Microbial Enumeration and Assessment of Lower Mahoning River Sediment

by

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MICROBIAL ENUMERATION AND ASSESSMENT OF LOWER MAHONING RIVER SEDIMENT

Erica Richards

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ABSTRACT

Youngstown area steel mills were once among the most productive in the United States. However, industrial and municipal output into the river have been radically reduced in the past thirty years due to regulations and output restrictions (MS Consultants 1993). These have been enforced through the Federal Clean Water Act and the National Pollution Discharge Elimination System (NPDES) (OEPA, 1996). As a result, water quality of the Mahoning River has improved greatly; however, the river's sediments remain highly contaminated (OEPA 1996).

My study involved a quantitative microbiological examination of the river's sediments. By examining the quantity of bacterial communities present using both total and viable count techniques, an estimation of the river's benthic bacteria was gained. This estimation was performed by comparing the bacterial communities in severely impacted river sites with those in unimpacted ones. Sediment samples were collected in triplicate from June through December 1998, at five sites along the lower Mahoning River, and analyzed for total bacteria using a DAPI- fluorescence technique, and aerobic viable bacteria counts using modified nutrient agar. Physical characteristics at sampling sites were recorded on each sampling date.

Site C, a severely impacted site, had significantly higher counts of total bacteria when compared with the four other sites. Site E, a relatively unimpacted site, had significantly less total bacteria than the other four sites. We found higher total and viable counts of bacteria during months with colder sediment temperatures. Sites with the highest percent volatile organic content (VOC) also had the highest levels of total PAH's, PCB's, and heavy metals. The same sites also had the highest numbers of total bacteria and the lowest numbers of aerobic viable bacteria, despite a lack of significant correlations. These results suggest that a relationship does exist between total and viable bacteria, and total PAH, PCB, and heavy metal levels, but remains to be elucidated.

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Last, but not least, I would like to thank my parents, family, and friends for their love and support of both me and my academic pursuits (6 years of school!), and to Eric Matheny for his love, support, and field assistance without which I would have never made it!!

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INTRODUCTION

I. Industrial History of the Mahoning River

The Mahoning River Basin has an extensive history of unregulated manufacturing and industry along its banks. From the late 1800's to the mid-1900's, Youngstown area steel mills were among the most productive in the United States (MS Consultants 1993). Mahoning River water was used in coking and other steel producing processes and returned to the river as wastewater (MS Consultants 1993). Industrial pumping of the lower Mahoning River exceeded 800 million gallons per day (Harnisch 1971). Much of this water was returned to the river with blast furnace flue dust and coke plant organic waste mixed in (Harnisch 1971). Steel mills along the Mahoning were not implemented with adequate sedimentation treatment to remove flue dust until 1965 (Harnisch 1971).

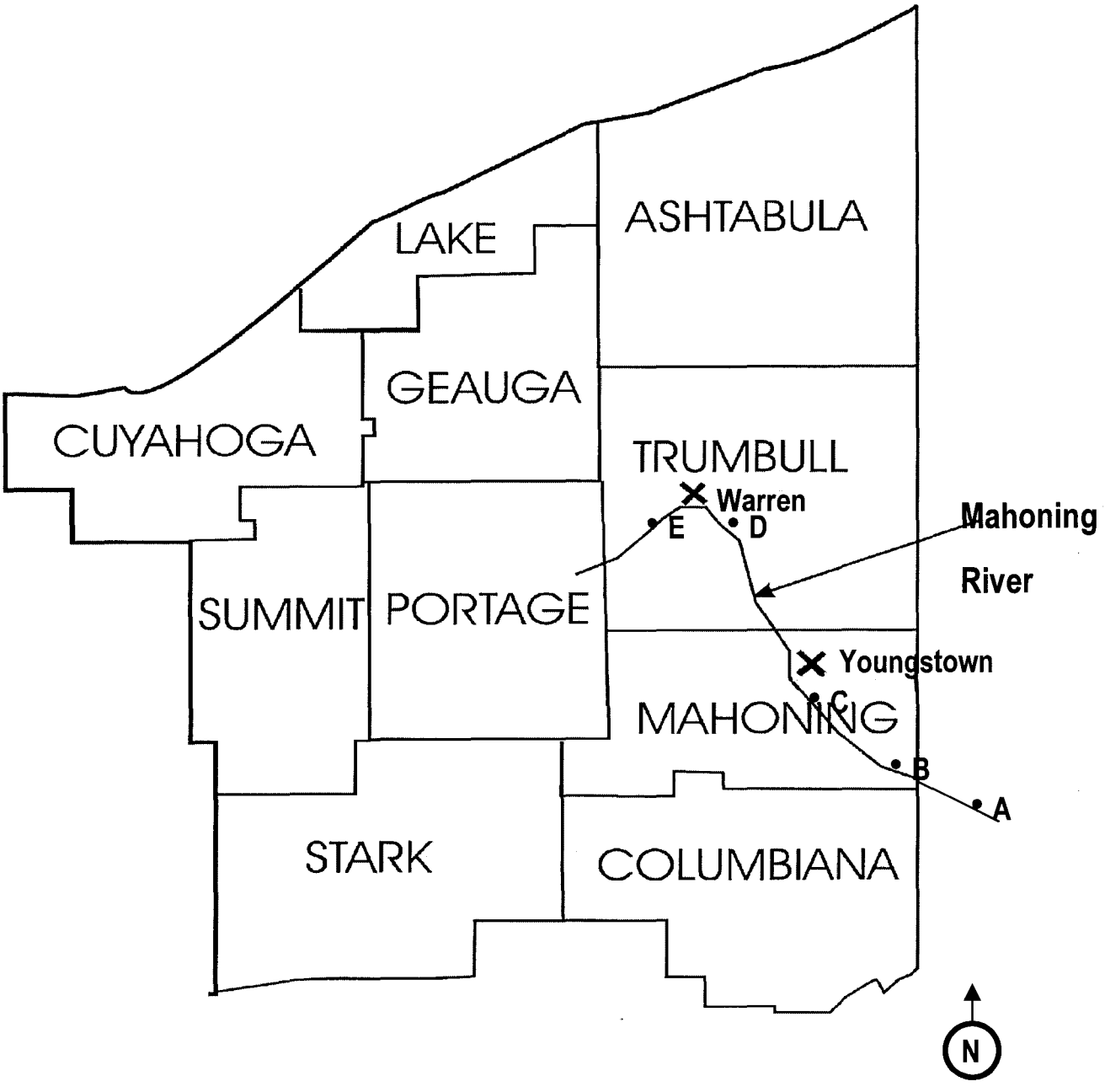
Coke plants used the river water to "quench" or cool the hot coal. This not only added large amounts of organic waste, particularly phenol, cyanides, and ammonia, but also increased the water temperature substantially (Harnisch 1971; Van Tassel 1970).

Before the implementation of primary sewage plants in the 1950's, raw untreated sewage flowed into the river, leaving it severely impacted by fecal coliform bacteria (OEPA 1996). Agreements were made between companies to allow industrial waste and sewage output to be expelled concurrently, as the industrial components helped to neutralize the odor of the sewage. Often times, municipal sewer lines carried both industrial wastes and plant sewage combined (Harnisch 1971). To compound these problems, nine lowhead dams were built to provide water for nine of the larger steel facilities along the lower Mahoning. These dams held the sediment back by impounding the streamflow, and they also concentrated the pollutants (OEPA 1996).

Nearly a century of this type of industrial activity has taken its toll on the river. However, industrial and municipal output into the river has been reduced for the past

thirty years due to new regulations and output restrictions through the Federal Clean Water Act and the National Pollution Discharge Elimination System (NPDES) (OEPA 1996). Although the river has greatly improved in water quality, much of the pollution has settled into the river's bottom to form black, contaminated, pudding-like sediments (OEPA 1996). The impact of this delineation in quality between the Mahoning River's sediments and water has been illustrated by the lack of any significant improvements in *Warm Water Habitat (WWH) aquatic life use attainment* status between 1981 and 1994 (OEPA 1996). *WWH aquatic life use attainment* designation defines the normal or typical assemblage of warmwater organisms that are expected to be found in Ohio rivers and streams, and also designates water quality suitable for these organisms (OEPA 1996). Despite the improvements in water quality, there have not been significant improvements in the *aquatic life use attainment* status of the Mahoning River. A total of 39.4 of 45.5 lower Mahoning River miles evaluated by the OEPA were found in non-attainment status of *WWH aquatic life use attainment* in both 1981 and 1994 (OEPA 1996). Non-attainment status means there were very poor levels of both fish and macroinvertebrates expected to be common in a warmwater habitat, and is thought to be due to the contaminated sediments (OEPA 1996).

