2. Tilton, D. L., and R. H. Kadlec. "The Utilization of a Freshwater Wetland for Nutrient Removal from Secondarily Treated Wastewater Effluent," *J. Environ. Qual.* 8:328–334 (1979).
3. Peverly, J. H. "Stream Transport of Nutrients Through a Wetland," *J. Environ. Qual.* 11:469–472 (1982).
4. Simpson, R. L., R. E. Good, R. Walker, and B. R. Frasco. "The Role of Delaware River Freshwater Tidal Wetlands in the Retention of Nutrients and Heavy Metals," *J. Environ. Qual.* 12:41–48 (1983).
5. Jenkins, M. C., and W. M. Kemp. "The Coupling of Nitrification and Denitrification in Two Estuarine Sediments," *Limnol. Oceanog.* 29:609–619 (1984).
6. Patrick, W. H., and K. R. Reddy. "Nitrification-Denitrification in Flooded Soils and Water Bottoms: Dependence on Oxygen Supply and Ammonium Diffusion," *J. Environ. Qual.* 5:469–472 (1976).
7. Coultas, C. L., and M. J. Duever. "Soils of Cypress Swamps," in *Cypress Swamps,* H. T. Odum and K. C. Ewell, Eds. (Gainesville: University of Florida Press, 1984).
8. Coultas, C. L. "Soils of the Apalachicola National Forest Wetlands, Part 2, Cypress and Gun Swamps," *Soil and Crop Sci. Soc. of Fla. Proc.* 37:154–159 (1977).
9. Jackson, M. L. *Soil Chemical Analysis* (Englewood Cliffs, NJ: Prentice Hall, Inc., 1958).
10. "Technicon AutoAnalyzer II, Method No. 696-82W. Total Kjeldahl Nitrogen," Technicon Industrial Systems, Tarrytown, NY (1979).
11. "Technicon AutoAnalyzer II, Method No. 696-82W. Total Phosphorus," Technicon Industrial Systems, Tarrytown, NY (1979).
12. "Technicon AutoAnalyzer II, Method No. 696-82W. Ammonia in Water and Wastewater," Technicon Industrial Systems, Tarrytown, NY (1979).
13. Gosselink, J. G., and R. Hatton. "Relationship of Organic Carbon and Mineral Content to Bulk Density in Louisiana Marsh Soils," *Soil Sci.* 137:177–180 (1984).
14. Henriksen, K. "Measurement of In Situ Rates of Nitrification in Sediment," *Microb. Ecology* 6:329–337 (1980).
15. Focht, D. D. "The Effect of Temperature, pH and Aeration on the Production of Nitrous Oxide and Gaseous Nitrogen—a Zero-Order Kinetic Model," *Soil Sci.* 118:173–179 (1974).
16. Focht, D. D., and W. Verstraete. "Biochemical Ecology of Nitrification and Denitrification," in *Advances in Microbial Ecology,* M. Alexander, Ed. (New York: Plenum Press, 1977), pp. 135–214.
17. Schmidt, E. L. "Nitrification in Soil," in *Nitrogen in Agricultural Soils,* F. J. Stevenson, Ed., Agronomy Monograph No. 22 (1982), pp. 253–288.

CHAPTER 38

Efficiencies of Substrates, Vegetation, Water Levels and Microbial Populations

38a Relative Radial Oxygen Loss in Five Wetland Plants

Susan Copeland Michaud and Curtis J. Richardson

INTRODUCTION

Drainage from coal mining and washing facilities frequently has a pH as low as 2.2, high concentrations of dissolved metals such as iron (Fe) and manganese (Mn), and high suspended solid concentrations.[1,2] Precipitation that falls on coal piles and slurries leaches ions from the exposed rock.[3] These pollutants contaminate streams where hydrogen and metal ions reduce water quality and inhibit plant and animal life.[4,5]

Recently, it has been demonstrated that constructed wetlands can be used to improve water quality.[2,6] The combination of chemical, hydrological, and biological processes in wetlands are able to precipitate Fe, Mn, and other metal ions, to reduce suspended solids and moderate pH.[7,8] Constructed wetlands can be built to mimic biogeochemical characteristics of natural wetlands that perform these functions.

