant geochemical process at the root surface is Fe deposition, such that the quantities of Zn deposited on the roots are determined not by Zn geochemistry *per se* but rather by the amount of Fe deposition. Indeed the Zn/Fe ratios in the root plaque were related to the Zn/Fe ratios in the surrounding sediments (NH<sub>2</sub>OH•HCl extract).

On a concentration basis ( $\mu\text{g/g}$ ), more Fe, Mn and Zn was found outside the root, in the iron plaque, than inside the root tissues. For all 3 metals, significant relationships were observed between the metal concentrations in the plaque and those inside the roots. For Zn, however, the best statistical relationship was not with [Zn]<sub>plaque</sub>, but rather with the [Zn]/[Fe] ratio in the plaque. It is hypothesized that the Zn/Fe ratio in the root plaque reflects the free Zn<sup>2+</sup> concentration adjacent to the root surface, and that this in turn affects Zn uptake by the plant root. For a given value of Zn in the sediments or in the root plaque, the Zn content of the root is inversely related to the concentration of Fe oxyhydroxides, implying that Fe plays a protective role in regulating Zn bioavailability.

## Introduction

The occurrence of iron oxyhydroxides as coatings on roots has been widely observed in aquatic plants and terrestrial plants subjected to flooding (Crowder & St-Cyr 1991). Two main factors are thought to explain the accumulation of iron oxyhydroxides on these roots: (i) the greater availability

of soluble ferrous iron species in submerged soils (Ponnamperuma 1972) and (ii) the radial loss of oxygen from the roots. To circumvent the problem of having roots growing under reducing conditions, aquatic plants have developed a ventilation system or aerenchyma that acts as a conduit for the transport of  $O_2$  from the shoot to the root tissues, where it supports root respiration (Williams & Barber 1961); some of this  $O_2$  leaks outside the root, to the rhizosphere (Armstrong 1979). When ferrous iron encounters  $O_2$  in the rhizosphere, it is subject to oxidation and precipitation as ferric oxyhydroxide on the root surface (Armstrong 1982). In emergent and floating-leaved plants, the source of  $O_2$  for the roots is the atmosphere whereas in submerged species the source of  $O_2$  is photosynthesis (Sand-Jensen et al. 1982; Thursby 1984).

The extent of iron oxyhydroxide accumulation on roots as affected by environmental factors has been studied in emergent plants (*Typha latifolia*: Macfie & Crowder 1987; *Phragmites australis*: St-Cyr & Crowder 1989), but to our knowledge no such data have been reported for submerged aquatic plants; previous reports have focused on visual and microscopic observations (St-Cyr et al. 1993). In this study, we have evaluated the extent of iron plaque deposition on the roots of two submerged aquatic plants, *Vallisneria americana* Michx. and *Heteranthera dubia* (Jacq.) MacM., in fluvial lakes of the St. Lawrence River, Québec, Canada. Relations between metals in root plaque (Fe, Mn, Zn) and environmental factors are examined and comparisons made with root plaques from emergent plants. The possible effects of the root plaque of submerged plants on the geochemical behaviour of metals and nutrients in freshwater lake sediments are discussed.

## Materials and methods

### *Sampling*

The rosette-type submerged plant *Vallisneria americana* was collected at sampling sites in lakes St. Louis and St. Pierre, in August 1990 (total: 17 stations), and in these two lakes plus lakes St. François and des Deux Montagnes in August 1991 (total: 13 stations); during the 1991 sampling, *Heteranthera dubia* was also collected at 8 field stations. Lakes St. Louis, St. Pierre and St. François are fluvial lakes of the St. Lawrence River system while Lake des Deux Montagnes corresponds to a widening of the Ottawa River, an important tributary, just upstream of its confluence with the St. Lawrence River (Québec, Canada; Fig. 1).

At each sampling station, plants (above- and below-ground parts) were carefully disengaged from the sediments by SCUBA divers and taken to the boat where the plants were put in plastic bags filled with lake water,

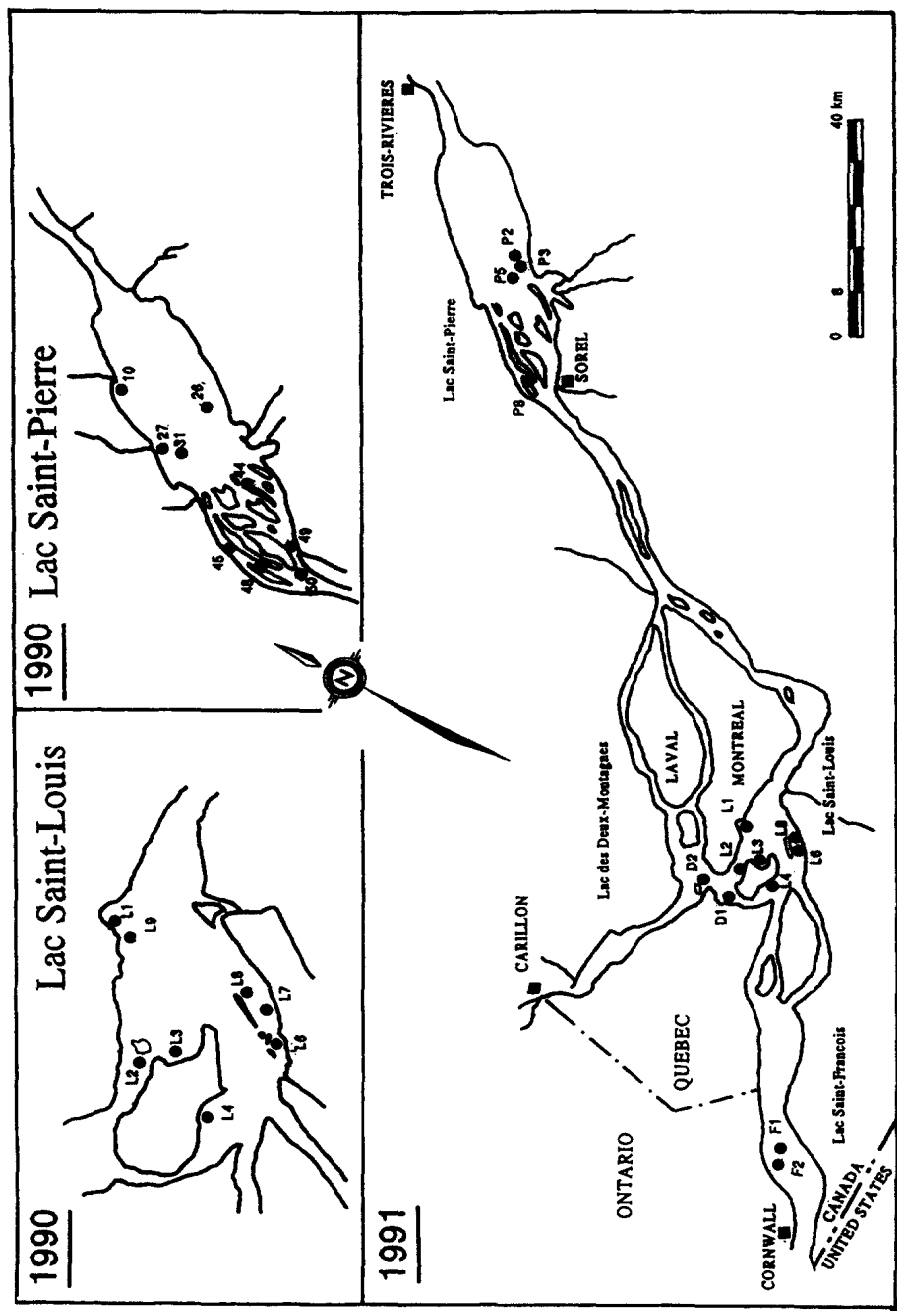


Figure 1. Map of the St. Lawrence River system showing sampling stations visited in 1990 and 1991.

placed in a cooler and transported to the laboratory. Plants were then carefully washed with tap water, rinsed with deionized water and were either processed immediately for the iron plaque extraction (1990 sampling) or frozen until analysis (1991 sampling).

The SCUBA divers also collected superficial bottom sediments at each sampling site, near the plants to be collected for iron plaque analysis, using a polypropylene corer. In the boat, the oxic superficial layer of the core (about the first cm) was removed using a Teflon sheet and placed in a 500 mL polypropylene bottle, previously soaked with acid and rinsed with deionized water. A composite sample from four cores was prepared at each sampling site, in order to obtain sufficient sediment for the analyses. Lake water was added to the bottles to suspend the sediments and maintain them in an oxic state. The bottles were placed in a cooler and transported to the laboratory where they were frozen until analysis. To evaluate within-site variations in iron plaque deposition, sediment and plant samples were collected in August 1991 at three different locations within the defined site area (about 4 m × 4 m) at stations L6 and P2, and were analyzed separately.

### *Sediment analysis*

Sediments were thawed, pebbles, shells, roots and other foreign material were removed and samples were centrifuged at 8000 rpm for 20 min. The supernatant was discarded. The sediments were then homogenized and a small portion weighted for water content determination by oven-drying overnight at 60°C. All labware was soaked in 15% HNO<sub>3</sub> for 24 h and rinsed with deionized water prior to use.

Metals in sediments were subdivided into four main fractions (F1 → F4), following the sequential extraction procedure of Tessier et al. (1979). An amount of wet sediment yielding approximately 1 g dry weight was analyzed. The four fractions obtained were: **F1**) exchangeable metals (MgCl<sub>2</sub> 0.5 M, pH 7.0); **F2**) metals bound to carbonates or specifically adsorbed (NaOAc 1.0 M/HOAc, pH 5.0); **F3**) metals bound to reducible Fe- and Mn-oxides (NH<sub>2</sub>OH•HCl 0.04 M in 25% HOAc) and **F4**) metals bound to organic matter and sulfides (H<sub>2</sub>O<sub>2</sub>/HNO<sub>3</sub> and NH<sub>4</sub>OAc 3.2 M). A second subsample was analyzed for total metals after digestion with HF and HClO<sub>4</sub>. To test the accuracy of the procedure, sediment samples certified for total metal concentrations (MEIS-I; National Research Council of Canada) were analyzed in parallel. Measured total metal concentrations compared well with the certified values for Fe, Mn and Zn.

