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# Examination of effects of acid and metals on benthic algae in streams using chemical diffusing substrata

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ABSTRACT

Acid mine drainage environments have multiple stressors, making it difficult to isolate effects of individual contaminants on benthic algal communities *in situ* in order to develop more specific indicators. Additionally, responses to AMD contaminants may be dependent on the abiotic and biotic conditions within the affected stream. We examined the efficacy of using chemical diffusing substrata (CDS) to determine how acid and metals interact to affect benthic algae in 2 streams with different trophy. CDS containing Mn, Fe, Al, acid, Mn + acid, Fe + acid, and Al + acid were deployed in a flow-through system to determine flux from the liquid media. Flux values for metals ranged from 1.0 to 18.1 μg/cm$^2$/d for 5 days, and 0.5–7.4 μg/cm$^2$/d for 21 days. Flux values for $H^+$ were 467 (5 day) and 212 (21 day) μg/cm$^2$/d. Corresponding water column concentrations that would produce the same flux as CDS metal treatments were estimated to be in the low to mid toxicity range for Al, low range for Fe, and nontoxic range for Mn. Acid treatments were estimated to be equivalent to a stream pH of 2.1–2.5. Five replicates of each CDS treatment, and a water control, were placed into a high-alkalinity, eutrophic stream (Wolf Creek) for 5 days and a low-alkalinity, oligotrophic stream (Silver Creek) for 21 days, respectively. In Silver Creek, all treatments except circumneutral Fe and circumneutral Mn had significantly lower algal cell densities than control treatments. Cell densities were significantly lower on Al + acid and Mn + acid than on the acid-only treatment, indicating synergistic effects. Cell densities were not significantly different among treatments in Wolf Creek. Higher alkalinity, nutrients, and productivity in this stream likely increased neutralization and detoxification processes within the biofilm. However, significantly lower chlorophyll *a* and higher phaeophytin concentrations of communities on treatments containing acid indicated sublethal effects. Significantly more acid tolerant taxa occurred on treatments with acid in Silver Creek, and significantly more metal-tolerant taxa occurred on metal treatments in Wolf Creek. However, treatment effects on taxonomic composition appeared to be constrained by the species pool within each stream, as these indicator taxa made up less than 4% of any community. Although equating CDS flux of contaminants to water column concentrations requires assumptions, and flux decreases over the exposure period, this study indicates that *in situ* experiments using CDS can separate out effects of individual toxic components on benthic algae, and determine context-dependent responses.

## 1. Introduction

Metal and acid contamination of freshwater environments from mining activities is a worldwide problem, whose devastating biological effects are well documented (Millennium Ecosystem Assessment, 2005; Hogsden and Harding, 2012). A critical component of these ecosystems is benthic algae, which often are the major primary producers at the base of the autochthonous food web (Cummins, 1974). A number of studies have documented that acid mine drainage (AMD) limits benthic algal communities to a reduced number of tolerant taxa, which in turn can affect biomass and productivity (Verb and Vis, 2005; Bott et al., 2012; DeNicola et al., 2012). As a result, AMD impacts on benthic algae can affect higher trophic levels through food availability. Additionally, benthic algae can affect consumers through direct consumption of metals that have accumulated in the biofilm (Kiffney and Clements, 1993).

The AMD environment contains a complex mixture of acid and various metals that are ultimately determined by the geology of the

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watershed (Younger et al., 2002). Community composition of benthic algae has been shown to be a good predictor of the degree of general AMD impact (Zalack et al., 2010), but it remains difficult to assess sites for remediation because taxonomic indicators for specific contaminants are not well developed. Separating biological effects of AMD contaminants requires them to be examined individually or in controlled combinations in order to determine target concentrations that would reduce ecotoxological impacts. In addition, responses of aquatic communities to contaminants in natural ecosystems depend on the abiotic and biotic conditions of the affected site (Clements et al., 2012). Traditionally, laboratory tests have been used to assess the toxic level of contaminants, yet these systems often employ single organisms in very simple, artificial conditions. While mesocosm experiments use more realistic communities, their environments are usually quite different than natural conditions. Field surveys develop correlations between contaminants and biota, but don't employ an experimental design to separate effects of multiple contaminants, and are restricted to the concentration gradient among the sites (Clements, 1991). Field experiments that add contaminants under controlled conditions have the advantage of permitting examination of community and ecosystem effects under natural environmental conditions, but they can be problematic if they require adding large amounts of the contaminant to a natural system. For benthic algal studies, this problem can be overcome by the use of chemical diffusion substrata (CDS), in which small amounts of chemicals diffuse out of media in a sealed container through a porous substrata surface that the biofilm grows on (Arnegard et al., 1998; Costello et al., 2016). An additional advantage is that the small size of CDS allow for controlled experiments with substantial replication *in situ*. Nutrient diffusing substrata (NDS) are used extensively to examine nutrient limitation of periphyton (Francoeur, 2001), but to our knowledge there have been only 2 published studies that used CDS to examine effects of potential AMD contaminants (Arnegard et al., 1998; Hirst et al., 2004). Challenges to using CDS for assessing contaminants are that it is difficult to relate chemical flux from CDS to stream water concentrations, and the species composition of the algal community cannot be controlled (Costello et al., 2016).

