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Substrate Effects on Metal Retention and Speciation in Simulated Acid Mine Wetlands

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In the past decade constructed wetlands have been increasingly used as an inexpensive treatment for acid-mine drainage (AMD) and other waste waters. Constructed wetlands have shown potential to lower acidity and total metal concentrations in these polluted waters (Erickson et al. 1987; Kleinmann 1985; Hammer 1989). However, since constructed wetlands are a relatively new technology, knowledge of how they function to reduce pollutants is limited. Most of the available information consists of comparisons of inflow/outflow metal concentrations (Stark et al. 1990; Brodie et al. 1988). Only a few attempts have been made to determine metal species distributions in effluents or substrates and evaluate the metal retention processes (Henrot and Wieder 1990).

Further understanding of metal attenuation in the wetland and identification of the solid forms of the retained metals are important for maintenance and improvement of constructed wetland treatments (Plankey and Patterson 1987; Lake et al. 1984). Factors which are likely to influence the efficiency of constructed wetlands include the types of wetland substrate and aquatic plant species involved in the treatment. Some plant species and substrates appear to be more effective than others in complexing certain metals without changing the total metal concentrations of the effluent (Hargrove and Thomas 1981). The distribution of dissolved toxic vs non-toxic metal species in the effluent can impact human health, wildlife and plant life (Florence 1983). However, significant quantities of organically bound metals may not be toxic at all (Stevenson and Fitch 1986).

Simulated wetlands employing various substrates and Typhia latifolia were subjected to a synthetic acid mine drainage solution under greenhouse conditions. Substrate effects, toxic metal species in effluents, and metal-retention processes were investigated. The objectives of the study were (1) to evaluate the efficiency of six different wetland substrates for metal removal from simulated AMD effluents, (2) to determine the distribution of organically complexed and inorganic metal species in treated surface and subsurface effluents, and (3) to determine the distribution of the solid metal forms retained in the substrates.

MATERIALS AND METHODS

Twelve simulated wetland chambers were established in 50x32x30-cm (LXWXH) polyethylene containers. Six replicated substrate mixtures, each

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receiving simulated AMD at a flow rate of 0.5 L/h were utilized. The substrates were 2:1 volume mixtures of ground pine needles with surface soil, peat moss with subsoil, peat moss with surface soil, ground hay with surface soil, *Sphagnum* moss with surface soil and a 1:1:1 volume mixture of peat moss, mine spoil and surface soil. Peat and *Sphagnum* moss mixed with various soil materials have been extensively used as constructed wetland substrates. The pine needles were selected because of their strong Fe and Al chelating capacity observed in spodosols, while the hay was chosen as an inexpensive, convenient alternative. The mixture of peat with subsoil vs. surface soil was used to compare the effect of increased clay content vs organic matter increase, respectively. The surface soil was collected from the Ap horizon of a Woolper soil (fine, mixed, mesic Typic Argiudoll) and the subsoil from the Bt horizon of a Maury soil (fine, mixed, mesic Typic Paleudalf). The mine spoil was a mixture of spoil materials collected at surface mine sites in Kentucky. The soil and mine spoil materials were finely ground to pass a < 2 mm sieve. Each wetland chamber contained a 10-cm base of crushed limestone covered by 20 cm of the substrate mixture. Five cattail (*Typha latifolia*) plants of 30-50 cm height were planted in each container. Thirty-liter tanks were filled with a solution representative of AMD in Kentucky. The simulated mine water consisted of a mixture of sulfate and chloride compounds which gave concentrations of Ca=200, Mg=200, Fe=70, Al=50, NH_4-N=35, Cl=28, Mn=20, Na=20, Si=12, K=10, H_2PO_4-P=8, Zn=5, and Cu=5 mg/L, with the pH adjusted to 3.4 with H_2SO_4. The solution was allowed to saturate the substrates for one week prior to beginning the experiment. A flow rate of 0.5 L/hr, was established for each chamber and the acid solution was allowed to flow through the substrates for five months. Outlets for the effluent solutions were placed 1 cm above the substrate surface and 5 cm below the surface.

