IDENTIFICATION OF DIATOMS IN A HEALTHY PENNSYLVANIA STREAM COMPARED TO THREE DOWNSTREAM SITES IMPACTED BY ABANDONED MINE DRAINAGE

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KEYWORDS

- diatom
- abandoned mine
- drainage
- borehole
- *Eunotia exigua*

ABSTRACT

Life in a healthy stream can be severely impacted by changes in pH and other water quality parameters. This study reveals differences in diatom diversity and water quality characteristics in a central Pennsylvania stream. One healthy site was compared to three nearby sites affected by abandoned mine drainage during a July sampling in 2013. Permanent slides were made and microscopically assessed for diatom identification. The healthy stream contained eleven diatom genera while the site most impacted by mine drainage showed only one diatom, *Eunotia exigua*. Data were analyzed for Shannon diversity index and species richness. Water samples showed differences in pH, aluminum, sulfate, and iron. This work demonstrates the use of diatoms as bioindicators of stream health.

INTRODUCTION

Abandoned mine drainage (AMD) is a prominent source of pollution in currently and previously mined areas throughout the United States. AMD impacted water is saturated with metals such as iron and may be very low in pH making it an inhospitable environment for the majority of aquatic life. Hughes borehole is a source of AMD pollution that flows into the Little Conemaugh River near Portage, Pennsylvania. The borehole was drilled in the 1920s to release water from miles of flooded underground coal mines in the area. The borehole was capped in 1950s only to blow out due to underground pressure some twenty years later. Since then, water with a pH as low as 3.08 has been bubbling out of the borehole at a rate of 800-3500 gallons per minute and blanketing the surrounding six acres with a reddish brown iron precipitate. (2)

Mine drainage occurs in areas where water comes in contact with exposed rocks that have a high concentration of sulfide minerals. Pyrite, also known as fool's gold, is a common mineral found with coal in the eastern United States. The oxidation of pyrite and other sulfide-rich minerals causes the release of sulfuric acid and metal ions. If a stream has a limited buffer capacity, the pH will continue to decrease, thus increasing the oxidation reactions and the precipitation of metals. When the temperature of the water increases in the summer months, gases such as oxygen become less soluble and salts become more soluble. (1)

Diatoms are unicellular, photosynthetic algae which can survive in a wide variety of aquatic environments. Each diatom species has a specifically shaped silica cell wall, called a frustule, which is used for microscopic identification. Diatom species are found in two different microenvironments, they are either suspended in water (planktonic) or growing on a substrate (benthic). Environmental factors such as pH, light availability, and temperature may cause teratology in frustule morphology, and particularly in harsh environments can have an effect on overall growth characteristics of the diatom. These factors, most importantly temperature, can have an effect on solubility of salts and gases found within waters especially those impacted with AMD, thus leading to large overall changes in water chemistry. Fluctuations in water chemistry throughout the year due to temperature change can have an effect on diatom species present as well as seasonal variation in diatom populations. Each diatom species has specific growth parameters and morphology giving us the ability to identify them by their frustule, making them good bioindicators of water quality. (6)

This study was undertaken to assess diatom diversity in a healthy stream and three sites downstream from the AMD outflow. The first site is the healthy stream, 40 m upstream of the AMD discharge with a pH of 7.12. The second site is at Hughes borehole, 5 m below the source of AMD discharge due to safety fencing, with a pH of 3.36. The borehole sits uphill and is completely devoid of vegetation. The third site is a naturally formed settling pond, 50 m below the discharge, where the flow of polluted water slows and has a pH of 3.24. The fourth site is at a bridge 600 m below the AMD discharge; roughly 30 m from where the healthy and low pH waters mix, with a pH of 6.68. Fig. 1 illustrates the four sampling sites.