In 1988, Dr. Ronald Fletcher of the Ohio Department of Health placed a ban on swimming, fishing, and wading in the lower Mahoning River, from Northwest Bridge Road in Warren, Ohio to the Pennsylvania-Ohio border (Fig. 1), due to dangerous levels of polycyclic aromatic hydrocarbons (PAH's), mirex, pthalate esters, and polychlorinated biphenyls (PCB's), (Fletcher 1988). Sediment contact and consumption of benthic fish and invertebrates have been strongly advised against. In 1997, the Ohio Department of Health advisory was reissued with a continuation of the ban on swimming and wading to avoid contaminated sediment contact, and several new contingencies on fish consumption, based on risk assessment.


LAKE
ASHTABULA
GEAUGA
CUYAHOGA
TRUMBULL
Warren
E
D
Mahoning
River
SUMMIT
PORTAGE
Youngstown
C
MAHONING
B
A
STARK
COLUMBIANA
N

II. Previous Mahoning River Research

Ohio Environmental Protection Agency (OEPA) performed chemical and biological surveys of the Mahoning River's water, sediment, fish, and macroinvertebrates in 1980, 1983, and 1986. More recently, in 1994 and 1995, OEPA performed the most extensive survey to date on the Mahoning River (OEPA 1996). Of the 28 lower Mahoning River sites examined for *aquatic life use attainment*, 23 were considered in non-attainment of qualities necessary for aquatic life. All 23 of these sites were at or below Warren, Ohio. Nineteen sites along the lower Mahoning River were tested for sediment heavy metals, and seventeen of these same sites were examined for sediment PAH's (OEPA 1996). To avoid bias and obtain a general overview of the river basin quality, individual sediment study sites were not chosen to pinpoint any particular pollutant sources. Four to six centimeter sediment grab samples were collected along the sides of the river channel at each of the above mentioned nineteen sites (OEPA 1996). A stainless steel scoop was used, and samples were taken five to ten feet from the bank of the river.

The Ohio EPA analyzed sediment levels for toxicity using the Kelly and Hite Guidelines (OEPA 1996). The Kelly and Hite Guidelines (1984) were established by the Illinois EPA based on an evaluation of seven years of stream sediment data, from 1974 to 1980. The guidelines were established to grade sediment quality from low concentration, non-elevated pollutant levels, at relatively unimpacted sites; to high concentration, slightly, highly, or extremely elevated pollutant levels, at heavily impacted sites (Kelly and Hite 1984). They are used to measure both PCB and heavy metal concentration levels, and any levels exhibiting higher than four standard deviations above the mean are considered significantly elevated (Kelly and Hite 1984).

In addition, Mahoning River sediment sampling was completed by the United States Environmental Protection Agency (USEPA) in late 1997. The Army Corps of Engineers (USACE) also performed an extensive examination of the sediment, including topographic mapping of the sediment's location along the river bottom, during the summer of 1998.

III. Project Introduction

Bacteria can be used as a bioindicator of the health of a river or other aquatic system (Lemke *et al.* 1996). By examining quantities of bacteria at both heavily impacted and unimpacted sites, effects of the pollutants on benthic bacterial assemblages can be estimated.

Bacteria found in the sediment are important in the cycling of bioelements (Stolp 1988). Several important methods of nutrient cycling common to benthic bacteria are the reduction of nitrates to nitrogen, an important nutrient source, and the reduction of sulfates to metal-binding sulfides, which decrease the accessibility of deleterious heavy metals (Droop & Jannasch 1977).

Up to this point, there has been no quantitative microbiological examination of the river's sediments. By examining the quantity of bacterial communities present using both total and viable techniques, an estimation of the river's benthic bacterial assemblages may be gained. This estimation can be performed by comparing the bacterial assemblages in a site with severely elevated pollution levels with those at a site with relatively non-elevated levels of pollution.

Also, as potential bioremediators of polluted systems, bacteria are an important indicator of the potential for less invasive return of the polluted river to within WWH attainment status (Aamand et al. 1989). This can be an important alternative or

supplement to dredging river sediments. Whether bacteria have the ability to adapt to contaminants and degrade them will certainly demonstrate the importance of examining the microbial system.

Bacterial abundance can be measured two different ways. Bacterial abundance is most commonly measured by performing total counts of bacterial cells present in a sample using epifluorescent staining techniques. The most widely accepted practice of estimating bacterial abundance involves using 4'6-diamidino-2-phenylindole fluorescent stain (DAPI) (Porter and Fieg 1980). DAPI stain allows counts of all microorganisms present, living or dead, by fluorescently dying nuclear, mitochondrial, and chloroplast DNA of all cells under a wide variety of conditions (Porter & Fieg 1980).

Another way of examining bacterial abundance is growing bacteria on prepared culture media, modified nutrient agar, and then counting the number of colony-forming units (CFU's) or individual bacteria cells that emerge (Leff 1994). This technique can be used to examine both aerobic and facultatively anaerobic bacteria. Viable counts, for CFU's, are on average at least one or more orders of magnitude lower than total bacterial counts, sometimes as much as ten times lower, as many bacteria enter a viable, non-culturable state when removed from their natural habitat (Leff 1994). Many bacteria, including strict anaerobes and some fragile aerobes, won't survive leaving the delicate balance of their natural environment. This selects for only the more hearty aerobes and facultative anaerobes to survive and be grown on nutrient agar.

Examining both total and viable counts could show population differences between polluted and unpolluted Mahoning River sites. Insight can also be given into whether physiological changes are occurring within the bacterial assemblage (Leff 1994). For example, if total density using the DAPI technique remains constant from site to site, and there are large fluctuations in CFU growth, it may be concluded that changes may be occurring in bacterial population diversity. These fluctuations between the proportions or

types of bacteria present may or may not affect total density. Both viable and total counts are important in examining population changes in Mahoning River benthic bacteria.

Goal: To determine quantities and characteristics of Mahoning River sediment bacterial communities.

Objectives:

1. To estimate bacterial abundances at study sites using total and viable count techniques.
2. To explore whether total or viable bacterial numbers are significantly different in highly polluted as opposed to less polluted sites.
3. To examine correlations between PAH's, PCB's, and total heavy metals, with bacterial abundance.
4. To examine correlations between total and viable bacterial abundance with sediment volatile organic content, sediment temperature, sediment pH, and dissolved oxygen in the river water.

METHODS

I. Experimental Design

Sediment samples were collected in triplicate, at each of five sites (Table 1, Fig. 1). Sampling was conducted in accordance with OEPA standards as listed in the OEPA Sediment Sampling Guide & Methodologies (1996-Draft). Sampling began June 1998 and continued monthly through December 1998. Five study sites were chosen based on their PAH, PCB, and heavy metal profiles as obtained from OEPA and the United States Army Corps of Engineers (USACE).

II. Study Site Selection

Five study sites were chosen based on distinctions in their PAH, PCB, and heavy metal profiles as obtained from OEPA and the USACE. All five sites are currently being monitored by OEPA. They were chosen not only for the above characteristics, but were also easily accessible from the riverbank. Sites were at several different types of locations. Two sites were near point sources in free-flowing areas of the river: wastewater treatment plant, site D in Warren, Ohio; and a steel mill, site C in Campbell, Ohio. One site was located above a lowhead dam: site B in Lowellville, Ohio. Another site was located below a lowhead dam in free-flowing water: site E in Leavittsburg, Ohio. This site was particularly less polluted as it was above the Ohio Department of Health advisory. The fifth site was in an unspecific free-flowing water site along the river near New Castle, Pennsylvania (site A). No official bans or advisories have been issued by the state of Pennsylvania. By examining a variety of different areas along the Mahoning

Table 1. Pollutant Data at Sampling Sites along Mahoning River, Northeastern Ohio and Western Pennsylvania. Site A is located at river mile 1.4, near the Route 108 bridge in New Castle, Pennsylvania. Site B is located approximately 75 m above the Lowellville dam in Lowellville, Ohio. Site C is located at river mile 16.4, behind an abandoned LTV steel plant in Campbell, Ohio. Site D is at river mile 35.5, behind the Warren wastewater treatment plant in Warren, Ohio. Site E is located at river mile 45.5, below the Leavittsburg dam in Leavittsburg, Ohio. Pollutant values are designated as to their data source with 1 corresponding to data from OEPA (1996), and 2 corresponding to USACE data (1998). Pollution levels are from the Kelly and Hite classification system and are ranked as follows: a = non-elevated levels, b = slightly elevated levels, c = elevated levels, d = highly elevated levels, e = extremely elevated levels, and -- means the parameter was analyzed but was below lab detection limits.

