GROWTH RESPONSES AND IRON UPTAKE IN *SPHAGNUM* PLANTS AND THEIR RELATION TO ACID MINE DRAINAGE TREATMENT[1]

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Abstract.-- Increasing interest in the use of *Sphagnum* wetlands to treat acid mine drainage (AMD) has prompted study of the tolerance of *Sphagnum* species to the various constituents of AMD waters. In this study, *S. fallax* and *S. henryense* plants were grown in the laboratory for 33 days in synthetic bog water to which $FeCl_2$ was added to achieve Fe^{2+} concentrations ranging from 0 to 10,000 mg/L. Upon exposure to Fe concentrations $\geq$ 100 mg/L, both species exhibited a significant reduction in growth after 33 days relative to controls, but this reduction was proportionately less for *S. fallax* than for *S. henryense*. Final Fe concentration in the plant tissue was negatively correlated with growth and chlorophyll concentration in both species, and greatly exceeded the total cation exchange capacity for *Sphagnum*, suggesting nutrient cation deficiency as a mechanism of Fe toxicity. Based on data from a previous study of net primary productivity of *Sphagnum* species in Big Run Bog, estimates were made for Fe accumulation by the growing plants on an areal basis. Projected areal accumulation of Fe was greatest at high Fe treatment concentrations (1,000-10,000 mg/L) for both species, despite significant decreases in plant growth. These estimates reinforce the conclusion that Fe uptake by growing *Sphagnum* plants can play only a relatively minor role in Fe retention in wetland systems constructed for mine drainage treatment. Results of this study also indicate that the viability of *Sphagnum* wetlands constructed for AMD treatment may be dependent on the species composition.

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INTRODUCTION

As a result of several studies suggesting that chemical and biological processes in freshwater wetlands may remove acidity, sulfate, and heavy metals from acid coal mine drainage (Wieder and Lang 1982, Kleinmann et al. 1983, Burris et al. 1984, Tarleton et al. 1984, Wieder and Lang, 1984, Gerber et al. 1985, Wieder et al. 1984a, 1985, McHerron 1986, Brodie et al. 1987), there has been an increasing interest in using constructed wetland systems as a low-cost, low-maintenance alternative to traditional chemical treat-

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ment of acid mine drainage (AMD). Before the wetland approach to AMD treatment becomes feasible, however, the effects of AMD on wetland vegetation, including Sphagnum, must be evaluated. The vegetation in man-made wetland systems serves not only as a mechanism of metal retention by the process of plant uptake, but also as a mechanism of stabilization of the organic substrate within the wetland, minimizing the potential for erosion.

Under natural conditions, Sphagnum growth and species distributions are affected by environmental factors such as pH, moisture, and nutrient availability (Clymo 1973, Vitt et al. 1975, Andrus et al. 1983, Titus et al. 1983, Wagner and Titus 1984, Andrus 1986). Sphagnum species can accumulate Fe and other metals with no apparent inhibition of growth when subjected to chronic inputs of low concentrations of metals via atmospheric deposition (Pakarinen and Tolonen 1976, Pakarinen 1978, Aulio 1980, Lembrechts and Vanderborght 1985). Accumulation under these conditions is species specific (Aulio 1982) and is not necessarily correlated with plant growth (Pakarinen and Rinne 1979).

In contrast to the considerable amount of information available on Sphagnum growth in relatively undisturbed environments, comparatively little is known about the response of Sphagnum to the low pH and high metal concentrations typical of AMD. One study has demonstrated that the discharge of chemically treated mine drainage (pH values from 6 to 9) into what was once a naturally acidic Sphagnum wetland (surface water pH near 4.0) resulted in both the death of the vegetation and erosion of the organic peat down to the underlying mineral soil (Wieder et al. 1984b).

In light of these findings, the objectives of this study were to assess the growth responses of two commonly occurring Sphagnum species in solutions with Fe concentrations typically found in AMD, and to assess the apparent uptake of Fe from solution by these two Sphagnum species. Also, the potential contribution that uptake of Fe by growing Sphagnum could make to Fe retention within a wetland is compared to the potential contribution that other chemical and biological processes could make to Fe retention.