Effluent samples were collected weekly from surface and subsurface flows and influent samples were collected monthly. The collected solutions were filtered through 0.25 mm filters and following pH measurements, they were acidified with 1*M* HCl and refrigerated in polyethylene bottles until analysis. Total Al, Fe, Mn, Zn, Cu, Ca, Mg, Na, and K concentrations were determined by atomic absorption spectroscopy (AAS) or colorimetry (Al, Fe) if concentrations were below the AAS detection limits of 5 and 1 mg/L, respectively (Page et al. 1982). The solutions were also analyzed for SO_4 turbidimetrically (Page et al. 1982). Surface and subsurface effluent data for pH, Al, Fe, Mn, Zn and Cu over the five-month period were statistically analyzed by one way ANOVA and the treatment (substrate) means were compared by using Duncan's multiple range test at the 0.05 probability level.

Soluble organically-complexed metal species were separated from soluble inorganic metal forms by passing filtered solution subsamples through a Chelex 100 cation-exchange resin equilibrated with a solution containing Ca^{2+}, Mg^{2+}, and H^+ concentrations similar to those in the collected effluents. The collected aliquot was analyzed by AAS or colorimetry and comprised the non-exchangeable metal load, which is organically complexed (Campbell et al. 1983). The concentration of the exchangeable metal load adsorbed by the resin was obtained by subtraction of the organically complexed value from the total filterable metal load. Equilibrium metal speciations of the surface and subsurface effluents during the last week of the experiment were calculated with EPA's MINTEQA2 geochemical equilibrium model (Allison et al. 1990).

Following completion of the greenhouse experiment, substrate samples were taken from 0-5 cm and 5-15 cm depths. The samples were sequentially extracted with 0.5 M KNO_3, distilled water, 0.5 M NaOH, 0.1 M Na_2EDTA and 4M HNO_3 (Emmerich et al. 1982) to determine the forms (exchangeable, sorbed, organic or residual) of metals bound to the substrate. A second extraction with 4M HNO_3 provided total concentrations of bound metals. Solution extracts were analyzed by AAS.

RESULTS AND DISCUSSION

The flow of simulated acid mine solution over the surface of the simulated wetland chambers produced changes in pH, Al, Fe, Cu and Zn effluent concentrations. There were significant differences among substrates. After five months of operation the hay-surface soil substrate and the pine needle-surface soil substrate proved to be the most efficient in reducing acidity and maintaining pH levels between 6 and 8 in the surface effluents. The mean surface effluent pH for substrates containing peat moss, Sphagnum moss or spoil materials was not significantly different from the mean influent pH (Fig. 1a).

Aluminum concentrations in the surface effluent showed an inverse relationship with pH values. The hay and pine needle substrates produced surface effluents with the lowest mean Al levels, representing an average reduction of about 88% of influent concentrations (Table 1). The Al retention efficiency of the Sphagnum substrate was about half that of the hay and pine needle substrates and was lowest for the mine spoil and peat-subsoil substrates. Iron retention efficiencies within treatments varied during the experiment, but were similar between treatments (60-71%) except the mine spoil mixture which showed about one half the efficiency of the others (Table 1). Manganese concentrations remained at levels near or in excess of influent concentrations, apparently due to Mn dissolution from Mn-concretions present in the soil matrix. This is reflected in the negative retention efficiencies for Mn for all substrates except hay (Table I). Copper removal from the surface effluents was variable, with hay and pine needle substrates showing the highest retention efficiencies (Table 1). Similar trends were exhibited for Zn (Table 1).