Sites	Total PAHs	Total Heavy Metals	Total PCBs
	(mg/kg dry wt)	(mg/kg dry wt)	(µg/kg dry wt)
A	21.9 [1]	159529.4 [1,e]	477.7 [1,d]
B	13510 [2]	190251.3 [2,e]	1900 [2,e]
C	95.2 [1]	135230.7 [1,e]	1360.6 [1,d]
D	35.7 [1]	350231.3 [1,e]	3375.8 [1,e]
E	-- [1]	9942.1 [1,a]	-- [1]

River, it was hoped to get an overview of the bacteria in the river sediments. Two of the sites chosen were less contaminated, with significantly less quantities of pollutants found (sites A & E). The other three sites selected were considered significantly contaminated in either their levels of PAH's and/or PCB's and/or heavy metals by OEPA or USACE (OEPA 1996; USACE 1998).

III. Sediment Sampling Methodology

Grab samples were collected 2-3 meters from the riverbank using a 3 m long PVC sampler, each sample ranging from 4-6 cm in depth. Specimens were taken from the side of the river channel, along the bank, and placed individually into sterile Whirlpack bags. Care was taken to find areas of fine sediment as rocky, gravelly samples contain less actual sediment and organic material and therefore fewer bacteria. Bacteria in a biofilm are known to preferentially attach to finer grain particles of sediments as opposed to more course particles, which supports the collection of finer grain sediments (Holm *et al.* 1992; Lutz-Arend Meyer-Reil 1994). These samples were kept at 4°C in a cooler during transport back to the lab until further analyzation. Physical characteristics were also gathered at the time of sampling, including sediment pH and temperature, water pH and temperature, dissolved oxygen, and ambient air temperature at the site. Sterile technique was used throughout the experimental procedure to prevent contamination of samples.

IV. Sediment Treatment

A 5 g (wet mass) sample was weighed out from each sample bag and added to a sterile vial of 10 ml filtered deionized water (0.2 μm pore size) (Schallenberg *et al.* 1989). Samples were mixed with 2 ml of filter sterilized (0.2 μm pore size) 0.01M

sodium pyrophosphate, a detergent, and agitated in a lab Enviro-shaker for 30 min (Durant *et al.* 1995). Halfway through this process, samples were removed and cooled by placing in ice water for 3 min. To aid particle separation, several drops of Tween 80 were added to the remaining solution (after completion of 30 min agitation in the Enviro-shaker) and the solution was then allowed to settle (Glazebrook *et al.* 1995). Ten µl of supernatant was removed from each sample to make dilutions for viable counts. The remaining supernatant was decanted to a sterile glass scintillation vial with a Teflon cap and preserved with 10 ml of 10% Formalin to give a 1:1 dilution (Lemke, McNamara, & Leff 1997). This process was repeated for all subsamples.

Both mechanical (Enviro-shaker) and chemical (detergent) processing of samples was necessary since the majority of sediment bacteria are contained within a biofilm, which means they are physically attached to sediment particles. As bacteria multiply and grow, they become immobilized on sediment particle surfaces (Lutz-Arend Meyer-Reil 1994). The entire matrix is embedded in a bacterial secretion of extracellular polysaccharides, which binds them together so they may function in a much more powerful way than a single organism alone (Lutz-Arend Meyer-Reil 1994). This can prove challenging for enumeration as it can make individual cells difficult to distinguish and can mask epifluorescent-stained cells (Lutz-Arend Meyer-Reil 1994). By taking the precautions listed above, enumeration techniques have been noted to result in much higher total counts of bacteria than using dilutions alone (Glazebrook *et al.* 1996).

V. Ash-Free Dry Mass Calculation

Five grams of the original sample (wet mass) was also used to calculate ash-free dry mass (AFDM) by drying at 105°C for 24 h, weighing, ashing at 550°C for 4 h in a muffle furnace, and weighing again (APHA 1992). AFDM is sediment with the water

and volatile organic content (VOC) removed. This permits expression of total bacterial counted as number per gram AFDM. It is useful to calculate bacteria in this way because volatile organic content, particularly organic matter resulting from primary production, is an important nutrient source for the sediment bacterial population (Stolp 1988).

VI. Bacterial Growth Procedure

Ten µl of each concentration of the unpreserved supernatant sample was plated on modified nutrient agar (Leff & Meyer 1991). The only exception to the modified nutrient agar was the omission of cycloheximide, a fungal deterrent, until the October sampling date. Dilutions of 10^{-3}, 10^{-4}, and 10^{-5} were used to culture the microbes. These concentrations were based on preliminary data examining one Mahoning River site for viable counts between 30 and 300 colony-forming units (CFU's) per plate. During the latter portion of the research (October-December), dilutions were made from 10^{-4} to 10^{-10} to compensate for larger numbers of bacteria emerging. Larger quantities of bacteria emerging were thought to be partially due to temperature changes in the environment (Leff *et al.* 1998) and partially due to the addition of cycloheximide, a fungal deterrent, to the growth media. The cycloheximide is thought to be a factor due to the inhibiting properties of the fungus to bacterial growth. A total of 3 plates were grown aerobically and incubated at 24°C for 3 days; and also another 2 plates grown anaerobically in a BBL anaerobic gas chamber at 24°C for 7 days.

VII. DAPI Staining Procedure

In a dark hood, a 25 mm diameter glass filter stand was assembled with a pre-dampened 0.22 μm black Nucleopore polycarbonate filter. All glassware was washed with 10% nitric acid and rinsed with filter-sterilized water before and between uses. Five ml of sterile, deionized water was passed through the filter to minimize hydrophobicity of the filter and "clumping" of bacterial cells on the dry filter (Glazebrook *et al.* 1996). One ml of deionized water was then added to the filter stand, followed by 25 μl of the preserved sample, and DAPI stain to a final concentration of 5 μg/ml (approximately 0.4 ml). The cells were stained in the filter stand for no less than 5 minutes in the dark and then gently suction-filtered through. Another 2 ml of sterile, deionized water was then passed through to remove any excess DAPI stain remaining. The damp polycarbonate filter was placed on a drop of low-fluorescence immersion oil on a large glass slide (50 x 75 mm). This was covered with another drop of low-fluorescence immersion oil and finally covered with a 25 mm round, glass coverslip. This technique is consistent with that designed by Porter and Fieg (1980) with the exception of the final DAPI concentration increase to 5 μg/ml (Tso & Taghon 1996). The stronger DAPI concentration has been found to be more effective and result in higher bacterial counts when working with sediment bacteria due to the large amount of detritus present (Tso & Taghon 1996).

Prepared slides were stored at 4°C in dark cardboard boxes to control for DAPI's light sensitivity. Slides were stored for no more then 3 days in these conditions. However, there is shown to be no degradation of DAPI-stained slides over the course of several months when kept in the dark at 4°C (Porter & Fieg 1980).

Using a DAPI-adapted microscope, 30 fields were counted per slide at 1000x magnification (Porter & Fieg 1980). DAPI-stained cells fluoresce a bright blue against

the black background, as DAPI binds with cellular DNA (Porter & Fieg 1980). Any remaining sediment particles and detritus fluoresce a pale yellow, if at all (Porter & Fieg 1980).

VIII. Numerical Conversions

The raw numbers of bacteria counted using the DAPI microscopic technique had to be converted to the number of bacteria per gram AFDM of each sediment sample. In order to do this the amount of AFDM in each sediment sample was calculated by drying sediment samples at 105°C for 24 h. This was followed by ashing the samples in a 550°C muffle furnace to remove volatile organic content. The final weight of the sample after ashing was the ash-free dry mass (AFDM).

