PREDICTING METAL UPTAKE BY WETLAND PLANTS UNDER AEROBIC AND ANAEROBIC CONDITIONS

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Abstract—Metal pollution can be a serious threat to ecosystems at a global scale. Although the bioavailability of potentially toxic metals is determined by many biotic and abiotic factors, including pH and redox potential, total metal concentrations in the soil are used widely to assess or predict toxicity. In the present study we tested the effect of desiccation of soils differing in acidification potential and total heavy metal contamination on the growth and metal uptake of three typical, common wetland species: Caltha palustris, Juncus effusus, and Rumex hydrolapathum. We found that plant growth in wet soils mainly was determined by nutrient availability, though in dry soils the combined effects of acidification and increased metal availability prevailed. Metal uptake under anaerobic conditions was best predicted by the acidification potential (sediment S/(Ca + Mg) ratio), not by total metal concentrations. We propose that this is related to radial oxygen loss by wetland plant roots, which leads to acidification of the rhizosphere. Under aerobic conditions, plant metal uptake was best predicted by the amount of CaCl₂-extractable metals. We conclude that total metal concentrations are not suitable for predicting bioavailability and that the above diagnostic parameters will provide insight into biogeochemical processes involved in toxicity assessment and soil policy.

Keywords—Acidification  Bioavailability  Metal pollution  pH  Redox potential

INTRODUCTION

Metal pollution of soils and water is a worldwide problem. Although metals occur naturally in many ecosystems, they usually do not reach toxic levels without human interference [1]. The main sources of metal contamination are industrial waste, mining activities, traffic, and agricultural pesticides. Metal pollution can be a serious threat to ecosystems, because metals can be toxic and even carcinogenic to organisms. It is therefore very important to know how metals behave in natural systems and which factors determine bioavailability.

Many studies in the past have tried to predict the bioavailability of metals to plants (e.g., [2–5]). Most of these studies used soil extraction techniques to predict bioavailability. However, not all of these studies proved equally successful. In fact, it has even been concluded that chemical extractions do not add to the prediction of metal availability when compared to the traditional determination of total metal content of the soil [2].

In the present study we used a different tool to predict metal availability to wetlands plants: The acidification potential (S/(Ca + Mg)) ratio. According to Lucassen et al. [6], this ratio is closely related to pH change and metal concentrations in the pore water after desiccation. In addition, we tested more traditional methods, including one-step and sequential extractions. The acidification potential is a tool to predict the amount of protons released from sediment during desiccation. It has been known for a long time that pH plays an important role in the mobility, and thus bioavailability, of metals [7–9]. It is therefore possible that metal availability is related to the acidification potential and can predict plant metal uptake.

Another important factor determining metal speciation and bioavailability is the redox potential [7,8,10,11]. Speciation of iron and manganese in particular is known to be highly dependent on the redox conditions in the soil [12]. In wetlands, which often have fluctuating water levels, redox potentials may fluctuate throughout the year. These changes in redox potential could strongly influence metal mobility and therefore its accumulation by wetland plants.

In the present study, we investigated the effects of changing redox conditions on soils that differed in acidification potential and total metal concentrations. We tried to predict metal uptake by three common wetland plant species using different extraction methods. We hypothesized that the acidification potential would be a useful tool to predict metal uptake by wetland plants and that biogeochemical parameters like redox potential and pH would be more important factors determining the bioavailability of metals than total concentrations in the soil.

