Environ. Sci. Technol. **2002**, *36,* 3997−4002

Critical Role of Macrophytes in Achieving Low Iron Concentrations in Mine Water Treatment Wetlands

LESLEY C. BATTY* AND
PAUL L. YOUNGER
Department of Civil Engineering, University of Newcastle, Newcastle upon Tyne, NE1 7RU, U.K.

Aerobic wetlands are increasingly being included in mine water treatment systems which need to achieve low residual iron concentrations (<0.5 mg L^{-1}) in final discharges. Traditionally the macrophyte components of such systems have been thought to be insignificant sinks for major contaminants such as iron. However, we report high rates of plant uptake of iron where the latter is present at relatively low concentrations, suggesting that macrophytes may well be critical to achieving low residual iron concentrations in final effluents from such systems. The wetland macrophyte *Phragmites australis* was grown in waters with a range of iron concentrations (0−50 mg L^{-1}). At an Fe supply of 1 mg L^{-1} almost 100% of the Fe was taken up into plant tissues. The majority of iron was stored in and around the roots of the plants, which helps allay fears of possible release of contaminants during seasonal die-back of emergent shoots and leaves. The 1 mg L^{-1} threshold also proved to be important in terms of plant growth, with significant inhibition (evident in root length and in dry weights of shoots and roots) in plants grown in waters with Fe above this concentration. No direct causal relationship between iron content in aerial tissues and growth inhibition was found, which strongly suggests that iron toxicity cannot explain these results. These results have implications for the design of mine water treatment wetlands, particularly with regard to initial establishment of vegetation and achieving sufficient Fe removal in "polishing" applications (i.e. where it is intended to remove the last few mg L^{-1} of Fe).

Introduction

The release of water from abandoned mine sites constitutes a major source of water pollution in many parts of the world (e.g., ref *1*). Iron is the major contaminant at many sites, commonly occurring at concentrations within a range from 3 to 200 mg L^{-1} (*1*) with extreme concentrations reaching tens of thousands of mg L^{-1} (e.g., ref *2*). During the last two decades constructed wetlands have increasingly been developed for the treatment of polluted mine waters (e.g., refs *3*−*7*). Compost wetlands which encourage bacterial sulfate reduction processes are typically recommended for the treatment of acidic mine waters (*3*, *7*). By contrast, so-called "aerobic wetlands" are the most appropriate wetland system for "net-alkaline" mine waters which are still contaminated with Fe (*3*, *7*). (Net-alkaline mine waters are defined (*3*) as those in which the total alkalinity (typically dominated by HCO_3^- content) exceeds the total acidity (which in mine waters is typically dominated by the content of hydroxide-forming metals such as Fe, Al, and Zn, with only minor contributions from $[H^+]$)). Aerobic wetlands are basins of shallow water (typically in the range 15−50 cm) in which the waters are made to flow through dense stands of reeds and rushes, among which Fe removal occurs by means of oxidation, hydrolysis, and sedimentation (*7*). Besides being applied in "stand-alone" mode to provide completely passive treatment of naturally net-alkaline mine waters, aerobic wetlands are increasingly being used to provide final "polishing" of originally acidic mine waters which have already been neutralized using conventional, active alkali-dosing techniques (*6*, *7*). Experiences in the U.S.A. and U.K. have demonstrated that Fe concentrations of < 0.5 mg L^{-1} can be attained in such applications, meeting the regulatory requirements for discharges to cold water fisheries or high quality streams. In typical polishing applications, aerobic wetlands may receive influent waters containing only 1−2 mg L^{-1} Fe^{2+}. Given that abiotic oxidation of Fe^{2+} in circum-neutral waters is first-order (*3*, *7*−*9*) removing such low initial concentrations of Fe down to a residual less than 0.5 mg L^{-1} might seem a daunting challenge. Yet it is increasingly observed that residual concentrations of Fe in the effluents from heavily vegetated wetlands are far lower than would be predicted from first-order Fe^{2+} oxidation kinetics: a recent review (*7*) cites two cases in point: (1) At Woolley Colliery (West Yorkshire, U.K.) the iron removal efficiency of an aerobic wetland receiving about 5 mg L^{-1} Fe^{2+} was on the order of only 70% when the emergent vegetation was first planted, but it increased steadily as the plants grew, reaching and maintaining rates in excess of 95% once the wetland had a mature and dense vegetation cover. (2) A densely vegetated wetland at Edmondsley, County Durham, U.K. has been found to remove Fe^{2+} to residual concentrations as low as 0.1 mg L^{-1} in an area of only 2000 m^2, whereas a model based on the first-order oxidation rate of Fe^{2+} (*9*) predicted that such levels would be reached only after flow through nearly 2 ha (*7*).

