# Polycyclic Aromatic Hydrocarbons and Microbial Community Structure in the Mahoning River Bank Sediments

by

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**Abstract:**

Sediments in the Lower Mahoning River are among the most polluted in the United States. Polycyclic aromatic hydrocarbons (PAHs) are the most extensively found contaminants posing the greatest human and ecological risks in the Mahoning River sediments. Fatty acid methyl esters (FAMEs) were used to determine microbial community structure in bank sediments and to determine possible correlations among microbial biomass, microbial activity, PAHs and physical sediment characteristics. Nine sediment cores were collected from two sites (top core samples were taken at 5 centimeters and bottom core samples were taken at 2 meters) from the Mahoning River banks during March 2005. Leavittsburg was a no-to-low PAH site and Lowellville was a high PAH site. There were PAHs detected in all samples and all depths but, they were only quantifiable in highly contaminated sediments from Lowellville. Both bottom locations had very similar biomass and FAMEs, with 27-37% of the microbial community structure being composed of sulfate reducing and other anaerobic bacteria. The highest measured PAH concentrations found in Lowellville bottom sediments (61.8 $\mu g/g\ g^{-1}$) correlated with the highest measured microbial activity (195 nmol INTF $g^{-1}$), indicating potential for bioremediation. Multivariate ordination (Principal Components Analysis) indicated opposite loadings (negative vs. positive). Sediment samples tended to cluster by core depth, more than by site.

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# Chapter 1: Introduction

## 1.1 Mahoning River History:

The Lower Mahoning River has been one of the most polluted rivers in Ohio. From Leavittsburg, Ohio, the river flows southeasterly into Pennsylvania through the cities of Warren, Niles, Girard, Youngstown, Struthers, and Lowellville (Figure 1). It ultimately joins the Ohio River by first joining with the Shenango River to form the Beaver River, which empties into the Ohio River.

The Mahoning River has been significantly altered by the construction of numerous large reservoirs and low-head dams. This has created an alternating series of free-flowing and impounded segments throughout the Mahoning River mainstem. The dams were created to provide a reservoir of cooling waters for the steel industries (Ohio EPA, 1996). Types of industrial waste discharged to the river included petroleum, lubricating oils, and many various chemicals. Between 1900 and the 1970's the Mahoning received up to and over 70,000 lbs of oil and grease each day (USACE, 1999).

Today, water quality in the river has improved due to the tapering off of unregulated waste and the decline of the steel industry. However, the sediment quality has not improved. Levels of polycyclic aromatic hydrocarbons (PAHs) are elevated in sediments because of their tendency to bind to the sediment particles, and their low solubility rates (MRC, 2005). When introduced into aquatic environments, the sediments serve as a repository for the majority of these toxins (OEPA, 1996).