The diameter of the portion of the polycarbonate filter used was 19 mm. Each viewing field on the 1000x microscope was 0.2 mm. The area of a viewing field (A_{VF}) was $(0.1)^2 * \pi = 0.0314$ mm^2. The total filter area (A_{TF}) used was $(9.5)^2 * \pi = 283.5287$.

Therefore:

$$\frac{A_{TF}}{A_{VF}} = \frac{283.5287 \text{ mm}^2}{0.0314 \text{ mm}^2} = 9025 \text{ viewing fields/ filter}$$

25 µl, or 1/40 ml sample was filtered for each slide and the contents of a 5 g wet mass sediment sample was used for each 20 ml of total fluid in a sample. The mean number of bacteria counted per viewing field in this example was 13.3667 (n=30).

Sample Calculation Example:

$$40 \times 13.3667 \times 9025 \times 20 \text{ ml} = \frac{96507574}{4.0154 \text{ g AFDM}} = \frac{2.4 \times 10^7 \text{ bacteria}}{\text{g AFDM}}$$

IX. Statistical Analyses

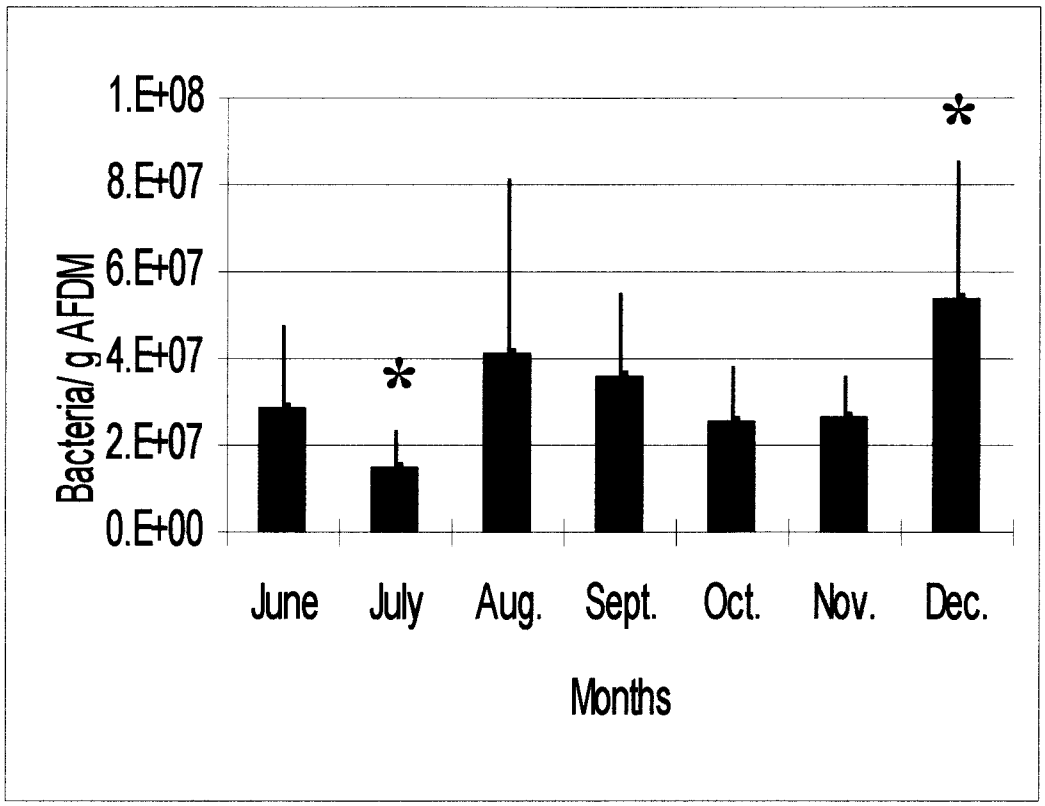
Data generated from total and aerobic viable densities were tested for normality using linearity and Levene homogeneity of variance tests. Data were log transformed to account for non-normal distribution. A one-way ANOVA was performed examining total bacteria counted by site and time, followed by Tukey's Honestly Significant Difference test when necessary. Pearson's Correlation test was used to explore relationships between total bacteria and total PAH's, PCB's, heavy metals, volatile solids, dissolved oxygen, sediment temperature, and sediment pH. Linear regression was also performed to compare log total bacteria with sediment temperature, sediment pH, volatile solids, and dissolved oxygen. SPSS v. 8.0 statistical software was used for all statistical calculations and statistics were performed in accordance with methods outlined in Zar's Biostatistical Analysis (1996).