The point is demonstrated further by Figure 1, which presents new data from one of many U.K. treatment wetland systems being monitored by the authors. The data displayed were selected without prior inspection (they are simply the most recent data available), to avoid any danger of interpretational bias. The wetland in question is a small (1900 m^2) aerobic treatment wetland at St. Helen Auckland (County Durham, U.K.), which receives net alkaline water (pH 7.15) containing an average of 2.74 mg L^{-1} Fe^{2+}. The wetland is heavily vegetated with three common plant species: *Typha latifolia*, *Phragmites australis*, and *Juncus effusus*. The St. Helen Auckland wetland allows a particularly robust test of the contention (*9*) that the rate of Fe^{2+} oxidation is the treatment-limiting step in such systems. This is because the lack of head difference on this site (essentially the elevation difference between the collar of the overflowing shaft from which the water emanates and the top of the wetland bund) precludes cascade aeration, which would be the normal first step in transforming soluble Fe^{2+} to insoluble Fe^{3+}. Hence the dissolved oxygen content of the water as it enters the wetland is below detection limits, which means that all Fe^{2+} oxidation must occur within the wetland itself. As can be seen in Figure 1, the observed removal of Fe^{2+} in this wetland always exceeds that predicted from the first-order kinetics of Fe^{2+} oxidation, usually by at least a factor of 2 and often by an entire order-of-magnitude.

* Corresponding author phone: 0191 2227942; fax: 0191 2226613; e-mail: L.C.Batty@ncl.ac.uk.

10.1021/es020033+ CCC: $22.00
Published on Web 08/16/2002

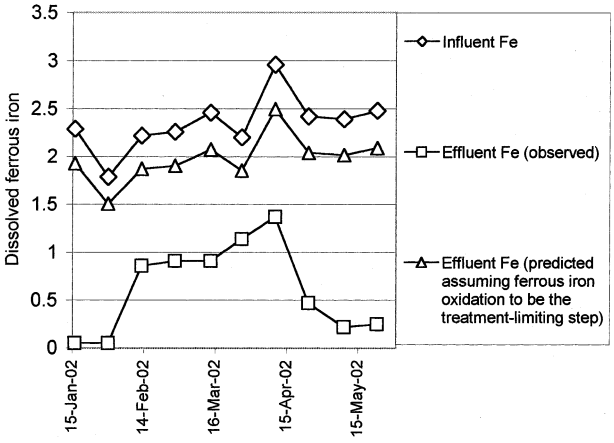

FIGURE 1. Fe removal in the St. Helen Auckland aerobic mine water treatment wetland for the period Jan−May 2002, illustrated by influent and effluent dissolved Fe^{2+} data. "Predicted" values are model results obtained using a solution (*9*) which assumes Fe^{2+} oxidation kinetics assumed to be the treatment-limiting factor.

How can such apparent overperformance be explained? Possible contributory factors include the following: (1) improved hydraulic performance over simple plug-flow hydraulics (which is implicit in the first-order kinetics based model (*9*)) due to baffling of flows by plant stems (e.g., ref *7*); (2) the high surface areas presented by dense stands of shoots, which will favor sorptive reactions, and in turn surface-catalyzed oxidation of Fe^{2+}, which occurs at a rate considerably faster than the open-water rate (*10*); (3) the encouragement of favorable microbial processes by provision of metabolites derived from root exudates and/or the hydrolysis of shoot and leaf litter which accumulates in the wetland (e.g., ref *7*); and (4) direct uptake of Fe by plants, a process which is most likely to occur in the rhizosphere, which is the principal zone in which actively growing plant material can be expected to be in intimate contact with dissolved Fe^{2+} present in pore waters. For this process to quantitatively affect the overall balance of iron entering and leaving a treatment wetland, there must be brisk exchange of water between the shallow pore waters and the overlying water column. This in turn implies that bed geometry and hydraulic conductivity are favorable for such exchange to occur. In the mine water treatment wetlands which we routinely monitor, bed surfaces typically have considerable relief (several centimeters) which favor hyporheic flows, and rhizosphere hydraulic conductivities are sufficiently high ($>1 \times 10^{-4}$ m s^{-1}) that advectional transport of solutes dominates over molecular diffusion (i.e. Peclet numbers typically > 100). Thus rhizosphere pore waters represent a *continuum* with open waters, and removal of Fe^{2+} from pore waters can be expected to contribute substantially to overall removal of Fe^{2+} within the wetland.

This paper concentrates on the last of these postulated processes, which we have experimentally examined in one of the most common plants used in treatment wetlands (*Phragmites australis* (Cav.) Trin. Ex Steudel). We have done this by examining the relationship between plant uptake of Fe as a function of dissolved Fe concentration. By discriminating between destinations of Fe taken-up (i.e. into roots, shoots and leaves) we are also able to judge whether seasonal deposition of litter derived from subaerial portions of wetland plants might contribute to seasonal re-release of metals within treatment wetlands. Finally, since uptake of metals by plants is likely to be most vigorous where plant growth is itself brisk, we have examined the effects of various concentrations of Fe on the growth rate of *P. australis* seedlings (as reflected in metrics such as biomass production rates for roots, shoots and leaves). Our findings help to explain behavior of real treatment wetlands, such as that shown in Figure 1, and thus appear to have direct relevance to the development of design and management guidelines for mine water treatment wetlands.