MATERIALS AND METHODS

Sampling was conducted in July 2013. Diatoms were collected by harvesting biofilms from benthonic sediments by scraping a three centimeter square area into a sterile 15mL polypropylene disposable centrifuge tube (Fisherbrand). Two samples per site were gathered and processed in a ventilation hood by placing 25-30 mL of the sample into a 150mL beaker on a hotplate, then adding 10-15mL nitric acid (Flinn Scientific Inc.). Samples were then boiled to remove organic matter, leaving behind only diatom frustules per Sgro and Johansen (7). Centrifugation, decanting of liquid waste, and suspension in 10-15mL of distilled water was performed six times. Permanent slides were created by suspending diatom frustules in 70% denatured ethyl alcohol (Fisher Chemical) until a cloudy suspension was achieved. Samples were diluted to approximately 400 frustules per field of view on low magnification (100X total magnification) to allow clear observation on permanent slides. Approximately 1mL of solution was placed on a glass coverslip and the alcohol was allowed to evaporate overnight, leaving behind only diatom frustules fixed to the coverslip. Coverslips were permanently mounted on slides using naphrax mounting medium (Brunel). Slide sets of 24 slides were created for use in laboratories such as general microbiology and water ecology.

Diatoms were identified to the genus level using an online database, Diatoms of the United States, (8) and a diatom identification text (6). From each site, 400 total diatoms were identified under oil immersion (1000X total magnification). Diatom images were captured with a Zeiss Axiostar Plus light microscope and SPOT imaging system with an in-sight camera and edited with SPOT version 5.0 software (SPOT Imaging Solutions).

Water analysis was sent to G and C Coal Analysis Lab (Summerville, PA) for testing of the suspended solids, dissolved metals and pH. These data were used in conjunction with the Shannon diversity statistical analysis data. Relationships between water quality and diatom diversity were examined.

RESULTS

Diatom samples from the healthy stream showed the most genera present, eleven in total. The healthy stream site contained low species diversity due to one genus (*Navicula*) dominating the population (Table 1). Shannon diversity index data for this site showed a fairly low diversity rating of 0.855 and a low richness (evenness) rating of 0.356. Diatoms of the genus *Navicula* made up 78% of the population in this location followed by *Surirella* at 12% and *Eunotia* at 3.5%. One other notable genus found in the sample, *Staurosirella*, was present in trace amounts but only found at this particular site (Fig. 2).

The Hughes borehole sampling site contained only one genus, *Eunotia* sp. Within in this genus however, we noted morphological size differentials. We observed either short and fat or long and narrow frustule morphology (Fig. 3). At all other sampling sites *Eunotia* was observed as being uniform in length and width. Because only one species was present, the Shannon diversity index was 0 and evenness could not be calculated.

The settling pond site exhibited the poorest water quality from which we sampled but showed eight genera of diatoms in total. The population here was dominated by *Eunotia* sp. which accounted for 79.9% of the population. *Pinnularia* sp and *Navicula* sp. made up 15.4% and 2.45% of the population respectively. The remaining species of diatoms were present in trace amounts (Fig. 4). Shannon diversity index data from this site were similar to that of the healthy stream with a lower diversity score of 0.670 and a similar evenness score of 0.344.

The bridge sampling site 600m downstream showed the most diverse and evenly distributed population of all four sites. The Shannon diversity score here was 1.01 and this was the most evenly distributed population with a richness score of 0.439. *Navicula* sp. made up 73.2% of the sample but three other genera made up more than 5% of the population, *Surirella* (9.97 %), *Eunotia* (6.81 %), and *Planothidium* (6.08%). (Fig. 5)

Water quality data, comparing pH, suspended solids, total iron, sulfate, and total aluminum concentration in mg/L (Table 2) were taken at each site. The healthy stream (pH 7.12), had the lowest measured concentrations of suspended solids, total iron, sulfate and total aluminum at 8 mg/L, 0.16 mg/L, .68 mg/L and 40.9 mg/L respectively. At the bridge site (pH 6.68), water quality data was very similar to that of the healthy site; total suspended solids were 9 mg/L, total iron was 1.58 mg/L, aluminum was 70.9 mg/L and sulfate was 1.16 mg/L.