METHODS

Living plants of two Sphagnum species, Sphagnum fallax and S. henryense, were collected at Big Run Bog, WV, a Sphagnum-dominated wetland not affected by AMD. In the laboratory, individual plants of each species were cut to an initial length of 5 cm and placed in groups of 7 or 8 into 2.5-cm-diameter, 3.5-cm-tall PVC cylinders (Wieder et al. 1984b). Three of these cylinders were placed in an 8-cm-diameter fingerbowl to which a particular treatment solution was added. Treatment solutions contained either 0, 1, 10, 100, 1,000, or 10,000 mg/L Fe, thereby spanning the range of Fe concentrations typical of AMD (Braunstein et al. 1977). Ferrous chloride, rather than $FeSO_4$, was used in preparing the treatment solutions. A previous study of the growth of S. fallax and S. henryense in AMD waters demonstrated that growth was negatively correlated with SO_4^{2-}, but not with Cl^-, concentrations in AMD (Kearney, 1986). The treatment solutions were prepared by adding $FeCl_2$ to synthetic bog water containing, in mg/L: 0.88 Ca^{2+}, 0.19 Mg^{2+}, 0.47 K^+, 0.23 Na^+, 0.16 NH_4^+, 0.07 NO_3^-, 3.44 SO_4^{2-}, and 0.62 Cl^-. The synthetic bog water approximates the ion concentrations found in Big Run Bog surface waters (Wieder 1985). Treatment solutions were added to each bowl so that the water table was at the tops of the PVC cylinders. Every 2-3 days, additional treatment solution was added to maintain the water at the tops of the cylinders, and at weekly intervals the entire volume of water in each bowl was replaced. Plants were grown for 33 days beginning on December 23, 1985, for S. fallax and on January 8, 1986, for S. henryense.

Plant growth was assessed by removing individual plants from their cylinders and measuring their length with a metric ruler at 11-day intervals. Since growth of Sphagnum is indeterminate and occurs along the long axis of the plant, increase in length is an appropriate measure of growth over time (Clymo 1970).

After 33 days, determinations of chlorophyll concentration were made. Using approximately half of the plants from each bowl, the uppermost 1 cm of tissue (capitulum) was excised and weighed. Chlorophyll was extracted from the fresh plant tissue with 80% aqueous acetone (Dolphin 1978). Absorbance of the extract at 649 and 665 nm was measured using a double-beam spectrophotometer, and chlorophyll concentration was calculated using equations given in Dolphin (1978).

The plants remaining in each bowl were weighed and then dried for 24 hours at 55° C for fresh mass/dry mass ratio determinations. The dried plant material was ashed in a muffle furnace at 300° C for 1 hour and at 800° C for 3 hours. The ash was extracted with 6 M HCl and brought to final volume with distilled water (Likens and Bormann 1970). Iron concentrations in the extract solutions were determined by atomic absorption spectrophotometry. For more detailed methodology, see Kearney (1986).

For each species, effects of Fe concentration in the treatment solution on growth (increase in length over the 33-day period) and chlorophyll concentration were assessed using Friedman's tests conducted on the data from day 33. Correlations between growth, final chlorophyll concentration, final tissue Fe concentration, and Fe concentration in the treatment solutions were determined using Spearman's rank correlation tests. Differences between the species with regard to growth and final tissue Fe concentration were evaluated with Mann-Whitney tests. A significance level of p=0.05 was used in all tests.

RESULTS

The two Sphagnum species exhibited both similarities and differences in growth responses to increasing Fe in the treatment solutions. For *S. fallax*, after 33 days growth in the 1 mg/L Fe solution was significantly greater than that in the control solution (0 mg/L), and growth in the 10 mg/L Fe solution did not differ significantly from that in the control solution. However, as solution Fe concentrations increased from 100 to 10,000 mg/L, plant growth progressively decreased (fig. 1). In contrast, for *S. henryense* growth at all Fe concentrations was significantly less than growth in the control solution (fig. 2). For each species, the increase in length achieved over 33 days was significantly negatively correlated with treatment solution Fe concentration (Spearman's rho values of -0.82 and -0.93 for *S. fallax* and *S. henryense*, respectively). Also for both species, at solution Fe concentrations ≥ 100 mg/L not only was growth reduced relative to controls, but also no significant increase in growth was observed after the first 11 days. Regardless of treatment solution Fe concentration, the inhibition of growth relative to control plants was proportionately greater for *S. henryense* than for *S. fallax* (fig. 3).