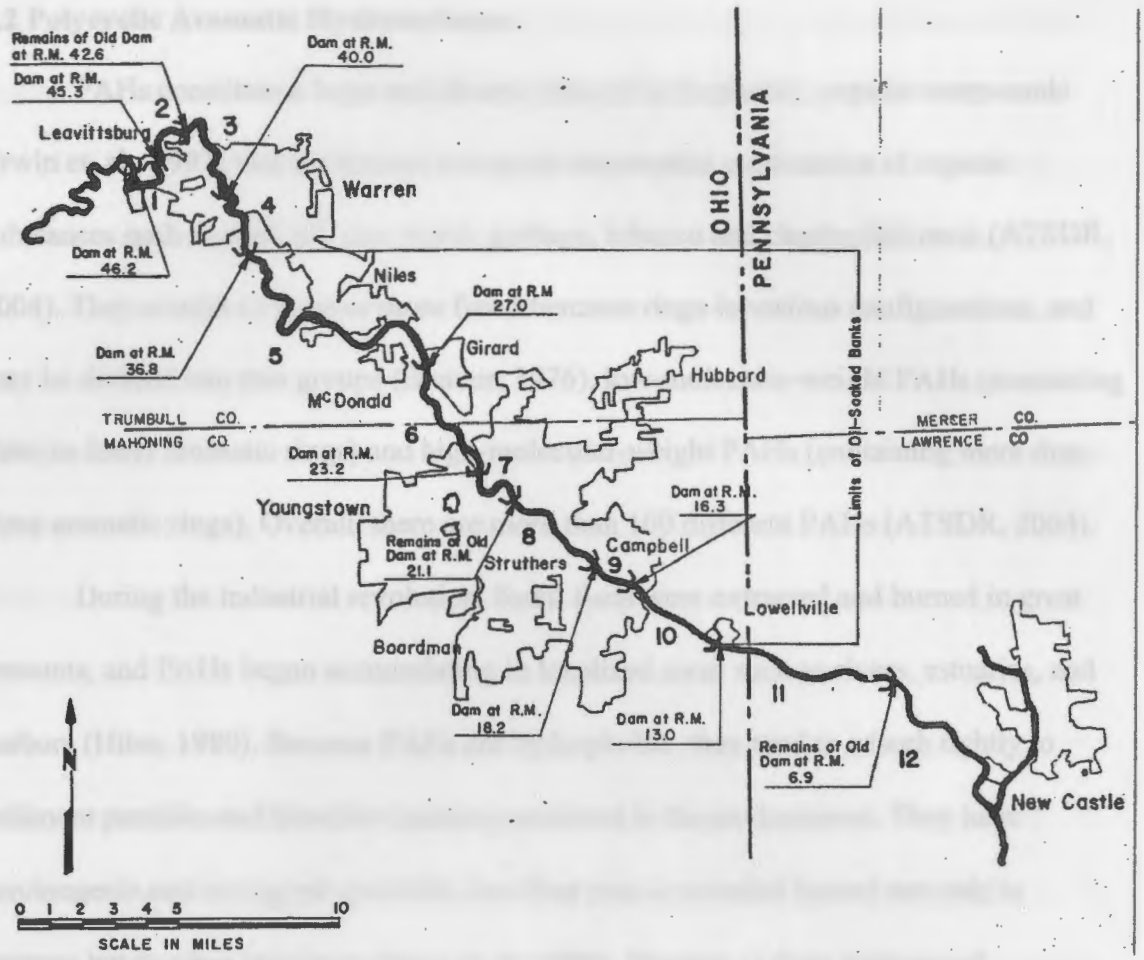


**Figure 1: Map of the lower Mahoning River.**

**1.2 Polycyclic Aromatic Hydrocarbons:**

PAHs constitute a large and diverse class of hydrophobic, organic compounds (Irwin et. al., 1997) that are formed during the incomplete combustion of organic substances such as coal, oil, gas, wood, garbage, tobacco and charbroiled meat (ATSDR, 2004). They consist of three or more fused benzene rings in various configurations, and may be divided into two groups (Blumer, 1976), low-molecular-weight PAHs (containing three or fewer aromatic rings) and high-molecular-weight PAHs (containing more than three aromatic rings). Overall, there are more than 100 different PAHs (ATSDR, 2004).

During the industrial revolution, fossil fuels were extracted and burned in great amounts, and PAHs began accumulating in localized areas such as rivers, estuaries, and harbors (Hites, 1980). Because PAHs are hydrophobic, they tend to adsorb tightly to sediment particles and therefore become persistent in the environment. They have carcinogenic and mutagenic potential, and thus pose a potential hazard not only to humans but to other life forms (Fang et. al, 1996). Because of their widespread distribution, environmental persistence, and harmful effects on human health, there is a need for a safe and effective way to remediate PAHs in the Mahoning River sediments (USACE, 1999).

**1.3 Microbial Community Structure:**

There are many methods that can be used to study microbial community structure. These methods include phenotypic methods such as Biolog substrate utilization profile analysis and fatty acid methyl ester (FAME) analysis. Genomic methods such as guanine-plus-cytosine composition analysis and newer genomic methods based on PCR amplification.

Newer genomic methods based on PCR amplification include ribosomal DNA restriction analysis, cloning and sequencing, denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) analysis and length heterogeneity PCR (LH-PCR) analysis (Kirk et al., 2003). T-RFLP identifies different sizes of PCR products based on restriction site variability. LH-PCR utilizes variations in the length of 16S ribosomal DNA sequences for species identification. T-RFLP analysis has been used successfully for a variety of environments. Use of the LH-PCR method has only been used to study microbial diversity for aquatic environments (Ritchie et al., 2000). DGGE and temperature gradient gel electrophoresis (TGGE) are two very similar methods for studying microbial diversity. For both of these methods, DNA is extracted from environmental samples and PCR amplified using universal primers for 16S or 18S rRNA. These two methods reliable, reproducible, rapid, somewhat inexpensive, and allow analysis of multiple samples making it possible to follow changes in microbial populations. Disadvantages include PCR biases, laborious sample handling, which could influence the microbial community, and variable DNA extraction efficiency. Additionally, DGGE may only detect 1–2% of the microbial population representing dominant species present in an environmental sample (Kirk et al., 2003). Also, one of the concerns with all PCR-based methods is biased representation of the community (Ritchie et al., 2000).

