APPLIED ISSUES

Prevention of sulphide accumulation and phosphate mobilization by the addition of iron(II) chloride to a reduced sediment: an enclosure experiment

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SUMMARY

1. In an enclosure experiment carried out in a ditch receiving sulphate-enriched seepage water, iron(II) chloride was added to the sediment. In the sediment pore water of the iron-treated enclosures sulphide levels decreased to very low values (<1 μmol L\(^{-1}\)) immediately after the iron addition while in the control enclosures sulphide reached values up to 500 μmol L\(^{-1}\).

2. The sulphide levels in the sediment pore water were also strongly correlated with temperature. In summer, phosphate mobilization was observed in the non-treated enclosures while in the iron-treated enclosures phosphate levels remained low.

3. Total phosphate levels increased greatly in the water layer of the non-treated enclosures, coincident with an algal bloom and increased turbidity. It is suggested that phosphate mobilization in summer is caused by the reduction of iron(III) phosphate complexes and in this high sulphate water body probably also by the reduction of iron(III) by sulphide and the consequential precipitation of iron(II) sulphide.

4. Iron addition appeared to prevent sulphide toxicity in Potamogeton acutifolius Link which was planted in the enclosures immediately after iron(II) addition. In the non-iron-treated enclosures P. acutifolius plants decayed within a few weeks probably as a result of sulphide toxicity.

Introduction

Smolders & Roelofs (1993) hypothesized that greatly decreased iron levels in sediment pore water are at least partly responsible for the dramatic decline of aquatic macrophytes in the Netherlands over recent decades. Due to reduced groundwater levels resulting from water extraction, seepage, which generally contains much iron, has dramatically decreased over large areas, while sulphate-enriched River Rhine water is introduced to prevent these areas from drying out. Decreased iron input and increased iron sulphide precipitation due to increased sulphate reduction can be expected to lead to a rapid exhaustion of the free iron(II) pool in the sediment.

Decreased iron levels appear to be correlated with increased phosphate levels in sediment pore water (Smolders & Roelofs, 1993, 1995). Furthermore, low iron:phosphate ratios in the upper sediment layer increase the exchange of phosphates to the water layer (Baccini, 1985; Smolders & Roelofs, 1993). Besides increased phosphate mobilization, highly toxic sulphide tends to accumulate in iron-depleted sediments (Ponnampenuruma, 1972; Yoshida & Tadano, 1978; Smolders & Roelofs, 1993). Sulphide accumulation can have deleterious effects on rooting plants as the roots of many (semi-)aquatic plants cannot survive high sulphide levels for long (Yoshida & Tadano, 1978). Furthermore, iron depletion can lead to iron deficiency in aquatic macrophytes (Smolders & Roelofs, 1993; Smolders, Roelofs & van der Velde, 1994).

In order to decrease the internal phosphate loading...
Table 1 Some sediment characteristics of the ditch where the enclosure experiment was carried out. All values, except percentage water and percentage organic matter, in mmol kg\(^{-1}\) dry weight, \(n = 6\).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>% water</td>
<td>54.7 ± 3.0</td>
</tr>
<tr>
<td>% organic matter</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>142 ± 25</td>
</tr>
<tr>
<td>Magnesium</td>
<td>52 ± 8.2</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Manganese</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Sodium</td>
<td>10 ± 4</td>
</tr>
<tr>
<td>Iron</td>
<td>202 ± 19</td>
</tr>
<tr>
<td>Calcium</td>
<td>61 ± 9</td>
</tr>
<tr>
<td>Aluminium</td>
<td>425 ± 79</td>
</tr>
<tr>
<td>Potassium</td>
<td>32 ± 5</td>
</tr>
<tr>
<td>Zinc</td>
<td>2.3 ± 0.2</td>
</tr>
</tbody>
</table>

of the water layer several techniques have been applied. In the deep Lake Dollar in the U.S.A., for instance, aluminium was introduced to the hypolimnion and successfully reduced phosphate levels for at least several years (Cooke et al., 1986). Iron(III) chloride injection into the sediments has been used in Lake Groot Vogelenzang, the Netherlands (Boers, 1991), but with limited success as phosphorus and chlorophyll levels in the water layer only declined for a few months.