Final chlorophyll concentration in *S. fallax* generally decreased with increasing treatment solution Fe concentration (fig. 1). For *S. henryense*, final chlorophyll concentration in treatment solution Fe concentrations ≤ 10 mg/L were not significantly different from those plants grown in 0 mg Fe/L, but as Fe concentrations in treatment solutions increased to 100 mg/L and greater, chlorophyll concentration decreased (fig. 2). In each species, chlorophyll concentration in plant tissues after 33 days was positively correlated with increase in length (Spearman's rho values of 0.68 and 0.82 for *S. fallax* and *S. henryense*, respectively). Moreover, at

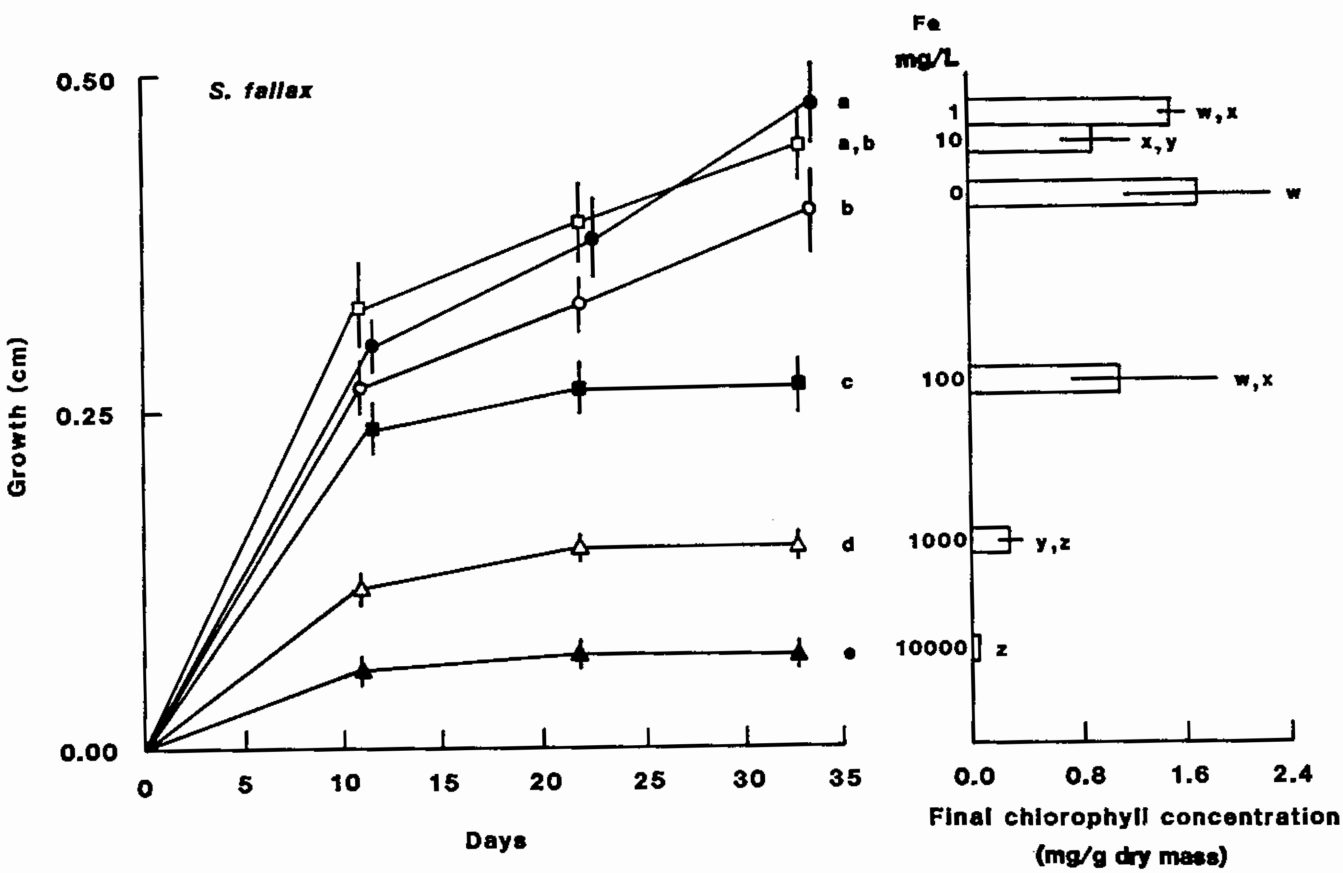

Figure 1.--Growth and final chlorophyll concentration of *Sphagnum fallax* in Fe solutions. (For growth, each symbol represents the arithmetic mean of 72 values ± 1 standard error. Values for mean length after 33 days with the same letter (a-e) do not differ significantly. For chlorophyll concentration, the mean and range of 3 measurements are shown. Values with the same letter (w-z) do not differ significantly. Iron concentrations in the treatment solutions are indicated to the left of the chlorophyll bars).