Microbial community structure can be determined by analyzing phospholipid fatty acids (PLFAs) which then can be analyzed as fatty acid methyl esters (FAMEs). Lipids are useful biomarkers that are an essential component to every living cell and can give a profile of the community in the environment (Table 1). By extracting the

phospholipids from the cell membrane, the fatty acid tail can be extracted and analyzed as FAMEs by GC or GC/MS. A methyl group is added to the fatty acid to increase volatility for GC analysis (Langworthy et. al., 2002). There are many advantages to using FAME analysis. It has been proven to be relatively simple, fast, and effective for assessing community structure accurately. The method is also reproducible and uses small sample sizes to simultaneously recover PAHs, FAMEs, and biomass in the same sample from a direct soil extraction (Fang and Findlay, 1996). However, disadvantages of this method are that individual fatty acids cannot be used to represent individual species. This is because individual species may contain numerous fatty acids and those same fatty acids can occur in more than one species (Kirk et al., 2003).

Fatty acids are named according to the total number of carbon atoms, the number of double bonds, and the position of where the first double bond is encountered from the methyl end of the molecule (Langworthy et al., 2002). For example, 16:0 would indicate 16 carbons in length and no double bonds (Figure 2), 20:5w3 would indicate 20 carbons in length, 5 double bonds, and the first double bond occurring at the number 3 carbon. Fatty acids may also contain a prefixes, which are shown in Table 2.

**Table 1. Common FAME biomarkers (Findlay, 2004)**

| Group | Fatty Acids |
|---|---|
| Green Algae & Higher Plants: | 16:1w13t, 18:1w9, 18:3w3, 18:2w6 |
| Heterotrophic microeukaryotes: | 18:1w9, 18:2w6, 18:3w6, 20:2w6, 20:3w6, 20:4w6 |
| Fungi: | 18:1w9, 18:2w6, 18:3w6, 18:3w3 |
| Aerobic bacteria and microeukaryotes: | 16:1w5, 16:1w7, 17:1w6, 17:1w9, 18:1w7, 18:1w9, 18:2w6 |
| Bacteria: | 15:0, i15:0, a15:0, 16:1w5, 16:1w9, i17:0, a17:0, cy17:0, 17:0, 18:1w5, i19:0, a19:0 |
| Aerobic bacteria: | 16:1w7, 18:1w7 |
| Gram-positive bacteria: | mid-branched fatty acids |
| Gram-positive bacteria and some gram-negative anaerobic bacteria: | 14:0, a15:0, i15:0, 15:0, i16:0 |
| Methanotrophs: | Type I: 16:1w8c, 16:1w5c , 16:1w6<br>Type II: 18:1w8c, 18:1w8t |
| Sulfate reducing bacteria and other anaerobic bacteria: | 16:0, 10Me16:0, a17:0, i17:0, 17:0, 18:0, cy9:0 |

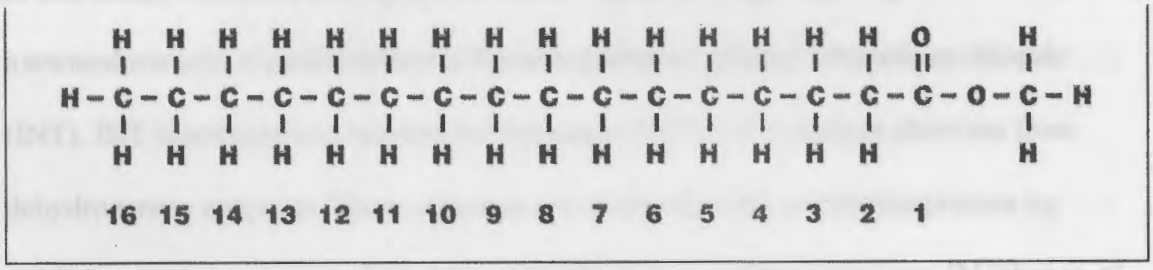


**Figure 2. The above represents the fatty acid methyl ester 16:0.**

**Table 2. Fatty acid designations (Langworthy et. al., 2002)**

| | |
|---|---|
| "i" | iso-branched (methyl branch on the 2nd carbon from the methyl end) |
| "a" | anteiso-branched (methyl branch on the 3rd carbon from the methyl end) |
| "10Me" | methyl branch on the 10th carbon from the carboxylate end |
| "cy" | cyclopropyl |
| "br" | Methyl branching at undetermined positions |
| "c" and "t" | *cis* and *trans* geometric isomers |

**1.4 Microbial Biomass:**

Microbial biomass is determined by measuring the lipid-bound phosphates from microbial cells (Vestal and White, 1989). The phosphates are extracted by organic solvents from phospholipids following the same extraction process as for FAME analysis. The phosphate is removed from the lipid by a potassium persulfate digestion and is then oxidized to orthophosphate which reacts colormetrically with malachite green and detected in a spectrophotometer (Findlay, 2004). The biomass is expressed as mass of microbes per gram of sediment (Vestal and White, 1989).