Concentrations of Fe, Mn and Zn in each fraction were measured using either a flame atomic absorption spectrophotometer (AAS) or an inductively coupled plasma atomic emission spectrophotometer (ICP-AES). Calibration

curves were obtained using, as a matrix, solutions of the same composition as those used for each extraction.

The pH of the wet sediment was measured in 0.01 M CaCl<sub>2</sub> (Peech 1965), using a 1:1 ratio of extractant/dry sediment.

### *Iron plaque extraction*

Roots were separated from the shoot. Roots from 3 to 5 individual plants collected over the entire sampling site were combined in a single composite sample to give enough root material for analysis. Sub-samples of these composite samples (1 to 6 per site) were analyzed. Three extraction methods were compared.

Following the 1990 sampling, the iron deposited on the plant roots was extracted using the cold DCB (dithionite-citrate-bicarbonate) technique of Taylor & Crowder (1983a). Roots (about 1 to 3 g fresh weight) were mechanically agitated at room temperature for 3 h in 40 mL sodium citrate (0.3 M), 5 mL sodium bicarbonate (1.0 M) and 3 g of sodium dithionite (gradually added). Roots were then removed from the resulting solution, rinsed with deionized water, and the rinse added to the DCB extract; the solution was then completed to 100 mL with deionized water (method 1).

Following the 1991 sampling, along with the Taylor & Crowder (1983a) procedure used the previous year, a modified DCB method reported in Otte et al. (1989) was also tested. In this latter method, roots of *V. americana* and *H. dubia* (about 1 to 3 g fresh weight) were incubated at 60°C for 20 min in a solution of 17.8 mL sodium citrate (0.03 M), 2.2 mL sodium bicarbonate (0.125 M) and 1.5 g sodium dithionite. Roots were then removed from the resulting solution, rinsed with deionized water, and the rinse added to the DCB extract; the solution was then completed to 30 mL with deionized water (method 2). In method 3, using the same extraction solution and general procedure as in method 2, the roots were mechanically agitated at room temperature during 15 min instead of being heated. Method 1 was employed at all sites; if the quantity of available root material was sufficient, methods 2 and 3 were tested in parallel.

The concentrations of Fe, Mn and Zn in the leachates were measured using inductively coupled plasma – atomic emission spectroscopy (ICP-AES). Calibration curves were obtained with, as a matrix, solutions of the same composition as those used for each extraction. In 1990, the dithionite used was found to be highly contaminated with Zn, as has frequently been reported for this reagent in the literature (Jenne et al. 1974). In 1991, however, the contamination from the dithionite reagent used was negligible.

Potassium concentrations in the leachates were also determined by ICP-AES as a measure of the possible damage done to the root cells by the

reagents used in the extraction methods. Potassium levels were compared with controls where roots of plants from the same sampling station were agitated in deionized water for the same extraction time.

After iron removal, the roots were oven-dried at 60°C for 24 h to obtain the root dry weight. Iron measured in the plaque can thus be expressed as  $\mu\text{g}$  of iron extracted per unit dry weight of root. This measure is referred to as the concentration of iron in the iron plaque.

The dry DCB-washed roots were then milled/ground to a powder with liquid nitrogen, and digested in concentrated nitric acid (Aristar grade) in Teflon bombs in a microwave oven. Sample digests were analyzed for Fe, Mn and Zn using ICP-AES. Certified reference material from the U.S. National Institute of Standards and Technology (Citrus leaves SRM 1572) was digested during each analytical run. Measured metal concentrations compared well with the certified values for Mn and Zn; measured values for Fe tended to be slightly lower ( $\sim 20\%$ ) than the certified values.

### *Statistical analysis*

Pearson's correlation test and multiple regression analysis were used to identify potential relations between metal concentrations in the root plaque and metals in sediments or roots. The data were logarithmically transformed to normalize the distributions and equalize the variances. In the graphs, each letter represents a particular sampling site. The statistical tables used were those in Jolicoeur (1991).

## **Results**

### *Methods of iron plaque extraction*

The amounts of Fe extracted from *V. americana* roots in 1991 using the DCB method of Taylor & Crowder (1983a) and the modified method of Otte et al. (1989) are reported in Table 1 for sampling sites where at least two different methods were tested in parallel. Analytical variability for a given extraction technique was appreciable (coefficients of variation, CV, varying from 1% (station P2(1)) to 53% (station F1), with an average CV of about 19%). Given this inherent variability, all three methods seem equally efficient in extracting the iron oxide coating from the tiny fibrous roots of *V. americana*. Comparisons of the results obtained by the different methods, using Student's paired t-test measurements, indicated no statistically significant differences between them. However, the third method (see Table 1) is less time consuming, with an extraction period of 15 min instead of 3 h as in the

*Table 1.* Measured Fe concentrations ( $\mu\text{g/g}$  root dry weight  $\pm$  standard deviation when available) on the roots of *Vallisneria americana* collected in 1991, as extracted using three different DCB (dithionite-citrate-bicarbonate) methods. Number of replicates in parentheses.

Station	Method (1) DCB	Method (2) DCB mod. 60°C	Method (3) DCB mod. 25°C
L2	144000 $\pm$ 12600(2)	105000 $\pm$ 13100 (2)	125000
L6(2)	65900 $\pm$ 14500(2)	51100	—
L6(3)	40400 $\pm$ 9330(2)	30500 $\pm$ 6310 (2)	30300
L8	12800 $\pm$ 3200(2)	11800 $\pm$ 2810 (2)	1700 $\pm$ 600 (2)
F1	8580 $\pm$ 3030(3)	—	7570 $\pm$ 4040 (3)
F2	18200 $\pm$ 850(2)	—	14700 $\pm$ 760 (4)
P2(1)	84000 $\pm$ 730(2)	143000	—
P3	163000 $\pm$ 58300(2)	192000 $\pm$ 21100 (2)	223000
P5	70300	71100	95800
D2	25500	20500	15000

(1) Roots were mechanically agitated at room temperature for 3 h in 40 mL  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  (0.3 M), 5 mL  $\text{NaHCO}_3$  (1.0 M) and 3 g of  $\text{Na}_2\text{S}_2\text{O}_4$ . Completed to 100 mL.

(2) Roots were incubated at 60°C during 20 min in 17.8 mL  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  (0.03 M), 2.2 mL  $\text{NaHCO}_3$  (0.125 M) and 1.5 g  $\text{Na}_2\text{S}_2\text{O}_4$ . Completed to 30 mL.

(3) Roots were mechanically agitated at room temperature for 15 min; solution as in (2).

original method of Taylor & Crowder (1983a). The resulting solution is also less dilute (30 mL vs. 100 mL), which affords a better operational detection limit for trace metals present in small amounts in the plaque, as for Zn. Similarly, based on limited comparisons with controls (roots swirled in deionized water), none of the methods appeared to aggravate potassium leakage due to tissue damage. Statistical comparisons between means at stations where replicates were available, using the Student-t test for small samples, revealed no differences between controls and roots processed to remove iron plaque, following method 3 (potassium concentrations in the leachates are expressed as  $\mu\text{g/g}$  root dry weight  $\pm$  standard deviation; station L8: 2270  $\pm$  310 vs. 1980  $\pm$  380; station F1: 3110  $\pm$  400 vs. 3370  $\pm$  240; station F2: 2330  $\pm$  320 vs. 2610  $\pm$  560;  $P > 0.05$ ). Note that appreciable loss of K was observed even for the controls, presumably due to leakage from the cut part of the root.

Results for extractable Fe and Mn, as obtained from the three methods, were used in further statistical analysis. For Zn, however, concentrations in the DCB solutions were only measurable with the modified extraction methods (total solution completed to 30 mL). Zinc values in the plaque of *V.*

Table 2. Pearson's correlation coefficients between the concentrations of Fe or Mn in plaque ( $\mu\text{g/g}$  root dry weight) from the roots of *Vallisneria americana* collected in fluvial lakes of the St. Lawrence River system, and the concentrations of Fe or Mn ( $\mu\text{g/g}$  dry weight) in various geochemical fractions in the adjacent superficial sediments, after a logarithmic transformation to normalize the distributions.

A	Fe					
Plaque	N	F1 <sup>1</sup>	F2	F3	F4	Total
August 1990	17	0.262	0.679**	0.416	-0.012	0.388
August 1991	17	0.405	0.713**	0.543*	-0.148	0.214
Both years	34	0.151	0.663***	0.536**	0.041	0.232

B	Mn					
August 1990	17	0.689**	-0.227	-0.232	0.159	-0.052
August 1991	17	0.678**	0.355	0.522*	0.319	0.448
Both years	34	0.522**	-0.021	0.157	0.258	0.029

<sup>1</sup> F1: exchangeable; F2: bound to carbonates or specifically adsorbed; F3: bound to reducible Fe- and Mn-oxides, and F4: bound to organic matter and sulfides.

\*  $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

*americana* were thus obtained only for the 1991 sampling, at the following stations: L1, L3, L6, L8, F1, F2, P2, P3, P5, P8, D2.

#### *Relations between metal concentrations in the plaque and in the sediments*

For the 1991 sampling, when the results of all three extraction techniques were combined, the coefficient of variation for the quantities of DCB-extracted Fe on the roots of *V. americana* varied from 13% at station F2 to 40% at station F1. At two sites (L6 and P2), rather than analyze composite root samples representative of the entire sampling site, three sub-samples were prepared representing three sub-sites within the confines of the same nominal sampling site (4 m  $\times$  4 m). Results from the extraction and analysis of these sub-samples revealed an important within-site variability (coefficients of variation ranging from 22% to 78%, average CV = 39%). This variability presumably reflects micro-scale heterogeneity in sediment parameters and possible differences in rhizosphere chemistry. To identify relations between metal concentrations in the plaques and in the sediments, we thus decided to compare root plaque from adjacent plants with sediment cores taken at exactly the same location.

