Mine Water and the Environment (2004) 23: 44–53 © IMWA Springer-Verlag 2004

Technical Article

What Limits the Productivity of Acid Mine Drainage Treatment Ponds?

Jeffrey A. Simmons, Jonah M. Long, and Joshua W. Ray

Biology Dept, WV Wesleyan College, 59 College Ave, Buckhannon, WV, USA 26201; corresponding author's e-mail: simmons@wvwc.edu

Abstract. Acid mine drainage (AMD) treatment ponds are very common in the U.S. Appalachian coal region and are the main source of many headwater streams. Though the water that discharges from these ponds generally meets state and federal water quality standards, there is a distinct lack of productivity in most of these ponds. Our first objective was to compare the productivity of chemically-treated, biologically-treated, and untreated AMD ponds with uncontaminated (reference) ponds. Next, we used principal component analysis and multiple regression of 20 physicochemical characteristics of these ponds to resolve which factor(s) were responsible for inhibiting productivity. We discovered that chemically-treated AMD ponds and untreated AMD ponds exhibited significantly less gross primary productivity (GPP) than reference ponds; biologically-treated ponds (containing AMD that has passed through a wetland) did not vary significantly from reference ponds. Chemically-treated ponds also had significantly less net primary productivity (NPP) than reference ponds. Community respiration did not vary among the pond types. Our test results indicated that soluble reactive phosphate concentration explained most of the variance in both GPP and NPP. Apparently, phosphate availability, not metal toxicity, regulated phyto-plankton productivity in these ponds.

Key words: Heavy metals; mine drainage; phosphorus; phytoplankton; primary productivity

Introduction

There are literally hundreds of acid mine drainage (AMD) treatment ponds distributed throughout the Appalachian region of the U.S.A. Since the passage of the Surface Mining Reclamation and Control Act in 1977, construction of treatment ponds has been required at all coal mining operations where AMD occurs. AMD treatment ponds serve as holding tanks to allow mine operators to reduce the acidity and heavy metal concentrations before the water is discharged into streams. Although the water in many of these ponds has been successfully treated, visual inspection reveals that algal productivity remains low. So what limits primary productivity in AMD treatment ponds in which acidity and heavy metals have been reduced?

AMD treatment ponds are usually constructed in series, with the water quality improving with each successive stage of treatment. Most commonly, treatment consists of adding alkaline chemicals, such as lime ($CaCO_3$), anhydrous ammonia (NH_3), or sodium hydroxide (NaOH). Constructed wetlands have also been used successfully to reduce acidity and contaminant levels, particularly Fe, Mn, and Al (Hedin and Nairn 1993; Skousen et al. 1995; Karathanasis and Johnson 2003). Sulfate reduction and limestone dissolution in wetlands generate alkalinity that neutralizes acidity in mine drainage.

As the pH and redox potential of the mine drainage are increased, metals tend to form insoluble hydroxides, precipitate out of solution, and settle to the bottom of the pond. The slow-moving water and long residence times of treatment ponds and wetlands facilitates the precipitation of Fe, Mn, and Al hydroxides. At the last pond in the series where the water is discharged into a nearby stream, the water quality must meet permitted effluent standards, which usually include a circumneutral pH and low metal concentrations.

Theoretically, phytoplankton and aquatic macrophytes should thrive in the sunny, warm waters of these final treatment ponds during the growing season, but usually there is no visually discernible difference between these ponds and untreated AMD ponds. Apparently, some factor inhibits the growth of aquatic plants in these circumstances. This observation is troubling because it suggests that treated pond water may continue to prevent algal growth after it is discharged into streams, thereby reducing stream productivity and altering the stream carbon cycle. Considering that approximately 10% of the streams in Appalachia receive either treated or untreated AMD inputs, this is a potentially serious threat to our stream ecosystems (Herlihy et al. 1990). Although some pond discharges are quickly diluted upon entering a stream, in other cases, the pond is the sole or predominant source of a stream.

One possible reason for the apparent inhibition is that even though the heavy metal concentrations in pond water are low enough to meet state or federal water quality criteria (usually based on epidemiological and fish toxicity studies), they are high enough to be toxic to phytoplankton. Metals such as Cu, Pb, Al, and Hg can be phytotoxic, especially at low pH where metals are more soluble (Leland and Carter 1984; Crowder 1991; MacFarland et al. 1997). Studies have also shown that some metals have a greater toxicity toward algae at circumneutral pH like that encountered in treated AMD ponds (Hargreaves and Whitton 1976; Campbell and Stokes 1985; Bortnikova et al. 2001). Furthermore, it is possible that phytoplankton could be more sensitive to combinations of heavy metals in solution than to single heavy metals used in typical toxicity tests (Crowder 1991; Okamura and Aoyama 1994).

A second possible reason for the lack of productivity could be a lack of nutrients such as ammonium, nitrate, or phosphate. Phosphate is the nutrient that most commonly limits productivity in freshwater systems (Guildford and Hecky 2000) and this limitation could be exacerbated in AMD treatment ponds because phosphate co-precipitates with both Fe and Al hydroxides at higher pH values (Lijklema 1980; Olsson and Pettersson 1993; Kopáček et al 2000).

As a first step in addressing the question of productivity in AMD treatment ponds, we chose to conduct a field study to gauge the extent of the variability among the ponds and to narrow down the list of possible causal factors. Our objectives were to first determine if productivity was indeed lacking in AMD treatment ponds, then to determine which chemical factors were limiting primary productivity. We focused on the process (i.e., productivity) rather than which organisms were present because we were more interested in the production of organic carbon, which serves as the base of the food chain in heterotrophic surface water systems, than in the community structure of these ponds. There is an abundance of literature on the effects of heavy metals and AMD on individual species of aquatic organisms, but there are fewer studies that examine the ecosystem-level effects of AMD.

