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Plant-derived phenolic compounds impair the remediation of acid mine drainage using treatment wetlands

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ABSTRACT

The use of wetlands to remediate acid mine drainage has expanded rapidly since the realisation that acid coal mine drainage running into natural sphagnum wetlands undergoes an increase in pH and a precipitation of metals. However, our study suggests that the inclusion of plants in the acid mine drainage treatment system may be questionable, due to inefficiencies caused by exudation of dissolved organic carbon (DOC), and in particular its phenolic constituents. They complex with iron, causing increased solubility, the exact opposite of what is required to facilitate amelioration. The addition of minewater to planted wetland mesocosms initially caused a decline in Fe concentrations, typically from over 1100 to a low of 75 mg L^{-1}. However, it increased higher than 300 mg L^{-1} after 15 days. The rise in iron occurred concurrently with DOC and phenolic increases; 15–69 and 5–15 mg L^{-1}, respectively, for *Eriophorum angustifolium*. Removal of DOC by precipitation with calcium lowered the DOC abundance, but without a simultaneous decrease in iron concentration. The concentration of one fraction of the DOC, phenolic compounds, did not decline, and we propose that the Fe was complexed with that phenolic DOC pool. The proposal was confirmed by enzymic depletion of the phenolic compounds using phenol oxidase. Our findings suggest that phenolic complexation represents a potent constraint on wetland-based bioremediation of iron in acid mine drainage.

1. Introduction

The UK, like many countries, experiences substantial environmental damage from abandoned mines, tailings and mine spoil. These affect 85 km of rivers due to 150 discharges at 135 locations (Ranson et al., 1998). Before mine abandonment, water table levels are closely monitored and pumping prevents acid mine drainage (AMD) release. After closure, however, the pumps are deactivated and the water table is allowed to rise, culminating in pyrite mineral dissolution. Pyrite oxidation and the hydrolysis of metals within minerals produce waters characterised by high concentrations of ferrous iron, sulfate and other heavy metals, often of low pH, due to the proton production involved with the oxidation of pyrite (Banks et al., 1997). This process is mediated by the presence of chemolithotrophic bacteria, which accelerate the oxidative process (Johnson et al., 2002). The release of these waters into streams has devastating effects on stream organisms by covering the stream bed with an iron hydroxide layer.

Various approaches to eliminate the problem have been attempted. The potential value of wetlands for remediation of acid mine drainage (AMD) was first recognised where acid coal mine drainage ran into natural *Sphagnum* wetland systems. This led to a decrease in harmful properties; an increase in pH and the precipitation of metals (Hancock, 1973; Hedin et al., 1994). The remedial process is microbiologically mediated; the capacity of indigenous microorganisms to catalyse the oxidation and reduction of iron and sulfur has been well established (Hallberg and Johnson, 2001). Wetland soils provide very diverse habitats for microorganism due to its unique characteristics (Faulkner and Richardson, 1989) for which various biogeochemical processes can occur.

Various plant species have been utilised in treatment of AMD, including *Typha latifolia* (Cooper et al., 1996), *Phragmites australis* and *Eriophorum angustifolium* (Stoltz and Greger, 2002a). Wetland planting was thought to aid the precipitation of metals via oxidation of the rhizosphere foremost, but also via uptake (Stoltz and Greger, 2002b). It was reported that Typha wetlands filtering coal mine drainage were able to remove 50% of the incoming iron (Kolbash and Romanoski, 1989). In addition, other valuable functions of wetland vegetation such as activation of denitrification process have widely been reported (Hernandez and Mitsch, 2007).

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However, the importance of planting in this process has recently been questioned; Iron accumulation by *T. latifolia* actually only accounted for 0.07% of the total iron in inflowing acid drainage in a case study (Mitsch and Wise, 1998). Furthermore, the planted aerobic cells of the Wheal Jane Wetland Project in Cornwall, UK actually underwent an increase of iron in solution, opposing remediation objectives (Johnson and Hallberg, 2002). The increase in iron in the Wheal Jane Wetland Project was partly attributed to the insufficient functioning of sulfate reduction bacteria (Whitehead et al., 2005). In addition, Tarutis and Unz (1995) have stated that while the abiotic reduction by organic acids produced by plants and microorganisms may contribute to metal solubility, the biotic reduction is much more important.

In addition to this possibility, we propose that this phenomenon can be attributed to the presence of phenolic compounds, a component of dissolved organic carbon (DOC). Significantly greater concentrations of DOC have been associated with planted wetlands (Goulet and Pick, 2001) and high DOC concentrations and metal availability have been correlated by various authors (Beining and Otte, 1996; Peiffer et al., 1999). A recent study has shown clear mechanism in a way that organic matter, humic material in particular, can increases solubility of iron oxides (Weber et al., 2006). The objective of this study was to reveal the role of DOC and phenolics in Fe removal efficiency of wetlands with emergent vegetation.