Correlation analysis was used to examine relations between the concentrations of iron extracted from the root plaque of *V. americana* and the concentrations of iron in the various geochemical fractions in the adjacent sediments (Table 2A). For the data from 1990 (lakes St. Louis and St. Pierre), a significant correlation was obtained only with Fe-Fraction 2. Similarly, for the



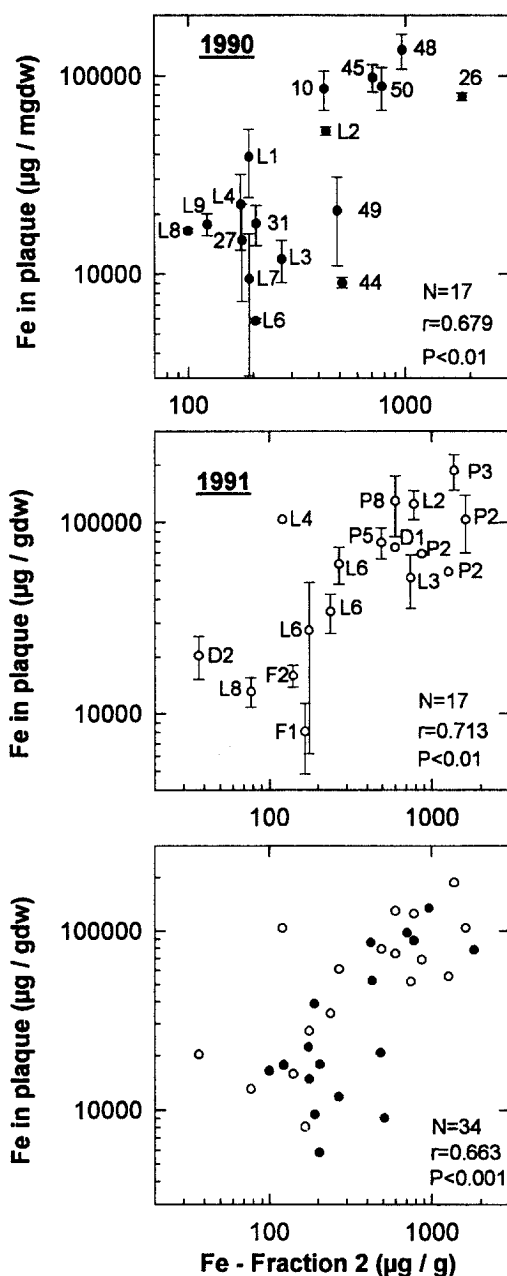


Figure 2. Relationship between the concentrations of Fe ( $\mu\text{g/g}$  root dry weight  $\pm$  standard deviation) in the root plaque of *Vallisneria americana* and the concentrations of Fe-Fraction 2 ( $\mu\text{g/g}$  dry weight) in the adjacent superficial sediments, from fluvial lakes of the St. Lawrence River system. Scales are logarithmic. The upper two panels correspond to samples collected in 1990 and 1991 respectively; the lower panel presents the data for both sampling campaigns. Each point represents an individual sampling site, as identified in Fig. 1.

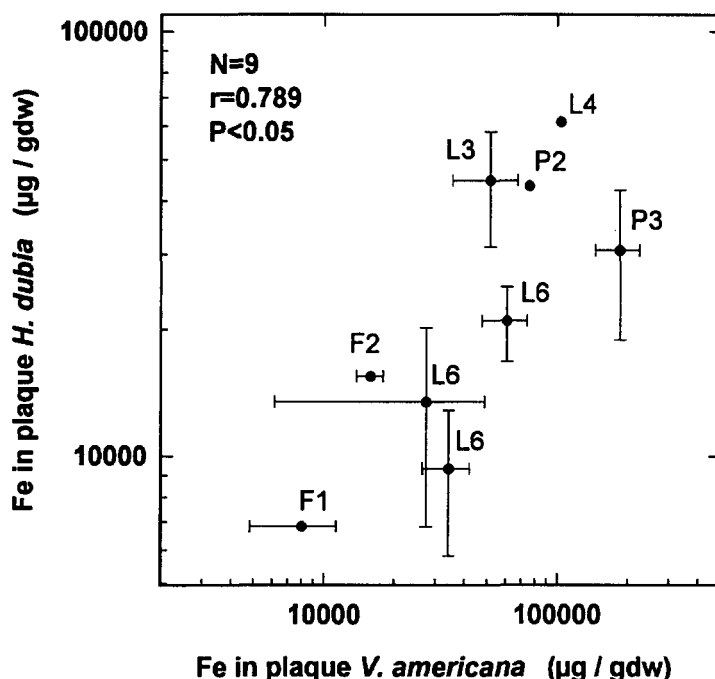


Figure 3. Relationship between the concentrations of Fe ( $\mu\text{g/g}$  root dry weight  $\pm$  standard deviation when available) in the root plaque of *Heteranthera dubia* and *Vallisneria americana* collected in 1991 in fluvial lakes of the St. Lawrence River system. Scales are logarithmic. Each point represents an individual sampling site, as identified in Fig. 1.

1991 data (lakes St. Louis, St. Pierre, St. François and des Deux Montagnes), the most significant correlation was again with Fe-Fraction 2 ( $r = 0.679$ ,  $P < 0.01$  in 1990;  $r = 0.713$ ,  $P < 0.01$  in 1991;  $r = 0.663$ ,  $P < 0.001$  for both years combined – see Fig. 2). Significant relations were also obtained with Fe-Fraction 3 (Table 2A). *Heteranthera dubia*, frequently found with *V. americana* in the St. Lawrence River, was collected in 1991. At sites where these two species co-existed, concentrations of Fe on the roots of *H. dubia* were positively correlated with those on the roots of *V. americana* (Fig. 3).

Examination of relationships between the concentrations of Mn in the plaque from the roots of *V. americana* and sedimentary Mn concentrations revealed highly significant correlation coefficients with Mn-Fraction 1 ( $r = 0.689$ ,  $P < 0.01$  in 1990;  $r = 0.678$ ,  $P < 0.01$  in 1991;  $r = 0.522$ ,  $P < 0.01$  for both years combined: Table 2B). Graphs showing these relationships are presented in Fig. 4. A similar relation was obtained between these two variables for *H. dubia* root plaques (1991,  $N = 10$ ,  $r = 0.675$ ,  $P < 0.05$ ).

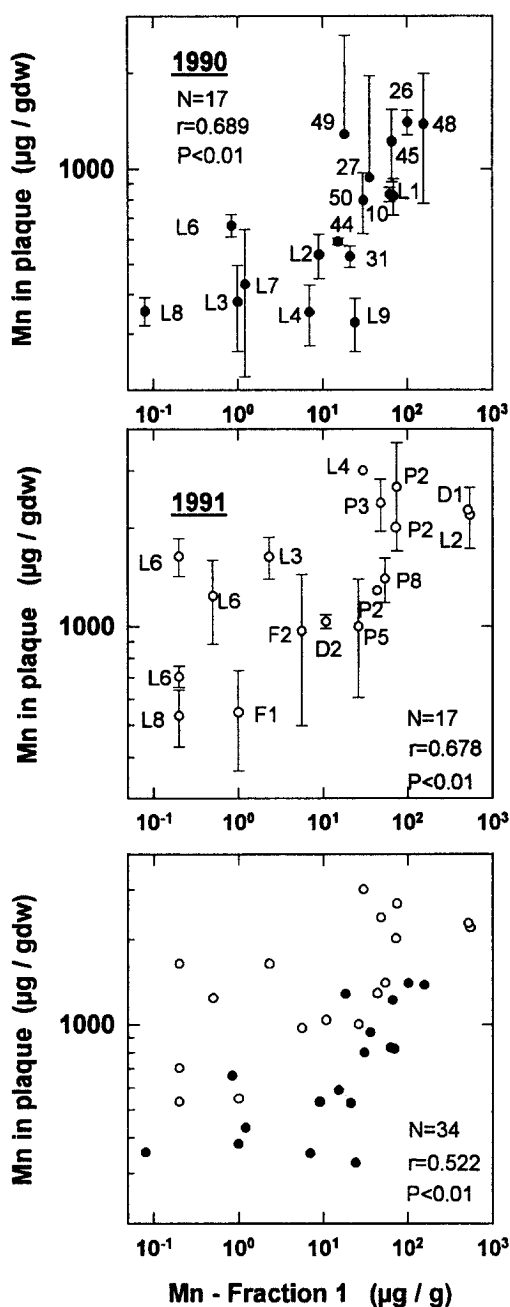


Figure 4. Relationship between the concentrations of Mn ( $\mu\text{g/g}$  root dry weight  $\pm$  standard deviation) in the root plaque of *Vallisneria americana* and the concentrations of Mn-Fraction 1 ( $\mu\text{g/g}$  dry weight) in the adjacent superficial sediments, from fluvial lakes of the St. Lawrence River system. Scales are logarithmic. The upper two panels correspond to samples collected in 1990 and 1991 respectively; the lower panel presents the data for both sampling campaigns. Each point represents an individual sampling site, as identified in Fig. 1.