MATERIALS AND METHODS

Plant and soil collection

We collected soils from four different locations: Ronde Venen (RV), a peat meadow area in the center of The Netherlands; Roeventerpeel (RP), an alder carr in a heavily polluted area in the south of The Netherlands; Beesel (BB), an alder carr in the southeast of The Netherlands; and Kelmis (KM), an alder carr in the northeast of Belgium (Fig. 1). All soils consisted of anaerobic peaty sediment, which had been formed in an alder carr vegetation. Although the alder carr vegetation in RV has now disappeared, remnants of alder trees are still clearly visible in the peat. The soils differed in the level of metal pollution and in acidification potential (based on previous research by Lucassen et al. [6] and M. Van der Welle, Radboud University, Nijmegen, The Netherlands, unpublished data). The soils were transported to the laboratory in airtight con-
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Three common wetland plant species were used in this experiment: Caltha palustris L. (marsh marigold), Juncus effusus L. (soft rush), and Rumex hydrolapathum Huds. (water dock). These plants are referred to below by their genus names (Caltha, Juncus, and Rumex). Juncus plants were obtained from seeds collected at RV (see Table 1), Caltha plants were bought from a commercial gardener, and Rumex plants were collected from a river floodplain close to Nijmegen, The Netherlands (51°52’N, 5°53’E). All plants were grown on vermiculite for three weeks prior to the experiment. Five plants of each species were kept apart to determine initial nutrient concentrations and morphological parameters as described below.

Experimental set-up

We filled eight glass containers (25 × 25 × 30 cm) per soil type with approximately 20 cm of soil. Half of the containers were filled with anaerobic, wet soil and were kept anaerobic during the entire experiment. The other half were filled with the same amount of soil but the soil had been dried naturally, by spreading it out to allow aeration for 21 d, prior to the start of the experiment. Four additional containers were filled with soil from RP and were acidified artificially with the same amount of soil but the soil had been dried aerobic during the entire experiment. The other half were filled with soil from RP and were acidified artificially with sulfuric acid to a pH of 4, to test the effect of acidification.

From seeds collected at RV (see Table 1), was kept at a constant temperature of 16°C without aeration. The containers were put in a water bath that was filled with soil from RP and were acidified artificially with sulfuric acid to a pH of 4, to test the effect of acidification.

In each container, a 10-cm soil moisture sampler (Rhizon Soil Moisture Sampler, Eijkelkamp Agrisearch Equipment, Giesbeek, The Netherlands) was placed to collect pore-water samples. Pore water was collected every month by connecting 100-ml vacuum flasks to the samplers. The first 5 ml collected were removed from the syringes to exclude stagnant water. From each container we collected a soil sample to determine organic matter content, soil moisture content, and nutrient concentrations.

We planted one individual of each species in each container. Before planting, the number of leaves, root length, width of the leaves, plant height, and number of inflorescences were determined. The plants were harvested after 12 weeks and the same morphological characteristics were determined again. Dead leaves and roots were removed and the plants were divided into aboveground parts, roots, and rhizomes. The leaves were washed with chloroform for 30 s to remove metals attached to the wax layer. The parts were dried for 48 h at 70°C and processed as described below.

Chemical analysis

A 10-ml subsample of pore-water samples was used to measure pH and alkalinity. Alkalinity was estimated from the amount of HCl needed to titrate the sample to a pH of 4.2. The remaining sample was divided into two parts. One part was frozen with 0.5% HNO₃ until analysis for Ca, Mg, Fe, Al, P, S, Cr, Ni, Cd, Pb, Cu, and Zn with an inductively coupled plasma mass spectrometer (X-series, Thermo, Waltham, MA, USA). The other part was frozen with 0.125 g/L of citric acid and analyzed for o-Po₄, NO₃⁻, and NH₄⁺ using an auto analyzer (AA 3, Bran + Luebbe, Norderstedt, Germany) and for K using flame photometry (FLM3 flame photometer, Radiometer, Copenhagen, Denmark; see Van der Welle et al. [13]). The SO₄²⁻ concentrations were determined by capillary ion electrophoreses (capillary ion analysis, Water, Milford, MA, USA).

The dried plant samples were ground and approximately 200 mg of dried plant material was weighed exactly and digested with 4 ml nitric acid (65%) and 0.9 ml 35% hydrogen peroxide, using an ETHOS D microwave labstation (Milestone, Sorisole, Italy). The digested sample was diluted and analyzed with inductively coupled plasma mass spectrometry as described above.

A subsample of fresh roots was weighed and extracted with an anaerobic bicarbonate-dithionite solution as described by Christensen and Sand-Jensen [14] to remove root plaque and to allow the concentrations of metals in the root plaque to be determined. The supernatant was diluted and analyzed by inductively coupled plasma mass spectrometry as described above.