2. Materials and methods

2.1. Minewater and plant sampling

Minewater was collected from Parys Mountain, Anglesey, north Wales. Collected water was transferred to the lab, filtered with a Whatmann ashless filter, and then was maintained at 4 °C until a manipulation experiment was conducted. The water contains high concentrations of iron ($1100\,mg\,L^{-1}$).

Intact cores of *Juncus inflexus*, *E. angustifolium* or *T. latifolia* with a peat substrate were collected from wetlands below Parys Mountain by using PVC pipes (diameter 20 cm × height 20 cm) and a knife. Peat-only cores were also collected as a control. Total 6 cores for each species or a control were collected and maintained at 15 °C in the light. Theses cores all had prior exposure to AMD and were thus believed to be tolerant to minewater additions in the laboratory. To stabilise the samples from a transplant shock, they were maintained for 2 weeks before minewater was added. At the initiation of a manipulation experiment, minewater was added to the surface of each core.

2.2. Iron, DOC and phenolic concentrations

Iron concentrations were tracked throughout a 15-day period along with DOC and phenolic concentrations components. Samples for iron, DOC and phenolic compound analyses were collected every 24 h for the first eight days and then less frequently thereafter from 5 cm depth of the mesocosms. Samples were filtered through glass fibre and 0.2 μm filters and analysed by AAS (nitrous oxide/acetylene flame), Shimadzu TOC Analyser (TOC-500) and spectrophotometric measurements (Box, 1983) for iron, DOC and phenolics, respectively.

2.3. Manipulation experiments

Once the incubation experiment was completed, we conducted two sets of short-term manipulation experiments. The first one was 'DOC precipitation' experiment, where calcium ($Ca(NO_3)_2{\cdot}4H_2O$) was added as an agent to precipitate DOC. Calcium is known to be capable of precipitating DOC, and this experiment was conducted to determine whether depleting the DOC would allow iron to return to lower levels. Six replicate solutions from the mesocosms were prepared for calcium added samples and control samples (e.g., water addition only).

The second manipulation was about enzymic removal of phenolic compounds. Phenol oxidase was added to solutions from the mesocosms to oxidize and remove phenolic compounds to determine whether they were important in binding with iron and increasing its mobility. Phenol oxidase (EC 1.14.18.1) was added to filtered pH-adjusted (0.001 ml of $200\,g\,L^{-1}$ $CaCO_3$ added) soil solutions from the established mesocosm at a concentration of $100\,mg\,L^{-1}$. After 30 h, the samples were centrifuged and the iron and phenolic content analysed. The pH was raised to determine the iron concentration liable to remain in solution following export to more favourable conditions.

2.4. Statistical analysis

Differences in iron, DOC and phenolics among treatments were sought by a one-way ANOVA followed by Dunn's multiple comparison test. Differences between Ca^{2+} or phenol oxidase additions and controls were tested by Kruskal–Wallis test because the data were not normally distributed. Significance was reported at $P < 0.05$ and labeled with different letters.

3. Results and discussion

3.1. Mecocosm experiment

During the first 15 days of minewater addition to the planted mesocosms, trends in iron followed those of earlier studies (Dennison, 2002) with iron showing an initial rapid decline followed by a steady increase in concentration (Fig. 1-A). While *J. inflexus* and *E. angustifolium* were active over the experimental period, *T. latifolia* failed to survive once minewater was introduced.

Dunn's multiple comparisons test revealed no significant difference between the effect of *E. angustifolium*, *J. inflexus*, *T. latifolia* and soil only control mesocosms in terms of iron removed ($P > 0.05$). There was a significant difference in DOC between vegetation mesocosms and soil only mescosms (Dunn's multiple comparison test, $P < 0.001$). The chemical properties of minewater-only control did not change substantially over the experimental period (data not shown). A significant difference in DOC levels at the start of the experiment compared to the end was found in all the mesocosms using one-way ANOVAs; *J. inflexus* ($P < 0.001$), *E. angustifolium* ($P < 0.001$), control ($P < 0.001$). DOC changed throughout the experiments in the planted mesocosms, as would be anticipated from plant root secretions and biomass decomposition. These results suggest that DOC is of plant origin and is secreted through normal metabolic activities. This is further supported by the fact that DOC, iron and phenolic concentrations in *T. latifolia* that was unhealthy, exhibited similar trends with control. Likewise, phenolics exhibited similar trends as DOC in a way that the concentrations were higher in *J. inflexus* and *E. gnaustifolium* than those in control or *T. latifolia*. This again suggests that phenolic materials were originated from vegetation.

Iron concentration initially decreased by 73–92% in the mesocosms within 48 h from around $1100\,mg\,L^{-1}$; atmospheric oxygen diffusion and aerobic substrate oxidizing the soluble iron probably caused this precipitation event. Subsequently, each of the mesocosms underwent a resolubilisation of iron of between 25% and 70%. The trend for resolubilisation was established at the time that DOC began to accumulate in the soil water. This observation is consistent with the hypothesis that DOC complexes iron causing increased