To accomplish our objectives, we measured productivity in 16 ponds that exhibited a wide range of AMD contamination and attempted to correlate it with a variety of physicochemical factors. Our first hypothesis was that net primary productivity (NPP), gross primary productivity (GPP), and community respiration (CR) would all be lower in untreated and treated AMD ponds than in reference ponds. Our second hypothesis was that the variance in NPP, GPP, and CR could be explained by one or more physicochemical factors.

Methods

Sampling Design

Our aim was to sample the range of AMD contamination in ponds to maximize the effectiveness of the correlation and regression analyses and to quantify the extent of variation in productivity and chemistry. We used a stratified-random design to select ponds from three categories initially: ponds receiving untreated AMD, ponds receiving treated AMD, and ponds with no AMD contamination (reference ponds). We began by generating a list of accessible ponds within Lewis and Upshur Counties in north-central West Virginia, USA, using 1:24,000 scale U.S. Geological Survey topographic maps. With the help of the WV Abandoned Mine Lands and Reclamation Office, we attempted to divide these 81 ponds into three categories: untreated, treated, and reference. Eight ponds could not be confidently assigned to a category and were omitted from further consideration. Next we randomly selected three untreated, ten treated and five reference ponds from the list. A larger number of treated ponds were selected because they were expected to show the widest degree of variability.

Property owners were contacted to gain permission for access to the ponds. Permission was obtained for three untreated, nine treated, and four reference ponds for a total of 16 ponds. In May 1999, we conducted a preliminary sampling of the ponds to confirm our categorization. Upon inspection of the sites and examination of the preliminary chemistry data, we decided to further subdivide the treated category into six chemically-treated (addition of $CaCO_3$, NaOH, or anhydrous NH_3) and three biologically-treated (passive treatment by artificial, surface-flow, aerobic wetlands dominated by *Typha*) AMD ponds.

It was necessary that water samples be processed the same day they were collected, so we were unable to sample all the ponds simultaneously. Instead we adopted a rotating schedule in which samples were collected from four of the 16 ponds each week from June through August 1999. Thus, we sampled each of the ponds three times during the study period.

Sample Collection

Four replicate, 1 L samples of pond water were collected between 8:00 and 11:00 am for productivity analysis from near the edge of each pond using a 2 m long sampling pole. At the same time, three replicate, 1 L samples were collected for chemical analysis. Dissolved oxygen, temperature and specific conductance were measured at 10 cm depth at three locations near the pond edge using portable meters (YSI™, Yellow Springs, OH, USA). Sampling locations on the pond edge were spaced at least 10 m apart. Samples were placed on ice in a cooler for transportation to the lab and were analyzed for productivity within six hours and for chemical parameters within 24 hours. Subsamples for dissolved metal analysis were filtered through acid-washed membrane filters (0.45 μm) within 24 hours, preserved with nitric acid, and refrigerated until analysis.

Sample Analysis

We measured phytoplankton productivity using the light-dark bottle technique. One dark and two clear biological-oxygen-demand (BOD) bottles were filled with a pond water sample. One light bottle (the initial) was "fixed" immediately by adding a series of chemicals that stops all biological processes and chemically fixes the dissolved oxygen so that it can be analyzed by titration at a later date (the Winkler method, APHA 1995). The other two bottles were incubated for 48 hours in an environmental chamber (25ºC and 12h light - 12h dark cycle). The two incubated bottles were then fixed as described above and all three bottles were analyzed for dissolved oxygen content by titration (APHA 1995). GPP, NPP, and CR were calculated as mg O_2 produced L^{-1} d^{-1}. CR was defined as the amount of oxygen in the dark bottle at the end of the incubation period minus the amount of oxygen in the initial bottle. NPP was calculated as the difference in the amount of oxygen between the clear bottle at the end of the incubation and the initial bottle. GPP was the difference between NPP and CR.

Chemical parameters were measured in three replicate samples from each pond. Sample pH was determined using a pH meter (Corning, Inc., Corning, NY, USA). Total acidity and total alkalinity were measured by titration of stirred samples to pH 8.3 and 4.5, respectively (APHA 1995). Total hardness was measured by titration with EDTA (APHA 1995). Nitrite-N, nitrate-N, and sulfate were determined by diazotization, cadmium reduction, and turbidometric methods, respectively, using a HACH™ DR-2000 spectrophotometer (HACH Co., Loveland, CO, USA). Ammonium was determined using the phenol nitroprusside method and the molybdate blue method was used to quantify the soluble reactive phosphate (SRP) concentration (APHA 1995). Filtered and acidified subsamples were analyzed for dissolved Fe, Al, Mn, and Zn using atomic absorption spectroscopy (APHA 1995). Labile Zn, Cd, Pb, and Cu were determined using stripping voltammetry on samples buffered at pH 4.5 (Florence 1982). Labile forms of metals were defined operationally by the stripping voltammetry procedure and include only the dissolved ionic forms and the most weakly bound forms of the metals.

Quality control procedures were employed throughout, including deionized water blanks, method blanks, duplicate samples, spiked samples, and both internal and external calibration samples. In general, if blanks, duplicates, or calibration check samples fell outside the 95% confidence limits, then the batch of samples was reanalyzed.