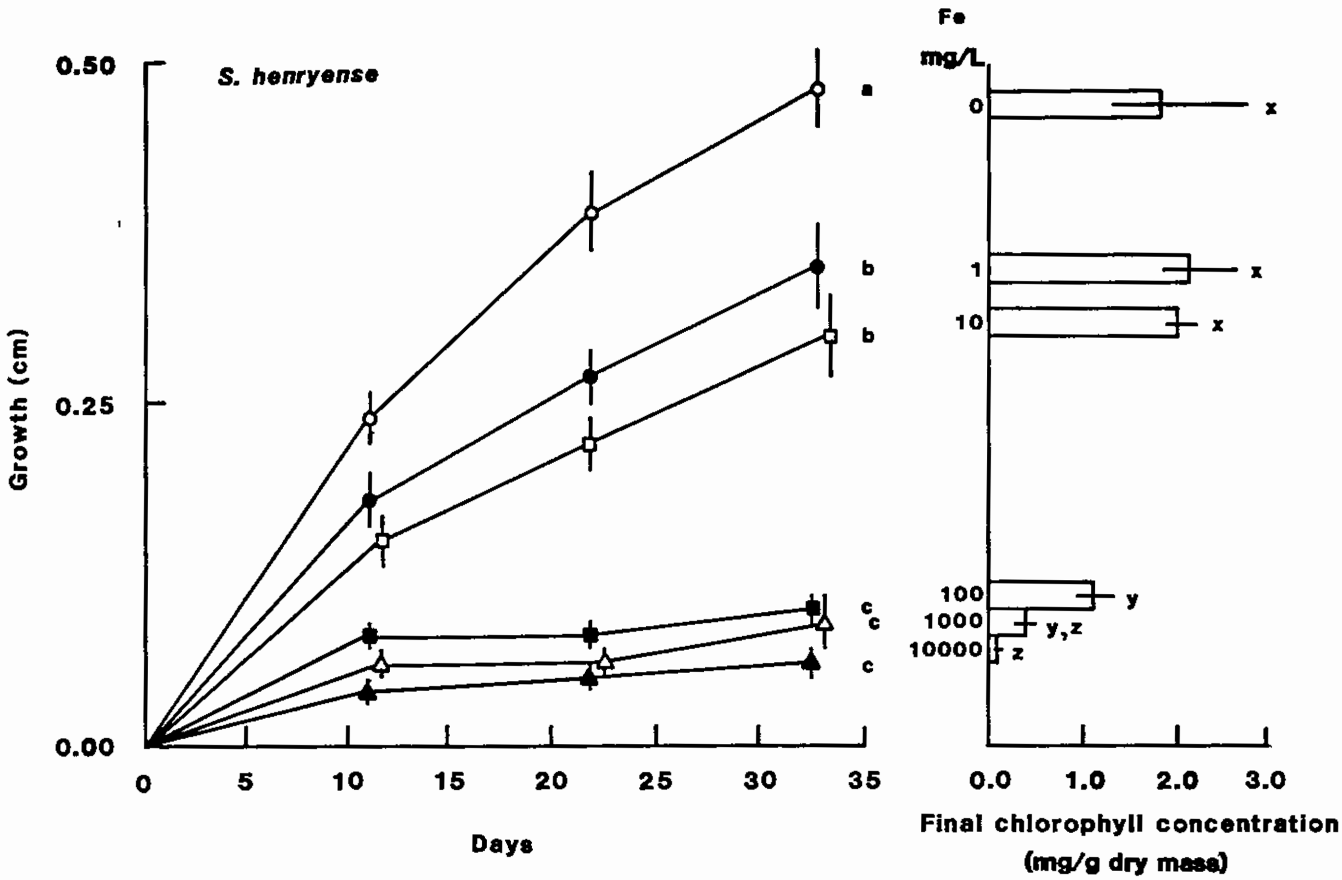

Figure 2.--Growth and final chlorophyll concentration of Sphagnum henryense in Fe solutions. (For growth, each symbol represents the arithmetic mean of 63 values ± 1 standard error. For chlorophyll concentration, the mean and range of 3 measurements are shown. For explanation of letter symbols, see Figure 1 legend. Iron concentrations in the treatment solutions are indicated to the left of the chlorophyll bars.)