Oxygen (O_2) diffusion to root tips is one of the physiological characteristics that permit wetland species to exist in flooded conditions.[9,10] Diffused O_2 not only supplies the roots but can oxidize the surrounding soil; this process is termed radial oxygen loss (ROL).[11,12] Increasing evidence suggests that O_2 transport provides a unique environment that can oxidize phytotoxic compounds such as ferrous iron.[12–14] This characteristic could be used to mitigate the concentration of toxic compounds in mining-related effluent. Theoretically, plants with the largest oxygenated rhizosphere and largest population of metal-oxidizing microbes would maximize the wetland's potential to remove toxic metals from the water column by oxidation. This research investigates the ability of five wetland plant species to diffuse O_2 into wetland soils.

Objectives of this study were (1) to develop a method to test for the presence of O_2 diffusion from plants' roots, (2) to determine species differences in O_2 loss to sediments and (3) to recommend plant species to use in constructed wetlands to maximize O_2 concentrations in the root zone.

MATERIALS AND METHODS

Plant Selection

Plants were obtained from a constructed wetland, TVA's Impoundment One, which receives effluent from a slurry pond. Total Fe concentration averaged 30 mg/L, Mn often exceeded 10 mg/L, dissolved oxygen (DO) was less than 2 mg/L, pH was 6.0, and suspended solids exceeded 98 mg/L.[2] Cattail (*Typha latifolia*), burreed (*Sparganium americanum*), spikerush (*Eleocharis quadrangulata*), woolgrass (*Scirpus cyperinus*), and rush (*Juncus effusus*), perennial emergent wetland plants, were selected because they were well established, healthy, and reproducing. Mature plants were collected during July and August 1987 from the third and fourth ponds of Impoundment One. *T. latifolia* and *S. americanum* grow in shallow to deep water (0.30 to 1.20 m). *E. quadrangulata* and *J. effusus* grow in shallower water (0.15 to 0.30 m), while *S. cyperinus* grows in the shallowest water (0.05 to 0.30 m).

All five species were tested with 20 replicates each. A control without plants tested the differences in DO due to O_2 diffusion down aerenchymal tissue and atmospheric diffusion. Dead plants and cut-off plants covered with paraffin to seal aerenchymal tissue were also tested to determine if O_2 presence was due to diffusion or plant enzymes.

Laboratory Procedure

To detect oxygen, we used an indigo carmine dye technique developed by Armstrong[15] for detecting ROL in some bog plant species. We deoxygenated deionized water with nitrogen gas. One-liter bottles were filled with 500 mL of deoxygenated water. Hydrogen sulfide (H_2S) saturated water (5 mL) was added to the deoxygenated solution to reduce the solution Eh below −250 mV. Five milliliters of 0.28% (weight/volume) indigo carmine dye was added to the deoxygenated solution.

The test plants' roots were carefully cleaned to remove sediment and decaying plant matter and placed in the reduced solution. Mineral oil was added to the surface to prevent O_2 from diffusing into the solution. At 6, 12, and 24 hours (h), samples were removed for spectrophotometric analysis to measure the percentage of light transmitted through the samples. Readings at 6, 12, and 24 h in conjunction with the plants' dry mass were used to determine differences in O_2 diffusion into solution. To reduce variation due to plant size, we developed a weighted index by dividing the spectrophotometer reading by the plants' total mass (% transmittance/total plant biomass = ROL index).