Materials and Methods

Uptake of Fe and plant growth was investigated using a factorial experiment. Data resulting from the experiment was analyzed using a one-way ANOVA followed by a Tukey-HSD test. The null hypothesis was rejected at 5%. Where data did not fit the assumptions of the statistical test, data transformations were carried out (either $\log_e$ or log).

Seeds of the common reed *Phragmites australis* Trin. Ex Steudel were collected from an uncontaminated field site (Felixstowe, U.K.) in 1997 and stored until required. The seeds were fully saturated with distilled water and chilled at 4 °C in the dark for 2 weeks before being transferred to a controlled environment growth chamber (8 h 14 °C night, 16 h 20 °C day, 250−300 μmol s^{-1} photon flux density). Seedlings were grown supported on polystyrene beads for 17 days in 10% Rorison's solution, which included 0.3 mg·L^{-1} Fe (*11*). Seedlings of a uniform size were selected, and one seedling was transplanted into each of 50 opaque PVC vessels (1 L capacity) containing 10% nutrient solution (pH 6.0). The total of 50 units allowed for five replicates of all treatments, and these were arranged in a randomized block design. The seedlings were grown for a further 27 d, the nutrient solution being changed every 3 d to maintain nutrient supply and compensate for any chemical changes in the solution. At the end of this period iron was added to the containers as $FeSO_4 \cdot 7H_2O$ (+ standard nutrient solution without FeEDTA) in appropriate quantities to give the following treatments: 0, 0.05, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, and 50.0 mg L^{-1} Fe. Metal exposure was continued for a total of 64 d, during which solutions were replaced every 3 d. Visual observations of plant conditions were made at every solution change. Measurements of longest root and shoot extension were also made every 3 d. At the end of the experiment (a total of 108 d) all plants were harvested and rinsed thoroughly in distilled water to remove potential surface contaminants. Each plant was divided into roots, rhizomes (horizontal underground stems), and shoots, each section placed in a labeled envelope and dried at 40 °C for 3 days. The resulting material was placed in a desiccator and cooled to room temperature before weighing. A subsample of plant material of known weight (0.1−0.3 g) was then acid digested in 5 mL of 30% HNO_3 at 90 °C for a minimum of 8 h (adapted from an accepted protocol (*13*)). Extraction efficiency was 98%; standard reference materials and digestion blanks were used throughout to test for contamination. The concentration of Fe in the digests was determined using an ATI Unicam 929 atomic absorption spectrophotometer.

Results

Visual Appearance of Seedlings. During the period prior to metal treatment all seedlings showed similar growth and development of both roots and shoots. Following initiation of Fe treatment a number of visual differences between the treatments became apparent. Seedlings exposed to concentrations of less than 0.5 mg L^{-1} continued to grow as before, with the exception of some yellowing of the leaves. Those seedlings supplied with 0.5 and 1.0 mg L^{-1} did not show any visual evidence of growth problem including leaf yellowing. Those seedlings supplied with 1 mg L^{-1} Fe and above developed an orange color on the roots, due to the formation of iron precipitates on the root surfaces. The depth of color increased with supply of iron. The precipitates were not present at the root tips but were evident along the root length from 1 cm behind the tip.