At Hughes borehole and settling pond sites extreme amounts of metals were detected. At Hughes borehole (pH 3.36), iron was found at a concentration of 82.45 mg/L and aluminum is was found at 571 mg/L as well as total suspended solids with a concentration of 10 mg/L and sulfate of 10.25 mg/L. At the settling pond (pH 3.24), sulfate concentrations are 9.64 mg/L much higher than both the healthy stream and bridge sites. Iron was 76.67 mg/L and aluminum at 9.64 mg/L slightly lower than the borehole site whereas the settling pond showed a higher concentration of total suspended solids at 14 mg/L.

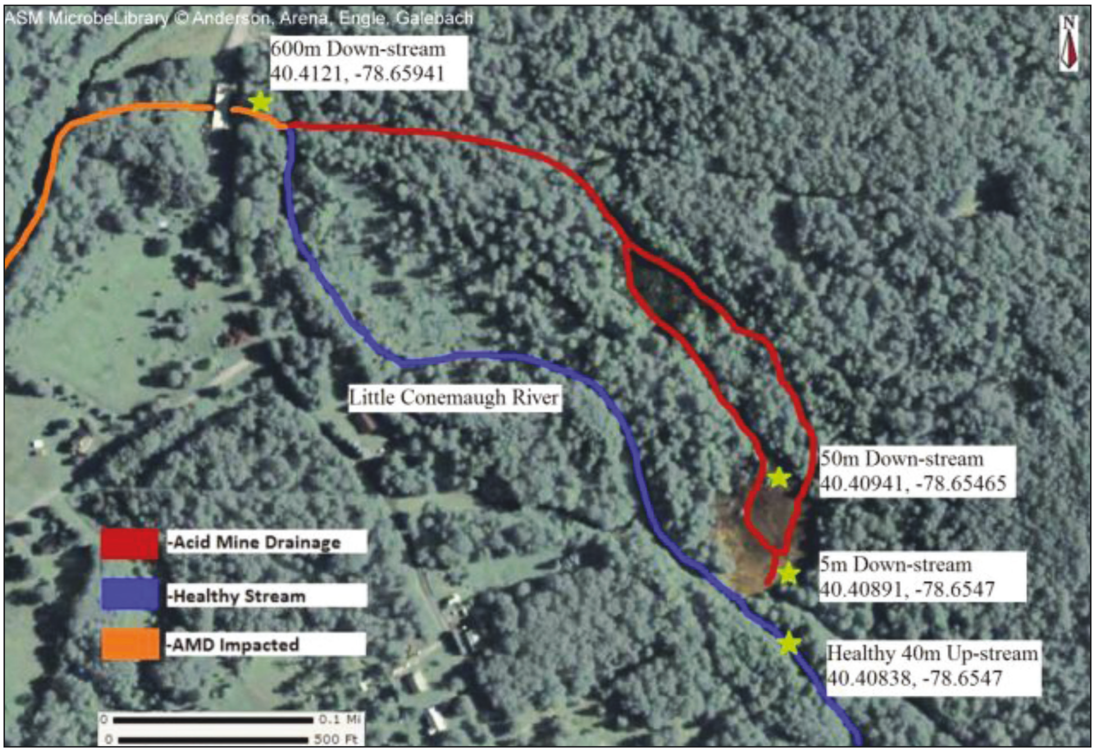
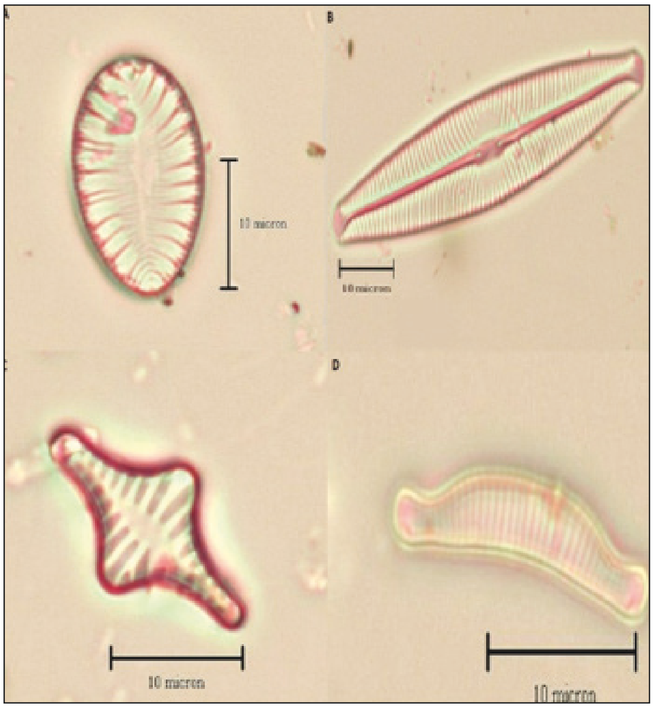