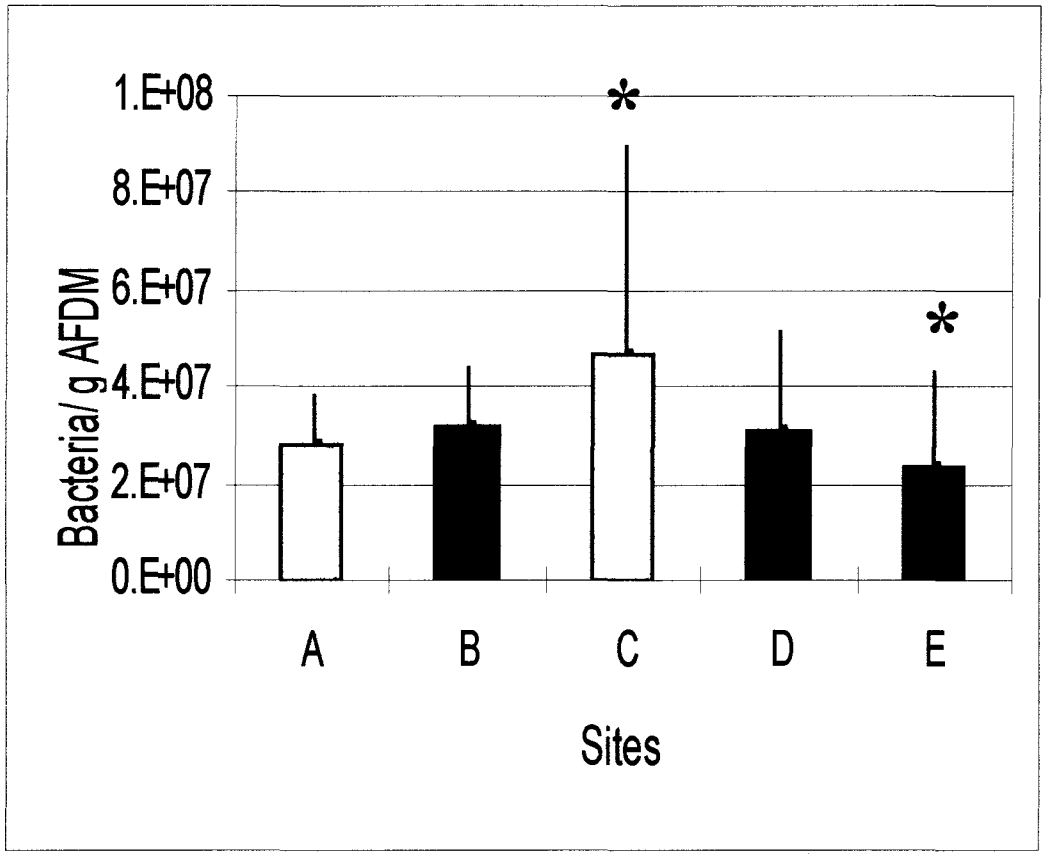
RESULTS

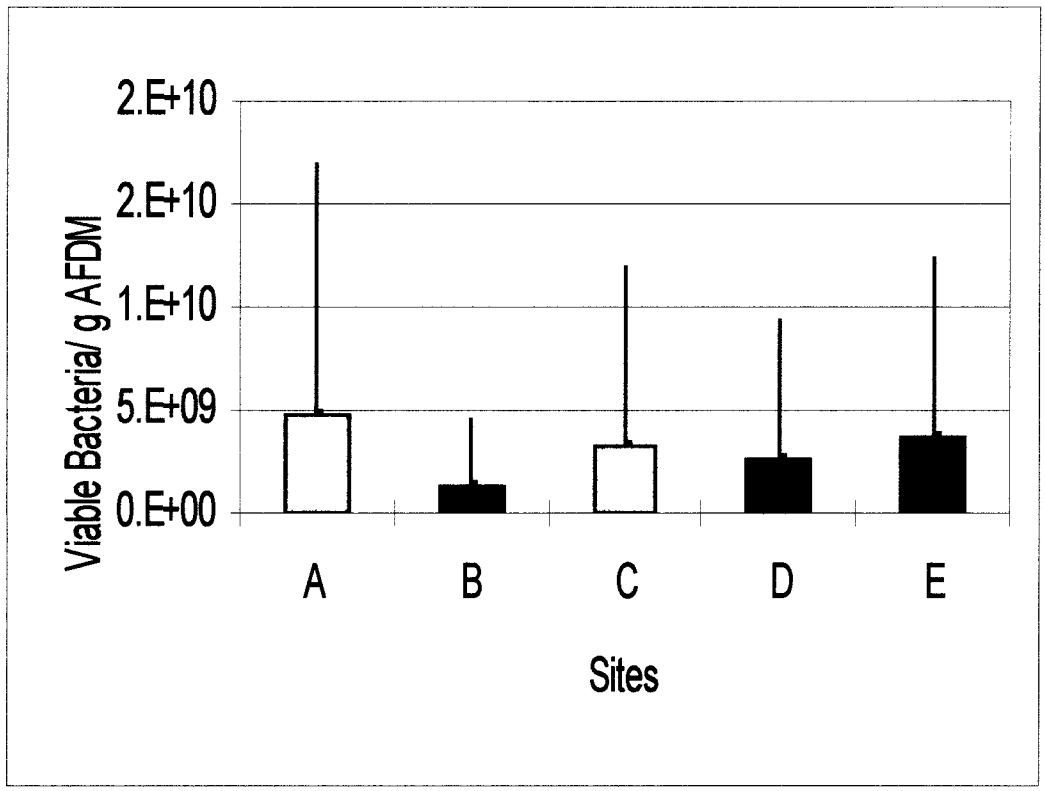
I. Bacterial Abundances

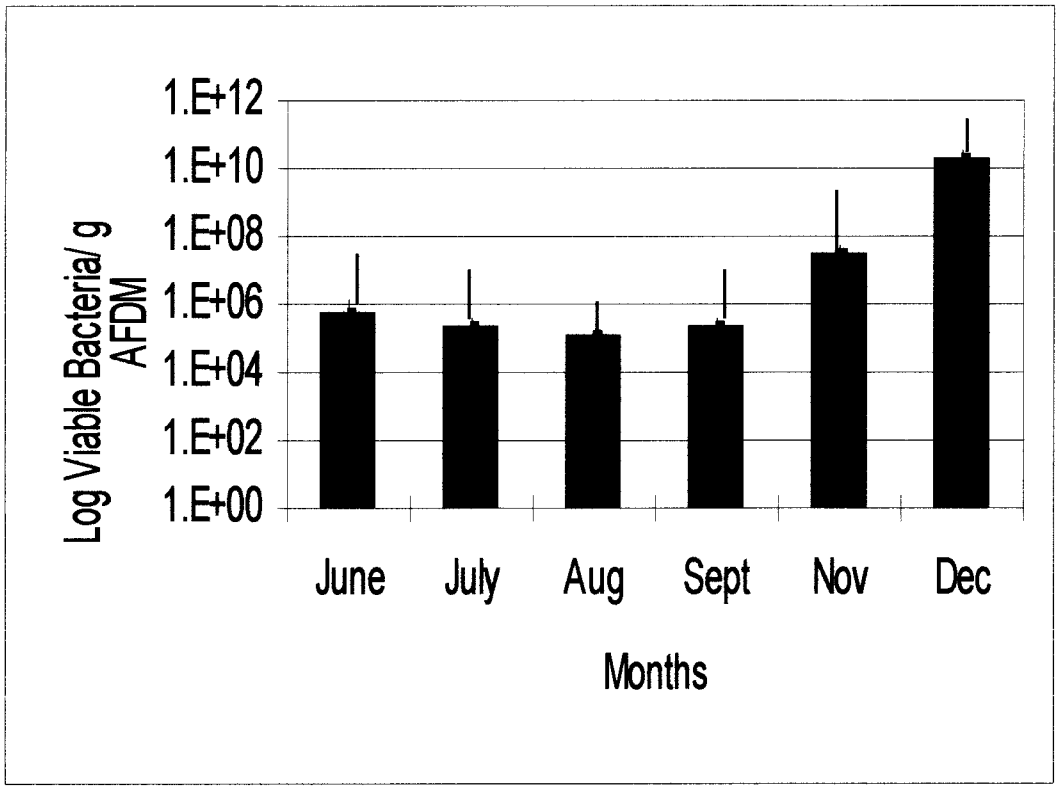
Sampling of the Mahoning River was performed in triplicate monthly from June through December to yield a total of 105 sediment samples. Total bacterial counts were within the range of those seen in similar studies on stream bacteria (Barkay & Pritchard 1988; Holm *et al.* 1992; Osgood & Boylen 1990). The greatest mean total bacteria counted was in December ($5.40 \pm 2.98 \times 10^7$ bacteria/ gram AFDM) (Fig 2). The month with the lowest mean total bacteria counted was July ($1.51 \times 10^7 \pm 7.98 \times 10^6$ bacteria/ gram AFDM) (Fig. 2). Site C in Campbell, Ohio, had the maximum mean total bacterial abundance per g AFDM at $4.73 \pm 4.16 \times 10^7$ (Fig. 3). The lowest mean total bacterial count per g AFDM, $2.30 \pm 1.94 \times 10^7$ was found at site E in Leavittsburg, Ohio, the site with the least amount of pollutants (Table 1, Fig. 3). Individual sediment samples ranged in mean total bacteria anywhere from 2.90×10^6 cells/ g AFDM at site E in July, to 1.60×10^8 cells/ g AFDM at site C in August (Appendix A).

Mean aerobic viable bacteria counted from individual sediment samples ranged widely; from 1.00×10^4 CFU's/ gram AFDM at site B in August, to 3.30×10^{10} CFU's/ gram AFDM at site C in December (Appendix A). The highest mean aerobic viable count was at site A, Route 108, Pennsylvania, a moderately polluted site, at $4.73 \times 10^9 \pm 1.19 \times 10^{10}$ CFU's/ gram AFDM (Fig. 4). Site B, Lowellville Dam, Lowellville, Ohio, the most significantly polluted site, had the lowest mean viable bacterial abundance at $1.16 \pm 3.30 \times 10^9$ CFU's/ gram AFDM (Fig. 4). Highest mean aerobic viable count was in December ($1.85 \pm 1.13 \times 10^{10}$ CFU's/ gram AFDM), while the lowest mean count was in August at $1.14 \times 10^5 \pm 6.93 \times 10^4$ CFU's/ gram AFDM (Fig. 5). This large amount of variation over time is most likely due to the addition of cycloheximide to the growth media beginning in October. October's bacterial growth was continuous and solid on the


1.E+08
8.E+07
6.E+07
4.E+07
2.E+07
0.E+00
Bacteria/ g AFDM
*
*
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Months


1.E+08
8.E+07
6.E+07
4.E+07
2.E+07
0.E+00
Bacteria/ g AFDM
*
*
A
B
C
D
E
Sites


2.E+10
2.E+10
1.E+10
5.E+09
0.E+00
Viable Bacteria/ g AFDM
A
B
C
D
E
Sites


1.E+12
1.E+10
1.E+08
1.E+06
1.E+04
1.E+02
1.E+00
Log Viable Bacteria/ g AFDM
June
July
Aug
Sept
Nov
Dec
Months

culture plates and impossible to count. Not only did this lead to a lack of viable count data for the month of October, the following two months of remaining sampling resulted in viable counts four to five degrees of magnitude higher than those recorded in the previous months. For this reason, the aerobic viable abundances are not consistent for the entire duration of the sampling period and analysis of variance was performed separately on results obtained before cycloheximide use, June through September, and after cycloheximide use, November and December.