In this paper we describe an experiment in which we tested whether it is possible to prevent sulphide toxicity and internal eutrophication by the addition of iron(II) chloride. Iron(II) was used because it is the most common form of iron in anaerobic sediments. Plants of Potamogeton acutifolius Link were introduced to iron-treated and non-iron-treated enclosures. \(P.\) acutifolius was selected as it is one of the species that often disappears after the introduction of sulphate-enriched Rhine-type water to aquatic habitats.

**Materials and methods**

**Study sites**

The experiment was carried out between March 1993 and April 1994 in ‘De Bruuk’, a small nature reserve near Nijmegen, the Netherlands, that is characterized by wet peaty grasslands intersected with ditches. The area is fed by seepage containing high amounts of sulphate and iron. In parts of the area sulphate reduction rates in the sediment are very high. As a result, iron is depleted in the upper sediment layers due to iron sulphide precipitation with the consequence that sulphide accumulates. In summer phosphate levels in the water layer are also increased and non-rooting \(Lemna\) species become dominant in the ditches. Rooting water plants are very scarce.

Table 1 shows some important sediment character-istics of the ditch in which the experiment was carried out. The sediment consists of a silty loam (1.7% clay, 78.9% silt and 19.4% sand) with a moderate organic matter content. Almost 66% of the phosphate appears to be iron and/or aluminium bound while 29% is calcium bound. The total iron content of the sediment is very high. Iron levels in sediment pore water, however, are very low.

**Experimental design**

Eight enclosures consisting of round polycarbonate cylinders (Fig. 1) with a depth of 2 m and a diameter of 1 m were used to isolate the water and top layer of the sediment from the surrounding water and sediment by pushing them 50 cm into the sediment. To four enclosures 75 g of iron(II) chloride were added to the sediment. The iron(II) chloride was dissolved in 5 l anoxic (N\(_2\)-flushed) deionized water. The solution was carefully injected into the top 15 cm of sediment by means of a big plant sprayer with nebulizer.

Sediment pore water samples were sucked from the upper 10 cm using porous ceramic cups which were connected to vacuum infusion flasks by airtight tubes (lysimeters). Two lysimeters were installed permanently in every enclosure. Subsamples of the sediment pore water samples were fixed immediately with a sulphide antioxidant buffer (SAOB) containing sodium hydroxide, sodium HDTA and ascorbic acid (van Gemerden, 1984). The redox potential of the sediment was measured at different depths and different locations in the upper 15 cm of the sediment with platinum wire electrodes (Pt cathode, saturated Ag/AgCl anode). Obtained values (\(n = 10\)) were averaged.

Water samples were collected in 500-ml iodated polyethylene bottles from mid-depth in the water layer. \(pH\) was determined using a Radiometer Combined \(pH\) electrode connected to a PHM82 Standard \(pH\) meter. Alkalinity was determined by titrating 50 ml with 0.01 m HCl down to \(pH\) 4.2. Another 50 ml subsample was passed through a Whatman GF/C filter and stored at ~28°C until analysis. Turbidity of the samples was determined with a Denton-type FN five turbidity meter. Water temperature was measured just above the sediment surface.

Six samples of sediment were collected with an Ekman sediment sampler from within the area where the enclosures were to be located. Subsamples were
Fig 1. The enclosure experiment in the nature reserve ‘de Bruuk’ near Nijmegen (province of Gelderland, the Netherlands).
dried at 105 °C for 24 h. Organic matter content was measured as loss on ignition, determined by heating 50 g of the dried sediment for 4 h at 550 °C.

Sediment samples were extracted stepwise with 1 M NH₄Cl, 0.1 M NaOH and 0.5 M HCl according to the fractionating scheme of Hieltjes & Lijklema (1980). Twenty-five millilitres of extractant was used per 250 mg of wet sediment. In this way P was fractionated into loosely adsorbed P (NH₄Cl), Fe- and Al-bound P (NaOH), and P incorporated mainly in Ca compounds (HCl).