Subsurface percolation of the simulated acid-mine solution through the substrates reduced acidity and metal concentrations to a greater extent than did surface flow, possibly due to increased residence time and interaction with the substrates or redox differences between surface and subsurface layers of the substrate. Subsurface effluent pH values rose above 6.0 for the pine needle, hay, and Sphagnum mixtures, and above 5.0 for the peat mixtures, but remained at the 3.0 to 3.5 level for the mine spoil material (Fig. 1b). As a result of the elevated pH, most subsurface effluents contained much lower levels of total Al than their surface counterparts. All substrates, except the mine-spoil, reduced Al in subsurface effluents by 71 to 99% of influent concentrations (Table 1). Total Fe concentrations of subsurface effluents were generally higher than those measured in surface effluents and in the cases of pine needle, hay and mine spoil substrates, in excess of influent levels. Iron removal

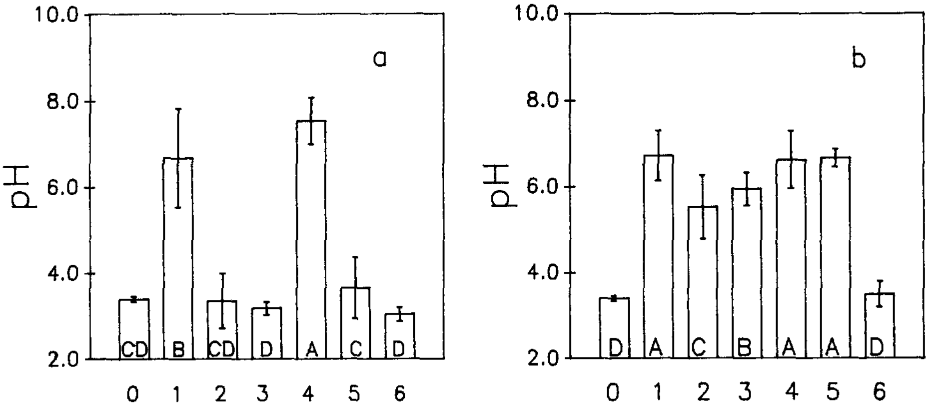

Figure 1. Mean values ± SD of surface effluent (a) and subsurface effluent (b) pH concentrations for the five months of treatment and mean separation by Duncan's multiple range test ($P = 0.05$). Bars containing the same letter are not statistically different. (0 = untreated influent; 1 = pine needle-surface soil mixture; 2 = peat subsoil; 3 = peat-surface soil; 4 = hay-surface soil; 5 = sphagnum-surface soil and 6 = spoil-peat-surface soil).

efficiencies fluctuated within treatments during the experiment. The negative efficiencies calculated for the pine needle and hay substrates, which also showed the highest variability in Fe content, are probably due to resolubilization of precipitated Fe under the prevailing reducing conditions and subsequent complexation by organic functional groups active in the substrate. All subsurface effluents showed higher total Mn concentrations than those of influents, and subsequently negative removal efficiencies, probably due to solubilization of Mn-concretions originally present in the soil mixture or due to resolubilization of secondary Mn retained by the substrates. Compared to surface effluents, all subsurface effluents showed further reductions in the levels of Cu (92-98%) (Table 1). The removal efficiencies for Zn also improved considerably in the subsurface flow of most substrates (80-98%) except the mine spoil mixture for which they remained very low (15%).

Because organically complexed metal species are generally considered less bioavailable than free metal ions or inorganic ion pairs, a partition of organic vs inorganic metal species was sought in surface and subsurface effluents. Although the large variability associated with these measurements did not always allow clear separation of the treatment means, the hay and pine needle substrates generally showed greater organic metal complexation affinities (except for Mn) than other substrates. For these two substrates, the organic metal complexation affinity sequence in surface effluents followed the order Cu > Al > Fe > Zn > Mn. With some exceptions, the percentages of organically bound metals in subsurface effluents increased substantially over those observed in surface flows for most treatments. Copper showed the highest overall tendency for complexation, followed by Al, Fe and Zn. Organically bound Mn

Table 1. Percent metal removal efficiencies {means of (influent-effluent)/(influent)} of different substrates for surface (s) and subsurface (b) effluents and mean separation by Duncan's multiple range test (P=0.05). Values followed by the same letter are not statistically different.