Fig. 1. This map shows the healthy stream and three downstream sites impacted by abandoned mine drainage.

Table 1. Summary of diatom and diversity findings at a healthy stream compared to three sites impacted by abandoned mine drainage.

Site	Shanon Diversity Index	Evenness	Number of diatom genera identified
Healthy	0.855	0.356	11
Hughes Borehole (5 m downstream)	0	0	1
Settling Pond (50 m downstream)	0.670	0.344	8
Bridge (600 m downstream)	1.01	0.439	11

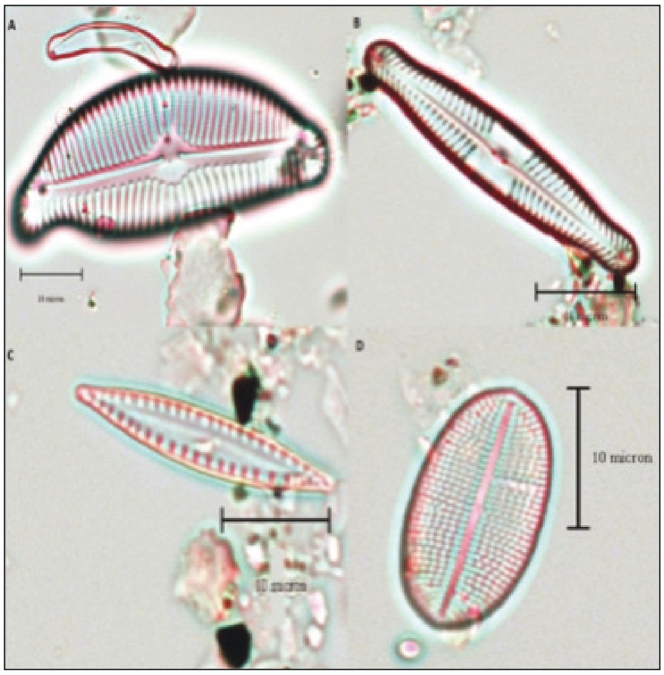


Genera	Healthy	Percent in Population
Eunotia	14	3.5
Navicula	313	78.25
Surirella	48	12
Cocconeis	1	.25
Diatoma	1	.25
Fragilaria	6	1.5
Meridion	2	.50
Melosira	7	1.75
Planothidium	7	1.75
Ulnaria	1	.25
Staurosirella	1	.25
Total	401	

Fig. 2. On the right, the total number of diatoms counted in our healthy stream sample. On the left, diatoms from the genera *Surirella* (top left), *Navicula* (top right), *Staurosirella* (bottom left) and *Eunotia* (bottom right).

Table 2. Water quality characteristics from the four sampling sites.

Site	pH	Suspended solids (mg/L)	Total Iron (mg/L)	Sulfate (mg/L)	Total Aluminum (mg/L)
Healthy	7.12	8	0.16	0.68	40.9
Hughes Borehole (5 m downstream)	3.36	10	82.45	10.25	571
Settling Pond (50 m downstream)	3.24	14	76.67	9.64	564.6
Bridge (600 m downstream)	6.68	9	1.58	1.16	70.9



Genera	Healthy	Percent in Population
Eunotia	326	79.9
Navicula	10	2.45
Encyonema	1	.24
Pinnularia	63	15.4
Surirella	2	.49
Cocconeis	4	.98
Diatoma	2	.49
Tabellaria	1	.24
Total	409	

Fig. 4. On the right, the total number of diatoms counted in the settling pond sample. On the left, diatoms from the genera *Encyonema* (top left), *Pinnularia* (top right), *Pseudostaurosira* (bottom left), *Cocconeis* (bottom right)

DISCUSSION

We hypothesized an association between diatom distribution and water quality data, predicting that AMD impacted sites would exhibit reduced diatom diversity compared to the healthy stream.