Statistical Methods

Data from each pond were pooled by replicate and date to yield a single "summer mean" value for each parameter that characterized the pond over the three-month study period. One-way analysis of variance (ANOVA) was employed to determine if there were differences in summer mean GPP, NPP, and CR among the four categories of ponds: reference, untreated, chemically-treated, and biologically-treated ponds. If the ANOVA was significant, then Fisher's least significant difference test was used to determine which categories were significantly different from each other.

Multivariate techniques like multiple regression and principal component (PC) analysis are powerful tools for relating ecological effects to mixtures of pollutants. They also highlight underlying patterns and clusters among multiple variables simultaneously. They do not prove cause and effect, but they nonetheless help researchers narrow down the list of potential causal factors, which is an important first step when dealing with complex mixtures of pollutants (see, for example, Pyle et al. 2001).

We used three methods to piece together a clear picture of the interactions between physicochemical variables and productivity. First, we used PC analysis on the physicochemical parameters to detect underlying trends among all of the variables. Second, we correlated the PCs against the physicochemical variables. Third, we used multiple regression analysis to determine the extent to which the physicochemical parameters contributed to the variability in the productivity variables (NPP, GPP, and CR). All statistical procedures were performed on SPSS 10.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Physical and Chemical Characteristics of Ponds

As intended, the ponds selected for this study exhibited a wide range of physical and chemical characteristics (Table 1). Ponds ranged in size from 204 to 2,360 m^2, but none were more than 3.5 m deep. NPP and GPP ranged from slightly negative values early in the summer to maximums of 6.78 and 5.79 mg O_2 L^{-1} d^{-1}, respectively. Microscopic examination of pond water showed that phytoplankton communities were dominated by Chlorophytes, with Chrysophytes and Cyanophytes occurring commonly. Because of the AMD in many of the ponds, maximum values of sulfate, acidity, dissolved metals and labile metals were elevated. For example, maximum dissolved Fe was 13.2, maximum dissolved Mn was 22.0, and maximum dissolved Al was 29.2 mg L^{-1}. Addition of alkaline chemicals, like $CaCO_3$, NaOH, and anhydrous ammonia (NH_3) to the chemically-treated ponds caused exceedingly high maximum values of alkalinity, hardness, and ammonium.

Productivity

Mean NPP varied from 0.102 in chemically-treated AMD ponds to 1.18 mg O_2 L^{-1} d^{-1} in reference ponds (Table 2). A one-way ANOVA followed by Fisher's least significant difference test showed that the difference between the chemically-treated AMD ponds and the reference ponds was significant ($p = 0.046$). NPP in untreated and biologically-treated ponds did not differ significantly from the reference ponds. Mean GPP paralleled NPP, ranging from 0.177 to 1.73 mg O_2 L^{-1} d^{-1}, with a significantly higher value in the reference ponds than in the untreated or chemically-treated ponds ($p = 0.005$). GPP in biologically-treated ponds did not differ from the reference ponds. CR rates did not vary significantly among the four pond types according to the ANOVA ($p = 0.224$; Table 2). Negative values for CR indicate that O_2 was consumed in the dark BOD bottles during the 48 hour incubation period. From these results, it is apparent that phytoplankton productivity in the chemically-treated ponds was much lower than in the reference ponds.

Principal Component Analysis

All of the physical and chemical parameters except pond area and depth (see Table 1 for list of parameters) were subjected to a PC analysis to elucidate the major patterns within the entire data set and to see which parameters were related to each other. The PC analysis groups together those parameters exhibiting similar trends and creates a surrogate trend line representing all of those parameters. The first three of these surrogate trend lines, or PCs, accounted for 81% of the variability among all of the factors. None of the remaining PCs accounted for more than 5% of the variability and so were excluded from the analysis. PC 1 alone explained

Table 1. Physical and chemical parameters of 16 ponds in Upshur and Lewis Counties, WV during June – August 1999; except for area and depth, the minimum and maximum values are from 48 samples (16 ponds x 3 dates)

	Units	Minimum	Maximum
GPP	(mg O_2 L^{-1} d^{-1})	-0.39	5.79
NPP	(mg O_2 L^{-1} d^{-1})	-1.40	6.78
CR	(mg O_2 L^{-1} d^{-1})	-1.76	3.82
Area	(m^2)	204	2,360
Max. Depth	(m)	1.4	3.5
Temperature	(C)	19.3	31.4
Dissolved Oxygen	(mg L^{-1})	1.61	11.1
Specific Conductance	(µS cm^{-1})	118	3,130
pH	(standard units)	3.00	10.07
Total Acidity	(mg $CaCO_3$ L^{-1})	0	309
Total Alkalinity	(mg $CaCO_3$ L^{-1})	0	614
Total Hardness	(mg $CaCO_3$ L^{-1})	88	1140
Nitrite-N	(mg L^{-1})	<0.002	3.73
Nitrate-N	(mg L^{-1})	0.142	6.29
Sulfate	(mg L^{-1})	<1.0	1,898
Ammonium-N	(mg L^{-1})	<0.005	240
Phosphate-P	(µg L^{-1})	<0.1	15.7
Fe, diss.	(mg L^{-1})	<0.1	13.2
Mn, diss.	(mg L^{-1})	<0.02	22.0
Al, diss.	(mg L^{-1})	<0.1	29.2
Zn, diss.	(mg L^{-1})	<0.01	5.96
Zn, labile	(µg L^{-1})	<1	303
Cd, labile	(µg L^{-1})	<2	13
Pb, labile	(µg L^{-1})	<2	110
Cu, labile	(µg L^{-1})	<2	69

Table 2. Mean (± standard deviation) net primary productivity (NPP), gross primary productivity (GPP) and community respiration (CR) of untreated, chemically-treated, and biologically-treated AMD ponds plus uncontaminated reference ponds in Upshur and Lewis counties, WV. Negative values mean that oxygen was consumed. The "summer mean" values for each pond in a category were averaged and N represents the number of ponds in each category.