Substrate	Effluent	Al, %	Fe, %	Mn, %	Cu, %	Zn, %
Pine needle & surface soil	s	83 ± 30 (A)	60 ± 54 (A)	- 58 ± 93 (B)	79 ± 29 (A)	75 ± 30 (A)
	b	93 ± 13 (A)	-565 ± 759 (B)	-696 ± 910 (C)	98 ± 2 (A)	98 ± 2 (A)
Peat & subsoil	s	10 ± 38 (CD)	65 ± 29 (A)	- 46 ± 55 (B)	2 ± 26 (D)	7 ± 27 (BC)
	b	89 ± 21 (AB)	93 ± 15 (A)	-307 ± 129 (AB)	92 ± 12 (C)	80 ± 26 (B)
Peat & surface soil	s	24 ± 14 (BC)	62 ± 37 (A)	- 28 ± 34 (AB)	27 ± 10 (BC)	18 ± 5 (B)
	b	96 ± 7 (A)	45 ± 56 (A)	-245 ± 145 (A)	97 ± 4 (AB)	93 ± 8 (A)
Hay & surface soil	s	93 ± 6 (A)	71 ± 23 (A)	16 ± 75 (A)	89 ± 9 (A)	90 ± 7 (A)
	b	71 ± 77 (B)	-400 ± 657 (B)	-584 ± 837 (BC)	98 ± 1 (A)	97 ± 5 (A)
Sphagnum & surface soil	s	39 ± 30 (B)	66 ± 51 (A)	- 46 ± 29 (B)	38 ± 28 (B)	16 ± 31 (B)
	b	99 ± 2 (A)	23 ± 62 (A)	-286 ± 207 (AB)	98 ± 1 (A)	98 ± 2 (A)
Peat & mine spoil & surface soil	s	-4 ± 53 (D)	31 ± 68 (B)	- 58 ± 143 (B)	22 ± 39 (C)	-3 ± 47 (C)
	b	39 ± 20 (C)	-30 ± 108 (A)	-241 ± 170 (A)	95 ± 5 (BC)	15 ± 18 (C)

remained very low as in surface effluents, and showed the least affinity for complexation. There were no statistical differences by treatment for organic complexation of Al, Fe, Mn and Zn, but hay and pine needles showed some superiority in organically complexing Cu.

MINTEQA2 speciations showed that with the exception of the high pH surface effluents of the pine-needle and hay mixtures, which were dominated by Al(OH) species, soluble Al was generally distributed evenly among Al^{3+}, $AlSO_4^{+}$, $Al(OH)_3^{\circ}$, $Al(OH)_4^{-}$, or $Al(OH)_2^{+}$ species. Soluble Fe was primarily present as Fe^{2+} or $Fe(OH)_2^{+}$ species, although some substrates contained small to moderate amounts of $FeSO_4^{+}$, $FeSO_4$ aq., $Fe(OH)_3^{\circ}$, $Fe(OH)_2^{+}$, or $Fe(OH)_4^{-}$ species. The majority of Mn was present in the Mn^{2+} form, with small amounts of $MnSO_4$ aq. present in the peat, Sphagnum and spoil mixtures. Soluble Zn followed distribution patterns similar to that of Mn. Except for the pine needle and hay mixtures in which $Cu(OH)_2$ aq. species predominated, Cu^{2+}, and to a lesser extent $CuSO_4$ aq., were the most common species in all other effluents.