The anaerobic viable bacterial counts were unreliable and sporadic, and were therefore not included in any results or analysis.

II. Sampling Sites and Months

Total and aerobic viable bacteria enumerated from Mahoning River sediment samples were statistically analyzed for normal distribution using a test for linearity. Data were found to be non-normally distributed and were suitably log-transformed (Zar 1996).

Analysis of the log of total bacterial abundances revealed site C, Campbell, OH as being significantly higher than the other sites and site E, in Leavittsburg, OH as being significantly lower than the other sites (ANOVA, $\alpha = 0.05$, $N = 105$, Tukey's HSD) (Fig. 3). Further examination of the log of total bacterial counts showed July's data was significantly lower than the data of August, September, November, and December; and December had significantly higher total counts than June, October, and November (Fig. 2).

Physical characteristics at sampling sites were recorded on each sampling date (Tables 2, 3). Linear regression of the log total number of bacteria with sediment temperature was highly significant ($T = -2.770$, $p = .0067$, $N = 105$) (Table 6). There was

Table 2. Monthly Mean Physical Characteristics Observed at Sampling Sites along Mahoning River in Northeastern Ohio and Western Pennsylvania, 1998

Months	Water Temperature (°C)	Water pH	Dissolved Oxygen (mg/l)	Sediment Temperature (°C)	Sediment pH	% Moisture Saturation of Sediment
June	20.58 ± 4.29	7.68 ± 0.21	7.06 ± 1.40	21.40 ± 1.62	6.62 ± 0.26	71 ± 15
July	25.20 ± 1.17	6.64 ± 0.47	6.68 ± 0.44	21.8 ± 1.17	6.32 ± 0.72	63 ± 19
August	27.20 ± 0.93	7.31 ± 0.12	7.22 ± 0.52	23.00 ± 1.10	6.50 ± 0.35	80 ± 17
Sept	25.10 ± 1.50	7.51 ± 0.11	7.22 ± 0.56	21.20 ± 1.60	6.28 ± 0.79	65 ± 11
Oct	20.10 ± 1.20	7.73 ± 0.18	7.96 ± 0.60	15.00 ± 0.63	6.20 ± 0.35	59 ± 6
Nov	13.00 ± 1.38	7.55 ± 0.26	7.28 ± 1.14	9.90 ± 1.66	6.20 ± 0.70	55 ± 3
Dec	10.20 ± 1.17	7.98 ± 0.17	9.28 ± 0.93	5.80 ± 1.94	6.25 ± 0.17	63 ± 5

Table 3. Mean Physical Characteristics Observed at Sampling Sites along Mahoning River in Northeastern Ohio and Western Pennsylvania, 1998. Site A is located at river mile 1.4, near the Route 108 bridge in New Castle, Pennsylvania. Site B is located approximately 75 m above the Lowellville dam in Lowellville, Ohio. Site C is located at river mile 16.4, behind an abandoned LTV steel plant in Campbell, Ohio. Site D is at river mile 35.5, behind the Warren wastewater treatment plant in Warren, Ohio. Site E is located at river mile 45.5, below the Leavittsburg dam in Leavittsburg, Ohio.

Sites	Water Temperature (°C)	Water pH	Dissolved Oxygen (mg/l)	Sediment Temperature	Sed pH	% Moisture Saturation of Sediment
A	20.07 ± 5.98	7.51 ± 0.35	7.31 ± 0.83	17.00 ± 6.78	5.99 ± 0.69	70 ± 15
B	19.64 ± 6.83	7.36 ± 0.25	6.59 ± 1.16	17.14 ± 5.84	6.61 ± 0.27	74 ± 18
C	21.43 ± 5.80	7.48 ± 0.37	7.43 ± 0.63	17.50 ± 5.97	5.89 ± 0.54	59 ± 7
D	20.77 ± 6.24	7.55 ± 0.79	8.03 ± 1.26	16.93 ± 7.07	6.64 ± 0.28	67 ± 15
E	19.07 ± 6.32	7.54 ± 0.34	8.29 ± 1.06	15.79 ± 6.32	6.62 ± 0.20	55 ± 4

Table 6. Multiple Linear Regression Analysis of Log Total Bacteria Compared with Physical Variables Measured Along the Mahoning River in Northeastern Ohio and Western Pennsylvania (N=105). ** Designates significance at the 99% confidence level.

Variable	T value	Significance
Sediment Temperature (°C)	-2.770	.0067**
Sediment pH	-0.720	.4731
% Volatile Organic Content (g)	0.614	.5406
Dissolved Oxygen (mg/l)	-0.127	.8991

also a significant linear regression between the log of total bacteria and the percent of volatile organic content in samples ($T = 3.477$, $p = .001$, $N = 105$) (Tables 4, 5, 6).

Analysis of variance of the log aerobic viable bacteria showed no significant differences between sites ($\alpha = 0.05$, $N = 90$) (Fig. 4). Log aerobic viable counts were analyzed over time (months) in two groups: the four months prior to the use of cycloheximide in the media, and the two months with the addition of cycloheximide. In the first four months, June had significantly higher viable counts than the other sites and August had significantly lower viable counts than the other sites (Fig. 5). November and December, the last two months, did not significantly differ from each other (Fig. 5). Linear regression analysis of the log aerobic viable bacteria with data on physical characteristics recorded was not significant.

III. Pollutant Correlations with Bacterial Abundances

There were no significant correlations between mean total bacterial abundances and total PAH's, PCB's, or total heavy metals (Table 7). There were also no significant correlations between mean aerobic viable bacteria and total PAH's, PCB's, or heavy metals.

IV. Physical & Chemical Characteristic Correlations with Bacterial Abundance

Mean physical characteristics were recorded by month and by site in Tables 2 and 3. Mean percent water and volatile organic content were recorded in Tables 4 and 5. Mean volatile organic content was highest at site B, in Lowellville, Ohio, and lowest at site E, in Leavittsburg, Ohio. This was reflected in the physical textures of the sediments—as site B was very humus-like and site E was very sandy.

Table 4. Mean Percent Water and Percent Volatile Organic Content in Mahoning River Sediment Samples from Northeastern Ohio and Western Pennsylvania, Shown by Site. Site A is located at river mile 1.4, near the Route 108 bridge in New Castle, Pennsylvania. Site B is located approximately 75 m above the Lowellville dam in Lowellville, Ohio. Site C is located at river mile 16.4, behind an abandoned LTV steel plant in Campbell, Ohio. Site D is at river mile 35.5, behind the Warren wastewater treatment plant in Warren, Ohio. Site E is located at river mile 45.5, below the Leavittsburg dam in Leavittsburg, Ohio.

Site	% Water	% Volatile Solids
A	25.36	1.91
B	48.94	14.75
C	38.9	10.93
D	41.31	11.46
E	25.76	1.77

Table 5. Monthly Mean Percent Water and Percent Volatile Solids in Mahoning River Sediment Samples, 1998

Months	% Water	% Volatile Solids
June	35.57	8.78
July	29.96	7.6
August	40.48	11.39
September	36.71	7.15
October	36.28	7.65
November	35.14	6.9
December	38.26	7.73

Table 7. Correlations with Log Total and Log Aerobic Bacteria Enumerated from Mahoning River sediments, 1998.
* Signifies Pearson correlation coefficient is significant at the 95% confidence level. ** Signifies Pearson correlation coefficient is significant at the 99% confidence level.

		Log Total Bacteria	**Log Aerobic Bacteria**	**Volatile Organic Content** (g)	**Dissolved Oxygen** (mg/l)	**Sediment Temp** (°C)	**Sediment pH**	**PAH** (mg/kg dry wt)	**PCB** (µg/kg dry wt)	**Heavy Metals** (mg/kg dry wt)	**Sites**	**Months**
Log Total Bacteria	*Pearson Correlation Coefficient*	N/A	.311**	.324**	0.137	-.230*	.059	.134	.157	.192	-.197*	.321**
	Sig.		.003	.001	.162	.018	.554	.207	.138	.070	.044	.001
	N		90	105	105	105	102	90	90	90	105	105
Log Aerobic Bacteria	*Pearson Correlation Coefficient*	0.311	N/A	-.095	.642**	-.915**	-.142	-.117	.000	.003	.026	.816**
	Sig.	.003		.372	.000	.000	.190	.316	.997	.977	.811	.000
	N	90		90	90	90	87	75	75	75	75	90

Results of all correlation analysis can be found in Table 7. Log total bacterial abundance was positively correlated with percent volatile organic content in samples (Pearson Correlation Coefficient = .324, $\alpha = 0.01$, N = 105). As sediment temperature decreased, the log total number of bacteria increased (Pearson Correlation Coefficient = -.230, $\alpha = 0.05$, N = 105). Log of total bacteria enumerated was also positively correlated with log of aerobic viable bacteria counted, meaning both increased over time. This may not be reliable due to discontinuity in the aerobic viable bacteria over time.