The 2 studies employing CDS to examine responses of benthic algae to acid and/or metals have given conflicting results when compared to expectations based on water column exposure. Arnegard et al. (1998) found periphyton exposed to Cu using CDS had identical responses to communities exposed in laboratory streams, whereas Hirst et al. (2004) found that the magnitude of periphyton response from exposure to Mn and $H^+$ via CDS was less than for reference communities transplanted to streams high in these constituents. It could be that the CDS were more effective at creating a response for Cu because of its greater toxicity. Our overall objective for this study was to further test the efficacy of using diffusion substrata to examine effects of metals and acid on benthic algae in streams. We used CDS that added acid, as well as 3 metals that are commonly the most abundant in AMD from abandoned Appalachian coal mines in the USA, Fe, Mn and Al (Herlihy et al., 1990). Specific objectives were: 1) to measure the flux of acid, Fe, Mn and Al from the CDS, and relate the values to stream water concentrations that would provide the same flux to an algal biofilm; 2) to deploy CDS in a field experiment to determine if the above contaminants affect benthic algal cell density, chlorophyll *a* concentrations and taxonomic composition; and 3) to determine whether algal responses to CDS treatments are influenced by alkalinity and trophic status of the receiving stream, based on the assumptions that higher alkalinity would buffer acid stress, and that higher nutrients would reduce limitation associated with potential binding of P by metals, as well as increase cellular resources for ameliorating physiological damage.

## 2. Materials and methods

### 2.1. CDS construction and determination of flux rates

Chemical diffusion substrata were similar in construction to those described in Rugenski et al. (2008) and Capps et al. (2011). 50 mL Nunc centrifuge tubes (Thermo Fisher Scientific, Waltham, MA) were filled with liquid solutions to create 8 different treatments: acid-only (AC); 34 mg/L $Fe^{2+}$ + acid (AF), 24 mg/L $Fe^{2+}$ + $NaHCO_3$ buffer (Fe), 32 mg/L $Mn^{2+}$ + acid (AM), 18 mg/L $Mn^{2+}$ + buffer (Mn), 4.1 mg/L $Al^{3+}$ + acid (AA), 3.4 mg/L $Al^{3+}$ + buffer (Al), and deionized water (DI). Acidified solutions contained 0.5% sulfuric acid and had an initial pH of 1.0–1.1. Buffered CDS solutions had 0.84 mg/L of $NaHCO_3$ and a circumneutral pH (6.4–7.6). We wanted to maximize dissolved metal concentrations in the CDS solutions, thus the higher solubility of metals in acid permitted higher concentrations in those treatments than for metal treatments in circumneutral solutions. In addition, ethylenediaminetetraacetic (EDTA) was added to the Fe (0.10 mg/L) and Al (0.04 mg/L) treatments as a chelating agent to increase the solubility of these metals in non-acidified solutions. The tubes were capped with fritted glass covers (4.9 $cm^2$; Leco Corporation, St. Joseph, MI, USA). The outer rim of each individual fritted glass disk was wrapped with PTFE thread seal tape, and secured to the centrifuge tube using a silicon sealant. To determine any decreases in concentration not due to diffusion, we also constructed CDS for each treatment that were sealed with caps and Parafilm®.

A flow-through system in the laboratory consisting of 3 round, 19-L buckets was used to measure flux rate from CDS over 21 days. Each bucket held approximately 5 L of water, with 5 cm of sand in the bottom to secure CDS. A peristaltic pump maintained a continuous flow of deionized water separately into each bucket at a rate of 1.10 L/h, which drained by gravity out of a port 15 cm from the bottom. The residence time within each bucket was approximately 4 h. Water temperature was maintained between 20–23 °C. Each bucket held 3 CDS for each of the 8 treatments, and 1 sealed CDS for each treatment. Thus there were a 32 CDS per bucket (3 × 8 treatments + 1 sealed × 8 treatments). Conductivity and pH of the water in the flow-through system was monitored throughout the duration of the 21-day diffusion study. For each treatment, solutions from 3 randomly selected CDS were sampled on days 7, 14 and 21 (n = 3 for each treatment on each date). Solutions within the sealed control CDS were sampled only on day 21 (n = 3 for each treatment). pH was determined for the CDS sample solutions, which were then acid fixed with 1.0 L of $HNO_3$. Metal concentrations of fixed solutions were measured using a 710-OES inductively coupled plasma spectrophotometer (Varian/Agilent, Santa Clara, CA, USA), following standard procedures that assure accuracy and precision (American Public Health Association, 1998).

The change in Fe, Mn, Al or $H^+$ concentration in the CDS treatment solutions as function of the square root of time was analyzed using a log-linear regression model. These transformations correspond to diffusion theory, and have been used in previous studies that analyzed chemical release from diffusion substrata (Fairchild et al., 1985; Rugenski et al., 2008). The concentration decrease of the chemical constituents over time was used to calculate their flux from treatment solutions based on Fick's 1st Law. We then used the flux values for metals or $H^+$ to estimate an equivalent water column concentration in a stream that would provide the same dose to benthic algae, with an assumed diffusive boundary layer thickness of 1 mm, growing on a substratum surface (Costello et al., 2016). Differences in productivity (benthic algal growth rate on CDS) for the 2 streams used in the field bioassay necessitated different CDS incubation times (see below). Because flux from CDS decreases over time, respective flux values and associated stream concentration estimates were calculated for the 2 incubation time-periods. Slow algal growth on CDS in the less productive stream (Silver Creek) required a 21-day bioassay, thus flux values were based on the change in CDS solution concentrations