**1.5 Microbial Activity:**

Microbial activity is used to measure respiration in microbial cells. This provides useful information to contaminated sites such as the Mahoning River for bioremediation. In this study, microbial activity was measured by estimating dehydrogenase activity using a tetrazolium salt, 2-(*p*-Iodophenyl)-3(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT). INT is reduced to a red-colored formazan (INTF) as it accepts electrons from dehydrogenase enzymes. These enzymes are involved in the respiration process by oxidizing organic compounds and quantified by using spectrophotometry (Mosher et. al., 2002).

Another method uses 5-cyano-2, 3-ditolyl tetrazolium chloride (CTC) and 5-(4,6-dichlorotriazinyl) aminofluorescein (DTAF) to quantify total microbial biomass and microbial activity. CTC is a tetrazolium dye that is reduced to a fluorescent formazan by the electron transport system and/or dehydrogenase enzymes. DTAF is used to quantify microbial biomass by using a fluorescein-based fluorochrome that stains the bacterial cell walls

The CTC/DTAF and the INT method both have been shown to be effective for quantifying the metabolic activity of aerobic and anaerobic bacteria. They also have been proved to be rapid, reliable, and inexpensive methods (Mosher et al., 2002).

Other methods to determine microbial activity have been done by adding radiolabeled carbon substrates. The assimilation of radiolabeled $^{14}C$ acetate or $^{14}C$ glucose into lipids then can be measured (Vestal and White, 1989).

**1.6 Objectives:**

The objective of this research was to study the relationships between concentrations of PAHs, microbial community structure, microbial biomass, and microbial activity. Microorganisms respond to modifications in the environment and under stress the structure of the community may change. Therefore by analyzing the microbial ecology including microbial communities, biomass, and activity, it may be possible to offer a responsive biomonitoring tool to measure environmental stress (Fang et. al., 1996).

# Chapter 2: Methods

## 2.1 Sample Collection:

Sediment samples were collected to analyze PAHs, phospholipids, phosphate biomass, microbial activity, total organic carbon, sediment particle size distribution, and percent moisture. The sediment samples were collected using an AMS manual auger from 2 locations along the Mahoning River bank, Leavittsburg (river mile 46.3) and Lowellville (river mile 13.3) (Figure 3 and 4). The Leavittsburg location is known to contain the lowest levels of PAHs and is referred to as the control reach for the Mahoning River Restoration Project. The Lowellville location is known for its high concentrations of PAHs. Nine cores each were taken from both sites upstream from the dam at two depths, one at approximately 5 centimeters below the surface and the other one at the visibly contaminated layer located at approximately 5 meters. Samples were placed in plastic bags and stored on ice after collection. Leavittsburg samples were collected on March 21, 2005, with an air temperature of 34.2 degrees and sediment samples ranged from 35.4-35.6 degrees in both sediment layers. Lowellville samples were collected on March 28, 2005, with an air temperature of 47.9 degrees and sediment samples ranged from 42.7 – 44.8 degrees. Microbial activity was quantified immediately upon returning to the lab along with three of the core samples for the lipid and PAH extraction. The remainder of the cores were weighed and stored at -70°C until ready for use.

**Figure 3. Sample collection at Leavittsburg**

**Figure 4. Creating core samples with the auger at Leavittsburg**

**2.2 Simultaneous Polycyclic Aromatic Hydrocarbon and Lipid Extraction:**

Lipids were extracted using the Fang and Findlay (Findlay, 2003) extraction method for organic pollutants and microbial lipids based on the Bligh and Dyer method. A mixture of 7.5 ml of Optima grade dichloromethane (DCM), 15 ml of Optima grade methanol, and 4.5 ml of 50 mM phosphate buffer was added to a 50 ml glass tube with Teflon cap along with 50 µl of a surrogate solution. Approximately 0.65 grams of sediment were added to the mixture, shaken, and let stand overnight in the refrigerator at 4°C. Two blanks with no sediment were treated identically. The next day, the aqueous phase was removed, transferred to a 50 ml tube and stored in the refrigerator at 4°C. DCM was added in three 2.5 ml aliquots to the original sediment tube through a Pasteur pipette used for transfers; another 15 ml methanol and 4.5 ml of 50mM phosphate buffer was added to the sediment tube, shaken and let stand overnight again in the refrigerator. The aqueous phase ($2^{nd}$ time) was transferred again to a 50 ml tube. Seven and a half milliliters of DI water were added to separate phases, shaken and let stand overnight in the refrigerator at 4°C. A final 7.5 ml of DCM and 7.5 ml of DI water were added to the aqueous phase in the 50 ml tubes, shaken and let stand in the refrigerator at 4°C overnight.