To achieve sediment digestion 100 mg of dried sediment sample was dispersed in 5 ml concentrated H₂SO₄, incubated at room temperature for 24 h, heated to 150 °C and digested by slowly adding 2 ml 30% H₂O₂. The destruates were diluted to 100 ml with bidistilled water and stored at 4 °C until analysis. Whatman filters, through which water had been filtered, were digested in the same way as sediment samples to determine the amount of nutrients accumulated in the algae.

Ca, Mg, Al, and Fe were measured with an Inductively Coupled Plasmaspectrophotometer, type IL Plasma 200. K and Na were measured using a Technicon Flame Photometer IV. The following were determined colourimetrically using a Technicon AAI-system (Technicon Corporation, 1969): NO₃⁻ according to Kamphake, Hannah & Cohen (1967); NH₄⁺ according to Grasshoff & Johannsen (1977), Cl⁻ according to O'Brien (1962) and SO₄²⁻ according to Technicon Auto Analyser Methodology (1981). Sulphide was measured using an Orion 94-16 A sulphide ion-specific silver electrode with a double junction calomel electrode serving as a reference (Roelofs, 1991). Two weeks after iron(II) addition, known amounts of healthy-looking P. acutifolius plants, with healthy root systems, were carefully planted in two iron-treated and two control enclosures. After 6 weeks the plants were harvested and divided into healthy shoots, dead shoots and healthy roots and weighed.

Results

Sediment pore water

Iron(II) concentrations in sediment pore water of the control enclosures were very low and did not exceed a value of 5 µmol l⁻¹. Iron(II) addition resulted immediately in a maximum mean iron level of approx. 1100 µmol l⁻¹. After a sharp decrease in the first month to levels of 220 µmol l⁻¹, iron levels gradually decreased over the next months and at the end of the experiment reached values comparable with those measured in the control enclosures (Fig. 2).

In the control enclosures, sulphide levels in sediment pore water showed a seasonal variation with highest mean sulphide level (490 µmol l⁻¹) in summer and lowest (70 µmol l⁻¹) in winter (Fig. 2). Fig. 3 shows that sulphide levels in sediment pore water of the non-treated enclosures were strongly correlated with water temperature. Sulphide levels in sediment pore water decreased very sharply in the iron-treated enclosures. Immediately after iron addition sulphide levels became lower than 1 µmol l⁻¹ and remained low until the end of September when they began to increase gradually. At the end of the experiment sulphide concentrations were 80 µmol l⁻¹; that is still about half as high as those measured in the control enclosures.

Immediately after iron addition calcium as well as magnesium levels increased sharply (Fig. 2). In the next 2 months calcium and magnesium levels decreased to values comparable with those of the control enclosures. In the control enclosures calcium and magnesium levels did not change very much over the year (Fig. 2).

Ammonium and potassium levels showed seasonal variation with highest values in the summer months (Fig. 2). After iron addition potassium levels were increased somewhat but after a few months were again more or less comparable with values measured in the control enclosures. In the iron-treated enclosures ammonium levels remained somewhat lower then in the control enclosures while the observed seasonal variation showed the same pattern.

In the control enclosures phosphate levels in sediment pore water were generally low and did not exceed values of 4 µmol l⁻¹ (Fig. 2). From June until September, however, mean phosphate levels strongly increased and reached a maximum of 16 µmol l⁻¹ in August. At the end of August phosphate levels in sediment pore water dropped sharply to the values observed throughout the rest of the year. In the iron-treated enclosures phosphate levels decreased immediately after iron addition and remained low during the experiment.