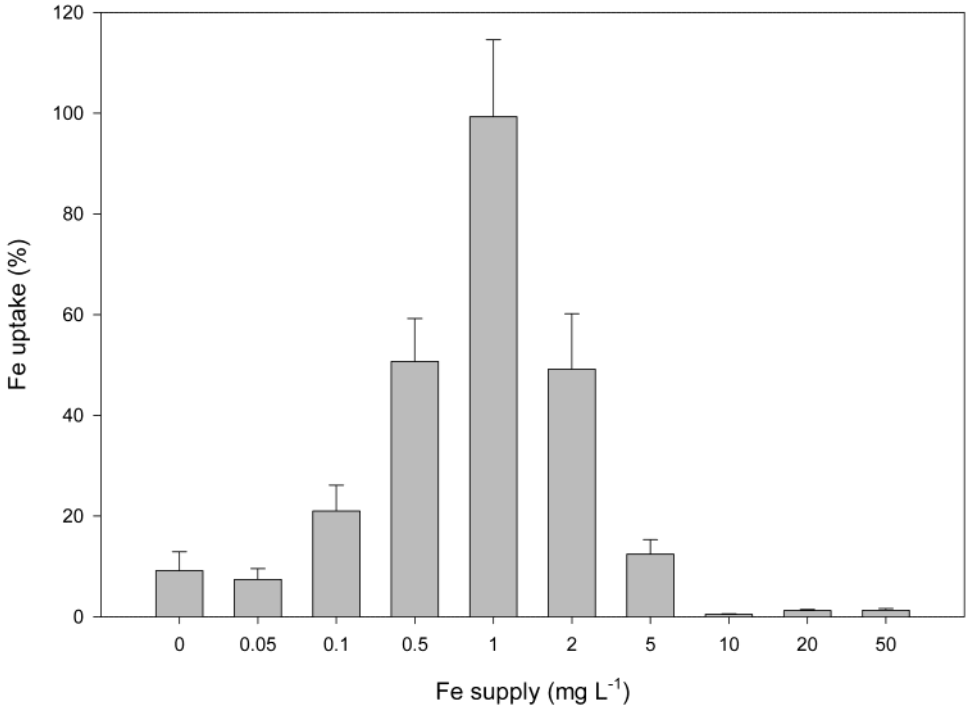

FIGURE 2. Fe uptake by *Phragmites australis* seedlings as a percentage of total iron supplied ($n = 5$).

Seedlings exposed to 2 mg L^{-1} Fe and above showed various changes in morphology including stunted shoot growth, browning, and/or die-back of the leaves, brown patches on the leaves, stunted root growth, lack of branching of roots, and root flaccidity. The severity of the changes were greatest in those seedlings exposed to concentrations of 20 and 50 mg L^{-1} Fe, which displayed complete die-back of shoots in the majority of the replicates.

Effects of Fe Concentrations on Fe Uptake. The uptake of Fe into the whole plant as a percentage of the total Fe supplied in solution is shown in Figure 2 and was calculated according to the following equation

$$((\text{rhizome Fe (g)} + \text{root Fe (g)} + \text{shoot Fe (g)})/\text{total solution Fe (g)}) \times 100$$

The total solution Fe included any Fe provided in the standard nutrient solution prior to the main metal dosing.

Absolute concentrations of Fe in roots, shoots, and leaves as functions of solution Fe concentration are shown in Figure 3. While it is clear that the absolute Fe content in plant tissues increases monotonically with solution Fe concentration, the proportion of the total supplied Fe which the rates of uptake represent peak at only 1 mg L^{-1} Fe, at which concentration almost 100% of the total iron supplied in solution is taken up by the plants.

Shoot Fe content was greatest in seedlings exposed to 50 mg L^{-1} Fe. The maximum average shoot content ($n = 5$) was 729 mg kg^{-1} dry wt (Figure 3.1). Seedlings exposed to 2 mg L^{-1} Fe or more had significantly greater root Fe content than those exposed to 0.5 mg L^{-1} or less (Figure 3.2). The maximum average ($n = 5$) root Fe content (76913 mg kg^{-1} dry wt) was found in those seedlings grown in 50 mg L^{-1} Fe and the minimum (568 mg kg^{-1} dry wt) in those grown in 0.05 mg L^{-1} Fe. There were few significant differences in Fe contents of rhizomal material between seedlings grown in solutions with different total Fe concentrations (Figure 3.3), although those seedlings grown in 0.05 mg L^{-1} Fe clearly have lower rhizomal Fe contents than those grown in 50 mg L^{-1} Fe.

Effect of Fe Supply on Seedling Growth. In seedlings exposed to 0.5 mg L^{-1} and below, the rate of root growth (expressed in units of mm d^{-1}) was notably increased after Fe treatment was initiated (Figure 4). Those seedlings exposed to 1 mg L^{-1} Fe showed a gradual increase in root growth after initiation of Fe treatment, whereas those subjected to more than 2 mg L^{-1} Fe showed an immediate decrease in rate of root growth. All seedlings showed a decline in the rate of growth after the 10th measurement.

Root lengths were significantly longer in seedlings exposed to less than 2 mg·L^{-1} Fe than in those exposed to 2 mg L^{-1} and above (Figure 5.1). This pattern was also reflected in shoot:root length ratios (Figure 5.2), with seedlings exposed to more than 2 mg L^{-1} Fe displaying lower ratios than those exposed to less. In addition seedlings grown in 0 mg L^{-1} Fe showed a lower shoot:root ratio than those in 0.5 mg L^{-1} Fe.

Dry weights of seedlings were also significantly affected by Fe solution concentration. Seedlings grown in concentrations of 5 mg L^{-1} Fe and above had lower shoot dry weights than those grown in 1 mg L^{-1} Fe and below (Figure 6.1). This was also true for root dry weights except that seedlings grown in 2 mg L^{-1} Fe also yielded weights significantly lower than those for seedlings grown in 1 mg L^{-1} Fe and below (Figure 6.2). The effect of Fe concentration on rhizome dry weight was less clear; however, plants grown in 20 and 50 mg L^{-1} Fe had significantly lower dry weights than those grown in less than 2 mg L^{-1}.