After 24 hours, the aqueous samples were centrifuged at 1500 rpm for 5 minutes and the upper water/methanol phase was removed with a Pasteur pipette connected to an aspirator. A 5 ml pipette was used to transfer the organic phase to sodium sulfate columns into 15 ml conical tubes using the Supelco Visiprep. The sodium sulfate columns are 6 ml glass columns with Teflon frits containing 1 gram of dry sodium sulfate and packed with 2 ml of DCM such that the sodium sulfate was always covered with

DCM without. One ml of DCM was then added to the original 50 ml tube, vortexed, centrifuged, and the organic phase transferred onto the sodium sulfate column. This step was repeated two more times without vortexing or centrifuging. Aliquots were transferred and loaded to the column without letting it run dry. The combined eluates from the sodium sulfate column were concentrated in a nitrogen evaporator at 35- 40 °C to 1 ml. The column was then rinsed with two 1 ml aliquots of DCM and finally pulled to dryness. The sample was concentrated in the 15 ml tube under nitrogen at 35-40°C to one drop.

To analyze phosphate biomass, the sample was brought up to volume with 1 ml of chloroform. Two 100 µl samples were taken from each vial and placed into 1 ml ampules. The ampules were rinsed with 100 µl of DCM and dried under nitrogen at 37°C. Four-hundred and fifty microliters of saturated potassium persulfate were added to the ampules, which were then flame sealed and let stand for 24 hours at 105°C. The ampules were cooled to room temperature and 100 µl of ammonium molybdate were added. After 10 minutes, 450 µl of malachite green were added and let stand for 30 minutes. The sample was transferred to a disposable cuvette and read on a Biomate 5 spectrophotometer at 610 nm.

For the separation of the sample into the PAH, neutral lipid, glycolipid, and phospholipid fractions, silica columns were used. Before use they were rinsed with 2 ml of chloroform and 2 ml of hexane. Copper filings were then added to the column, which was cleaned with 2 rinses of 1 N HCl, 2 rinses of methanol, 2 rinses of DCM, and 2 rinses of hexane and dried under nitrogen.

PAH Fraction: A clean test tube was placed in the column apparatus to collect hexane. The total lipid fraction was transferred from chloroform to 200 µl hexane using solvent exchange and a drop of chloroform was added, vortexed and transferred to column. The transfer was repeated three more times using 100 µl of hexane. The PAH fraction was eluted from the column with 5 ml of hexane in three aliquots (1 ml, 2 ml, 2ml) and let go dry by gravity. The PAH fraction was then stored and 0.5 g of dry copper wire was added.

Neutral lipid fraction: One milliliter of chloroform was added to the column to elute the remaining hexane. A test tube was then placed in the Visiprep apparatus to collect the neutral lipid fraction which was eluted with four 1 ml aliquots of chloroform using a vacuum. The vacuum was released when the air/chloroform interface reached the frit.

Glycolipid fraction: One milliliter of acetone was then added. As the solvent front approached the 2/3 mark, the column was closed and the test tube containing chloroform and the neutral lipids was removed. Two aliquots of 2 ml of acetone were added to elute the glycolipids. The acetone was pulled to the frit surface without drying it out. The acetone fraction with the glycolipids was discarded.

Phospholipid fraction: One milliliter of chloroform:methanol:DI water (5:5:1) solution was added and let drip until most of the acetone was washed off the column. The eluate was discarded. Two aliquots of 2 ml of 5:5:1 solution were added to the column and the eluate collected in a 15 ml conical tube. A vacuum was used to pull the remaining solution off the column. The eluate was concentrated to dryness under nitrogen at 35-40°C. If not immediately undergoing methanolysis, samples were stored in 0.5-1.0 ml DCM at –20°C for short term or at –70°C for long term.

For the cleanup of the PAH fraction, 3 ml aminopropyl columns were used. Prior to their use, they were cleaned with 3 ml of Optima grade chloroform and 2 ml of hexane, pulled through at 1 drop/second without letting the column run dry. The PAH fraction was dried to one drop and brought up to 200 µl with hexane in a conical tube. One drop of chloroform was added, vortexed, and added to the column. The process was repeated three more times using 100 µl hexane. The PAHs were eluted from the column with 5 ml of hexane in 3 aliquots (1 ml, 2 ml, 2 ml). The column was let go to dryness and the eluate concentrated to 980 µl under the nitrogen evaporator at 35-40°C. The sample was then transferred to an autosampler vial with the addition of 20 µl of internal standard before being read on the GC/MS.