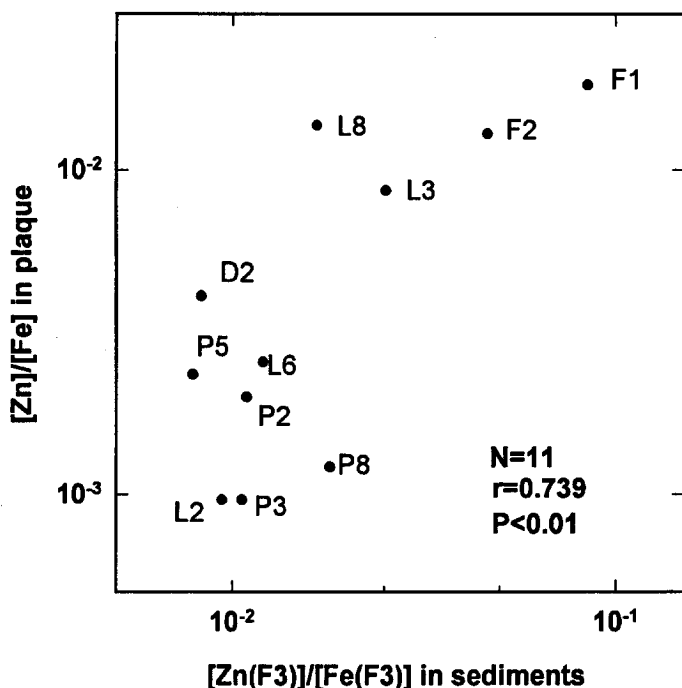


Figure 5. Relationship between the Zn/Fe ratio in the root plaque of *Vallisneria americana* and the ratio ZnF3/FeF3 in the adjacent superficial sediments, from fluvial lakes of the St. Lawrence River system. Scales are logarithmic. Each point represents an individual sampling site, as identified in Fig. 1.

In contrast to Fe and Mn, Zn concentrations in the plaque were not significantly correlated with the Zn concentrations in any of the geochemical fractions in the adjacent sediments. However, the Zn/Fe ratio of the plaque was significantly correlated with this same ratio in the sediment (ZnF3/FeF3,  $r = 0.739$ ,  $P < 0.01$ ; all Zn data from station L6 were combined – see Fig. 5). This relation was not statistically significant for *H. dubia*; however, Zn concentrations in the plaque were obtained at only 7 stations for this species.

#### *Metals within the plaque*

The concentrations of Fe and Mn in the root plaque of *V. americana* were significantly correlated, giving  $r = 0.671$ ,  $P < 0.01$  in 1990;  $r = 0.699$ ,  $P < 0.01$  in 1991;  $r = 0.723$ ,  $P < 0.001$  for both years combined (Fig. 6). A similar relationship was obtained in the plaque of *H. dubia* ( $N = 10$ ;  $r = 0.891$ ,  $P < 0.001$ ). Zinc concentrations in the plaque of *V. americana* (determined for the 1991 survey) were not significantly correlated with either Fe ( $r = 0.198$ ,

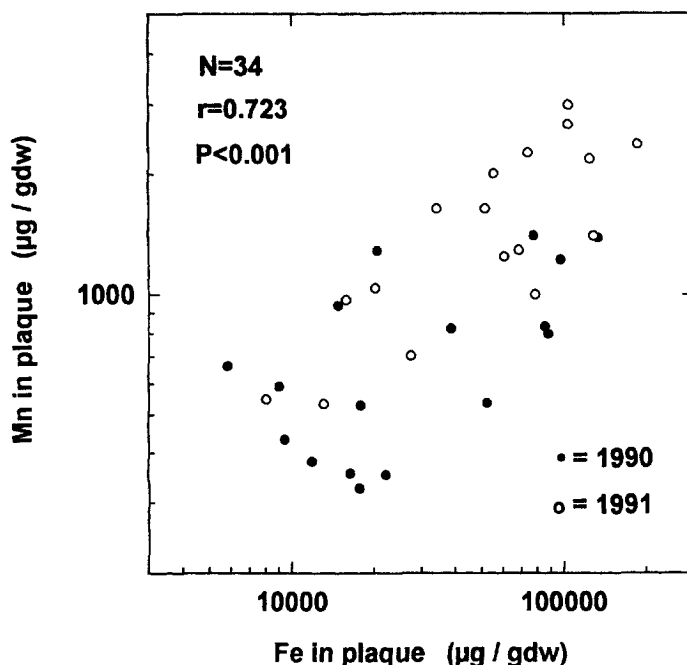


Figure 6. Relationship between the concentrations of Mn and Fe ( $\mu\text{g/g}$  root dry weight) in the root plaque of *Vallisneria americana* from fluvial lakes of the St. Lawrence River system. Scales are logarithmic.

$P > 0.05$ ) or Mn ( $r = 0.282$ ,  $P > 0.05$ ) in the plaque, but these relations were statistically significant in the root plaque of *H. dubia* ( $N = 9$ ; Fe vs. Zn,  $r = 0.808$ ,  $P < 0.01$ ; Mn vs. Zn,  $r = 0.844$ ,  $P < 0.01$ ).

#### *Relations between root internal and external metal concentrations*

The concentrations of Fe, Mn and Zn in the root plaque and inside the roots were determined. Metals on the roots were measured in the DCB extract, whereas metals inside the roots were determined by digesting these same DCB-washed roots and analyzing the metals in the resulting solution. The proportion of external metals was then calculated. For *V. americana* in the 1991 sampling, with the exception of two stations (P2 and D1), the percentage of Fe outside the roots was of the order of 95% or more (Table 3); this percentage was  $\geq 91\%$  in 1990. The percentage of Mn outside the roots compared to the concentrations inside the roots, for each station, was  $\geq 91\%$  in both 1990 and 1991 (Table 4). For zinc, this proportion was somewhat more variable; 54% at station P8, 72 and 78% at stations in Lake St. François (F1 and F2) and  $\geq 85\%$  at the other sites (Table 5). Similar results were obtained

*Table 3.* Mean concentrations of Fe in  $\mu\text{g/g}$  root dry weight (standard deviation given in parentheses when available) in and on the roots of *Vallisneria americana* collected in August 1991 in fluvial lakes of the St. Lawrence River system. (1) Fe on the roots was determined following a DCB wash. (2) Fe in the roots was determined by digesting the DCB-washed roots.

Station	(1) Fe on roots	(2) Fe in roots	(1) + (2)	% Fe on roots
L2	125000 (21400)	870 (17)	126000	99%
L3	51800 (16000)	1320 (401)	53100	98%
L4	104000	1130	105000	99%
L6(1)	27500 (21400)	1020 (1030)	28600	96%
L6(2)	61000 (13300)	1210 (496)	62200	98%
L6(3)	34400 (7850)	470 (66)	34900	99%
L8	13100 (2330)	410 (13)	3500	97%
F1	8080 (3240)	330 (92)	8410	96%
F2	15900 (2100)	770 (588)	16600	95%
P2(1)	104000 (34100)	37000 (50900)	141000	74%
P2(2)	69000	67900	37000	50%
P2(3)	55600	37900	93500	60%
P3	187000 (39900)	1520 (62)	188000	99%
P5	79100 (14500)	2620 (2280)	81700	97%
P8	130000 (45100)	3680 (769)	133000	97%
D1	74400	51000	125000	59%
D2	20400 (5230)	570	21000	97%

for *H. dubia* in 1991, where metals outside the roots compared to total root metals represented (means) 94% for Fe (excluding station P2: 66%), 92% for Mn and 84% for Zn (excluding station F1: 34%).

For *V. americana*, there was a strong relationship between the concentrations of Fe in the plaque and those inside the roots ( $r = 0.779$ ,  $P < 0.001$ , Fig. 7 for 1990–1991 data combined; stations P2 and D1 were excluded from the statistical analysis, Fe concentrations in roots from these stations being much higher ( $\sim 50\times$ ) than at the other 11 stations: Table 3). A similar positive correlation was observed between the concentrations of Mn in the plaque and those inside the roots ( $r = 0.521$ ,  $p < 0.01$ , Fig. 7 for 1990–1991 data combined).

A significant relationship was also obtained between Zn concentrations in the roots and in the plaque ( $r = 0.648$ ,  $P < 0.05$ ). However, a more significant correlation was obtained between Zn concentrations in the roots and the Zn/Fe ratio in the plaque ( $r = 0.785$ ,  $P < 0.01$ ; Fig. 8). Also, the Zn concentrations in the roots were significantly correlated with the ZnF3/FeF3 ratio of the sediments ( $r = 0.888$ ,  $P < 0.001$ ). Note that station P8 was removed from

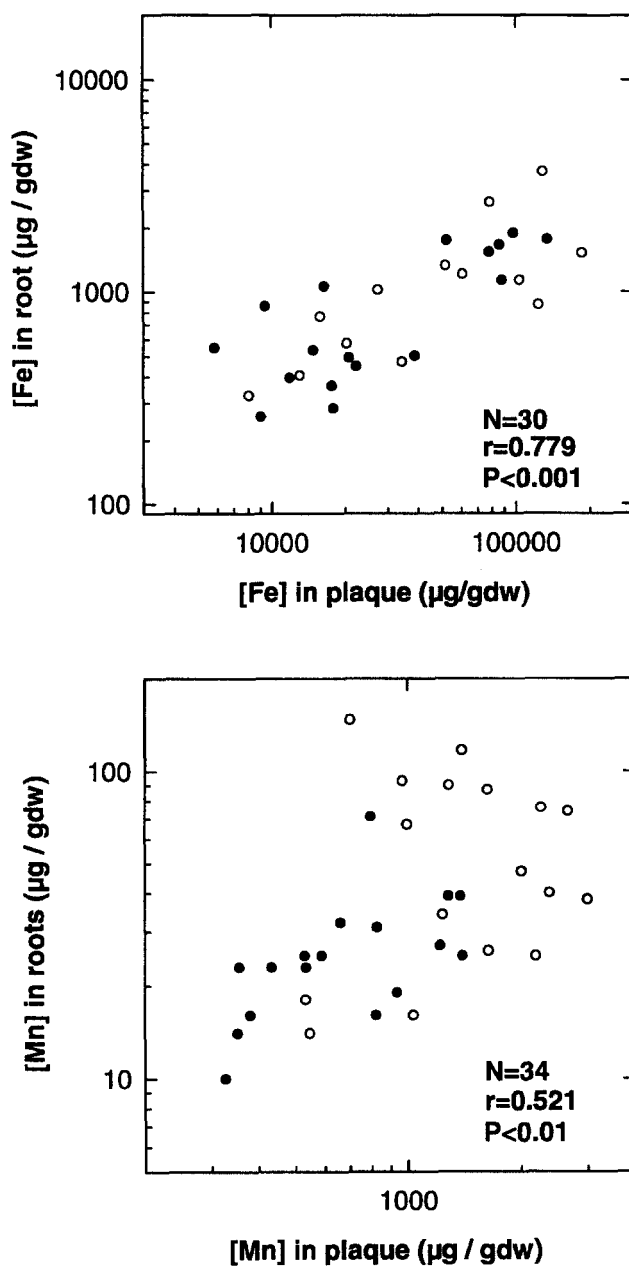


Figure 7. Relationship between the concentrations of Fe and Mn inside the roots ( $\mu\text{g}/\text{g}$  root dry weight) and in the root plaque ( $\mu\text{g}/\text{g}$  root dry weight) of *Vallisneria americana* from fluvial lakes of the St. Lawrence River system. Scales are logarithmic.