Sediment samples were dried for 48 h at 105°C to determine the moisture content and then heated to 550°C for 4 h to estimate the organic matter content. Dried soil samples were digested with 4 ml nitric acid (65%) and 0.9 ml 35% hydrogen peroxide and analyzed with an inductively coupled plasma mass spectrometer as described for the plant samples.

In addition, an amount of fresh sediment was extracted with bidistilled water with 0.1 M CaCl₂, 2 g/L ammonium citrate, and 0.1 M sodium nitrate to determine potentially bioavailable metal fractions in the soil (see Table 2 and Chojnacka et al. [5]). Extraction with 0.5 M NaHCO₃ was used to determine plant-available phosphate concentrations [15]. A sequential ex-

![Fig. 1. Map of The Netherlands showing the locations where soil was collected for the experiment. BB = Beeselsbroek; KM = Kelmis (Belgium); RP = Roeventerpeel; RV = Ronde Venen.](Image 55x432 to 281x738)
tration of the soils was performed according to Twardowska and Kyziol [16], to estimate the different available metal fractions in the soil.

Data analyses

Possible differences between soils and plant species were analyzed with analysis of variance using a Tukey’s post hoc test. Possible differences between aerobic and anaerobic treatments were analyzed using an independent samples t-test. Correlation analysis was used to test for relationships between nutrient concentrations in the extractions and plant tissue concentrations. Multiple step-wise regression was used to determine which fractions in the sequential extractions were taken up by the plants. All statistical analyses were performed with SPSS® 13.0 (SPSS, Chicago, IL, USA).

RESULTS

Desiccation and soils

Desiccation of the soils led to significantly lower moisture contents (t-test, \( t = 13.33, p < 0.000 \)). The anaerobic, wet soils had average moisture content of 87% (standard deviation = 2.6), and the aerobic, drier soils had an average moisture content of 55% (standard deviation = 11). No significant differences were found between the moisture contents of the different soils in the aerobic treatment (analysis of variance, \( p = 0.157 \)). The anaerobic soils from RP and RV had a slightly, but probably not ecologically relevant, higher moisture content than the soils from BB and KM (\(~84 \text{ and } ~88\%\), respectively).

In addition, desiccation led to acidification in some of the soils. This was especially the case in soil from BB, of which pH dropped to values below 3 and, to a lesser extent, in the soil from RP, which had a much lower pH in the aerobic treatments than in the anaerobic treatments. Figure 2 displays acidification as the net production of protons during desiccation and concomitant acidification. The pH of the soils was significantly correlated with the acidification potential (Spear-
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Fig. 3. Correlation between the acidification potential and iron uptake by Calthta, Juncus, and Rumex. All correlations were significant. The $r^2$ values (Spearman correlation coefficients) were 0.82 for Calthta, 0.62 for Juncus, and 0.53 for Rumex (see also Table 4).

Fig. 4. Relative growth of Calthta, Juncus, and Rumex in the different soils and treatments (biomass: initial biomass). Root biomass is indicated by black bars, shoot biomass by white bars. Error bars indicate the standard error of the mean ($n=100$). Letters indicate significant differences between treatments (lowercase for aerobic [aer.], capitals for anaerobic [anaer.] treatments). BB = Beeselsbroek (The Netherlands); KM = Kelmis (Belgium); RP = Roeventerpeel (The Netherlands); RV = Ronde Venen (The Netherlands).

Man’s correlation coefficient = $-0.539, p = 0.001$), which had been defined previously as the sediment total S/total(Ca + Mg) ratio (see also Lucassen et al. [6]). The net amount of protons produced during desiccation also was correlated significantly with the acidification potential (Spearman’s correlation coefficient = 0.549, $p = 0.001$).