For the phospholipid to fatty acid methyl ester conversion, the dry phospholipid was dissolved in 0.5 ml of methanol:toluene (1:1) and 0.5 ml of 0.2 N KOH in methanol was added. The sample was vortexed, heated at 37°C for 15 minutes, and then allowed to cool to room temperature. 0.5 ml of 0.2 N acetic acid was added and the solution was vortexed for 2 seconds. Two milliliters of chloroform and 2 ml of DI water were added, vortexed for approximately 1 minute, and then centrifuged at 1500 rpm for 5 minutes. The chloroform phase (bottom layer) was transferred to a test tube with a Pasteur pipette. One milliliter of chloroform was added to the original tube and the process repeated. Another 1 ml chloroform was added without vortexing or centrifuging and transferred to the tube. The internal standard EE 20 was added to the 4 ml of chloroform along with 2 - 3 calibration tubes. The calibration tubes receive only the ethyl ester standard. All samples including the calibration tubes were dried under nitrogen at 35-40°C. 1 ml fresh

chloroform or DCM was added after drying if the samples were not processed immediately and were stored at –20°C (short term) or –70°C (long term).

C-18 columns were used for the purification of the FAMEs. The C-18 columns were washed with 2 ml of DI water and pulled to dryness with vacuum and then rinsed with methanol to completely dry column. Two milliliters of methanol were added and pulled through at 1 drip/ second, without letting the column go to dryness. The columns were closed and washed subsequently with 1 ml of chloroform, two 1 ml aliquots of acetonitrile (ACN), and 2 ml of ACN:water (1:1). The flow was stopped such that a small amount of ACN:water remained over the packing. If FAMEs were stored in DCM, they were dried under the nitrogen evaporator at 35-40°C. Small pieces of glass and 250 µl of ACN to facilitate dissolution were added to the FAMEs. The mixture was let stand for 10 minutes while vortexing several times. 250 µl of DI water were added, the mixture vortexed, and transferred onto the column. The process was repeated three more times without the 10 minute wait. The column was then washed with 1 ml of ACN:water, pulled to dryness, and dried for 5 minutes with nitrogen and the Visiprep drying attachment. The column was then washed with 2 ml of DI water and pulled to dryness. The combined eluates were discarded. To elute the FAMEs, the columns were closed and 750 µl of hexane:chloroform (95:5) was added. The vacuum (15 mmHg) was opened long enough to pull the hexane:chloroform mixture into packing without releasing any hexane:chloroform into the collection tube. It was let stand for 2 minutes. Three 500 µl aliquots of hexane:chloroform were used to elute the FAMEs. After the last addition, the column was let go to dryness. The FAMEs were dried under nitrogen at 35-40°C. Two hundred and fifty microliters of hexane was added for the GC/MS analysis. If not

analyzed immediately, 0.5 ml DCM was added and the solution stored at –20°C (short term) or –70°C (long term) (Figure 5).

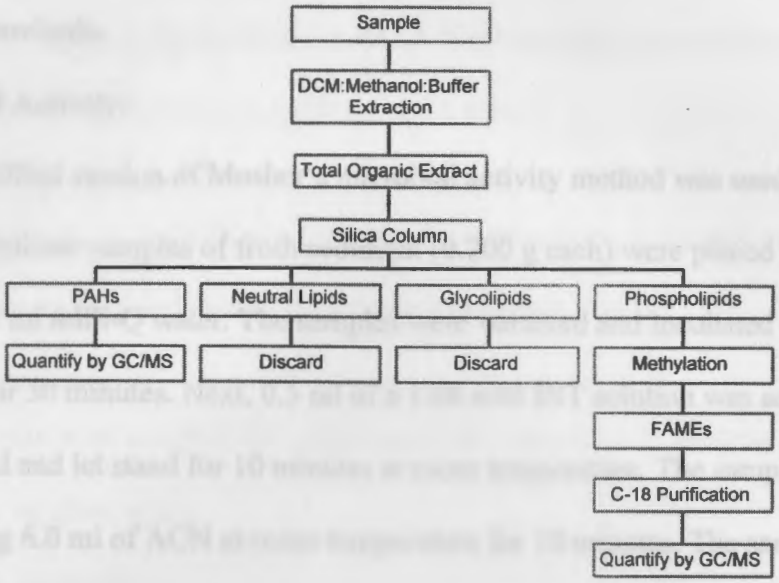


**Figure 5. Organization chart of PAH and FAME extraction method**

**2.3 Analysis of PAHs and FAMEs:**

The PAHs and FAMEs were analyzed on a Hewlett Packard 5890 Gas Chromatograph/5970B Mass Spectrometer. The GC was fitted with a DB-5 column 30 M, 0.32 mm ID, and .25 μm film thickness. The samples (1.0 μl) were injected splitless using a Finnigan-Mert A 2005 autosampler.