After the addition of iron(II), pH and alkalinity of sediment pore water dropped sharply (Fig. 2). Although in the course of the differences with
the control enclosures decreased gradually, pH as well as alkalinity remained lower in the iron-treated enclosures until the end of the experiment. Sulphate levels were fairly constant over the entire period of the experiment and amounted to approx. 1000 µmol l\(^{-1}\). Only in the summer months did sulphate levels show a dip and become very low. In the iron-treated enclosures sulphate levels dropped immediately following
Fig. 2 Some important chemical and physical characteristics of the water layer and sediment in iron(II)-treated and control enclosures. All concentrations in μmol l⁻¹ except pH, redox potential (mV), temperature (°C) and alkalinity (meq l⁻¹). Vertical bars represent SD. n = 4 for the water layer and n = 8 for the sediment. * indicates the moment at which four of the enclosed were treated with iron(II) chloride. □ represents iron-treated enclosures, ▲ represents control enclosures. The inset in the iron (sediment) graph also represents the iron levels in sediment pore water on a more detailed scale.
Table 2 Growth of *Potamogeton acutifolius* in two iron-treated (A and B) and two control (C and D) enclosures in 'de Bruuk

<table>
<thead>
<tr>
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<th>Iron-treated</th>
<th>Control</th>
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<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Initial biomass</td>
<td>42.91</td>
<td>31.50</td>
</tr>
<tr>
<td>after 6 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (g FW)</td>
<td>59.86</td>
<td>47.35</td>
</tr>
<tr>
<td>Biomass increase</td>
<td>39.50%</td>
<td>50.52%</td>
</tr>
<tr>
<td>Healthy shoots</td>
<td>40.05</td>
<td>27.99</td>
</tr>
<tr>
<td>Dead shoots</td>
<td>13.31</td>
<td>12.25</td>
</tr>
<tr>
<td>Healthy roots</td>
<td>6.50</td>
<td>7.11</td>
</tr>
</tbody>
</table>

Fig. 3 Relationship between the temperature of the water layer (measured 1 cm above the sediment surface) and the sulphide levels in sediment pore water of the control enclosures.

iron addition. In the control enclosures sulphate levels started to decrease a month later.

Redox potential (Fig. 2) increased sharply by c. 80 mV following iron addition. In autumn and winter redox potentials in the iron-treated enclosures decreased again and reached values comparable with those measured in the control enclosures. Redox potentials were lower in the summer months than in winter.

**Water layer**

Turbidity of the water layer was relatively low in the enclosures at the onset of the experiment but increased sharply in the control enclosures in the summer months (Fig. 2). A maximum value of 55 p.p.m. was reached in September and thereafter decreased gradually until January (Fig. 2). In the iron-treated enclosures turbidity also increased somewhat but remained much lower than in the controls.

Due to enclosure effects the pH of the water layer increased from a mean value of 6.5 to values of ≈7.5 in the first 2 months of the experiment. In the control enclosures pH also increased sharply to values well above pH 8 as soon as the turbidity of the enclosures increased.

Phosphate concentrations were higher in summer, particularly in the control enclosures. The values dropped again in November but remained higher than at the onset of the experiment. Ammonium levels in the enclosures showed a clear seasonal variation and increased to values that were four to five times higher than during winter. The turbidity in the non-treated enclosures was caused by algae, as illustrated by the much higher chlorophyll levels in the non-treated enclosures. In August 1993 the chlorophyll a level in the seston fraction of the water layers was 208 (± 66) ng l⁻¹ in the four control enclosures and 31 (± 23) ng l⁻¹ in the four iron-treated ones. Furthermore, an important proportion of the phosphorus in the water layer was incorporated in the algal fraction (18.5 (± 5) μmol l⁻¹ in the iron-treated enclosures and 2.0 (± 0.8) μmol l⁻¹ in the control enclosures). The algal growth in the enclosures coincided with the development of a *Lemma* blanket in the surrounding ditch; the dominance of algae instead of *Lemma* species in the enclosures is apparently an enclosure effect.

**Effects of iron(II) addition on *Potamogeton acutifolius* growth**

Table 2 shows the effects of iron(II) addition on the growth of *Potamogeton acutifolius*. In the control enclosures *P. acutifolius* did not grow very well. Total biomass decreased by 50–60% over a period of 6 weeks and the major part of the plant tissue appeared to be dead. The shoots decayed at the base, releasing plant parts which floated at the water surface. These floating parts are not included in the total biomass in Table 2. Furthermore, few living roots were present while most of the root system had decayed.