	NPP* ($mg\ O_2\ L^{-1}\ d^{-1}$)	GPP ($mg\ O_2\ L^{-1}\ d^{-1}$)	CR ($mg\ O_2\ L^{-1}\ d^{-1}$)	N
Untreated	0.090^{ab} (± 1.11)	0.503^{ab} (± 1.05)	-0.405 (± 0.077)	3
Chemically-Treated	0.102^{a} (± 0.252)	0.177^{a} (± 0.357)	0.012 (± 0.360)	6
Biologically-Treated	1.06^{b} (± 0.105)	1.20^{bc} (± 0.270)	-0.168 (± 0.406)	3
Reference	1.18^{b} (± 0.904)	1.73^{c} (± 0.469)	-0.491 (± 0.498)	4
F value (3,13)	3.60	7.21	1.68	
p-value	0.046	0.005	0.224	

*Means in a column followed by different letters indicate significant differences according to Fishers least significant difference test ($p < 0.05$)

47% of the variance and was significantly correlated ($r > 0.50$; $p < 0.05$) with the parameters that represent the degree of AMD contamination. Specifically, PC1 was negatively correlated with pH and alkalinity and positively correlated with acidity, sulfate, labile Zn and dissolved Fe, Al, Mn, and Zn.

PC 2 explained 21% of the variance and was positively correlated with the nutrient ions nitrate, nitrite, and ammonium, and negatively correlated with soluble reactive phosphate ($r > 0.50$). The opposing trends between SRP and the nitrogen compounds were created by the type of treatment used in some of the ponds. Anhydrous ammonia was added to some of the chemically-treated ponds to raise the pH. As a result, ammonium, nitrate, and nitrite concentrations in these ponds were artificially elevated by as much as 10,000 times compared to the other ponds. SRP on the other hand exhibited a narrower range and tended to be higher in reference and biologically-treated ponds for reasons explained below.

PC 3 explained 13% of the variance. The only significant contributors to this vector were SRP and labile Cd. The remaining PCs improved the model by less than 6% each and so were excluded from the analysis.

Ordination plots of the PCs successfully clustered the four types of ponds (Figure 1a). The PC 1 axis (the AMD parameters) clearly separated the untreated ponds from the others and the PC 2 axis (the nutrient ions) differentiated the reference ponds. Plotting PC 2 versus PC 3 (SRP and Cd concentrations) clearly separated the two types of treatment ponds (Figure 1b). The four principal components were then correlated with GPP and NPP to see if any of them might be able to explain the variance in these biological indicators. Only PC 2 (nutrient ions) was significantly correlated with NPP and GPP ($r = -0.53$ and -0.65, respectively). The negative correlation of productivity with this proxy of nitrogen compound concentrations seems counterintuitive, but can be explained by the extremely high and potentially toxic levels of ammonia present (up to 240 mg NH_4-N L^{-1}) in some of the chemically-treated ponds. At the same time, this correlation implies a positive relationship with SRP concentration since phosphate was inversely correlated with PC 2. Surprisingly, PC 1, which represented the degree of AMD contamination, was not correlated with NPP or GPP.

Multiple Regression

The four principal components represent only generalized trends among groups of physicochemical parameters, so we also used stepwise multiple regression to determine which individual parameters could best explain the variance in NPP and GPP. For GPP, the first variable that the software program included in the model was SRP concentration (Table 3), indicating that it was the parameter that was most strongly correlated with GPP ($r^2 = 0.65$). No additional parameters were included in the model, meaning that the variance around the SRP regression line could not be adequately explained by any other variables. This result implies that a simple linear regression with SRP was the best model for predicting GPP (Figure 2).

For NPP, soluble reactive phosphate was again the first variable added ($r^2 = 0.69$). The addition of dissolved zinc to the model increased the predictive ability by 14% and adding alkalinity increased it another 6%. No other variables would have increased the accuracy of the model by more than 5%. Altogether, the three explained 89% of the variability.

Discussion

As expected, untreated AMD ponds had significantly lower GPP than unpolluted ponds, but surprisingly, NPP and CR were not significantly lower. The NPP