*Table 4.* Mean concentrations of Mn in  $\mu\text{g/g}$  root dry weight (standard deviation given in parentheses when available) in and on the roots of *Vallisneria americana* collected in August 1991 in fluvial lakes of the St. Lawrence River system. (1) Mn on the roots was determined following a DCB wash. (2) Mn in the roots was determined by digesting the DCB-washed roots.

Station	(1) Mn on roots	(2) Mn in roots	(1) + (2)	% Mn on roots
L2	2190 (460)	25 (1)	2215	99%
L3	1640 (240)	87 (86)	1727	95%
L4	3000	38	3038	99%
L6(1)	700 (50)	150 (180)	847	83%
L6(2)	1240 (360)	34 (1)	1274	97%
L6(3)	1640 (210)	26 (3)	1666	98%
L8	530 (110)	18 (2)	548	97%
F1	550 (190)	14 (6)	564	98%
F2	970 (470)	93 (120)	1063	91%
P2(1)	2670 (970)	74 (39)	2744	97%
P2(2)	1290	90	1380	94%
P2(3)	2010	47	2057	98%
P3	2380 (430)	40 (1)	2420	98%
P5	1000 (390)	67 (64)	1067	94%
P8	1400 (220)	120 (1)	1517	92%
D1	2270	76	2346	97%
D2	1040 (50)	16	1056	99%

these statistical analyses as an outlier; root Zn concentrations at this station were  $\sim 5$  to 13 times higher than those at the other sites, and in the scattergram (Fig. 8) this station clearly does not belong to the statistical population.

## Discussion

### *Methodological concerns*

Most root iron was found on the surface as iron plaque (Table 3). The same phenomenon was reported for emergent plants under wetland conditions (*Phragmites australis*: St-Cyr & Crowder 1989; *Typha latifolia*: Macfie & Crowder 1987). Similar trends hold for Mn (Table 4) and Zn (Table 5).

In aquatic plants, roots are frequently reported to contain higher concentrations of most metals than above-ground parts (Outridge & Noller 1991). However, elevated metal concentrations in roots of aquatic plants, as reported in the literature, will tend to reflect both internal concentrations of metallic



Table 5. Mean concentrations of Zn in  $\mu\text{g/g}$  root dry weight (standard deviation given in parentheses when available) in and on the roots of *Vallisneria americana* collected in August 1991 in fluvial lakes of the St. Lawrence River system. (1) Zn on the roots was determined following a DCB wash. (2) Zn in the roots was determined by digesting the DCB-washed roots.

Station	(1) Zn on roots	(2) Zn in roots	(1) + (2)	% Zn on roots
L2	120 (10)	8.7 (0.2)	129	93%
L3	447	49.7 (0.2)	497	90%
L6(1)	55	9.7 (2.4)	65	85%
L6(2)	152	12.9 (2.4)	165	92%
L6(3)	111 (10)	20.1 (14.0)	131	85%
L8	179 (86)	27.1 (2.0)	206	87%
F1	147 (38)	57.6 (16.6)	205	72%
F2	204 (66)	56.5 (1.9)	261	78%
P2(1)	208	17.5 (5.0)	226	92%
P3	180 (30)	13.3 (9.0)	193	93%
P5	186 (38)	14.9 (2.0)	201	93%
P8	158 (6)	133 (159)	291	54%
D2	83 (8)	6.1	89	93%

ions and external contamination, including metals associated with the plaque. These high amounts of metals outside the root tissues can potentially confound relationships between root metal content and sediment metal content, if not properly removed. This is particularly true if these relations are sought at the end of the growing season, since the iron plaque accumulates on the roots throughout the summer (Crowder & Macfie 1986).

The effectiveness of various techniques for the extraction of iron plaque from plant roots was tested by Taylor & Crowder (1983a) and McLaughlin et al. (1985). The DCB washing removed all the root external precipitate, whereas other methods, such as rinsing roots with deionized water, washing with EXTRAN-300 (a non-phosphate detergent), use of an ultrasonic probe or treatment with the synthetic chelates EDTA and DTPA, were all revealed to be inefficient, leaving considerable Fe on the surface of the washed roots. The DCB technique was originally designed for the extraction of iron oxides from soil material (Aguilera & Jackson 1953), and it has been commonly used to extract iron plaque on emergent plant roots (Chen et al. 1980; Otte et al. 1989). The technique involves the use of sodium dithionite ( $\text{Na}_2\text{S}_2\text{O}_4$ ) as a strong reducing agent ( $\text{Fe(III)} \rightarrow \text{Fe(II)}$ ), sodium citrate as a chelating agent to maintain the extracted metals in solution and sodium bicarbonate as a buffer. DCB method is very efficient in removing the iron oxyhydroxide

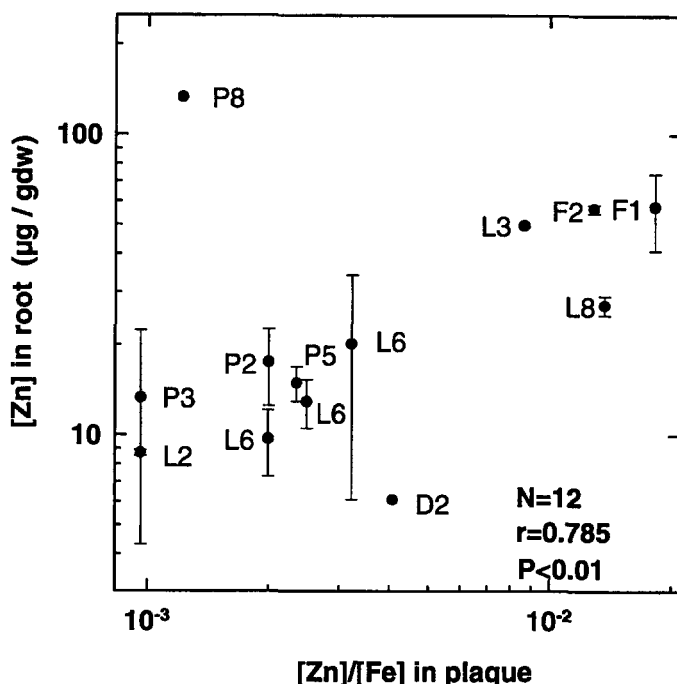


Figure 8. Relationship between the concentrations of Zn ( $\mu\text{g/g}$  root dry weight  $\pm$  standard deviation, when available) inside the roots and the Zn/Fe ratio of the plaque for *Vallisneria americana* from fluvial lakes of the St. Lawrence River system. Scales are logarithmic. Each point represents an individual sampling site, as identified in Fig. 1.

coating without damaging root tissues (Bienfait et al. 1984; Otte et al. 1989). In the present study, using ICP–AES as the technique for metal quantitation, we did not encounter any of the problems previously reported in the literature concerning the analysis of Fe in the DCB solutions, attributed mainly to their high sodium content (Koch & Mendelssohn 1989; Taylor & Crowder 1983a). However, the detection limits of the ICP–AES instrument are too high to allow the determination of trace metals possibly present in the plaque on plant roots collected in the field. In principle, graphite furnace atomic absorption spectrophotometry offers better detection limits, but the DCB extracts are so concentrated that very severe matrix effects effectively preclude its use. Alternative extraction methods (e.g. Hudson & Morel 1989; Otte et al. 1987) are needed if one wishes to study metals present in trace amounts in iron plaques.

The manner in which Fe concentrations in root plaques are expressed in this study ( $\mu\text{g}$  of Fe extracted per unit dry weight of roots) hinders the direct comparison of the extent of Fe deposition on roots of different plant

species, collected at the same field station – different plant species will tend to differ in root size, with different surface:volume ratios. Even for a particular species, root pattern, morphology and radial oxygen loss can differ as a function of the sediment conditions (Engelaar et al. 1993). *V. americana* has slender filiform roots with a high surface area:volume ratio, compacted beneath the rosette. Visual observations show a higher surface:volume ratio for *V. americana* roots, and possibly a more localized oxidized area around the compacted roots, than in *H. dubia*. In this study, root weight per individual plant was higher in *H. dubia* than in *V. americana*, whereas Fe accumulated on the roots at stations where both species were analyzed was higher in *V. americana* than in *H. dubia*. To compare directly the extent of Fe deposition on roots of species having different root surface:volume ratios, or roots of the same species growing under different sediment conditions, one needs a different way of expressing Fe concentrations on roots, e.g. as  $\mu\text{g Fe}/\text{cm}^2$  of root or  $\text{nmol Fe}/\text{cm}^2$  of root, using total root area as a basis for comparison (Otte et al. 1989).

### *Sediment – plaque – root relations*

In principle, rooted aquatic plants could accumulate trace metals either from the sediment interstitial water, via their roots, or from the overlying water column, via their shoots and leaves (Denny 1980). If plants behave towards trace metals as they do towards nutrients (e.g., P: Carignan & Kalff 1980), then one might expect the sediment source to dominate. In such a case, reproducible and geochemically explicable relationships should exist between metal partitioning in sediments and metal concentrations in plant underground parts (Campbell et al. 1985; Campbell & Tessier 1991). The data from the current study include metal concentrations in surface oxic sediments (Table 6), in iron oxide plaques on plant root surfaces, and in the root tissues – these combined results provide a unique opportunity to explore potential relationships between these different compartments (Fig. 9). In the following discussion we shall first consider relationships between sediments and the root plaque, and then discuss links between the root plaque and intracellular metals.