In the iron-treated enclosures *P. acutifolius* grew very well. Biomass increased significantly in the 6-week period and the plants appeared healthy when harvested.
Discussion

Iron(II) addition had a very pronounced effect on sulphide levels in sediment pore water. Immediately after iron(II) addition sulphide levels dropped to values below 1 µmol L⁻¹ and did not increase until iron(II) levels in sediment pore water had become very low again. These observations are in accordance with those of Smolders & Roelofs (1993) who found no accumulation of sulphide in sediments containing ample amounts of iron(II). Thermodynamic calculations confirm that sulphide accumulation is to be expected when iron(II) levels in sediment pore water become very low (Yoshida & Tadano, 1978).

The marked increase of calcium and magnesium following iron(II) addition is most probably caused by the exchange of these bivalent ions with iron(II) on the cation exchange sites of sediment particles (Ponnamperuma, 1972; Schachtschnabel et al., 1992), the formation of iron(II) carbonate (siderite) (Davison, 1993), and acid-neutralizing reactions such as:

\[ \text{CaCO}_3 + \text{H}^+ \rightarrow \text{HCO}_3^- + \text{Ca}^{2+} \] (1)

\[ \text{CaMg(CO}_3)_2 + 2\text{H}^+ \rightarrow \text{Ca}^{2+} + \text{Mg}^{2+} + 2\text{HCO}_3^- \] (2)

In the month following iron(II) addition, calcium, magnesium and iron levels decreased to normal values, probably due to the diffusion of these ions to deeper sediment layers and to (re)precipitation of the ions. The immediate decrease of phosphate levels in sediment pore water can most probably be attributed to iron(II) phosphate precipitation (Boström, Jansson & Forsberg, 1982).

Due to H⁺-generating processes such as iron sulphide precipitation (eqn (3) below) and the formation of iron carbonates (eqn (4) below) alkalinity and pH decreased substantially following iron(II) addition.

\[ \text{HS}^- + \text{Fe}^{2+} \rightarrow \text{FeS} + \text{H}^+ \] (3)

\[ \text{Fe}^{2+} + \text{HCO}_3^- \rightarrow \text{Fe(CO}_3)_2 + \text{H}^+ \] (4)

Ammonium, potassium and sulphide levels in sediment pore water showed a pronounced seasonal variation, probably reflecting a strong correlation with temperature-dependent microbiological processes (Brock et al., 1994). Sulphide levels were fairly constant. Only in summer was a strong dip in the sulphate level observed. In summer, decreased seepage might result in decreased sulphate input. Furthermore the dip can, at least partly, be explained by increased sulphate reduction rates because in the same period sulphide levels reached maximum values in the control enclosures. The sulphate dip was even more pronounced in the iron-treated enclosures. We assume, therefore, that sulphate reduction rates in the iron-treated enclosures were certainly not much lower, and perhaps even higher than in the control enclosures. Sulphide produced by sulphate reduction will easily precipitate with iron(II). In this way iron is constantly stripped from the sediment pore water while at the same time iron(II) desorbs from clay/silt particles. In the enclosures where iron(II) was added these processes ensured almost total precipitation of sulphide during the summer months. Sulphide accumulation did not start until late in the year when the fractions of free iron(II) and iron (II) adsorbed to clay/silt particles were exhausted.