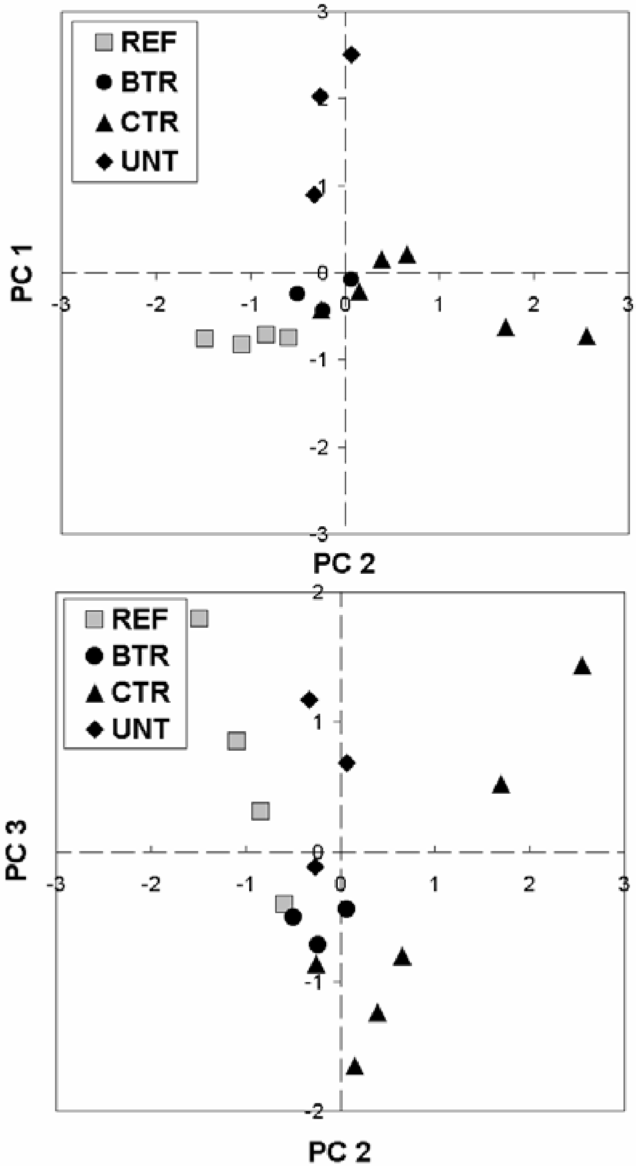

Figure 1. Ordination plots of (a) principal component (PC) 1 and 2, and (b) PC 2 and PC 3 of 20 physico-chemical parameters measured in ponds in central WV. Each point represents one of the 16 ponds. PC 1 was correlated with AMD parameters, PC 2 with nutrient ions, and PC 3 with soluble reactive phosphate and cadmium concentrations. REF = reference, BTR = biologically-treated, CTR = chemically-treated, and UNT = untreated ponds.

and GPP of chemically-treated ponds was also significantly lower, being about 10 to 15% of reference and biologically-treated ponds. The lack of productivity in these ponds was most likely caused by the harsh chemical environment. For example, untreated AMD ponds had mean pH values below 3.5 and metal concentrations often exceeding 5.0 mg L^{-1} and, although most of the chemically-treated AMD ponds had pH values near 7.0, they still exhibited elevated Mn concentrations (Table 4).

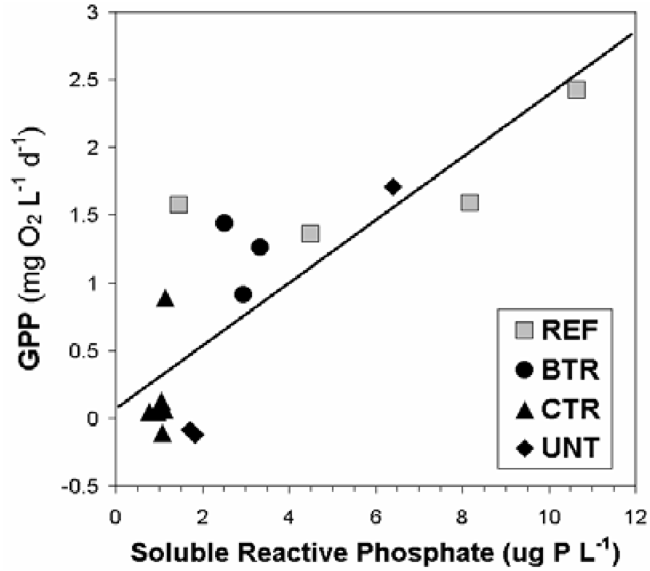

Figure 2. Linear regression of gross primary productivity (GPP; mg O_2 L^{-1} d^{-1}) vs. soluble reactive phosphate (SRP; µg P L^{-1}) in reference (REF), untreated (UNT), chemically-treated (CTR) and biologically-treated (BTR) AMD ponds; each point represents a different pond. The equation for the trend line is GPP = 0.208 * (SRP) + 0.265 (r^2 = 0.65).

NPP and GPP of biologically-treated AMD pond water (i.e. AMD that previously flowed through constructed, surface flow, aerobic wetlands) were no different than reference pond water and nearly 10 times greater than chemically-treated AMD ponds. At the same time, these two types of treatment ponds did not differ significantly in terms of pH or metal concentrations (Table 4). Thus, it appears that using constructed wetlands to treat AMD not only results in adequate water treatment but also in conditions that encourage growth of phytoplankton. One of the few consistent differences between biologically- and chemically-treated AMD ponds was that the former had significantly higher phosphate concentrations (Table 4).

Mean community respiration was not affected by the presence of AMD or AMD treatment methods (Table 2). Saksena and Sharma (1991) similarly found either no change or increased respiration in metal-contaminated pond water concurrent with dramatic decreases in GPP. The lack of response in community respiration may be caused by the contribution of non-phytoplankton species (namely bacteria, fungi, and protists) to respiration. If these other heterotrophic organisms were less sensitive to the chemical components of AMD, then CR would not be expected to change much in the presence of AMD.

In this study, we focused exclusively on phytoplankton productivity and did not attempt to

Table 3. Results of stepwise multiple regression of mean summer values of physicochemical parameters against gross primary productivity (GPP) and net primary productivity (NPP) in 16 ponds in Upshur and Lewis counties, WV. B = Coefficients of the model, SE = Standard error; β = Standardized coefficients of the model.