The acidification led to increased concentrations of Al, Zn, Cu, Cd, Fe, and Pb in the pore water. The concentrations of these metals were significantly correlated with pH (Pearson’s correlation coefficient $\geq 0.445, p < 0.05$). Figure 3 shows a typical example of the correlation between the acidification potential and Fe concentrations in the three plant species. Apart from acidification, redox potential also played an important role for Cu and in particular Fe. Iron availability decreased when soils became aerobic, but within the aerobic soils, it increased with decreasing pH. In aerobic soils, the correlation between Fe in pore water and the amount of protons produced during aeration and simultaneous acidification had a correlation coefficient of $r^2 = 0.955$ ($p < 0.000$). For Cu, we found a similar significant interaction between redox potential and acidification. The measured redox potentials (Eh) of the anaerobic soils were between $\pm 115$ mV and $+5$ mV, and differed significantly between soils from different locations. Redox potentials of the aerobic soils were all above $+250$ mV.

In addition to the biogeochemical changes mentioned above, desiccation led to a change in nutrient availability. Phosphate concentrations in the pore water decreased as a result of desiccation; nitrate concentrations increased, and ammonium concentrations simultaneously decreased. This is caused by changed oxygen intrusion and the resulting redox conditions, promoting iron oxidation and concomitant phosphate binding, as well as nitrification.

Desiccation and plant growth

Plant biomass was strongly affected by desiccation. For all plant species growing on all of the soils, relative growth was lower in the aerobic treatments than in the anaerobic treatments (Fig. 4). All three species had the highest aboveground biomass in soils from KM in the anaerobic treatment. In the aerobic treatment, aboveground biomass always was lowest in BB, which was also the treatment with the strongest acidification. However, there were no significant correlations between aboveground biomass and pH. For Calthta and Rumex, aboveground biomass was significantly negatively correlated with the ammonium concentration in water extracts (with Spearman’s correlation coefficients of $-0.921, p < 0.000$ and $-0.732, p = 0.001$, respectively). Ammonium concentration was in turn significantly correlated with pH. In addition, the aboveground biomass of Rumex in the aerobic treatment also was negatively correlated with metal concentrations in water extractions (Pearson’s correlation coefficient between $-0.577$ and $-0.618, p \leq 0.019$), which in turn were negatively correlated with pH (Pearson’s correlation coefficient between $-0.858$ and $-0.886, p < 0.000$).
In the anaerobic treatments, the aboveground biomass of *Caltha* and *Rumex* was negatively correlated with iron concentrations (with Spearman’s correlation coefficients of $-0.518$, $p = 0.04$, and $-0.532$, $p = 0.034$, respectively), and that of *Rumex* was negatively correlated with Al and Cd concentrations (with Spearman’s correlation coefficients of $-0.635$, $p = 0.008$, and $-0.593$, $p = 0.016$, respectively). In addition, aboveground biomass of *Rumex* was positively correlated with pH, which may have been linked to the negative effects of Al, because Al was significantly correlated with pH (Pearson’s correlation coefficient $= -0.754$, $p = 0.001$). For *Juncus*, we found a positive correlation between ammonium and aboveground biomass in the anaerobic treatment (Spearman’s correlation coefficient $= 0.706$, $p = 0.002$).

Root biomass showed similar patterns, with a few important exceptions. In the aerobic treatment pH was significantly correlated with the root biomass of *Juncus* and *Rumex* (with Pearson’s correlation coefficient $= 0.668$, $p = 0.005$ and Spearman’s correlation coefficient $= 0.668$, $p = 0.005$, respectively), and correlations between the root biomass of *Juncus* and Al, Fe, Zn, Cu, Cd, and Pb were similar to those for *Rumex* with aboveground and root biomass. For *Caltha*, we found no significant correlations between root biomass and concentrations in water extracts in the anaerobic treatment, whereas correlations in the aerobic treatment were similar to those found for aboveground biomass.

Phosphate concentrations in the pore water were not related to plant growth, nor to P concentrations in the plant. Moreover, Olsen-extractable P was not related to plant growth or tissue P concentrations either and the patterns among the different soils were similar to those for pore water phosphate concentrations.