### *Plaque-sediment relationships*

#### *Iron*

Iron concentrations in the root plaque varied widely over the studied region, ranging from a minimum of  $8,080 \mu\text{g}/\text{g}$  to a maximum of  $187,000 \mu\text{g}/\text{g}$  (23-fold concentration range). At those sites where *V. americana* and *H. dubia* co-existed, the amounts of iron deposited on their respective root surfaces were positively correlated (Fig. 3), suggesting that sediment geochemistry

Table 6. pH values and concentrations of Fe, Mn and Zn ( $\mu\text{g/g}$ ) in various geochemical fractions of the superficial sediments from sampling sites of the St. Lawrence River system<sup>1</sup>.

Sites	Fe				Mn				Zn				pH			
	F1	F2	F3	F4	Total	F1	F2	F3	F4	Total	F1	F2		F3	F4	Total
1990																
10	9.2	420	3300	190	53300	63.0	86	70	8.0	1200	0.2	11.0	26	5.7	130	6.9
26	19.0	1800	7600	650	67200	100.0	200	130	32.0	950	0.7	88.0	86	31.0	260	7.2
27	6.8	180	2800	370	34800	36.0	120	50	8.6	780	0.1	13.0	34	7.4	130	6.9
31	14.0	210	1700	90	33900	21.0	24	30	5.0	730	0.3	8.8	21	7.7	87	7.0
44	22.0	510	4000	680	37300	15.0	140	100	18.0	760	0.1	23.0	49	22.0	180	7.2
45	65.0	710	3200	540	33700	67.0	41	60	14.0	660	0.3	18.0	41	36.0	170	6.6
48	21.0	970	5400	780	43600	160.0	130	150	26.0	900	0.7	44.0	99	64.0	320	6.6
49	7.4	490	1500	120	35100	18.0	37	40	6.9	700	<0.1	9.6	13	6.0	79	7.2
50	7.0	780	3500	640	36200	31.0	67	80	17.0	640	0.1	29.0	58	83.0	260	7.0
L2	0.2	430	8600	1600	47700	9.1	760	490	57.0	1700	0.3	73.0	144	28.0	360	7.0
L3	12.0	270	4400	680	39000	1.0	330	280	21.0	1300	0.3	45.0	74	13.0	270	7.1
L4	10.0	180	3700	230	32400	7.0	140	370	14.0	1200	0.7	12.0	46	5.3	180	6.7
L6	7.5	200	2800	920	27500	0.8	140	70	13.0	630	<0.1	25.0	29	9.1	130	7.1
L7	0.2	190	3100	1400	36000	1.2	220	90	17.0	930	0.4	24.0	43	13.0	220	7.1
L8	0.1	100	3000	2200	30300	0.1	160	80	18.0	620	0.1	33.0	47	15.0	190	7.2
L9	6.9	120	1800	74	36400	24.0	84	160	4.4	920	0.1	7.0	17	1.8	70	6.9
L1	6.6	190	10000	320	161000	70.0	680	1900	370.0	5400	0.2	18.0	97	15.0	310	7.2

Table 6. (continued)

Sites	Fe				Total	Mn				Total	Zn			pH	
	F1	F2	F3	F4		F1	F2	F3	F4		F1 + F2	F3	F4		Total
1991															
L2	7.5	780	10000	1400	28300	550.0	340	530	49.0	1200	16.0	78	15.0	130	6.0
L3	1.0	750	4600	670	38600	2.3	180	200	59.0	630	43.0	73	11.0	140	7.0
L4	0.4	120	4100	170	23600	30.0	110	190	5.4	840	4.5	14	3.2	59	7.1
L6(1)	< 0.7	180	4600	1600	16600	<0.3	200	120	21.0	400	28.0	33	12.0	74	7.3
L6(2)	2.1	270	5100	2000	17900	0.5	250	140	25.0	490	26.0	33	13.0	79	7.4
L6(3)	< 0.7	240	5100	1800	17800	<0.3	260	140	23.0	470	26.0	32	12.3	81	7.3
L8	< 0.7	80	2900	1000	22000	<0.3	90	60	8.8	550	17.0	31	7.7	94	7.5
F1	1.6	170	5000	4600	29000	1.0	58	90	28.0	440	120.0	290	43.0	550	7.1
F2	2.0	140	4500	1400	26400	5.6	79	100	13.0	460	53.0	156	16.0	340	7.3
P2(1)	5.0	1600	8900	980	60000	75.0	100	130	26.0	830	39.0	55	25.0	190	7.0
P2(2)	< 0.7	870	6700	760	60700	44.0	60	100	18.0	730	23.0	67	22.0	170	7.1
P2(3)	1.3	1300	10000	1000	61200	73.0	150	150	30.0	910	35.0	53	26.0	200	6.9
P3	0.8	1400	7300	1100	58600	48.0	76	110	28.0	750	29.0	48	24.0	170	7.0
P5	1.8	490	4200	420	56900	26.0	20	50	8.9	690	13.0	20	8.3	110	7.2
P8	46.0	600	5700	1400	36500	55.0	32	100	22.0	630	31.0	71	64.0	240	6.5
D1	3.4	600	14500	1500	53200	520.0	420	1700	140.0	2900	17.0	81	14.0	220	5.8
D2	< 0.7	40	1700	70	148400	11.0	37	200	5.9	2400	2.9	11	1.5	140	6.4

<sup>1</sup> Note: F1: exchangeable; F2: bound to carbonates or specifically adsorbed; F3: bound to reducible Fe- and Mn-oxides, and F4: bound to organic matter and sulfides. The extraction procedures for the different fractions and total metal determinations are described in the Materials and Methods section.

(i.e., the common factor at each site) exerts an important influence on plaque formation. Indeed, Fe concentrations in the root plaque showed a weak negative correlation with sediment pH over the range 5.8–7.5 ( $r = -0.374$ ,  $P < 0.05$ ,  $N = 34$ ). An increase in sediment pH in this range would be expected to influence the extent of plaque formation, though the overall direction of the pH effect is not intuitively obvious – at higher pH the rate of (abiotic) oxidation of Fe(II) to Fe(III) is greatly accelerated (Morel & Hering 1993), but the solubility of Fe and its availability in the sediment interstitial water will decrease. The weak negative correlation observed *in situ* suggests that the latter effect dominates, i.e. that at higher pH less Fe(II) is available for deposition at the root surface. Recall that the pH was measured on samples of the bulk sediment, not in the rhizosphere where the oxidation of Fe(II) is occurring. The pH in the rhizosphere would be expected to be lower than in the bulk sediment, due both to the respiration of the living roots and to the production of protons by the hydrolysis of Fe(III).

In addition to sediment pH, Fe concentrations in the root plaque were also related to iron partitioning in the adjacent sediment – a positive relationship was consistently observed between the concentrations of Fe in the root plaque of *V. americana* and the Fe concentrations in the sediment extract F2 (extracted with NaOAc 1.0 M/HOAc, at pH 5.0, after having removed fraction F1 using MgCl<sub>2</sub> 0.5 M at pH 7.0; Table 2A, Fig. 2). A similar correlation was also observed in an earlier field study of an emergent plant (*Phragmites australis*: St-Cyr & Crowder 1988). Multiple regression analysis, with Fe concentrations in the root plaque as the dependent variable and sediment pH and [Fe(F2)] as the predictors, yielded a highly significant regression:  $\log [\text{Fe}]_{\text{plaque}} = -0.29 \text{ pH} + 0.63 \log [\text{Fe}(\text{F2})] + 4.98$  ( $R^2 = 0.503$ ,  $P < 0.001$ ,  $N = 34$ ).

To interpret the relationship between concentrations of Fe in the root plaque and iron partitioning in the sediments, it is necessary to consider the geochemical significance of the metal partitioning results. The distribution of a metal among various sediment extracts does not necessarily reflect its association with discrete sediment phases, but rather should be considered as operationally defined by the methods of extraction (Tessier et al. 1979; Tessier & Campbell 1991). Nevertheless, tests in our laboratory have suggested that for oxic sediments fraction F2 represents mainly metals bound to carbonates or specifically adsorbed to Fe(III) iron oxyhydroxides (Tessier et al. 1982); in a sub-oxic sediment, amorphous FeS would also contribute to sediment extract F2 (Rapin et al. 1986). Thus, in the present case differences in [Fe(F2)] presumably reflect variations in the “pool” of available Fe(II) in the host sediments (Table 6) – as this pool increases in the bulk sediment, more Fe(II)

is available for oxidation in the rhizosphere, and greater amounts of Fe are deposited in the root plaque.

### *Manganese*

Manganese has been frequently reported to be present in the root plaque of emergent plants (St-Cyr & Crowder 1990). We recently reported microscopic observations of Mn oxyhydroxides for *V. americana* root plaque (St-Cyr et al. 1993). In the present study Mn concentrations in the root plaque varied over the studied region, but less so than for Fe; observed values ranged from a minimum of 530  $\mu\text{g/g}$  to a maximum of 3000  $\mu\text{g/g}$  ( $\approx 6$ -fold concentration range). At those sites where *V. americana* and *H. dubia* co-existed ( $N = 9$ ), the amounts of Mn deposited on their respective root surfaces co-varied, suggesting that sediment geochemistry exerts an important influence on Mn deposition; unlike the case for Fe, however, this correlation was not statistically significant.