Discussion

The current orthodoxy in treatment wetland research holds that plant uptake of Fe makes an insignificant contribution to overall removal of Fe from mine waters passing through aerobic treatment wetlands (e.g. ref *3* and *9*). This position seems logical, since the abiotic oxidation of Fe^{2+} at circumneutral pH is known to be more rapid than the "biotic" rate (*8*). Although the term biotic in the context of Fe^{2+} oxidation refers to microbial rather than macrophytic processes (*8*), the true role of macrophytes in encouraging immobilization of Fe in the ferric form has been little studied (*3*).

While indirect macrophyte influences (such as baffling of flows and providing a large surface area for sorptive reactions) will undoubtedly contribute, the data presented here from laboratory microcosms (in which these indirect influences

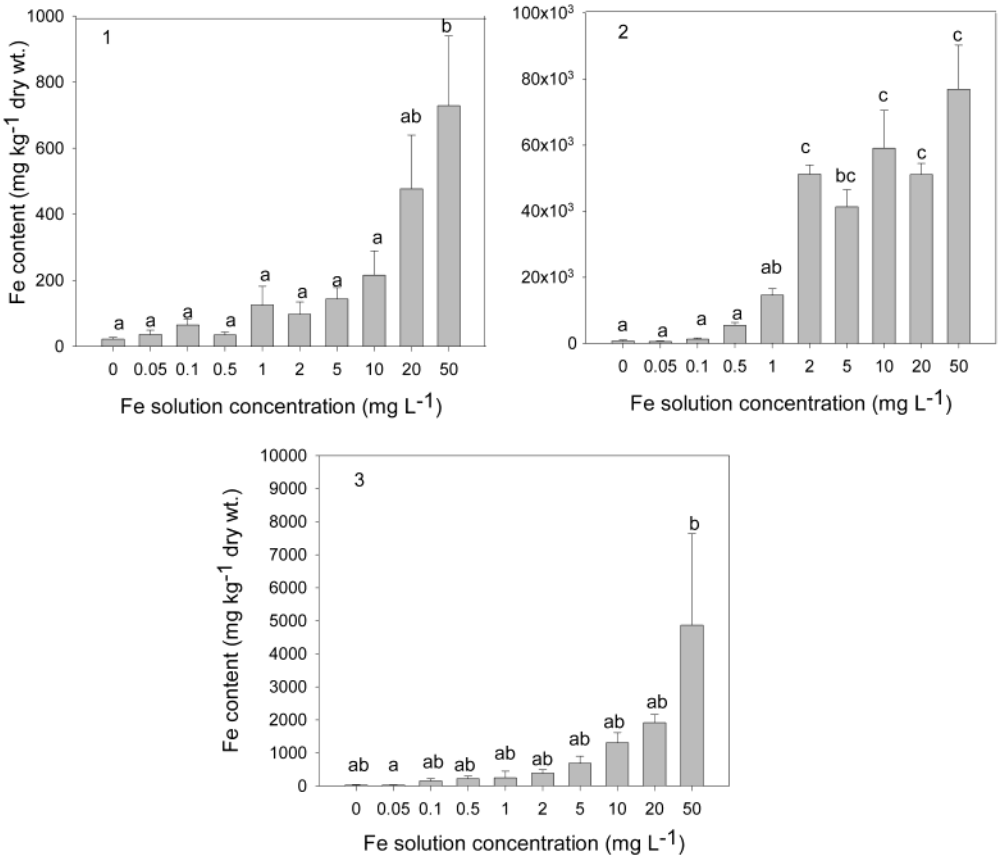

FIGURE 3. Concentrations of Fe in (1) shoots, (2) roots, and (3) rhizomes of *Phragmites australis* seedlings exposed to different concentrations of Fe. 1-way ANOVA, different letters on each graph indicate a significant difference at $p = 0.05$ ($n = 5$).