### Plant metal uptake and metal availability

Plants accumulated more metals when growing in aerobic soils. Zinc, Cu, and Cd concentrations were increased significantly in aboveground parts in all four soils and Al concentrations increased in plants growing in soils from BB and KM ($t$-test, $p \leq 0.05$). The roots of plants growing in the most acidified soils (RP and BB) had increased concentrations of Zn and Cd. Iron concentrations were decreased in aboveground parts and roots, but were higher when the pH of the soil was low. In the anaerobic treatment, plant iron concentrations were increased strongly in iron-rich sediments (RP and BB). Metal concentrations in the plants in the different treatments and soils are summarized in Table 3.

In the aerobic treatment, *Caltha* plants had higher metal concentrations than the same species growing in the same soil
in the field, especially in the soils with the highest acidification potential. We analyzed aboveground parts of *Caltha* from the field in BB, KM, and RV, where the species occurs naturally. Concentrations of Al, Zn, Cu, Cd, and Pb were all much (17–600 times) higher in the aerobic treatment in our experiment than those measured under wet conditions in the field (data not shown).

When plants were growing in aerobic soils, metal uptake in their aboveground parts was predicted best by CaCl₂ extractions. For *Juncus* and *Caltha* plant metal concentrations were correlated best with CaCl₂ extractions (Table 4). For *Rumex*, NaNO₃ extractions also predicted plant metal uptake, but usually not much better than the CaCl₂ extractions (data not shown). In general, the other single extractions described in the Materials and Methods section show much smaller and usually not significant correlations with plant concentrations.

In anaerobic soils, plant metal uptake was predicted best by the acidification potential (estimated by the total S/[Ca + Mg] ratio; [6]). We found significant correlations between Al, Fe, Ni, and Cd concentrations in the plants and the acidification potential, for all three plant species (Table 4). In addition, we found significant correlations with Zn and Cu for *Caltha* and *Rumex* and with Pb for *Caltha*.

Stepwise multiple regression analyses showed that the largest part of the uptake of metals (Al, Fe, Ni, Zn, Cu, Cd, and Pb) by the plants could be predicted from the water- and NH₄OH.HCl-extractable fractions [16], which represent the pore water and easy reducible fractions, respectively. We found that it was not possible to predict the uptake of Cu and Pb from the sequentially extracted fractions. Uptake of Al and Fe by *Juncus* was predicted best by the mobile (F2) and exchangeable (F1) fractions, respectively. Another exception was Pb uptake by *Rumex*, which was predicted best by the moderately reducible (F4) fraction.

### Root plaque

Root plaque was visibly present in all three species, especially in the iron-rich soils (RP and BB). The main components of root plaque were found to be iron oxides and/or iron-hydroxides. The largest amounts of such iron oxides and iron hydroxides were formed in the plants growing in soil from BB and, to a lesser extent, in those growing in soil from RP (Fig. 5), which also was the soil richest in iron (Table 1). Plants growing in soil from RV, the soil with the lowest iron content, hardly formed any root plaques.

The plant species were significantly different. In the iron-rich soils (RP and BB), *Juncus* and *Rumex* formed significantly more root plaque per gram of root than *Caltha* (Fig. 5). Iron concentrations in root plaque extracts from plants growing in aerobic soils were much lower than in those in anaerobic soils; this was true for most soils (~25 times lower), except for the soil poorest in iron (RV), where iron concentrations in root plaque extracts were only three times lower.

### DISCUSSION

**Desiccation and acidification**

Desiccation clearly led to acidification in some of the soils and was linked to the acidification potential, which proved to be a useful tool to predict acidification of soils and corresponded very well with the findings by Lucassen et al. [6]. They found that the amount of protons produced in a soil during desiccation was determined by the balance between the concentration of sulfur in the soil and those of calcium and magnesium. The latter two act as a buffer mechanism in soils with a pH between 4 and 6 by cation exchange at the adsorption complex [17]. In the type of soils that we used, one of the most important proton-generating processes during desiccation is the oxidation of sulfide to sulfate. This (partially) micro-biologically mediated process leads to acidification when the buffering capacity of the soil is insufficient.