Following the completion of the leaching stage of the experiment, substrate samples were extracted to determine the forms in which metal species were retained. Residual 1 (sulfates, sulfides, carbonates) and residual 2 (oxyhydroxides) forms dominated the retention process for every metal except Cu, for which organic forms were dominant (Fig. 2). Organic complexes also constituted a substantial portion of the Al forms (12-22%). The highest amounts of residual Al (75-85%) were retained in the peat-subsoil, Sphagnum, and spoil substrates (Fig. 2); intermediate amounts were retained by the peat-surface soil mixture and hay, while the pine-needle mixture contained the lowest residual Al forms (~ 50%). With the exception of the peat-subsoil substrate, where the oxyhydroxide metal forms dominated, the residual forms were split almost evenly between residual 1 and residual 2 components. Organic Al forms were similarly distributed in all substrates (12-22%), while sorbed Al species were prominent only in the pine-needle, the peat-surface soil and the lower depth of the hay substrate in the range of 26 to 42%. Measurable concentrations of exchangeable Al species were present only in the spoil substrate. More than 90% of the Fe in all substrates was retained in residual forms (Fig. 2). Oxyhydroxides were by far the most dominant form of retention. The pine-needle and hay substrates showed a higher affinity than the other substrates for retention as carbonate, sulfate, and organic forms. Sorbed and exchangeable Fe species were absent. In general, zinc retention showed trends similar to Fe, with more than 85% of the retained Zn forms being residual in all substrates (Fig. 2). Again the pine-needle and hay substrates, but only at the 0-5 cm depth, showed somewhat stronger preference than other substrates to form residual 1 (probably sulfate or sulfide) or organic species. Sorbed forms of Zn were negligible, but more than 5% exchangeable species were present in the spoil substrate and the lower depths (5-15 cm) of the peat substrates. The majority of Mn was also retained in residual forms (55-97%). Substantial amounts of exchangeable Mn, however, were present in most substrates (Fig. 2). The spoil and sphagnum substrates contained the highest amounts of exchangeable (25-35%) and sorbed (5-12%) Mn. Smaller amounts of exchangeable, organic and sorbed Mn were also present in other substrates. The pine needle and hay substrates again showed greater affinity than other substrates for sulfate or sulfide