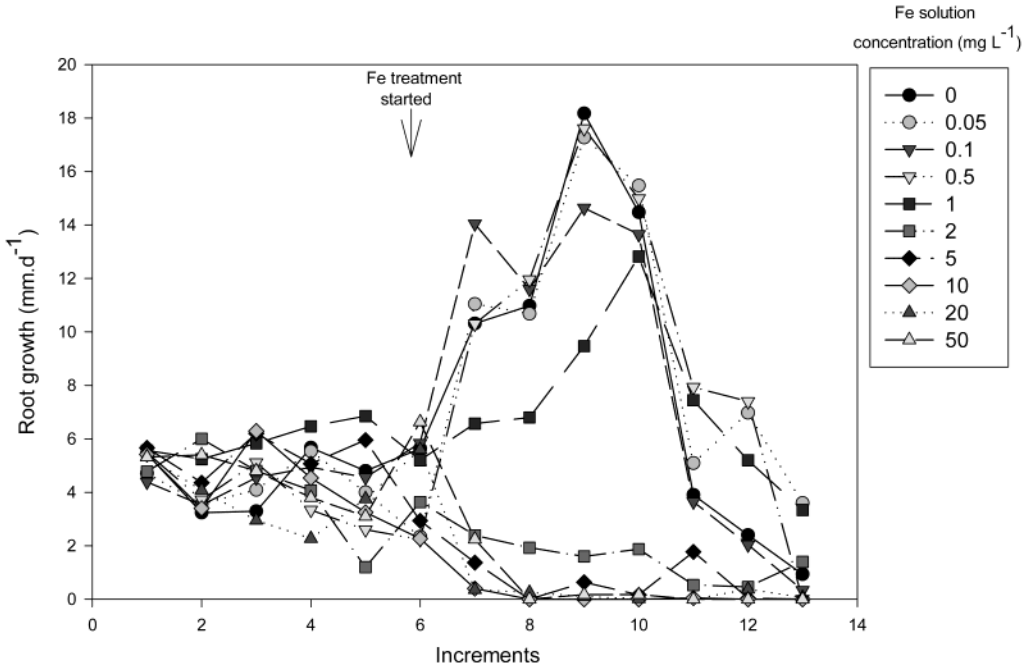

FIGURE 4. Root growth of *Phragmites australis* seedlings with age, exposed to different concentrations of Fe.

are inherently eliminated) suggest that direct Fe uptake by wetland plants can account for much of the Fe removal in parts of the wetlands in which dissolved Fe concentrations are relatively low. Figure 2 clearly illustrates that *P. australis* exposed to waters containing 1 mg L^{-1} Fe is capable of removing almost 100% of the supplied iron by means of uptake. In "polishing" treatment applications, therefore, where the last few mg L^{-1} of Fe are to be removed from a water, plant uptake may make a much more significant contribution to the overall rate of Fe removal within the wetland than has hitherto been appreciated.

If plant uptake of Fe is indeed a more significant process than is generally realized, it follows that seasonal die-back of vegetation might also release significantly greater quantities of Fe back into the dissolved phase than has previously been imagined. Concern over this phenomenon would be greatest

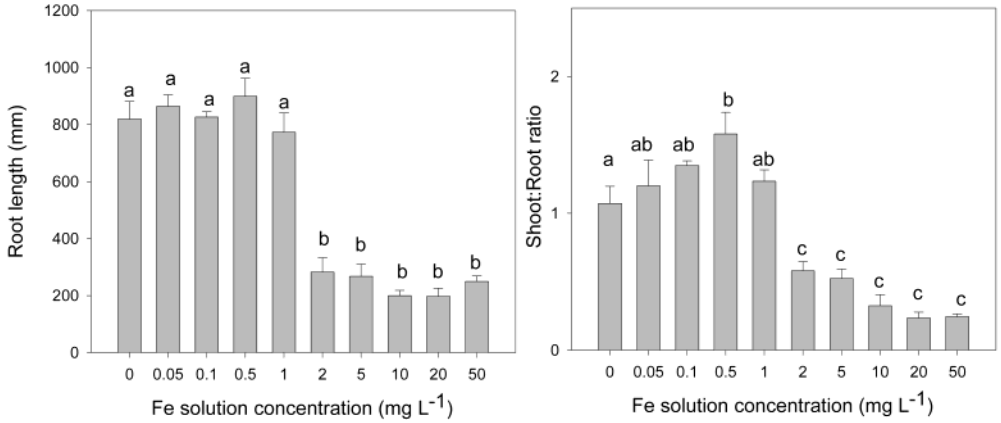

FIGURE 5. Longest root elongation and shoot:root ratio of *Phragmites australis* seedlings exposed to different concentrations of Fe. 1-way ANOVA, different letters indicate a significant difference at $p = 0.05$ ($n = 5$).