The healthy stream site essentially served as a positive control site for comparisons and showed eleven genera of diatoms present. This was to be expected considering it had a neutral pH of 7.12 and was low in dissolved metals, with aluminum measured at 0.68 mg/L and iron at 0.16 mg/L, both within normal ranges for stream health

Fig. 3. *Eunotia* sp. found at Hughes borehole.

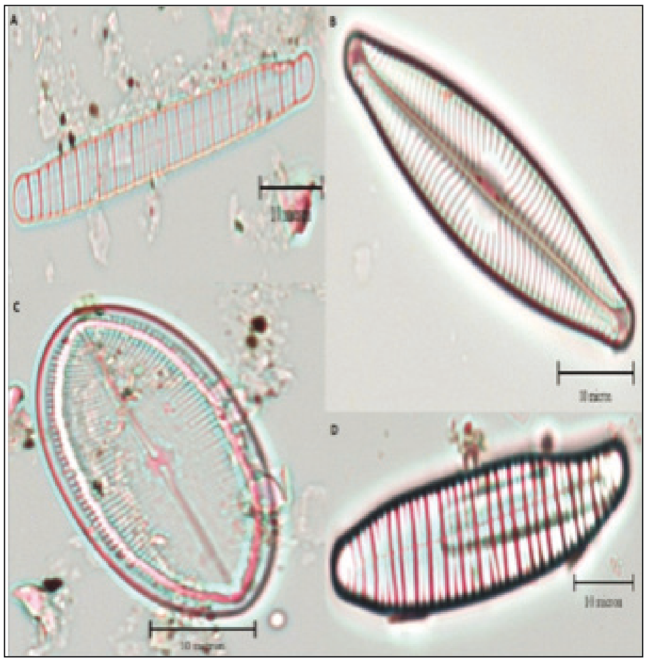


Fig. 5. On the right, the total number of diatoms counted at the bridge site. On the left, diatoms from the genera *Diatoma* (top left and bottom right), *Navicula* (top right), and *Cocconeis* (bottom left).

Genera	Healthy	Percent in Population
Eunotia	28	6.81
Navicula	301	73.2
Surirella	41	9.97
Diatoma	6	1.45
Tabellaria	1	.242
Fragilaria	3	.729
Meridion	1	.242
Synedra	1	.242
Melosira	4	.97
Planothidium	25	6.08
Total	412	

(4). The dominant diatom at this site was *Navicula* sp. making up 78% of the diatom population. *Navicula* was also found at the other three sampling locations; this not surprising considering *Navicula* can survive in a wide range of environments but will tend to thrive where water quality is the highest, making it valuable for assessing water quality and health of aquatic environments (3). The genus *Staurosirella* was also observed at the healthy site, but was not found at any other sites, leading us to hypothesize that it could be used as a bioindicator of good water quality. *Staurosirella* has been shown to thrive in moderate light and nutrient situations similar to the healthy stream we sampled from (5).

The two heavily AMD-impacted sites, Hughes borehole and the settling pond, are fairly similar in water quality. Both have an extremely acidic pH; the settling pond being slightly more acidic with a pH of 3.24 compared to a pH of 3.36 at the borehole. The two locations were also very high in concentrations of total dissolved solids and sulfates both of which are characteristic of AMD impacted waters that come in contact with organic sulfur compounds such as pyrite (FeS_2). As the AMD water emerges at the borehole, the slight increase in temperature changes the solubility of oxygen, removing it from solution and producing the iron precipitate found at the borehole and outlying areas.

There is a large "terrace" of precipitate as the water moves about 50m to the settling pond. Bottom dwelling organisms such as diatoms are particularly sensitive to AMD precipitates so we were surprised to find diatoms growing at both locations.