Phosphate did not show the typical seasonal pattern that was observed for nitrogen, potassium and sulphide, but produced a pronounced peak in the summer months. Increased phosphate mobilization due to organic matter breakdown is normally easily counteracted by the sorption of phosphates to clay particles (Stumm & Morgan, 1970; Boström et al., 1982) or iron hydroxides (Boström et al., 1982). Due to the strongly lowered sulphide concentration, the redox potential in the iron-treated enclosures is considerably higher than in the non-treated enclosures. As a result of the higher redox equilibrium in the iron-treated enclosures iron(III) reduction rates will be lower (Patrick, Gotoh & Williams, 1973; Roelofs, 1991). We suggest that the net phosphate mobilization in the control enclosures in the summer months can be explained by the lower redox potential. Furthermore FePO₄ might by reduced directly by sulphide (Sperber, 1958; Coleman et al., 1993). As phosphate fractionation (Table 1) shows that an important part of the phosphate in the sediment is present in iron/aluminium complexes, increased reduction of iron(III) will lead to an increased mobilization of phosphates. In the summer months the increased phosphate mobilization in the non-treated enclosures was apparently not sufficiently counterbalanced by phosphate sorption in the control enclosures.

Smolders & Roelofs (1993, 1995) have shown that decreased iron(II) input due to decreased seepage and the concomitant increased sulphate enrichment due to the inlet of sulphate-enriched river water leads to iron depletion, sulphide accumulation and eutrophication of aquatic ecosystems. The decline of many
rooting aquatic macrophytes in the Netherlands, and possibly elsewhere, is associated with increased sulphate levels in surface waters and decreased iron levels in sediment pore water (Smolders & Roelofs, 1993, 1995). The results of this enclosure experiment clearly illustrate the important interactions between iron, sulphate and phosphate households in aquatic ecosystems on reduced sediments. If sulphate reduction and iron(II) input are not in balance, sulphide accumulation and internal eutrophication can become serious threats for aquatic ecosystems.

Sulphide accumulation can lead to decreased vitality of *Stratiotes aloides* due to sulphide toxicity (Roelofs, 1991; Smolders & Roelofs, 1993), while greatly decreased iron levels can lead to iron shortage in aquatic macrophytes, as has been described for *S. aloides* (Smolders & Roelofs, 1993) and *Nymphoides peltata* (Gmel.) O. Kuntze (Smolders et al., 1994). Our results show that greatly increased sulphide levels affect the growth of *Potamogeton acutifolius* considerably. *Potamogeton acutifolius* is one of the aquatic macrophytes that has declined dramatically in recent decades in the Netherlands, especially in those waters where sulphate levels have increased greatly. Eutrophication did not play a role because the growth of *P. acutifolius* occurred before the algal bloom started in the control enclosures.

Apart from preventing phosphate mobilization, high iron levels in sediment pore water can also prevent phosphate exchange to the water layer (Baccini, 1985; Smolders & Roelofs, 1993). Oxidation of iron and concomitant iron(III) phosphate precipitation in the oxidative boundary layer at the sediment-water interface will reduce phosphate exchange to the water layer. Due to increased phosphate mobilization and consequential increased phosphate fluxes from the sediment in the summer, algal growth increased significantly in the non-treated enclosures. Apparently, phosphate was limiting algal growth in the enclosures as turbidity strongly responded to the increased phosphate mobilization. Iron(II) addition to the sediment clearly prevented internal eutrophication in the enclosures.

High sulphide levels will increase phosphate exchange as sulphide will greatly reduce or eliminate the oxidative boundary layer. In the control enclosures sulphide levels of up to 20 μmol l⁻¹ could be detected in the water layer 10 cm above the sediment surface; this indicates anaerobic conditions in the lower part of the water layer (data not shown).

Although our results illustrate that iron(II) addition can prevent sulphide accumulation and eutrophication in aquatic ecosystems with high sulphide levels in sediment pore water, it remains debatable whether beneficial effects of such additions can be achieved in the longer term. Our results indicate that the beneficial effects of iron(II) addition are only temporary when the system is still receiving high quantities of external sulphate. In the summer of 1994 all the enclosures became turbid, while sulphide and phosphate levels were very similar in the iron-treated and control enclosures (data not shown). Although our results reveal the important role of iron in reduced sediments, the temporal effects of iron addition make it rather unsuitable for nature conservation. Furthermore, iron addition is relatively expensive and application on a large scale might prove to be economically unsustainable. Restoration of the original hydrology seems the only sensible way to deal with internal eutrophication and sulphide accumulation.

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