In aerobic soils, phosphate and ammonium availability decreases, and nitrate availability increases. Phosphate can be immobilized by binding to ferric iron (Fe³⁺), forming immobile iron-phosphate compounds [18–20]. Aerobic nitrification of ammonium leads to increased nitrate concentrations at the expense of ammonium concentrations in the pore water. However, this nitrification process did not occur in soils from BB. In fact, ammonium concentrations in the pore water were even increased in the aerobic soils compared to the anaerobic soils. This is most probably the result of the strong acidification in

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**Table 4. Correlation coefficients between metal uptake by the plants and CaCl₂ extractions (aerobic treatment) and the acidification potential**

<table>
<thead>
<tr>
<th></th>
<th><em>Caltha</em></th>
<th></th>
<th></th>
<th><em>Juncus</em></th>
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<th></th>
<th><em>Rumex</em></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>CaCl₂</td>
<td>Acid. pot.</td>
<td>CaCl₂</td>
<td>Acid. pot.</td>
<td>CaCl₂</td>
<td>Acid. pot.</td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>0.735 (0.001)*</td>
<td>0.562 (0.012)</td>
<td>NS</td>
<td>0.774 (0.000)</td>
<td>0.579 (0.010)</td>
<td>0.521 (0.019)</td>
<td></td>
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<tr>
<td>Fe</td>
<td>0.856 (0.000)</td>
<td>0.821 (0.000)</td>
<td>NS</td>
<td>0.621 (0.005)</td>
<td>NS</td>
<td>0.526 (0.018)</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>0.923 (0.000)*</td>
<td>0.607 (0.006)*</td>
<td>0.961 (0.000)*</td>
<td>0.660 (0.003)*</td>
<td>0.929 (0.000)</td>
<td>0.471 (0.033)*</td>
<td></td>
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<tr>
<td>Zn</td>
<td>0.924 (0.000)*</td>
<td>0.506 (0.023)</td>
<td>0.791 (0.000)*</td>
<td>NS</td>
<td>0.887 (0.000)</td>
<td>0.765 (0.001)</td>
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<tr>
<td>Cu</td>
<td>0.867 (0.000)*</td>
<td>0.550 (0.014)</td>
<td>NS</td>
<td>NS</td>
<td>0.538 (0.016)</td>
<td>0.438 (0.045)</td>
<td></td>
</tr>
<tr>
<td>Cd</td>
<td>0.922 (0.000)*</td>
<td>0.756 (0.001)</td>
<td>0.794 (0.000)</td>
<td>0.594 (0.008)*</td>
<td>0.874 (0.000)*</td>
<td>0.796 (0.000)</td>
<td></td>
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<tr>
<td>Pb</td>
<td>0.773 (0.000)*</td>
<td>0.488 (0.028)</td>
<td>NS</td>
<td>NS</td>
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**Fig. 5.** Iron concentrations in root plaque extracts of the plant roots in the anaerobic treatment. Letters indicate significant differences between species; error bars indicate standard error of the mean (n = 4).
these soils, because a low pH will decrease nitrification rates. It indeed has been found that when the pH of heathland soils is increased by liming, nitrification is strongly stimulated compared to unlimed control soils with low pH [21].

Acidification also leads to increased mobility of all metals, but in some cases interacts with the redox potential. Speciation of iron especially, but also manganese, is known to depend strongly on redox conditions [12]. Iron oxides and iron hydroxides, which are formed under aerobic conditions, are insoluble and therefore less bioavailable. Increased mobility of metals as a result of acidification is a relatively well-known process, which has been described by many authors (for reviews see Gambrell [7] and Hesterberg [10]). When the pH drops below 4.5, which was the case in the aerobic soils from RP and BB, metal mobility greatly is increased ([10,22]; M. Van der Welle, Radboud University Nijmegen, The Netherlands, unpublished data). This effect of acidification proved to be independent of the redox effect, because metals were mobilized to the same extent in the artificially acidified soils (data not shown).