Variables	Cumulative r^2	B	SE of B	β	Significance
GPP					
Intercept	- -	0.265	0.194		0.196
Phosphate	0.65	0.208	0.044	0.809	<0.001
NPP					
Intercept	- -	0.721	0.283		0.025
Phosphate	0.69	0.182	0.029	0.654	<0.001
Zn, dissolved	0.83	-0.788	0.167	-0.662	0.001
Alkalinity	0.89	-0.007	0.003	-0.386	0.018

Table 4. Comparison of summer mean values of three biologically- and six chemically-treated AMD ponds in Upshur and Lewis counties, WV. Four replicate water samples were collected from each pond on three dates between June and August 1999. Mean pH values were back-calculated after determining mean H+ concentrations.

		Biologically-treated	Chemically-treated
GPP	($mg\ O_2\ L^{-1}\ d^{-1}$)	1.20	0.177
NPP	($mg\ O_2\ L^{-1}\ d^{-1}$)	1.06	0.102
pH		6.39	7.04
Acidity	($mg\ L^{-1}$)	23.0	18.8
Alkalinity	($mg\ L^{-1}$)	46.8*	103
Total Hardness	($mg\ L^{-1}$)	730	569
Phosphate	($\mu g\ L^{-1}$)	2.9*	1.0
Fe, diss.	($mg\ L^{-1}$)	0.49	0.04
Mn, diss.	($mg\ L^{-1}$)	6.08	4.54
Al, diss.	($mg\ L^{-1}$)	0.12	0.11
Zn, diss.	($mg\ L^{-1}$)	0.06	0.17

* Significantly different according to an unpaired t-test at alpha = 0.05

quantify productivity of aquatic macrophytes. However, we believe our conclusions would not change substantially if they were included. We observed very few macrophytes in or around untreated and chemically-treated ponds. In contrast, macrophytes were abundant around the edges of biologically-treated ponds and throughout the reference ponds. In fact, one reference pond was virtually choked with stonewort (*Chara spp.*) during the early part of the summer. Thus, we are most likely underestimating the differences in total productivity among the pond types.

The PC test verified that the ponds were successfully sorted into distinct categories and that several of the chemical factors, such as dissolved metals, varied collinearly. Principal component analysis is useful for detecting possible groupings of related variables in a large multivariate data set, but it is important to remember that the derived PCs are strictly hypothetical and that a PC that is significantly correlated with NPP and GPP may or may not represent an actual causal factor.

The PC analysis highlighted three major trends among the chemical variables. The strongest trend was the degree of AMD contamination (PC 1 in Figure 1a), which separated the untreated ponds from the others and highlighted the success of the treatment systems in increasing alkalinity and reducing dissolved heavy metal concentrations, acidity, and sulfate. However, this trend was not correlated with phytoplankton productivity, suggesting that neither low pH nor high metal concentrations were important controlling factors across the range of chemical conditions studied.

PC 2 was negatively correlated with both NPP and GPP, suggesting that the factors represented by this PC (ammonium, nitrate, nitrate, and phosphate) varied systematically with NPP and GPP. As discussed earlier, the correlation with the nitrogen variables is most likely an artifact of the anhydrous ammonia treatment systems. Whereas ammonium toxicity may well play a role in limiting productivity in some chemically-treated ponds, it was not a factor in biologically-treated or reference ponds where concentrations were always less than 0.10 mg NH_4-N L^{-1}. However, the principal components test does implicate phosphate as a possible controlling factor of NPP and GPP.

Like the PC test, multiple regression analysis revealed that metal concentrations were unimportant. In contrast, a strong correlation existed between SRP and both NPP and GPP. Furthermore, one of the few differences between biologically- and chemically-treated AMD ponds that could explain the difference in GPP was SRP concentration (Table 4). All of these results together suggest that SRP is the main controlling factor of productivity across all of these ponds. Fertilization experiments would be the best method to confirm this result and are planned. A positive correlation between phosphate and productivity is commonly reported in streams and lakes since phosphate is often a limiting factor in freshwater systems (Elser et al. 1990; Olsson and Pettersson 1993; Kopáček et al 2000).

Heavy metal concentrations were indeed high enough in many of the ponds to cause inhibition of growth to non-tolerant phytoplankton species. For example, in a review by Campbell and Stokes (1985), dissolved zinc concentrations at which toxic effects were observed ranged from 0.19 to 7.15 mg L^{-1}. Concentrations of 4 and 8 μg L^{-1} of Zn or Cd were sufficient to inhibit ^{14}C uptake in Ganges River water (Singh and Rai 1990). In our study, Zn and Cd concentrations in some of the ponds far exceeded these values (Table 1). Parent et al. (1996) demonstrated that 0.17 mg L^{-1} of Al inhibited growth of *Chlorella*, and Al concentrations in our AMD ponds were usually much higher.

If SRP is the factor limiting productivity in these ponds, what controls its availability, particularly the low SRP concentrations in chemically-treated AMD ponds? One likely explanation is the complexation of SRP with heavy metals. Phosphate is known to co-precipitate with Fe- and Al-hydroxides when the pH of AMD is increased (Hsu 1976; Christensen 1997; Lee et al. 2002) and to adsorb to metal hydroxide complexes (Lijklema 1980; McLaughlin et al 1981; Tate et al. 1995). In chemically-treated AMD ponds, metal hydroxide precipitates are continually forming and accumulating, thereby serving as a phosphorus sink. Another possibility is precipitation of calcium-phosphate complexes in those treatment ponds receiving lime additions.