The injection temperature was set at 250°C. For PAHs, the oven temperature was held at 45°C for 2 minutes then ramped at 20°C per minute to 310°C. The final temperature was held for 5.5 minutes. The total running time was 20.75 minutes. Responses were taken from the GC/MS software and used to determine final concentrations of PAHs.

For FAMEs, the oven temperature was set at 80 °C, then ramped at 4°C per minute to 250°C where it was held for 20 minutes. The total run time was 62.50 minutes. FAMEs were identified by comparing retention times and GC/MS spectra PUFA 1, 2, 3 and BAME standards.

**2.4 Microbial Activity:**

A modified version of Mosher's microbial activity method was used (Mosher et. al., 2001). Triplicate samples of fresh sediment (0.200 g each) were placed into conical tubes with 0.3 ml milli-Q water. The samples were vortexed and incubated at room temperature for 30 minutes. Next, 0.5 ml of a 1.08 mM INT solution was added, the solution mixed and let stand for 10 minutes at room temperature. The sample was extracted using 6.0 ml of ACN at room temperature for 10 minutes. The samples were then filtered through Whatman No. 40 filters using a vacuum. The sediment was washed with 6.0 ml of acetonitrile and the total filtrate was read using a spectrophotometer at 490 nm.

To calculate microbial activity, killed control samples were used. These were made by adding 6 ml acetonitrile prior to adding the INT solution. The controls were then extracted for 10 minutes and filtered. To calculate the net microbial activity, the absorbance from the killed controls were subtracted from the samples.

All activity measurements were performed in dark room conditions as the INT solution is light sensitive.

**2.5 Particle Size Distribution:**

A hydrometer method (Fisher Environmental) was used to analyze the particle size. Sediments were combusted at 550°C for 24 hours. Forty grams were placed into a 1000 ml flask. A detergent solution (50 g/l) was added in the amount of 100 ml along

with 300 ml of DI water. The mixture was homogenized and let stand overnight. The mixture was then poured into a 1000 ml graduated cylinder, brought up to volume with DI water, and homogenized again. A hydrometer was used to measure the specific gravity after 40s and then again after 7 hours. A blank reading was performed by using 200 ml of the detergent solution and 800 ml of DI water.

**2.6 Total Organic Carbon:**

Sediments dried at 105°C were weighed into aluminum boats and combusted at 550°C for 24 hours then weighed again (Tiessen and Moir, 1993).

**2.7 Percent Moisture:**

Sediments were weighed into aluminum boats and dried in an oven at 105°C for 24 hours, then weighed again.

**2.8 Statistical Analysis:**

SPSS was used to do a principal component analysis (PCA) to look for relationships among PAHs, FAMEs, microbial biomass, microbial activity, percent moisture, percent total organic carbon, and particle size distribution. All the parameters were used for the Lowellville location. However, because no PAHs were quantified in Leavittsburg, all the parameters except PAHs were used. The Shannon Diversity Index was used to look at FAME diversity as an indicator of microbial community structure.

# Chapter 3: Results and Discussion

## 3.1 PAH Results:

PAH concentrations were detected at higher amounts at the bottom depth in the Lowellville sediments than at the top surface sediments at the same location. PAHs were also detected in the Leavittsburg sediments, but, were not quantifiable because they were in such low concentrations. Lowellville surface sediments contained a total of seven different PAHs: anthracene, benzo(a)anthracene, benzo(b&k)fluoranthene, chrysene, fluoranthene, phenanthrene, pyrene. Lowellville bottom sediments contained eleven different types of PAHs: acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(b&k)fluoranthene, chrysene, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene (Table 3). Concentrations in µg/g of dry weight ranged from 6.4 to 20.5 in the top and 0.4 to 61.8 in the bottom (Figure 6). The highest amounts of PAHs found were fluoranthene and pyrene in both layers of sediments. In the Leavittsburg sediments, anthracene, fluoranthene, and pyrene were detected in a few surface sediments and in almost all of the bottom sediments. However, none were in high enough concentration to be quantified.

Overall, 249.9 µg/g of PAHs were detected in the Lowellville bottom sediments and 77.6 µg/g in the top sediments. In 1986, 77.7 µg/g of PAHs were detected and in 1994 83.3 µg/g of PAHs were detected near Lowellville (13.2 RM) in-river sediment (OEPA, 1996).