**Plant growth**

Plant growth was strongly affected by desiccation: All three species in all soils did not grow as well in the aerobic soils as in the anaerobic soils. This probably was not caused by the lowered availability of water, because the soil moisture content averaged around 55% in the aerobic soils, which should be sufficient for plant growth, even of wetland plants. In the field (Ronde Venen, see Materials and Methods section), *Caltha* and *Juncus* were found growing on soils with moisture contents between 40 and 80%, and *Rumex* plants appeared to persist at even drier locations along the river Waal (M. Van der Welle, Radboud University Nijmegen, The Netherlands, unpublished data). Moreover, all three species have been found growing under fluctuating redox conditions in the field (M. Van der Welle, personal observation) and therefore should be able to cope with drier conditions. It is more likely that the combined effects of low pH, high metal availability, and, perhaps most importantly, changed nutrient availability caused the decreased growth on aerobic soils. As described in the previous section, ammonium and phosphate availability decreased, and nitrate increased. In wetland soils, phosphate is often the growth-limiting nutrient [23,24], and decreased phosphate availability thus may have resulted in decreased growth. However, tissue P concentrations did not decrease and sometimes even increased, which does not point towards P limitation. Plant-available phosphate, as should be reflected by Olsen-extractable P [15], showed patterns similar to those of pore water phosphate concentrations, but was not related to plant growth or to tissue concentrations of P. This confirms that P was not limiting growth, because increased P availability did not lead to increased growth.

Under anaerobic conditions, plant growth most likely was determined by a combination of nutrient availability and the toxicity of metals, including Fe, and sulfide [25]. Plant growth was highest on soils from KM, which had the highest nutrient availability, especially of ammonium (Table 1), and lowest on soils from BB, which had the lowest availability of ammonium and phosphate. The BB soils not only had the lowest nutrient availability (N and P), but also had the highest sulfate concentration, which may have led to the production of toxic sulfide under anaerobic conditions. Sulfide is a very strong phytoxin, which can depress plant growth even at low concentrations [13,26–28]. Ammonium toxicity is not likely to have occurred, because the plants grew best in the soils with the highest concentrations of ammonium. This can be explained by the fact that wetland plants are well adapted to using ammonium as their main nitrogen source [29].

**Metal bioavailability**

In a study from 1993, Otte et al. [2] found that “Chemical extractions to determine the ‘bioavailable’ concentrations of heavy metal in plants are not better predictors for uptake of metals in plants than the total metal concentration in the soil.” The findings of the present study contradict this view. Despite equal or even higher total concentrations of Zn, Cd, and Pb in the soils from KM, plant uptake was much lower than in plants growing on BB soils. Similar patterns were found for Zn in RP and RV. Although total concentrations were similar, plants growing in RP took up more Zn than those growing in RV soils, especially under aerobic conditions. These findings confirm our hypothesis that soil biogeochemistry is much more important for metal bioavailability than total concentrations. This viewpoint is supported by other studies reported in the literature, which show that both pH [9,30] and redox conditions [11,30] are important factors determining plant metal uptake in wetlands.

We found that under anaerobic conditions, the acidification potential of the soils is a good predictor of the bioavailability of most metals, and under aerobic conditions, the CaCl₂-extractable fraction is a good predictor of biouptake. The fact that metal uptake by plants under anaerobic conditions strongly was related to the acidification potential most likely is linked to radial oxygen loss from the plant roots. Oxygen loss to the rhizosphere may cause local acidification, especially in soils vulnerable to acidification. Previous research by Jaynes and Carpenter [31] and Kostka and Luther [32] found that pH in vegetated soil was significantly lower than in similar unvegetated soil, as a result of oxygen loss from the roots and the resulting oxidation processes.

The fact that the acidification potential did not predict metal uptake under aerobic conditions in desiccated wetland soils can be explained by the fact that acidification already had taken place and metals therefore were already more mobile. In that case, radial oxygen loss hardly is relevant. Apparently, CaCl₂ extractions provided the best simulation of the metal uptake mechanisms of the wetland plants under aerobic conditions, which is well-known for terrestrial plants (e.g., [3,4]).