Fe concentrations ≥ 100 mg/L, final chlorophyll concentration was less than 1.2 mg/g dry mass and no additional growth was achieved after the first 11 days.

For both species, Fe concentration in the plant tissues after the 33-day period increased with increasing treatment solution Fe concentration (fig. 4), yet there was no significant difference between the two species in final tissue Fe concentration across all treatment solution Fe concentrations ($p=0.34$). For each species, growth achieved after 33 days was negatively correlated with final tissue Fe concentration (Spearman's rho values of -0.83 and -0.86 for S. fallax and S. henryense, respectively).

DISCUSSION

The observed reduction in Sphagnum growth with increasing Fe concentration in the treatment solutions may be related to a decreased ability of the plants to take up nutrient cations. Cation exchange on the cell walls of Sphagnum plants appears to be a major mechanism of cation uptake by the living cells of the plants (Clymo 1967). The maximum reported values for

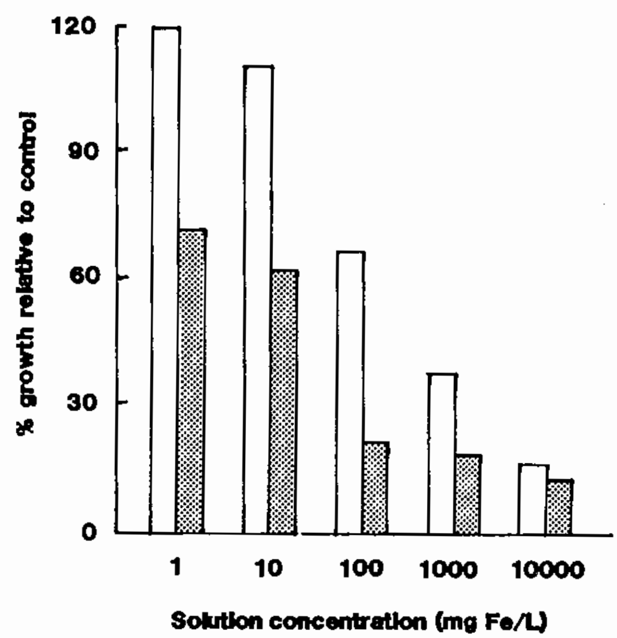

Figure 3.--Mean growth after 33 days of Sphagnum fallax (open bars) and S. henryense (stippled bars) in Fe solutions, expressed as percent relative to control treatments (0 mg Fe/L).

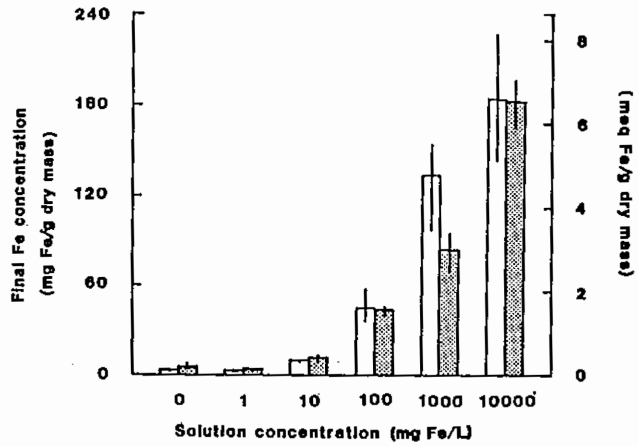

Figure 4.--Final concentrations of Fe in the tissues of Sphagnum fallax (open bars) and S. henryense (stippled bars). The arithmetic mean and range of 3 measurements are shown.

cation exchange capacity in Sphagnum species are close to 1 meq/g dry mass (Clymo 1967, Spearing 1972), and base saturation of Sphagnum (percent of total cation exchange capacity accounted for by the base cations Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}) has been measured as 8.7% (Braekke 1981). Cation exchange is a dynamic process in which equilibria between adsorbed cation and soluble cation concentrations are rapidly established (Clymo 1963). Therefore as Fe^{2+} concentration in solution increases, increasing quantities of base cations and protons will be desorbed from exchange sites because of competition from the Fe^{2+} ions. For both Sphagnum species, when grown in solutions with Fe concentrations $\geq$ 100 mg/L, not only did final Fe concentration in the plant tissues exceed the cation exchange capacity of Sphagnum (1 meq/g dry mass; cf. fig. 4), but also a dramatic reduction in both growth and chlorophyll concentration relative to controls was observed after 11 days (figs. 1,2). Thus, the observed reduction in plant growth may have been a result of a deficiency of nutrient base cations induced by competition from Fe^{2+} for exchange sites.