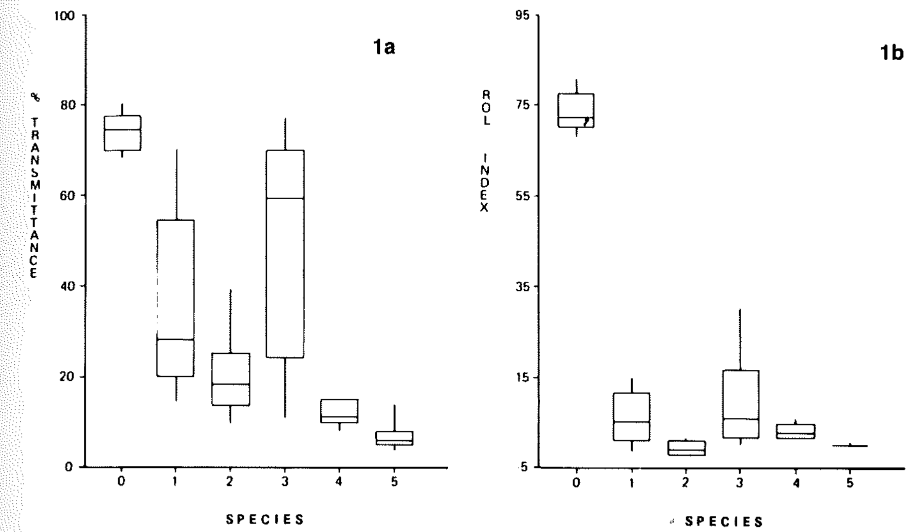

Figure 1. (1a) is a box plot of the percent light transmitted through a solution containing an oxygen-indicating dye for the control and five wetland plants tested for ROL after 12 h. Lower numbers on the y axis indicate a more oxidized solution. (1b) uses the ROL index, which divides the percent transmittance by dry weight after 12 h. 0 = control; 1 = *E. quadrangulata*; 2 = *J. effusus*; 3 = *S. cyperinus*; 4 = *S. americanum*; 5 = *T. latifolia*.

RESULTS

Transmittance taken during the experiment ranged from 80% to 7%. Controls had readings greater than 70% and only a small percentage of the dye had been oxidized, but complete dye oxidation with H_2O_2 permitted 7% light transmittance.

Figure 1a is a box plot showing relationships between species after 12 h. There is clearly a difference between controls and samples with plants. *S. cyperinus*, although statistically different from the control, did not diffuse as much O_2 into the sediments as other species tested.

Much of the variability in Figure 1a was removed by using mass as a scaling factor. Figure 1b shows relationships when transmittance readings are divided by the individual's total dry weight. Variation in *S. cyperinus* values was substantially greater than in other species.

Differences in transmittance for each plant and controls demonstrates an input of O_2 by the plants (Table 1). Experiments with cut and waxed plants had significantly higher readings than those for live, intact plants. However, waxed plant treatments did have more O_2 present than controls. This is likely due to some O_2 storage in the roots and subsequent diffusion into solution. Dead plants tested were able to transmit a significant amount of O_2 into solution but were not as efficient as live plants. For example, the mean value for dead *J. effusus* plants at six hours was 53.5; live *J. effusus* had a mean reading of 30.5

Table 1. Mean ROL from Five Wetland Plant Species Collected from a Constructed Wetland used for Coal Mine Drainage Abatement Tested with Indigo Carmine Dye as an Oxygen Indicator

		Percent Light Transmitted		
	n	6h	12h	24h
Control	15	73.8 (3.3)	73.2 (3.8)	71.1 (6.7)
Live				
E. quadrangulata	20	53.9 (15.8)	35.4 (19.2)	17.8 (2.7)
J. effusus	20	30.5 (18.9)	21.2 (10.8)	17.2 (2.2)
S. cyperinus	20	56.7 (20.3)	48.8 (23.6)	38.1 (20.8)
S. americanum	20	34.8 (20.3)	16.0 (14.6)	15.2 (4.7)
T. latifolia	20	43.2 (5.0)	27.2 (3.8)	18.1 (0.9)
Waxed				
E. quadrangulata	2	73.5	72.0	61.5
J. effusus	2	65.0	68.5	47.5
S. cyperinus	2	66.0	60.5	56.0
S. americanum	3	65.0	59.7	45.3
T. latifolia	2	61.5	58.5	45.5
Dead				
E. quadrangulata	2	67.0	67.0	30.0
J. effusus	2	53.5	31.5	23.5
S. cyperinus	—	—	—	—
S. americanum	3	36.0	31.0	21.0
T. latifolia	2	32.5	17.0	20.5
Live[a]				
E. quadrangulata	20	9.3 (4.8)	6.3 (3.9)	3.2 (1.4)
J. effusus	20	4.0 (2.1)	2.6 (4.04)	1.7 (1.4)
S. cyperinus	20	12.7 (7.9)	11.2 (12.2)	8.77 (10.1)
S. americanum	20	6.9 (6.8)	3.0 (3.5)	2.7 (1.7)
T. latifolia	20	0.04 (0.02)	0.02 (0.005)	0.01 (0.007)