Clear differences were noted in metal uptake between the three species. Under aerobic conditions, *Juncus* and *Rumex* had lower metal concentrations than *Caltha* (Table 3), especially on the potentially most acidic soils (BB and RP). This also may be related to differences in radial oxygen loss. Previous studies by Visser et al. [33] and Van der Welle et al. [13] have demonstrated that the radial oxygen losses of *Juncus* and *Rumex* are higher than that of *Caltha*. As a result of radial oxygen loss, root plaque consisting of oxides and hydroxides is formed on the outside of the roots [14,34–36]. This root plaque, which mainly consists of iron hydroxides, can prevent the plants from taking up essential nutrients, but also from taking up nonessential metals. *Rumex* and *Juncus* accumulate 2 to 3 times more iron in root plaque than *Caltha* (Fig. 5), which supports the theory that increased root plaque reduces the uptake of nutrients and metals. In addition, *Juncus* plants probably are better adapted to acidic environments, because they can occur naturally at locations with low soil pH [37].
and may have special adaptations to prevent excessive uptake of metals as a result of low pH. The difference between *Caltha* and *Juncus* in surface-to-volume ratio of the roots [33] cannot account for the observed lower amount of iron in the root plaque of *Caltha*. For *Rumex*, the lower surface-to-volume ratio probably increases the negative effects of root plaque formation.

The findings also may have been influenced by differences in uptake mechanisms between monocotyledonous (*Juncus*) and dicotyledonous species (*Rumex*, *Caltha*), which may influence metal uptake. Dicots excrete protons, thus increasing the reducing capacity of their roots and mobilizing essential metals. Monocotyledonous, graminoid species, on the other hand are known to excrete siderophores, which can mobilize metals from the soil [29,38]. The excretion of siderophores is combined with a highly specific transport mechanism in the plasma membrane. The siderophores specifically promote iron uptake, but also form complexes with other metals, although the uptake mechanism has much smaller affinity for the latter complexes [39,40]. This makes the uptake mechanism of graminoid species like *Juncus* much less specific than that of dicotyledonous species like *Rumex* and *Caltha* and may explain the differences in metal uptake between the species. The differences in uptake mechanisms provide an adequate explanation for the difference in metal concentrations between *Caltha* and *Juncus*: Uptake of Al, Zn, and Fe by *Juncus* was higher under the least acidic conditions and lower under the most acidic conditions than the metal uptake by *Caltha*. However, comparison of our findings for the dicotyledonous *Rumex* with *Juncus* did not show the abovementioned contrasts between dicots and monocots. This is probably a result of the presence of large rhizomes in *Rumex*, which can be used as a nutrient source under unfavorable conditions, resulting in lower metal accumulation than might be expected under acidic conditions. This is confirmed by the fact that *Rumex* plants growing in BB soils under aerobic conditions had significantly smaller rhizomes (5 times smaller) than those growing under anaerobic conditions, and rhizome size was not significantly different for the other soils (data not shown).

It is unlikely that increased metal concentrations are a result of the accumulation of metals in plant tissue due to decreased growth. Tissue analysis of dead material of the plants growing on BB soils under aerobic, acidic conditions, showed very high metal concentrations (data not shown). It seems likely, therefore, that plants accumulate metals in their tissue to a certain limit and that leaves start to die off when this is exceeded. Moreover, we observed that even in the treatments with the lowest biomass, the plants still formed new leaves. In addition, actual tissue concentrations are far more important for the study of bioaccumulation and biomagnification of metals than the uptake rates of metals, because tissue concentrations determine the amounts of metals that actually can accumulate at higher trophic levels.

**CONCLUSION**

Our study clearly demonstrated that biogeochemical changes as a result of both soil characteristics and plant-soil interactions determine metal availability for wetland plants. Despite physiological differences between wetland plant species, metal uptake could be well predicted by the acidification potential under aerobic conditions and by CaCl₂-extractions under anaerobic conditions. Nevertheless, current policy still is based on total metal concentrations in the soil. We showed that relatively simple measurements, like the acidification potential and single extractions, provide a much more reliable prediction of actual metal uptake. Therefore, it is essential to include biogeochemical parameters, including pH, acidification potential and redox potential, in the models that are used now as policy supporting instruments.

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