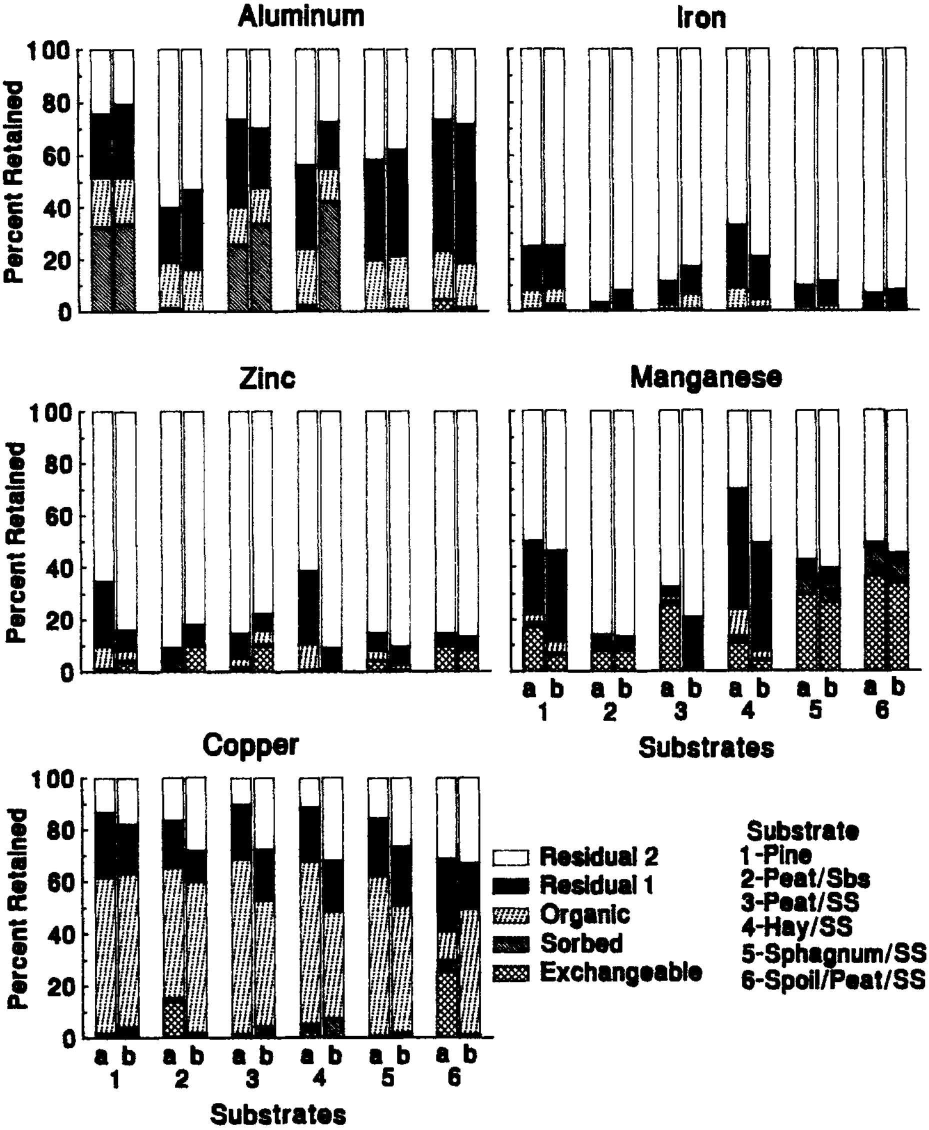

Figure 2. Distribution of Al, Fe, Mn, Cu, and Zn retained by the substrate in exchangeable, sorbed, organic,residual1 (sulfates, sulfides, carbonates, and residual 2 (oxyhydroxides) forms at the end of the experiment (ss = surface soil; sbs = subsoil).

retention. Copper was the only metal retained mainly in organic forms by most substrates (except for the 0-5 cm depth of the spoil mixture). Residual Cu species were also abundant in all substrates and were distributed evenly between residual 1 and 2 forms (Fig. 2). Minor amounts of sorbed Cu species were present in some substrates. Only the upper depths of the peat-surface and the spoil substrates retained substantial amounts of exchangeable species.

Based on MINTEQA2-calculated mineral saturation indices, alunite ($KAl_3SO_4(OH)_6$), gibbsite, boehmite, diaspore and hercynite ($Fe^{2+}Al_2O_4$) are the most likely Al bearing minerals to precipitate in most substrates. Jurbanite ($AlOHSO_4$) precipitation could also occur in the peat and spoil substrates, while basaluminite ($Al_4(OH)_{10}SO_4$) precipitates are likely in the pine-needle, hay and Sphagnum substrates. All effluents showed supersaturation with respect to goethite, hematite, lepidocrocite and jarosite, hence these minerals may potentially precipitate in all substrates. Ferrihydrite and $Fe_3(OH)_8$ could precipitate only in the pine-needle, hay, and Sphagnum substrates. Wustite (FeO) also showed a positive saturation index in the pine-needle and hay effluents. Cupriferrite ($CuFe_2^{3+}O_4$) and to a lesser extent tenorite (CuO) were the two Cu-bearing minerals most likely to precipitate in most substrates. Only the lower depths of the peat and sphagnum substrates showed supersaturation with respect to gypsum. None of the Mn or Zn minerals considered by the MINTEQA2 data base showed positive saturation indices. All metal carbonate species also appeared to be unstable.

In conclusion, the findings of this experiment suggest significant differences among substrates in their ability to buffer mine drainage acidity and in metal removal efficiency. Of the six substrates tested, the pine-needle and hay mixtures were the most efficient in reducing acidity and Al, Cu and Zn concentrations in surface effluents. The majority of the substrates (except the mine spoil-surface soil) showed similar efficiencies for Fe removal, but all substrates were a source rather than a sink for Mn in surface and subsurface flows. The increased residence time and interaction of the simulated AMD with the substrate material attained in the subsurface flow improved the efficiency of the peat and sphagnum substrates in reducing acidity, Al, Cu and Zn to the levels found in the surface effluents of the hay and pine-needle substrates. The greater affinity of the latter substrates to form soluble organic metal complexes under the more prevalent reducing conditons in the lower substrate caused them to act as sources of Fe in the subsurface flows. The overall superior performance of the hay and pine needle substrates may be attributed to their higher Ca and Mg content, which contributes to their buffering capacity, and to greater concentrations of fulvic acids which are enriched in O-functional groups that enchance metal-organic interactions. The high affinity of these two substrates to form soluble organic metal complexes, which are not as bioavailable as the inorganic free metal species to plants growing in the wetland, is an additional advantage that allows them to continue functioning well even under very low pH and high Al and Cu conditions. Finally, the distribution of metal forms retained by the substrates indicates that the dominant mechanism for metal attenuation in all substrates is precipitation, primarily in hydroxyoxide and secondarily in sulfate or sulfide forms. For most metals little attenuation occurred in exchangeable, sorbed, or organic forms, but Cu showed equal or greater preference for organic than for residual retention. These processes apparently are important only for a short time during the early stages of treatment. After they reach their plateaus, precipitation processes appear to dominate. This can be accomplished either by full saturation of all available sites or by blockage of the remaining sites by precipitate coatings. The speciation chemistry and retention patterns of field-constructed wetland ecosystems used for acid mine drainage treatment are likely to follow the trends observed in short-term greenhouse experiments, but long-term effects and efficiency predictions should be cautiously evaluated on the basis of extensive operation and testing under field conditions.