Note: Lower percent transmittance corresponds to higher ROL by the plants. Live, waxed, and dead individuals from each species were tested. (X.X = S.D.)
[a]Transmittance/total plant dry weight; S.D. in parenthesis.

for the same time. Sample size for dead plants was small, and a definitive statement that dead plants have less ROL was not possible.

Since preliminary tests revealed these data are not normally distributed, we used the nonparametric Kruskal-Wallis One-Way Analysis to rank each observation.[16] The test statistic for readings at 6 h was 49.68 at a significance of 4.22^{-10} ($p = 0.05$). The high test statistic and small significance level indicate that the 6-h readings differ significantly among species. The values for 12 and 24 h were significant as well (Table 2). *T. latifolia* transferred more O_2 to solution than any other species at all times. Pairwise analysis for unequal sample sizes[17] revealed that *J. effusus* and *S. americanum* were more efficient than *S. cyperinus* at 6 h. *S. americanum* transferred more O_2 than *S. cyperinus* at 12 and 24 h.

The Kruskal-Wallis analysis for species differences corrected for differences in biomass and showed significant species differences (Table 2). *T. latifolia* transferred the most O_2 at all three sampling times. *J. effusus* was better at transferring O_2 than *S. cyperinus* at 6 and 24 h.

Table 2. Percent Light Transmitted for Radial Oxygen Loss in Five Wetland Plant Species Ranked by the Kruskal-Wallis Method

	Average Rankings, Unadjusted for Biomass[a]		
	6 h	12 h	24 h
E. quadrangulata	69.35	59.33	55.65
J. effusus	44.40	44.44	53.79
S. cyperinus	74.05	65.41	75.28
S. americanum	47.90	31.95	39.88
T. latifolia	16.8	12.98	11.48
Test statistic	49.68	54.22	62.10
Significance level	4.22^{-10}	4.74^{-11}	1.05^{-12}

	Average Rankings, Adjusted for Biomass[b]		
	6 h	12 h	24 h
E. quadrangulata	72.00	61.89	63.23
J. effusus	42.45	39.74	40.21
S. cyperinus	70.25	61.75	69.75
S. americanum	57.50	45.73	54.35
T. latifolia	10.50	10.50	10.50
Test statistic	61.05	56.19	59.40
Significance level	1.74^{-12}	1.82^{-11}	3.87^{-12}

[a]*S. cyperinus* ranked highest for all three periods and *T. latifolia* ranked lowest. Higher ranks indicate less oxygen was transferred to solution than lower ranks.
[b]Percent light transmittance divided by the total dry weight. *E. quadrangulata* was highest at 6 and 12 h, while *S. cyperinus* was highest at 24 h. *T. latifolia* ranked lowest at all three times.

DISCUSSION

The role of dissolved oxygen in wetlands used for treating mining-related effluent is important in improving water quality. Oxygen is required for the removal of dissolved metals, to support aerobic microorganisms, and to reduce BOD_5. Results of this experiment indicate that O_2 concentration in the soil-water matrix can vary with plant species. By planting and managing for specific plants to maximize O_2 concentration, toxic metals can be oxidized more quickly and efficiently.

Our ROL experiments have shown the following trends on a per unit biomass basis:

T. latifolia > *J. effusus* > *S. americanum* > *E. quadrangulata* > *S. cyperinus*

However, individual growth characteristics, such as stand density, need to be considered when comparing ROL performance as a management tool.

J. effusus performed better in terms of ROL than *S. cyperinus* per unit biomass. Because both species grow in shallow water, planting *J. effusus* instead of *S. cyperinus* will result in greater O_2 contribution to the substrate. *T.*

latifolia and *S. americanum* both grow in deep water. *T. latifolia* consistently contributed more O_2, but *S. americanum* grows in much denser stands. Oxygen transfer per unit of substrate must be investigated before recommending which to plant. Because *E. quadrangulata* did not transfer O_2 as well as *J. effusus, S. americanum,* or *T. latifolia,* it might be advantageous to minimize the area of depth where *E. quadrangulata* grows (0.15–0.60 m).