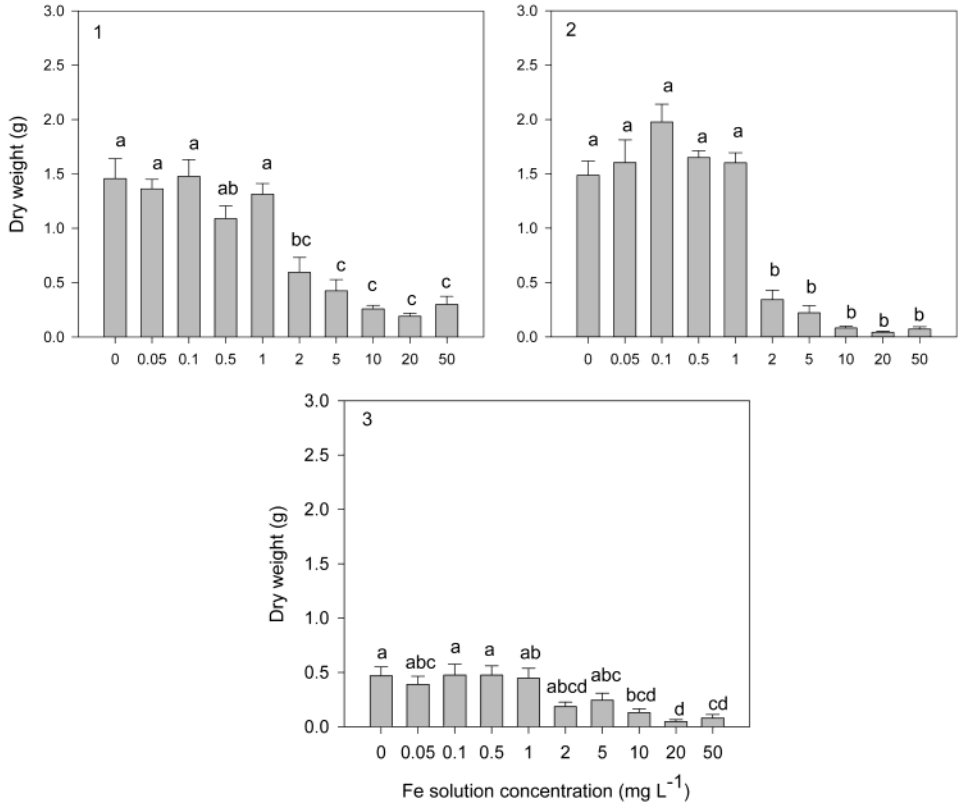

FIGURE 6. Dry weights of (1) shoots, (2) roots, and (3) rhizomes of *Phragmites australis* seedlings exposed to different concentrations of Fe. 1-way ANOVA, different letters indicate a significant difference at $p = 0.05$ ($n = 5$).

if much Fe were stored in the aerial portions of the plants, since die-back of roots alone is likely to leave the Fe in a relatively reducing environment, where it is readily immobilized as a sulfide (e.g., ref *3*). The data presented here make it clear that the majority of the Fe is actually contained within and around the plant roots, mainly as iron oxide plaque deposits. The turnover rate of roots varies greatly with species but is approximately every 2 years rather than annual. Hence any Fe remobilization in the rhizosphere is unlikely to occur simultaneously from all plants in a given wetland each winter. This is encouraging from an engineering perspective, albeit further investigations are still warranted.

It is evident from our data (Figures 4−6) that growth of *P. australis* seedlings (in terms of root length, dry weight of roots, and, to a lesser extent, dry weight of shoots) was significantly inhibited where Fe was supplied at a concentration greater than 1 mg L^{-1}. In addition plants exposed to elevated Fe concentrations (>10 mg L^{-1}) exhibited symptoms which are possibly attributable to iron toxicity (i.e. root flaccidity, reduced root branching, increased shoot die-back, and mottling of leaves). These findings are consistent with those of earlier studies (e.g., refs *11* and *12*).

Root growth rate measurements confirmed an inhibitory effect of elevated solution Fe concentrations. Upon initiation of Fe treatments on plants previously grown in nutrient media containing 0.3 mg·L^{-1} Fe, enhanced growth was encountered at concentrations up to 1 mg L^{-1} (presumably due to elimination of any growth problems attributable to Fe deficiency) but was noticeably reduced in seedlings grown in > 2 mg L^{-1} Fe, falling to 0 mm d^{-1} for those grown in 10 mg L^{-1} Fe and above. One practical implication of these results is that, where a constructed wetland must perform well from the first day of formal use, it may be worthwhile nurturing the initial growth of the wetland plants using an alternative water source with relatively low Fe, before introducing water with higher Fe concentrations once the

plants are mature. (A full-scale field trial of this concept is currently underway at the Whittle Colliery site in Northumberland, U.K. (*6*)). From a scientific standpoint, the possible causes of inhibition in plant growth with increasing Fe supply might include one or both of the following: (i) elevated concentrations of Fe result in iron toxicity within the plants and (i) high Fe concentrations impede the uptake of other nutrients by the plants, thus resulting in nutrient deficiency.

Visible signs of the presence of iron oxyhydroxide plaque deposits on the roots of the seedlings were found when Fe was supplied at concentrations of 1 mg L^{-1} and above. The depth of orange color on the roots (based on visual judgment) increased with increasing Fe supply. It has previously been suggested that these deposits could adsorb essential elements including phosphate and thus immobilize them, preventing their uptake by the plant (*15*). This could then result in deficiencies of nutrients and corresponding reductions in seedling growth.