A negative correlation was observed between log total bacteria and sites. This simply means, however, that as we progress from site A through site E, total bacterial counts decreased. Site A was the farthest site downstream, and site E was the farthest site upstream, so more bacteria were found as we traveled farther and farther downstream. Over time, a positive Pearson Correlation Coefficient of .321 was noted between log total bacteria and months, meaning the total bacterial counts increased with time, from June through December ($\alpha = 0.01$, N = 105).

Log aerobic viable bacteria also had significant correlations with several physical characteristics. Log aerobic viable bacteria was found to increase as the amount of dissolved oxygen in the river water increased (Pearson Correlation Coefficient = .642, $\alpha = 0.01$, N = 90). As sediment temperature decreased, the numbers of aerobic viable bacteria cultured was found to increase, suggesting a link between bacteria that grow in colder sediment with greater culture viability (Pearson Correlation Coefficient = -.915, $\alpha = 0.01$, N = 90). Over time, aerobic viable bacteria increased significantly. However, this correlation is only exploiting the large increase from cycloheximide's effect and is of no real value.

DISCUSSION

The focus of this study was to examine the bacteria present in a local polluted streambed and to examine physical and anthropogenic chemical factors that may be affecting them. Overall, relationships with bacteria over time showed peaks in both total and aerobic bacteria in December when both water and sediment temperatures were the coldest, and showed slowest growth in the hottest summer months, July and August. Other researchers such as Glazebrook et al. (1996) and Leff et al. (1998) have also noted sediment and stream bacteria thriving in cold temperatures. On the Mahoning River, sediment temperatures dropped by as much as 17°C between August and December.

However, mean volatile organic content, often associated with bacteria, peaked in August at 11.39% while it was only at 7.73% in December. Site B had the highest percent volatile organic content but the lowest mean aerobic viable count. This suggests that viable bacteria may not be thriving in areas of high sediment organic content, as we would have expected. A plausible explanation for this is that the areas of highest organic content are also those with the most significant sediment pollution. The aerobic viable bacteria may be more sensitive to pollutants than the unculturable anaerobes, which make up a large fraction of total sediment bacteria. All physical characteristics examined were relatively consistent, showing little variation from site to site.

Although there were no significant correlations between sediment bacteria and pollutants at the $\alpha = 0.05$ level, total bacterial counts were positively correlated with total heavy metals at the 0.10 level, suggesting that a relationship may exist but needs to be examined more thoroughly. Despite a lack of significant correlation, the areas with the most heavy historical pollution, which also happen to be the areas with the highest organic matter, have the lowest viable counts. Such an area in this study was site B,

above the Lowellville Dam, Lowellville, Ohio. Factories and cities located upstream of Lowellville expelled pollutants into the river which concentrated above this dam to form a hotspot of pollution with extremely elevated levels (according to Kelly & Hite guidelines) of heavy metals, PCB's and over 13,000 mg/kg dry wt of PAH's present. Total bacterial numbers did not seem to be affected by pollution to any significant degree, even though total bacteria were significantly higher in areas with greater volatile organic contents (Table 7).

Total sediment bacterial numbers were within the range of similar studies that state they have low bacterial counts (Barkay and Pritchard 1988; Holm *et al.* 1992; Osgood and Boylen 1990). Other studies obtained higher bacterial counts and considered counts similar to ours to be low (Kaplan and Bott 1983). There could be several reasons for this. Bacteria may not have been stained sufficiently by the DAPI. Although the literature suggests five minutes as sufficient staining time (Porter and Fieg 1980), the wide variety of sediment types dealt with in this research, ranging from severely polluted and pudding-like to non-elevated pollution and sandy, may have required longer staining and resulted in inadequately fluorescing cells. Another persistent problem is sediment masking of stained bacterial cells. Due to the large amounts of very fine grain sediments in most of the sediment samples, it was virtually impossible to collect bacteria in fluid samples without retaining some fine-grain particles. In December's samples, the mean aerobic bacteria, which should have been a percentage of the total bacteria, slightly exceeded the total bacteria count. This was most likely due to a combination of factors including insufficient staining, sediment masking, and the effect of fungal deterrent on increasing viable bacterial growth. This has been seen in the literature before, such as by Lemke, Brown, & Leff (1997), and is not altogether uncommon.

In both the culturable and total bacteria data, a lot of variation was present from sample to sample. This may be due to the large amount of variation in the Mahoning

River's sediments. The shape of the riverbed, depth of the sediment, and flow rate of the river water play a large part in the establishment of sediment settling patterns, and changes from month to month and site to site (USACE 1998). Because of this, even sampling the exact same one square meter of river every month would not necessarily result in sampling the same sediment, as it may have washed downstream or been buried under new debris. Bacteria have also been studied for their ability to move downstream with fine sediment particles carrying genes that may be adapted to different pollutants or disturbances from area to area within the river (Leff *et al.* 1992). Perhaps this is helping them to adapt to the pollutants that remain in the Mahoning River's sediments.

In conclusion, although no significant correlations were found between PAH's, PCB's, heavy metals, and total or viable counts of bacteria, a relationship may exist. The relationship between larger total counts and smaller viable counts of bacteria in areas of higher volatile organic content and higher volumes of pollutants is apparent. This relationship suggests pollutants are indeed affecting bacteria, but due to the large amount of variation within samples, and inadequacies in total bacteria eluting techniques, this relationship remains to be elucidated.

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APPENDIX A.

Table A.1. Mahoning River Sediment Aerobic & Total Bacteria Data (including Log Bacteria Data), Collected from northeastern Ohio and western Pennsylvania, 1998

Month	Site	Aerobic Bacteria /g AFDM	Log Aerobic Bacteria/g AFDM	Total Bacteria /g AFDM	Log Total Bacteria/g AFDM
June	A	2.0E+05	5.3010	2.4E+07	7.3808
June	A	3.6E+05	5.5563	1.7E+07	7.2222
June	A	7.9E+04	4.8976	2.0E+07	7.3010
June	B	8.8E+04	4.9445	3.0E+07	7.4800
June	B	6.7E+04	4.8261	4.7E+07	7.6721
June	B	1.1E+05	5.0414	3.3E+07	7.5185
June	C	5.0E+05	5.6990	8.6E+07	7.9345
June	C	6.1E+05	5.7853	3.4E+07	7.5315
June	C	4.8E+05	5.6812	3.8E+07	7.5798
June	D	5.2E+05	5.7160	1.2E+07	7.0792
June	D	3.0E+05	5.4771	1.4E+07	7.1461
June	D	5.7E+05	5.7559	1.7E+07	7.2304
June	E	9.1E+04	4.9590	1.5E+07	7.1761
June	E	3.1E+06	6.4914	2.0E+07	7.3010
June	E	1.2E+06	6.0792	1.3E+07	7.1139
July	A	1.1E+05	5.0414	2.2E+07	7.3424
July	A	5.3E+05	5.7243	2.2E+07	7.3424
July	A	2.3E+05	5.3617	1.8E+07	7.2553
July	B	9.1E+04	4.9590	2.1E+07	7.3222
July	B	1.1E+05	5.0414	2.7E+07	7.4314
July	B	1.3E+05	5.1139	1.0E+07	7.0000
July	C	1.6E+05	5.2041	8.1E+06	6.9085
July	C	4.7E+05	5.6721	8.1E+06	6.9085
July	C	4.9E+05	5.6902	5.8E+06	6.7634
July	D	7.5E+04	4.8751	2.0E+07	7.3010
July	D	2.3E+05	5.3617	2.7E+07	7.4314
July	D	2.6E+05	5.4150	1.9E+07	7.2788
July	E	4.2E+05	5.6232	8.8E+06	6.9445
July	E	2.9E+05	5.4624	6.5E+06	6.8129
July	E	7.0E+04	4.8451	2.9E+06	6.4624
Aug	A	8.4E+04	4.9243	1.1E+07	7.0414
Aug	A	1.8E+05	5.2553	3.4E+07	7.5315
Aug	A	1.5E+05	5.1761	5.5E+07	7.7404
Aug	B	1.2E+05	5.0792	1.5E+07	7.1761
Aug	B	1.0E+04	4.0000	2.6E+07	7.4150
Aug	B	1.7E+05	5.2304	3.1E+07	7.4914
Aug	C	2.4E+05	5.3802	8.8E+07	7.9445
Aug	C	1.1E+05	5.0414	1.6E+08	8.2041
Aug	C	8.5E+04	4.9294	6.9E+07	7.8388

Table A.1. continued.