As was the case for iron, both sediment pH and Mn partitioning in the sediments proved to be related to Mn concentrations in the root plaque of *V. americana*. The correlation with sediment pH was negative over the range 5.8–7.5 ( $r = -0.347$ ,  $P < 0.05$ ,  $N = 34$ ), whereas a positive relationship was obtained between the concentrations of Mn in the root plaque and the concentrations of Mn in sediment extract F1 (exchangeable metals; Table 2B, Fig. 4). In an earlier field study on the emergent plant *P. australis*, St-Cyr & Crowder (1990) observed a similar positive relationship but with Mn in sediment fraction F2. Unlike iron, Mn is often found in appreciable quantities in fractions F1 and F2, presumably in the reduced manganous form, Mn(II), given the extreme insolubility of the higher Mn oxidation states. Manganese concentrations in fraction F1 ranged from  $< 0.3 \mu\text{g/g}$  to 550  $\mu\text{g/g}$  in the study area (Table 6), reflecting variations in the pool of available Mn(II) in the host sediment. Multiple regression analysis, with Mn concentrations in the root plaque as the dependent variable and sediment pH and [Mn(F1)] as the predictors, yielded a significant regression:  $\log [\text{Mn}]_{\text{plaque}} = 0.07 \text{ pH} + 0.001 \log [\text{Mn}(\text{F1})] + 2.45$  ( $R^2 = 0.206$ ,  $P < 0.05$ ,  $N = 34$ ).

### *Zinc*

The variability of Zn concentrations in the root plaque was similar to that of Mn, covering an 8-fold concentration range (minimum 55  $\mu\text{g/g}$ ; maximum 450  $\mu\text{g/g}$ ). However, unlike Fe and Mn, Zn concentrations in the root plaque of *V. americana* did not show significant correlations with any of the individual sediment extracts, Zn(F1)–Zn(F4). The only significant relationship revealed by the statistical analysis was that between the Zn/Fe ratio in the plaque and the [Zn(F3)/Fe(F3)] ratio in the surrounding sediment (Fig. 5). This correlation is perhaps not surprising, in that the hydroxylamine reagent used to extract

fraction F3 is in fact designed to reduce labile Fe- and Mn-oxyhydroxides present in the bulk sediment (Tessier et al. 1979). Note, however, that the Zn/Fe ratio in the root plaque is consistently lower than that in the sediment extract (factor of  $\approx 0.3$ ; compare the x- and y-axes in Fig. 5). The lower concentrations of Zn in the plaque might reflect their authigenic origin and younger age – the Fe oxyhydroxides in the bulk sediment will in general be older and thus might well have accumulated more trace contaminants. An alternative explanation of the lower Zn/Fe ratios observed in the root plaque in the present study may simply be the anticipated lower pH of the rhizosphere as compared with the bulk sediment; zinc adsorption on both synthetic and natural iron oxyhydroxides is known to be sensitive to pH changes in the pH range 5–7 (Tessier et al. 1989; Tessier 1992).

The dominant metal in the root plaque is obviously iron (average molar ratios [Fe:Mn:Zn] : [500:10:1]), and the site-to-site variability of Fe concentrations deposited on the plant roots is greater than for the other metals. It would appear that the dominant geochemical process at the root surface is Fe deposition; since Zn itself is not redox-sensitive, and since the site-to-site variation of Zn/Fe ratios is much *less* than that noted for the absolute amounts of Fe deposited on the root surface, it follows that the quantities of Zn deposited on the roots are determined not by Zn geochemistry per se but rather by the amount of Fe deposition. It is thus not surprising that for zinc, unlike Fe and Mn, we do not see relationships between the metal concentrations on the root surface and metal partitioning in the adjacent sediment.

### *Plaque-root relationships*

Let us now consider relations between root internal and external metal concentrations. The oxidized rhizosphere of aquatic plants has been hypothesized to play a protective role, diminishing the exposure of the plant to the reduced forms Fe(II) and Mn(II) that tend to accumulate in anaerobic soils (Armstrong 1979). Once formed, the iron plaque might in turn be expected to bind other metals (e.g., Cu and Ni; Taylor & Crowder 1983b) and nutrients (e.g., P; Howeler 1973), rendering them less available for uptake. Contrary to this prediction, however, experimental studies have suggested that iron plaques do not impede plant uptake of Mn (Levan & Riha 1986) or Cu (Crowder et al. 1987). Otte et al. (1989), in a laboratory study, reported that whereas moderate amounts of Fe oxyhydroxide deposits on the roots of the emergent plant *A. tripolium* enhanced uptake of Zn by the roots, large amounts of Fe deposited on the root surface acted as a barrier.

In the present field study, *V. americana* rosettes were collected at the maximum of the growing season, in August, when Fe accumulation on the roots is normally the highest (Crowder & Macfie 1986). Virtually the entire



root surface is expected to be covered with iron plaque, with somewhat lesser coverage towards the root tip (St-Cyr et al. 1993); *V. americana* roots have abundant root hairs, but these also tend to be covered with iron plaque. In agreement with this presence of a ubiquitous iron oxyhydroxide coating, more Fe, Mn and Zn were consistently found outside the root than within the root tissue (on a  $\mu\text{g/g}$  rdw basis – see Tables 3–5).

For Fe, Mn and Zn, significant relationships were observed between the metal concentrations in the plaque and those inside the roots of *V. americana* collected in the St. Lawrence River system (Fig. 7). Similar relationships were reported for *P. australis*, for Fe, Mn and Cu (St-Cyr & Crowder 1987). Such relationships are intuitively reasonable, if metal concentrations in the root plaque are considered to be a reflection of the plant's *in situ* "exposure" to metals. For Zn, however, the most significant statistical relationship was not with  $[\text{Zn}]_{\text{plaque}}$ , but rather with the  $[\text{Zn}]/[\text{Fe}]$  ratio in the plaque (Fig. 8). As is shown below, there is some geochemical basis for this improved relation, in that the  $[\text{Zn}]/[\text{Fe}]$  ratio can under certain circumstances be considered as a surrogate measure for the free  $[\text{Zn}^{2+}]$  concentration in the interstitial water surrounding the root (Campbell & Tessier 1991).

For most metals, the key factor influencing metal bioavailability in the interstitial water will be the free aquo-ion concentration,  $[\text{M}^{z+}]$ , a difficult variable to measure. An alternative to the direct measurement of  $[\text{M}^{z+}]$  is the determination of the concentrations of metals in sorptive equilibrium with the sediment constituents  $\equiv\text{S}_n\text{-OM}$ , together with the concentrations of the solid phase(s),  $\equiv\text{S}_n\text{-OH}_x$ , responsible for binding the metal in these forms.



In the particular case of  $\text{Zn}^{2+}$  sorption on iron oxyhydroxides, the following relation can be derived (Tessier et al. 1989; Tessier 1992; see Appendix I):

$$[\text{Zn}^{2+}] = \frac{\{\text{Fe-OZn}\}}{\{\text{Fe-OH}_x\}} \cdot \frac{[\text{H}^+]^x}{N_{\text{Fe-Zn}} * K_a} \quad (2)$$

where:  $\{\text{Fe-OH}_x\}$  = the analytical concentration of iron oxyhydroxide;  $\{\text{Fe-OZn}\}$  = the analytical concentration of zinc bound to iron oxyhydroxide;  $N_{\text{Fe-Zn}}$  = the number of zinc binding sites per mole of the sediment constituent  $\{\text{Fe-OH}_x\}$ ;  $*K_a$  is an apparent overall equilibrium constant;  $x$  = the average number of protons released per zinc ion sorbed.

Examination of Equation (2) reveals that the free  $\text{Zn}^{2+}$  concentration is directly related to the quotient  $\{\text{Fe-OZn}\} \div \{\text{Fe-OH}_x\}$ , i.e. the Zn/Fe ratio in the iron oxyhydroxides, provided that the ambient pH does not vary appreciably (a reasonable assumption in the present case, where we are considering the pH of sediment interstitial water). In other words, the Zn/Fe ratio in the

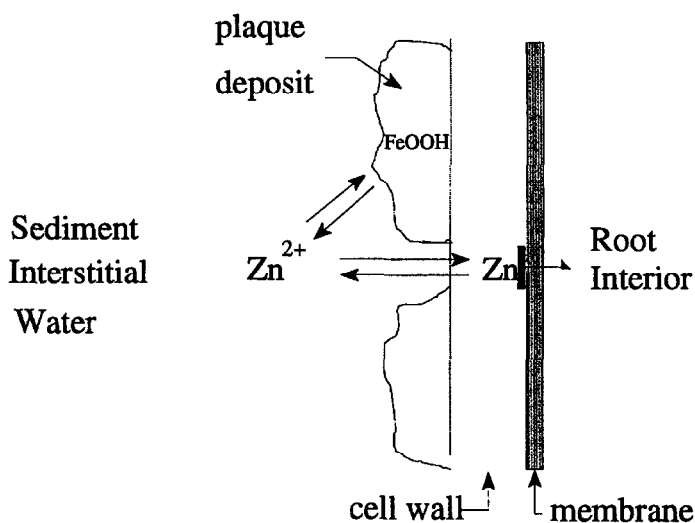


Figure 9. Hypothetical representation of Zn uptake at the sediment-root interface, with control of dissolved  $[Zn^{2+}]$  by iron oxyhydroxide. The hydrated oxyhydroxide plaque is considered to be porous, allowing access by the hydrated  $Zn^{2+}$  ion to the root surface.

root plaque reflects the free  $Zn^{2+}$  concentration adjacent to the root surface, and this in turn affects zinc uptake by the plant root (Fig. 9). The existence of a relationship between Zn concentrations in roots and the Zn/Fe ratio implies that, for a given value of Zn in the sediments or in the plaque, the Zn content of the root will be inversely proportional to the concentration of Fe oxyhydroxides, indicating that Fe plays a protective role in regulating Zn bioavailability (Campbell et al. 1985).