It is not clear why SRP concentrations are so much higher in biologically-treated ponds than in chemically-treated ponds (Table 4) since metal hydroxides are presumably precipitating in these wetland ecosystems as well. No organic amendments were added to these systems; however, it is possible that organic acids from the decomposing plant material compete with SRP for hydroxide surfaces. This phenomenon requires further investigation.

For NPP, dissolved zinc and alkalinity were also significant components of the multiple regression model that explained 89% of the variance, but unlike phosphate, these were negatively correlated with NPP. Zinc has been shown to inhibit phytoplankton growth at concentrations as low as 0.19 mg L^{-1}, which is within the range found in this study (Table 1; Hargreaves and Whitton 1976; Campbell and Stokes 1985; Sunda and Huntsman, 1998). Furthermore, Zn tends to be more toxic at circumneutral pH and so could still exert an effect in the chemically-treated or biologically-treated ponds (Michnowicz and Weaks 1984; Campbell and Stokes 1985). However, in an AMD-contaminated stream, Niyogi et al (1999) found that an acid-tolerant alga was not inhibited by dissolved Zn concentrations as high as 10 mg L^{-1}. Clearly, some species or strains of algae are able to tolerate high metal concentrations.

Alkalinity varied directly with the degree of AMD treatment, ranging from zero in untreated ponds to over 100 mg L^{-1} in chemically-treated ponds. We know of no reports in the literature describing direct negative impacts of alkalinity on aquatic plants, but in this case, alkalinity may serve as an indicator of the degree of chemical treatment and therefore of phosphate immobilization. The ponds with high alkalinity will have large quantities of metal oxides that can adsorb phosphate, leaving little left for phytoplankton uptake.

Although laboratory studies on the effects of heavy metals on aquatic plants abound, there are relatively few studies that examine the effects of AMD on phytoplankton *in situ*. Pan and Stevenson (1996) studied diatom assemblages in Kentucky wetlands, some of which received AMD inputs, and reported that total phosphorus was one of the best predictors of diatom community structure. In contrast, MacFarland et al. (1997) reported abnormalities in diatom cell structure that correlated with Zn, Cd, Fe, and Cu, suggesting that high metal concentrations negatively impacted periphyton in streams contaminated with mine waste. Bortnikova et al. (2001) documented short-term decreases in phytoplankton biomass in lake mesocosms to which AMD was added. Vinyard (1996) demonstrated reduced phytoplankton growth in AMD-impacted streams that seemed to be better correlated with Fe than with SRP concentrations. Clearly, heavy metals can limit phytoplankton growth in some AMD-impacted surface waters; however, our study and others suggest that phosphate may also be a dominant factor under certain conditions.

Because our study ponds were selected at random, we can cautiously extrapolate our results to AMD treatment ponds throughout northern West Virginia where the geochemistry of soils and bedrock are similar. Chemically-treated AMD ponds on average will generate only 9% of the NPP of unpolluted ponds and therefore will supply receiving streams with a much smaller influx of organic carbon. If the pond is a major water source for the stream, it may affect the population of filter-feeding macroinvertebrates and heterotrophic microorganisms that serve as a major source of food for the stream ecosystem. Studies like those by Brenner and colleagues (1987) and Pan and Stevenson (1996) further suggest that the species diversity of phytoplankton will be much lower as well. Thus, AMD treatment ponds, even when they meet governmental water quality criteria, cannot be considered unstressed ecosystems equivalent to unpolluted ponds. Furthermore, discharge water from these ponds may not be able to support a diverse phytoplankton community.

This underscores an important disconnect in how our aquatic systems are managed. Most regulations, such as those governing effluent discharges, are based on chemical criteria because they are easy to set, measure, and enforce. However, it could be argued that the ultimate goal of these regulations is to protect or restore ecological health, which would best be measured with ecological, not chemical, indicators. There is very little information available to assess whether current regulations are adequate to maintain the diversity and integrity of aquatic systems.

To summarize, phytoplankton NPP was substantially reduced in untreated and chemically-treated AMD ponds and GPP was reduced in chemically-treated AMD ponds. In contrast, productivity in biologically-treated ponds was no different than in reference ponds, suggesting that passive treatment using wetlands can adequately reduce acidity and metal loads while at the same time maintaining productivity rates similar to unpolluted ponds. Soluble reactive phosphate concentration was the best predictor of GPP and NPP across all pond types, even when heavy metals were present in high concentrations, which supports the hypothesis that phytoplankton in these ponds are limited by phosphate availability rather than by metal toxicity.

Acknowledgements

The authors thank E. Leamer and S. Houck for their help with field and laboratory work. Funding was provided by the Council for Undergraduate Research, the Appalachian College Assoc, and the WV Space Grant Consortium.

References

APHA (1995) Standard Methods for the Examination of Water and Wastewater. 19th ed. American Public Health Assoc, Washington, DC

Bortnikova SB, Smolyakov BS, Sidenko NV, Kolonin GR, Bessonova EP, Androsova NV (2001) Geochemical consequences of acid mine drainage into a natural reservoir: inorganic precipitation and effects on plankton activity. J Geochem Exploration 74: 127-139

Brenner FJ, Edmundson J, Werner M, McGrath T (1987) Plankton, chlorophyll characteristics and fishery potential of surface coal mine lakes in western Pennsylvania. Proc, Pa Acad Sci 61: 147-152

Campbell PGC, Stokes PM (1985) Acidification and toxicity of metals to aquatic biota. Can J Fisheries Aquatic Sciences 42: 2034-2049