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REFERENCES

Allison JD, Brown DS, Novo-Gradac KJ (1990) MINTEQA2/PRODEFA2, a geochemical assessment model for environmental systems: Version 3.0 users manual. Environmental Research Laboratory, U.S. E.P.A., Athens, GA 30613, 106 p

Brodie GA, Hammer DA, Tomljanovich DA (1988) Constructed wetlands for acid drainage control in the Tennessee Valley. Proc Mine Drainage and Surf Mine Reclam 1, 325. Bur Mines Inf Cir 9183

Campbell PGC, Bisson M, Bougie R, Tessier A, Villeneve JP (1983) Speciation of aluminum in acidic freshwaters. Anal Chem **55**:2246-2252

Emmerich WE, Lund LJ, Page AL, Chang, AC (1982) Solid phase forms of heavy metals in sewage sludge treated soils. J Environ Qual **11**:178-181

Erickson PM, Girts MA, Kleinmann RLP (1987) Use of constructed wetlands to treat coal mine drainage. Proc. National Western Mining Conference, Denver, CO, February 11-13, 1987

Florence TM (1983) Trace element speciation and aquatic toxicology. Trends Anal Chem **2**:162-166

Hammer DA (ed) (1989) Constructed Wetlands for Wastewater Treatment, Municipal, Industrial, Agricultural. Lewis Publishers, Inc. Chelsea, MI

Hargrove WL, Thomas GW (1981) Effect of organic matter on exchangeable aluminum and plant growth in acid soils. p. 151, In: Baker DE (ed) Chemistry in the soil environment, ASA Special Publ. No. 40

Henrot J, Wieder RK (1990) Processes of iron and manganese retention in laboratory peat microcosms subjected to acid mine drainage. J Environ Qual **19**:312-320

Kleinmann RLP (1985) Treatment of acid mine water by wetlands. In: Control of acid mine drainage. Bureau of Mines, Internal Circular 9027

Lake DL, Kirk PW, Lester JN (1984) Fractionation characterization and speciation of heavy metals in sewage sludge and sludge-amended soils: a review. J Environ Qual **13**: 175-183

Page AL, Miller RH, Keeney, DR (eds) (1982) Methods of soil analysis: Chemical and microbiological properties. 2nd edition. Agron No. 9. Am Soc Agron, Madison, WI

Plankey BJ, and Patterson HH (1987) Kinetics of aluminum fulvic acid complexation in acidic waters. Environ Sci Technol **21**:595-601

Stark LR, Stevens SE Jr, Webster HJ, Wenerick WR (1990) Iron loading, efficiency and sizing in a constructed wetland receiving mine drainage. In: Skousen J, Sencindiver J, Samuel D (eds) Proceeedings of the 1990 Mining and Reclamation Conference and Exhibition, April 23-26, 1990, Charleston, WV, Volume II, p. 393

Stevenson FJ, Fitch A (1986) Chemistry of complexation of metal ions with soil solution organics. p. 29. In: Huang PM, Schnitzer M (eds) Interactions of soil minerals with natural organics and microbes. SSSA Special Publ No 17

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