The concentration of Fe in the tissues of both species exceeded the total cation exchange capacity of the plants by as much as 650% in Fe treatment solution concentrations $\geq$ 100 mg/L (fig. 4). This excess Fe must have been incorporated into the plants as nonexchangeable Fe via specific binding to organic matter or by the formation of Fe oxides or oxyhydroxides (cf. Wieder et al. 1987). No obvious oxide deposits were discernible during examination of the tissues using light or scanning electron microscopy, suggesting that organic binding of Fe by the plants may have been a more important process in Fe accumulation than either cation exchange or oxide formation.

The potential contribution of growing Sphagnum plants to Fe retention in a wetland constructed for AMD treatment can be estimated based on the growth of the plants relative to controls (fig. 3) and measured Fe uptake (fig. 4). To estimate potential Fe uptake on an areal basis, field estimates of primary productivity of Sphagnum must be incorporated into the computation. The average annual net primary productivity of the two dominant Sphagnum species from Big Run Bog (S. fallax and S. magellanicum) is 5.75 g dry mass/dm^2/yr (Wieder and Lang 1983). Multiplying this value by the percent growth relative to controls (from fig. 3) and by final tissue Fe concentration (from fig. 4) for each species at each solution Fe concentration yields estimates of potential Fe accumulation by growing Sphagnum (table 1). The projected areal accumulation of Fe by S. fallax was 30 to 230% greater than that by S. henryense for any particular solution Fe concentration. This difference is accounted for by the difference between the species in growth relative to controls; in all solution Fe concentrations excluding 10,000 mg/L, reduction of growth of S. fallax was significantly less than the reduction of growth of S. henryense (fig. 3). The maximum Fe accumulation projected for the two species (29 g/m^2/yr) was more than 8 times greater than a previous estimate (3.5 g/m^2/yr) based on a lower Fe concentration in Sphagnum tissue (5.8 mg/g; Wieder et al. 1987). However, in achieving an Fe uptake value of 29 g/m^2/yr, the plants would be effectively dead. At Fe treatment solution concentrations at which plants were still growing and green, maximum Fe uptake is only 6.5 g/m^2/yr. Despite these new estimates of the potential maximum for Fe uptake by Sphagnum plants, the contribution that plant uptake would make to total Fe retention in a wetland system is still small in comparison to the contributions made by other chemical and biological processes within the peat, especially the binding of Fe to

Table 1.--Estimation of the potential Fe uptake, on an areal basis, by growing Sphagnum plants subjected to different Fe concentrations in AMD. Methods used to make these estimates are described in the text.

Treatment Solution Fe Concentration (mg/L)	Estimated Fe uptake (g/m^2/yr)	
	S. fallax	S. henryense
1	2.2	1.8
10	6.5	4.1
100	16.8	5.4
1000	29.0	8.8
10000	17.7	13.8

organic matter and the formation of Fe oxides, which together may account for as much as 97% of total Fe retention (Wieder et al. 1987).

These results show that Sphagnum plants can survive and grow in solutions with Fe concentrations up to 10 mg/L, but at 100 mg/L or greater, they turn brown and stop growing. In a wetland designed to treat low flows of AMD containing relatively low Fe concentrations, the plants could remain viable. In addition, although Sphagnum plants do accumulate more Fe when exposed to higher Fe concentrations, the potential contribution of Sphagnum plants to overall Fe retention in comparison to other processes contributing to Fe retention in a wetland constructed for AMD treatment appears to be minor. Nonetheless, the need to maintain a vigorous cover of vegetation in constructed wetland treatment systems is critical in terms of minimizing the potential for erosion of an organic substrate. We have demonstrated clear differences between species in their growth and chlorophyll responses to AMD; S. fallax would exhibit greater increase in length than S. henryense in Fe-rich water. Further study of Sphagnum species responses to other AMD constituents, particularly to metals, is warranted to determine which species of Sphagnum are most likely to survive when exposed to different types of AMD.

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