Christensen KK (1997) Differences in iron, manganese, and phosphorus binding in freshwater sediment vegetated with *Littorella uniflora* and benthic microalgae. Water Air Soil Pollut 99: 265-273

Crowder A (1991) Acidification, metals and macrophytes. Env Pollution 71: 171-203

Elser JJ, Marzolf ER, Goldman CR (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. Can J Fisheries Aquatic Sciences 47: 1468-1477

Florence TM (1982) Development of physico-chemical speciation procedures to investigate the toxicity of copper, lead, cadmium and zinc towards aquatic biota. Anal Chim Acta 141: 73-94

Guildford SJ, Hecky RE (2000) Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnol Oceanogr 45: 1213-1223

Hargreaves JW, Whitton BA (1976) Effect of pH on tolerance of *Hormidium rivulare* to zinc and copper. Oecologia 26: 235-243

Hedin RS, Nairn RW (1993) Contaminant removal capabilities of wetlands constructed to treat coal mine drainage. In: Moshiri GA (ed) Constructed Wetlands for Water Quality Improvement. Lewis Publ, Boca Raton, FL, pp 187-195

Herlihy AT, Kaufmann PR, Mitch ME, Brown DD (1990) Regional estimates of acid mine drainage impact on streams in the mid-Atlantic and southeastern United States. Water Air Soil Pollut 50: 91-107

Hsu PH (1976) Comparison of iron(III) and aluminum in precipitation of phosphate from solution. Water Res 10: 903-907

Karathanasis AD, Johnson CM (2003) Metal removal potential by three aquatic plants in an acid mine drainage wetland. Mine Water Environ 22: 22-30

Kopáček J, Hejzlar J, Borovec J, Porcal P, Kotorová I (2000) Phosphorus inactivation by aluminum in the water column and sediments: Lowering of in-lake phosphorus availability in an acidified watershed-lake ecosystem. Limnol Oceanogr 45: 212-225

Lee G, Bigham JM, Faure G (2002) Removal of trace metals by coprecipitation with Fe, Al and Mn from natural waters contaminated with acid mine drainage in the Ducktown Mining District, Tennessee. Appl Geochem 17: 569-581

Leland HV, Carter JL (1984) Effects of copper on species composition of periphyton in a Sierra Nevada, California, stream. Freshwater Biol 14: 281-296

Lijklema L (1980) Interaction of orthophosphate with iron(III) and aluminum hydroxides. Environ Sci Technol 14: 537-541

MacFarland BH, Hill BH, Willingham WT (1997) Abnormal *Fragillaria spp.* (Bacillariophyceae) in streams impacted by mine drainage. J Freshwater Ecol 12: 141-149

McLaughlin JR, Ryden JC, Syers JK (1981) Sorption of inorganic phosphate by iron- and aluminum-containing components. J Soil Sci 32: 365-377

Michnowicz CJ, Weaks TE (1984) Effects of pH on toxicity of As, Cr, Cu, Ni and Zn to *Selenastrum capricornutum* Printz. Hydrobiologia 118: 299-305

Niyogi DK, McKnight DM, Lewis Jr. WM (1999) Influences of water and substrate quality for periphyton in a montane stream affected by acid mine drainage. Limnol Oceanogr 44: 804-809

Okamura H, Aoyama I (1994) Interactive toxic effect and distribution of heavy metals in phytoplankton. Environ Toxicol Water Qual 9: 7-15

Olsson H, Pettersson A (1993) Oligotrophication of acidified lakes – a review of hypotheses. Ambio 22: 312-317

Pan Y, Stevenson RJ (1996) Gradient analysis of diatom assemblages in western Kentucky wetlands. J Phycol 32: 222-232

Parent L, Twiss MR, Campbell PGC (1996) Influences of natural dissolved organic matter on the interaction of aluminum with the microalga *Chlorella*: A test of the free ion model of trace metal toxicity. Environ Sci Technol 30: 1713-1720.

Pyle GG, Swanson SM, Lehmkuhl DM (2001) Toxicity of uranium mine-receiving waters to caged fathead minnows, *Pimephales promelas*. Ecotoxicol Environ Safety 48: 202-214

Saksena DN, Sharma SP (1991) Effect of heavy metals on the primary production of phytoplankton in a freshwater fish pond at Gwalior, Madhya Pradesh. J Environ Biol 12: 159-162

Singh AK, Rai LC (1990) Use of *in situ* structural and functional variables of phytoplankton of the River Ganga for assessment of heavy metal toxicity. Biomedical Environ Sciences 3: 397-405

Skousen JG, Sexstone A, Garbutt K, Sencindiver J (1995) Wetlands for treating acid mine drainage. In: Skousen JG, Ziemkiewicz PF (eds) Acid Mine Drainage Control and Treatment. WV Univ and the National Mine Land Reclamation Center, Morgantown, WV, USA, pp199-210

Sunda WG, Huntsman SA (1998) Interactions among Cu^{+2}, Zn^{+2}, and Mn^{+2} in controlling cellular Mn, Zn, and growth rate in the coastal alga *Chlamydomonas*. Limnol Oceanogr 43: 1055-1064

Tate CM, Broshears RE, McKnight DM (1995) Phosphate dynamics in an acidic mountain stream: interactions involving algal uptake, sorption by iron oxide, and photoreduction. Limnol Oceanogr 40: 938-946

Vinyard GL (1996) A chemical and biological assessment of water quality impacts from acid mine drainage in a first order mountain stream, and a comparison of two bioassay techniques. Environ Technol 17: 273-281

Received June 13, 2003; accepted February 2, 2004