#### *Possible effects of the root plaque on the sediments*

In oxidizing a micro-area around their roots, submerged plant beds can modify the redox profile of the sediment column (Jaynes & Carpenter 1986; Tessenow & Baynes 1975; Wium-Andersen & Andersen 1972). Aquatic sediments typically present a thin oxidized surface layer in which Fe and Mn are oxidized, overlying a deep, reduced horizon (Gambrell & Patrick 1978). At the root-sediment interface, due to the oxygen leaking from the aquatic plants' roots, a micro-environment similar to the one at the water-sediment interface is created (Good & Patrick 1987). The sediment oxidized surface layer can then become exceptionally thick. The oxygenation of lake bottom sediment by roots decreases the concentrations of dissolved Fe(II) and Mn(II) around the roots by the creation of Fe and Mn oxyhydroxides. This results in the formation of steep concentration gradients for Fe(II) and Mn(II) in the interstitial

water, between the reduced lower sediment layers and the oxidized upper layer, causing an upward diffusion from lower sediment layers (Tessenow & Baynes 1975, 1978). The presence of Fe and Mn oxyhydroxides would enhance retention of sediment P as well as several trace metals (Jenne 1977) over the summer growing season (Jaynes & Carpenter 1986). In the Upper Chesapeake Bay, *V. americana* altered the sediment redox potential, giving rise to enhanced retention of P, Fe and Mn (Wigand 1994).

We have estimated the amounts of Fe present as root coatings in *V. americana* beds at two stations (stations #48 and 50 – Lake St. Pierre - 1990). *V. americana* has a fibrous root system extending to a maximum of about 10 cm into the sediment, depending on the substrate type. The number of rosettes per unit surface area was estimated from the biomass of *V. americana*, according to the regression equation relating these two measures for Lake St. Pierre (St-Cyr et al. 1994). From the mean root biomass per rosette (station #48: 0.014 g dry weight; station #50: 0.019 gdw) and the mean of DCB-extracted Fe on roots (station #48: 135  $\mu\text{g}$  of Fe/g root dry weight; station #50: 88  $\mu\text{g}$  of Fe/g rdw), amounts of 0.25 g of Fe per square meter of lake bottom at station 48 and 0.52 g of Fe/m<sup>2</sup> at station 50 were calculated for the root coatings. Chen et al. (1980) tentatively estimated an amount of 500 kg of Fe/ha present as root coatings on rice plants (*Oryza sativa*; dry weight), giving 50 g of Fe/m<sup>2</sup>. This 100-fold difference with respect to our results reflects the fact that emergent plants have a much more extensive root system than do submerged ones. Chen & Barko (1988) reported a much greater oxidation of the sediment by the emergent *Sagittaria latifolia* than by the submerged *Hydrilla verticillata*, which has a relatively minor root system. Emergent plants will thus more plausibly alter sediment nutrient dynamics through rhizospheric oxidation and Fe oxyhydroxide accumulation than will submerged plants. Even within the category of submerged plants, species of *Isoetes*, *Lobelia* and *Littorella* are reported to be stronger oxidizers than other submerged species (Sand-Jensen et al. 1982), with high release of O<sub>2</sub> from their roots.

However, even though the quantities of diagenetic Fe oxyhydroxides added to the sediments by *V. americana* roots appear to be modest, one should recall that (i) Fe and Mn oxyhydroxides, as constituents of normal aquatic systems, are thought to play a dominant role in the cycling of many trace metals in fresh-water sediments despite the fact that they are highly diluted in the sediment matrix (typically 0.1–3.0% dry weight; Belzile et al. 1989) and thus any complementary amount can become important, and (ii) the Lake St. Pierre substrate is mostly sandy (station 48: 73% sand, 13% silt and 14% clay; station 50: 69% sand, 20% silt and 11% clay), and the amounts of diagenetic Fe oxyhydroxides in the superficial sediment layers would be low without the plants' contribution. Microscopic observations of the iron

plaque on *V. americana* roots revealed that the Fe oxyhydroxides formed in the plaque were morphologically very similar to diagenetic Fe oxyhydroxides formed *in situ* in oxic lake sediments and sampled on inert Teflon collectors (Fortin et al. 1993; St-Cyr et al. 1993). In superficial oxic layers of sediments from sites in lakes St. François and St. Louis where important sediment deposition occurs, Huerta-Diaz et al. (1993) have reported that an important proportion of total sediment trace metals (about 5% to 50%, depending of the element) can be associated with diagenetic Fe oxyhydroxides.

It has been suggested (Jaynes & Carpenter 1986) that vegetation shifts in acidifying lakes, from rooted vascular plants to bryophytes, could enhance sediment reduction and mobilization of solutes from particulate to pore-water phases; these solutes would then be readily exchangeable with the overlying water (Fe, Mn and their previously adsorbed elements). One can envisage the same phenomenon occurring at the end of the growing season, when plants undergo decomposition and no longer oxidize their rhizosphere. Wigand (1994) suggested that the elevated redox potential associated with *V. americana* roots in Upper Chesapeake Bay sediments may have caused the adsorption of inorganic phosphate to Fe oxyhydroxides and consequently reduced the level of pore-water phosphate, as measured in the sediments. It seems reasonable to suppose that this adsorbed phosphate would then be released back to the pore water when the plants died. The transient diagenetic Fe and Mn oxyhydroxides formed by submerged plants could in this manner alter sediment nutrient dynamics in shallow lakes.

## Acknowledgements

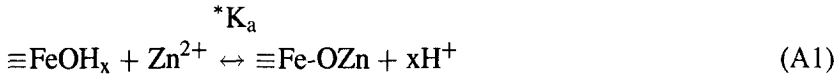
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## Appendix 1

### Derivation of the surface complexation model for zinc sorption on iron oxyhydroxides

In this approach it is assumed: (i) that iron oxyhydroxides play an important role in controlling the concentration of  $\text{Zn}^{2+}$  in the interstitial waters of oxic sediments and in the iron plaque (Tessier et al. 1989); (ii) that the sorption

sites on these oxyhydroxides can be treated as surface ligands; and (iii) that surface complexation concepts that have been developed for simple, well-defined systems in the laboratory can be applied to more complex sediment components. According to surface complexation theory, sorption of  $\text{Zn}^{2+}$  on iron oxyhydroxide can be expressed in a simplified manner as:



$$*K_a = \frac{\{\equiv\text{Fe-OZn}\}[\text{H}^+]^x}{\{\equiv\text{FeOH}_x\}[\text{Zn}^{2+}]} \quad (\text{A2})$$

where charges on the solid species are omitted for simplicity;  $\text{FeOH}_x$  = amorphous iron oxyhydroxides;  $*K_a$  is an apparent overall equilibrium constant; “ $\equiv$ ” refers to sorption sites, either free ( $\equiv\text{Fe-OH}_x$ ) or occupied by Zn; and  $x$  = the average number of protons released per metal ion sorbed.

At low sorption densities, i.e. when the concentration of occupied sorption sites is low relative to the free site concentration, the condition  $\{\equiv\text{Fe-OH}_x\} \approx \{\equiv\text{Fe-O}\}_T$  should apply, where  $\{\equiv\text{Fe-O}\}_T$  is the total concentration of sorption sites. This term can in turn be related to the analytical concentration of the sorbent,

$$\{\equiv\text{Fe-O}\}_T = N_S \cdot \{\text{Fe-ox}\} \quad (\text{A3})$$

where  $\{\text{Fe-ox}\}$  = the analytical concentration of iron oxyhydroxide;  $N_S$  = the number of moles of sorption sites per mole Fe-ox. If it is further assumed that each sorbed metal ion occupies a single site, then

$$\{\equiv\text{Fe-OZn}\} = \{\text{Fe-OZn}\} \quad (\text{A4})$$

where  $\{\text{Fe-OZn}\}$  = the analytical concentration of Zn sorbed to the iron oxyhydroxide. Combining equation (A2), (A3) and (A4) yields

$$[\text{Zn}^{2+}] = \frac{\{\text{Fe-OZn}\}}{\{\text{Fe-ox}\}} \cdot \frac{[\text{H}^+]^x}{N_{\text{Fe}} * K_a} \quad (\text{A5})$$

which shows how the free  $\text{Zn}^{2+}$  concentration at the sediment-water interface could be estimated, *provided that* the values of “ $N_{\text{Fe}} * K_a$ ” and “ $x$ ” are known. The symbols  $\{\}$  and  $[\ ]$  refer to concentrations of solids and dissolved species respectively. Note that if the ambient pH were relatively constant, as would normally be the case for the roots of an aquatic plant, the term  $[\text{H}^+]^x$  could be dropped from equation (A5) and the ratio  $\{\text{Fe-OZn}\}/\{\text{Fe-ox}\}$  used directly to estimate  $[\text{Zn}^{2+}]$ .

To estimate  $N_{\text{Fe}} \cdot K_a$  and  $x$ , equation (A5) can be rearranged as follows

$$K_{\text{Fe-Zn}} = \frac{N_{\text{Fe}} \cdot K_a}{[\text{H}^+]^x} = \frac{\{\text{Fe-OZn}\}}{\{\text{Fe-ox}\}[\text{Zn}^{2+}]} \quad (\text{A6})$$

where  $K_{\text{Fe-Zn}}$  is an apparent equilibrium constant which is a function of pH. Values of  $\{\text{Fe-OZn}\}$  and  $\{\text{Fe-ox}\}$ , needed to estimate  $K_{\text{Fe-Zn}}$ , can be obtained by selective extraction of the surficial sediments; values of  $[\text{Zn}^{2+}]$  can be obtained by *in situ* dialysis (porewater peepers) – see Tessier (1992) and Tessier et al. (1993).

Equation (A6) can then be linearized

$$\log K_{\text{Fe-Zn}} = x \text{ pH} + \log(N_{\text{Fe}} \cdot K_a) \quad (\text{A7})$$

and a plot of  $\log K_{\text{Fe-Zn}}$  vs. pH yields a slope of  $x$  and a Y-intercept of  $\log(N_{\text{Fe}} \cdot K_a)$ . Tessier (1992) reports values of  $x = 1.21$  and  $\log(N_{\text{Fe}} \cdot K_a) = 2.83$  ( $r^2 = 0.89$ ;  $n = 41$ ).

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