Both Fe toxicity (for monocotyledons) and nutrient deficiency have been invoked previously to explain decreases in the shoot:root ratio (*14*, *16*), and similar decreases were evident in the seedlings grown in higher Fe concentrations in the present study. In the data presented here, Fe uptake by plants generally did increase with increasing Fe supply, albeit this was not a clear relationship in all cases. In shoot tissues iron content did not significantly increase until Fe was supplied at a concentration greater than 10 mg L^{-1} (although there was some statistically nonsignificant increase above 5 mg L^{-1}). This evidently does not directly correlate with the inhibition of growth which occurred when Fe was supplied at a concentration greater than 1 mg L^{-1}. The Fe content in shoot tissues at which toxicity is reached varies with each species but is approximately 1100−1600 mg kg^{-1} dry weight of leaf tissues for most wetland plants (*16*). In none of the seedlings in the present study was this threshold reached. It is possible that the threshold may be lowest in young plants, as it has previously been demonstrated that less Fe is required to injure seedlings (5 days old) than mature plants (95 days old) (*17*). The seedlings in the present study were 42 days old when Fe treatment was started, and therefore it is conceivable that the toxicity threshold could have been lower. However Fe content of shoots was similar between seedlings grown in 1 mg L^{-1} (which showed no inhibition of growth) and those grown in 2 and 5 mg L^{-1} Fe (which showed definite inhibition). Hence it may reasonably be inferred that Fe toxicity is an unlikely cause of growth inhibition in this case.

In summary, the growth rate of *P. australis* in Fe-rich, net-alkaline mine waters peaks at solution Fe concentrations of around 1 mg L^{-1}, at which concentration uptake of Fe by this plant also peaks, at almost 100% of the Fe supplied in solution. These results are consistent with the interpretation that, contrary to prevailing assumptions, macrophytes can be expected to play a direct and critical role in the attainment of low residual Fe concentrations in mine waters treated using aerobic wetland systems.

Acknowledgments

This study was completed as part of PIRAMID (Passive In-situ Remediation of Acidic Mine/Industrial Drainage) (www.piramid.org) a research project of the European Commission Fifth Framework Program (Key Action 1: Sustainable Management and Quality of Water, Contract No. EVK1-CT-1999-000021). The authors would also like to thank Dr. A. Borland, Dept. of Agriculture and Biological Sciences, University of Newcastle for the use of controlled growth facilities.

Literature Cited

(1) Banks, D.; Younger, P. L.; Arnesen, R. T.; Iversen, E. R.; Banks, S. D. *Environ. Geol.* **1997**, *32*, 157−174.
(2) Nordstrom D. K.; Alpers C. N.; Ptacek C. J.; Blowes D. W. *Environ. Sci. Technol.* **2000**, *34*, 254−258.
(3) Hedin, R. S.; Nairn, R. W.; Kleinmann, R. L. P. *U.S. Department of the Interior, Bureau of Mines Information Circular 9389*; 1994.
(4) *Mine water Treatment Using Wetlands*; Younger, P. L., Ed.; Chartered Institution of Water and Environmental Management: London. 1997.
(5) Scholes, L.; Shutes, R. B. E.; Revitt, D. M.; Forshaw, M.; Purchase, D. *Sci. Total Environ.* **1998,** *214*, 211−219.
(6) Parker, K. *Trans. 1nst. Min. Met. (Sect. A: Mining Technol.)* **2000**, *109*, A219 − A223.
(7) Younger, P. L.; Banwart, S. A.; Hedin, R. S. *Mine Water: Hydrology, Pollution, Remediation;* Kluwer: Dordrecht, 2002.
(8) Singer P. C.; Stumm, W. *Science* **1970**, *167*, 1121−1123.
(9) Tarutis W. J.; Stark L. R.; Williams F. M. *Ecological Eng.* **1999**, *12*, 353−372.
(10) Burke, S.; Banwart, S. A. *Appl. Geochem.* **2002**, *17*, 431−443.
(11) Hewitt, E. J. *Technical Communication No. 22, Commonwealth Agricultural Bureaux*; Farnham Royal: Bucks, 1966.
(12) Allen, S. E.; Grimshaw, H. M.; Parkinson, J. A.; Quarmby, C. *Chemical Analysis of Ecological Materials;* Blackwell Scientific Publications: Oxford, 1989.
(13) Snowden, R. E. D.; Wheeler, B. D. *J. Ecol.* **1993,** *81*, 35−46.
(14) Snowden, R. E. D.; Wheeler, B. D. *New Phytol.* **1995,** *131*, 503−520.
(15) Christensen, K. K.; Sand-Jensen, K. *Can. J. Bot.* **1998**, *76*, 2158−2163.
(16) Marschner, H. *Mineral Nutrition of Higher Plants*, 2nd ed.; Academic Press Ltd: London, 1995; 889 pp.
(17) Foy, C. D.; Chaney, R. L.; White, M. C. *Annu. Rev. Plant Phys.* **1978**, *29*, 511−566.

Received for review February 18, 2002. Revised manuscript received July 2, 2002. Accepted July 8, 2002.

ES020033+