Aug	D	2.1E+05	5.3222	8.3E+06	6.9191
Aug	D	1.8E+05	5.2553	2.7E+07	7.4314
Aug	D	5.0E+04	4.6990	3.1E+07	7.4914
Aug	E	2.2E+04	4.3424	3.0E+07	7.4771
Aug	E	4.0E+04	4.6021	1.2E+07	7.0792
Aug	E	5.7E+04	4.7559	2.0E+07	7.3010
Sept	A	7.5E+04	4.8751	3.1E+07	7.4914
Sept	A	3.7E+04	4.5682	4.2E+07	7.6232
Sept	A	1.7E+05	5.2304	4.2E+07	7.6232
Sept	B	2.4E+05	5.3802	2.3E+07	7.3617
Sept	B	8.0E+04	4.9031	3.1E+07	7.4914
Sept	B	1.5E+05	5.1761	4.9E+07	7.6902
Sept	C	3.1E+05	5.4914	1.7E+07	7.2304
Sept	C	3.3E+05	5.5185	2.1E+07	7.3222
Sept	C	1.8E+05	5.2553	5.3E+07	7.7243
Sept	D	3.8E+05	5.5798	4.8E+07	7.6812
Sept	D	6.8E+05	5.8325	1.9E+07	7.2788
Sept	D	4.0E+04	4.6021	4.3E+07	7.6335
Sept	E	1.2E+05	5.0792	8.8E+07	7.9445
Sept	E	2.4E+05	5.3802	2.0E+07	7.3010
Sept	E	3.1E+04	4.4914	1.7E+07	7.2304
Oct	A	No Data	No Data	2.5E+07	7.3979
Oct	A	No Data	No Data	1.9E+07	7.2788
Oct	A	No Data	No Data	2.7E+07	7.4314
Oct	B	No Data	No Data	3.0E+07	7.4771
Oct	B	No Data	No Data	1.4E+07	7.1461
Oct	B	No Data	No Data	4.2E+07	7.6232
Oct	C	No Data	No Data	3.1E+07	7.4914
Oct	C	No Data	No Data	2.5E+07	7.3979
Oct	C	No Data	No Data	1.6E+07	7.2041
Oct	D	No Data	No Data	1.2E+07	7.0792
Oct	D	No Data	No Data	4.0E+07	7.6021
Oct	D	No Data	No Data	5.4E+07	7.7324
Oct	E	No Data	No Data	1.0E+07	7.0000
Oct	E	No Data	No Data	1.5E+07	7.1761
Oct	E	No Data	No Data	1.7E+07	7.2304
Nov	A	3.9E+07	7.5911	2.7E+07	7.4314
Nov	A	6.2E+07	7.7924	2.9E+07	7.4624
Nov	A	2.9E+07	7.4624	3.2E+07	7.5051
Nov	B	1.6E+07	7.2041	3.6E+07	7.5563
Nov	B	2.3E+07	7.3617	3.7E+07	7.5682
Nov	B	1.2E+07	7.0792	3.2E+07	7.5051

Table A.1. continued.

Nov	C	1.6E+07	7.2041	2.0E+07	7.3010
Nov	C	7.4E+06	6.8692	1.2E+07	7.0792
Nov	C	8.5E+06	6.9294	1.3E+07	7.1139
Nov	D	1.5E+07	7.1761	4.2E+07	7.6232
Nov	D	3.1E+07	7.4914	2.2E+07	7.3424
Nov	D	2.4E+07	7.3802	2.9E+07	7.4624
Nov	E	4.8E+07	7.6812	1.3E+07	7.1139
Nov	E	3.7E+07	7.5682	1.6E+07	7.2041
Nov	E	6.0E+07	7.7782	3.3E+07	7.5185
Dec	A	2.0E+10	10.3010	2.2E+07	7.3424
Dec	A	4.7E+10	10.6721	2.8E+07	7.4472
Dec	A	1.8E+10	10.2553	3.9E+07	7.5911
Dec	B	1.4E+10	10.1461	4.6E+07	7.6628
Dec	B	3.0E+09	9.4771	6.0E+07	7.7782
Dec	B	3.9E+09	9.5911	3.9E+07	7.5911
Dec	C	3.3E+10	10.5185	6.9E+07	7.8388
Dec	C	1.9E+10	10.2788	1.1E+08	8.0414
Dec	C	4.8E+09	9.6812	1.1E+08	8.0414
Dec	D	1.7E+10	10.2304	1.0E+08	8.0000
Dec	D	2.4E+10	10.3802	3.8E+07	7.5798
Dec	D	6.4E+09	9.8062	2.3E+07	7.3617
Dec	E	2.5E+10	10.3979	5.9E+07	7.7709
Dec	E	2.3E+10	10.3617	4.7E+07	7.6721
Dec	E	1.9E+10	10.2788	2.0E+07	7.3010

Table A.2. Physical Characteristics at Mahoning River Study Sites, in Northeastern Ohio and Western Pennsylvania, 1998

Date	Site	H_2O Temp (°C)	H_2O pH	Dissolved O_2 (mg/l)	Sediment Temp. (°C)	Sediment pH	% Saturation
June	A	20	7.65	6.9	20	6.6	0.68
June	B	12.5	7.46	4.4	19	6.9	1
June	C	22.5	8.04	8	22	6.2	0.7
June	D	23.9	7.74	7.9	23	6.9	0.58
June	E	24	7.5	8.1	23	6.5	0.59
July	A	25	6.86	6.4	22	6.9	0.5
July	B	26	6.91	7.1	21	6.1	1
July	C	26	6.81	7.3	23	5	0.5
July	D	26	5.7	6.2	23	6.8	0.6
July	E	23	6.94	6.4	20	6.8	0.55
Aug.	A	26.5	7.22	6.6	23	6	1
Aug.	B	27	7.25	7.2	24	6.9	0.7
Aug.	C	28	7.29	6.7	23	6.9	0.68
Aug.	D	28.5	7.54	7.8	24	6.4	1
Aug.	E	26	7.26	7.8	21	6.3	0.6
Sept.	A	25	7.56	7.6	23	5	0.8
Sept.	B	27	7.41	6.4	23	6.8	0.72
Sept.	C	26.5	7.39	6.7	21	5.7	0.5
Sept.	D	24	7.7	7.6	20	7	0.7
Sept.	E	23	7.5	7.8	19	6.9	0.55
Oct.	A	21	7.71	8	15	6	0.67
Oct.	B	21	7.52	7.2	15	6.5	0.6
Oct.	C	21	7.57	7.4	16	5.6	0.61
Oct.	D	19.5	7.98	8.4	15	6.4	0.6
Oct.	E	18	7.89	8.8	14	6.5	0.49
Nov.	A	14	7.5	6.8	13	5	0.57
Nov.	B	14	7.23	5.6	10	6.7	0.53
Nov.	C	14	7.4	7.3	9.5	5.8	0.58
Nov.	D	12.5	7.99	7.6	8.5	6.8	0.55
Nov.	E	10.5	7.64	9.1	8.5	6.7	0.5
Dec.	A	9	8.05	8.9	3	6.4	0.7
Dec.	B	10	7.75	8.2	8	6.4	0.6
Dec.	C	12	7.84	8.6	8	6	0.58
Dec.	D	11	8.23	10.7	5	6.2	No Data
Dec.	E	9	8.02	10	5	No Data	No Data