Oxygen transport varied significantly in the emergent wetland macrophytes evaluated, suggesting that plant species can influence the amount of O_2 in the sediments. The importance of O_2 in the water column and sediments and the poor understanding of plant-oxygen transport mechanisms warrant further research into oxygen transfer per unit area and effects of oxygen transport on BOD_5 and metal oxidation. This study points toward the continued research into constructed wetlands and their potential contribution in the remediation of contaminated water.

REFERENCES

1. Mitsch, W. J., M. A. Cardamone, J. R. Taylor, and P. L. Hill, Jr. "Wetlands and Water Quality Management in the Eastern Interior Coal Basin," in *Wetlands and Water Management on Mined Lands,* R. P. Brooks, Ed. (University Park, PA: Pennsylvania State University, 1985).
2. Brodie, G. A., D. A. Hammer, and D. A. Tomljanovich. "Man-Made Wetlands for Acid Mine Drainage Control," Eighth Annual National Abandoned Mine Lands Conference, Billings, MT (1986).
3. Forstner, U., and G. Wittman. *Metal Pollution in the Aquatic Environment* (New York: Springer-Verlag, 1981).
4. Parsons, J. W. "A Biological Approach to the Study and Control of Acid Mine Pollution," *J. Tn. Acad. Sci.* 24(4):304–310.
5. Riley, C. V. "The Ecology of Water Areas Associated with Coal Strip-Mined Lands in Ohio," *Ohio J. Sci.* 60(2):196–121 (1960).
6. Richardson, C. J., and J. A. Davis. "Natural and Artificial Wetland Ecosystems: Ecological Opportunities and Limitation," in *Aquatic Plants for Water Treatment and Resource Recovery,* K. R. Reddy and W. H. Smith, Eds. (Orlando, FL: Magnolia Publishing Inc., 1987), pp. 819–854.
7. Stone, R. W. "The Presence of Iron- and Manganese-Oxidizing Bacteria in Natural and Simulated Bogs," in *Treatment of Mine Drainage by Wetlands,* J. E. Burris, Ed. (University Park, PA: Pennsylvania State University, 1984), pp. 30–36.
8. Barlett, R. J. "Iron Oxidation Proximate to Plant Roots," *Soil Sci.* 92:6 (1961).
9. Armstrong, W. "Aeration in Higher Plants," *Adv. Bot. Res.* 7:225–332 (1980).
10. Sculthorpe, C. D. *The Biology of Aquatic Vascular Plants* (London: Edward Arnold Publishers Ltd., 1967), pp. 60–65.
11. Teal, J. M., and J. W. Kanwisher. "Gas Transport in the Marsh Grass *Spartina alterniflora,*" *J. Exp. Bot.* 17:355–361 (1966).
12. Armstrong, W. "Root Aeration in the Wetland Condition," in *Plant Life in Anaerobic Environments,* D. D. Hook and R. M. M. Crawford, Eds. (Ann Arbor, MI: Ann Arbor Science Publishers, Inc., 1978), pp. 264–297.

13. Sanderson, P. L., and W. Armstrong. "Soil Waterlogging, Root Rot and Conifer Windthrow: Oxygen Deficiency or Phytotoxicity?" *Plant and Soil* 49:185–190 (1978).
14. Moorhead, K. K., and K. R. Reddy. "Oxygen Transport Through Selected Aquatic Macrophytes," *J. Environ. Qual.* 17:138–142 (1988).
15. Armstrong, W. "The Oxidizing Activity on Roots in Waterlogged Soils," *Physiol. Plant.* 20:920–926 (1967).
16. STATGRAPHICS Statistical Graphics System, Rockville, MD (1986).
17. Hollander, M., and D. A. Wolfe. *Nonparametric Statistical Methods* (New York: John Wiley & Sons, Inc., 1973), p. 125.