The only genus found at Hughes borehole was *Eunotia*, most likely *Eunotia exigua*. However, there were variations in morphology between *Eunotia* frustules found at the borehole and those found at our other three sites. *Eunotia* frustules at the other three sites all appear to be consistent in length, but what we saw at the borehole was individual diatoms within the population varying greatly in length but still showing all other morphological characteristics of the genus. We believe this morphological variability is due to lack of nutrients present within the borehole environment specifically the availability of silica, a known limiting factor of diatom growth. It is otherwise possible that we have two separate species of *Eunotia* here. Further research into this phenomenon is ongoing using DNA sequencing and other molecular techniques.

At the settling pond site we observed eight different species, which was unexpected considering the similar water chemistry to the borehole. *Eunotia* sp. dominated this population making up 79% of the total diatoms found in our sample. *Eunotia* at the settling pond did not show variable morphology like we observed at the borehole. Possible explanations for this increased diatom diversity include changes in nutrient availability, inflow of diatoms from surrounding unimpacted environments, or creation of microenvironments from increased organic matter. Another factor possibly contributing to the increased diatom growth at the settling pond could be due to a slight decrease of dissolved metals in solution. The precipitate found near the borehole appears to be coarse and quickly accumulates as the waters hit the surface. As this water makes its way 50m downstream to the settling pond the precipitate appears more as a mud or fine silt. This in conjunction with the micronutrients being added to this sampling site could be a possible explanation for the increased diversity of diatoms observed.

The final site, the bridge, showed the most evenly distributed population of diatoms. The water at this site was still moderately impacted by AMD; white aluminum and orange iron precipitate was clearly be observed. Of the eleven species present, there were very few genera that differed from the settling pond site. The precipitate observed at the bridge, like the settling pond, was very fine in texture. The population was mainly *Navicula* sp. (73.2 %), but three other genera made up over 5% of the population. This possibly shows that the genera present, specifically *Surirella*, can survive in harsh environments at the borehole and settling pond sites but don't thrive in that environment like at the bridge site where water quality was more suitable for life.

The Shannon diversity index measures overall species diversity, as well as richness or the evenness of the population. A site with a low richness score is often dominated by one or two main genera with the others only being present in trace amounts. Shannon diversity data in the healthy stream and settling pond sites were very similar as both populations are dominated by *Navicula* and *Eunotia*. Each site contained different diatom genera, but the ecosystem structure appears to be similar based on Shannon diversity calculations at each site. This suggests that the particular diatom genera present may be a better indicator of water quality than the diversity and richness of the aquatic life present. The borehole, not surprisingly, had a diversity and richness score of zero due to only one genus being present. The bridge site, however, showed the most evenly distributed and diverse population of diatoms according to the Shannon diversity data. The population at the bridge site was still dominated by one genus, *Navicula*, but a few other diatoms emerged in numbers significant enough to balance the population. Although the bridge site was impacted by the AMD discharge, a healthy aquatic ecosystem was achieved according to Shannon diversity index and higher number of genera observed. This suggests that even a slight change in water chemistry and substrate can have a huge effect on aquatic life, meaning only a small amount of remediation is needed at AMD impacted sites to make a huge difference in overall stream health and ecology.

Samples for this study were collected in mid-July when water temperatures are warmer than other times of the year, particularly at the settling pond. We know from sampling in the winter months that some seasonal variation in diatom diversity does occur. Changes in temperature can impact water quality factors such as the solubility of salts. With warmer temperatures, more nutrients are found in solution which can be filtered and used by diatoms. Changes in temperature can also impact the solubility of gases such as oxygen. In the summer months oxygen can be removed from the solution more quickly due to decreased solubility. This leads to the reaction and precipitate formation occurring closer to the borehole and thus better living conditions in the settling pond conducive to increased diatom diversity. During the winter, this reaction may occur more slowly and have an effect on downstream diatom growth. Future research will focus on the amount of seasonal variation within our sites as well as the overall effects of precipitate formation on diatom growth.

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