**Table 3. PAH average concentrations (µg/g of dry weight), standard deviation, and relative standard deviation in Lowellville top and bottom sediments.**

| Lowellville Top Sediments | AVG | STDEV | RSD |
|---|---|---|---|
| 4) Naphthalene | 0 | 0 | |
| 7) Acenaphthylene | 0 | 0 | |
| 8) Acenaphthene | 0 | 0 | |
| 9) Fluorene | 0 | 0 | |
| 11) Phenanthrene | 10.8 | 11.0 | 102 |
| 12) Anthracene | 2.8 | 2.5 | 88 |
| 13) Fluoranthene | 20.5 | 15.0 | 73 |
| 14) Pyrene | 16.9 | 12.0 | 71 |
| 17) Benzo(a)anthracene | 9.5 | 7.6 | 80 |
| 18) Chrysene | 10.7 | 8.4 | 79 |
| 19) Benzo(b&k)fluoranthene | 6.4 | 9.7 | 153 |

| Lowellville Bottom Sediments | AVG | STDEV | RSD |
|---|---|---|---|
| 4) Naphthalene | 1.2 | 3.6 | 300 |
| 7) Acenaphthylene | 0.4 | 0.8 | 206 |
| 8) Acenaphthene | 4.9 | 5.8 | 119 |
| 9) Fluorene | 6.4 | 6.0 | 94 |
| 11) Phenanthrene | 27.1 | 21.6 | 80 |
| 12) Anthracene | 9.0 | 8.0 | 89 |
| 13) Fluoranthene | 60.0 | 25.6 | 43 |
| 14) Pyrene | 61.8 | 27.9 | 45 |
| 17) Benzo(a)anthracene | 32.5 | 21.2 | 65 |
| 18) Chrysene | 32.2 | 15.6 | 48 |
| 19) Benzo(b&k)fluoranthene | 13.8 | 17.8 | 129 |

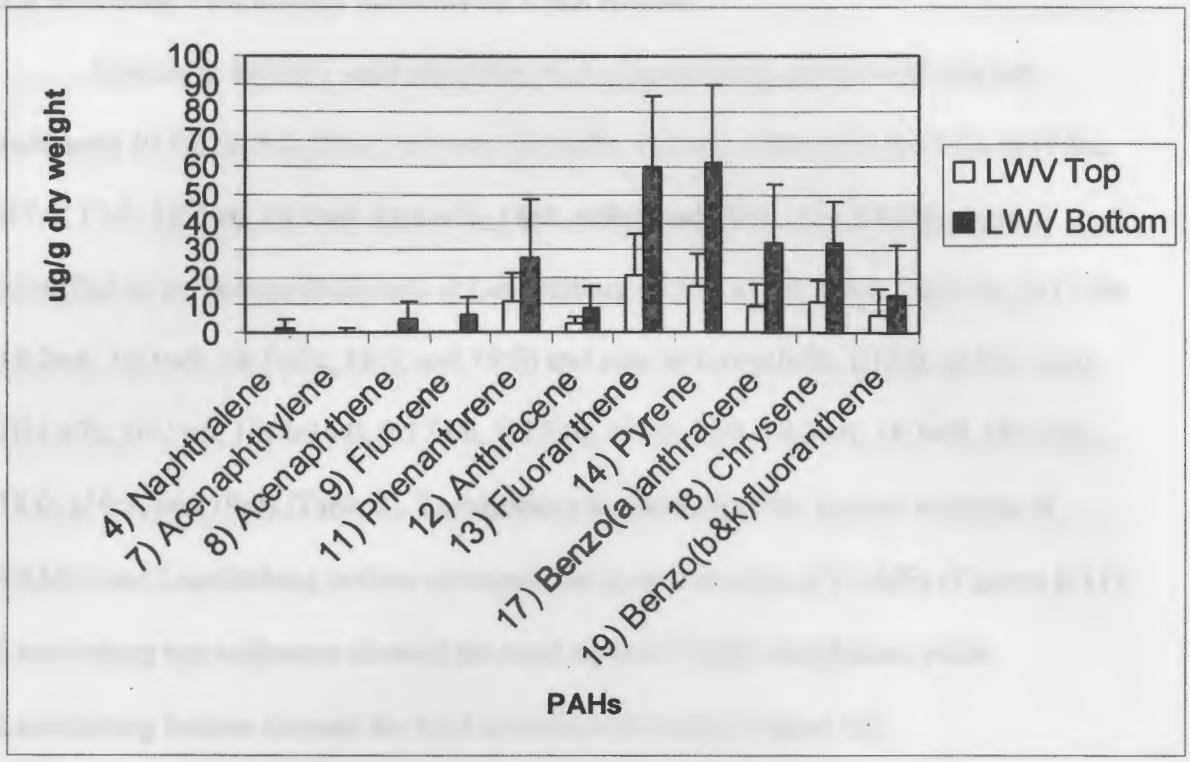


**Figure 6. Average PAH concentrations (µg/g dry weight) in Lowellville (LWV) top and bottom sediments.**

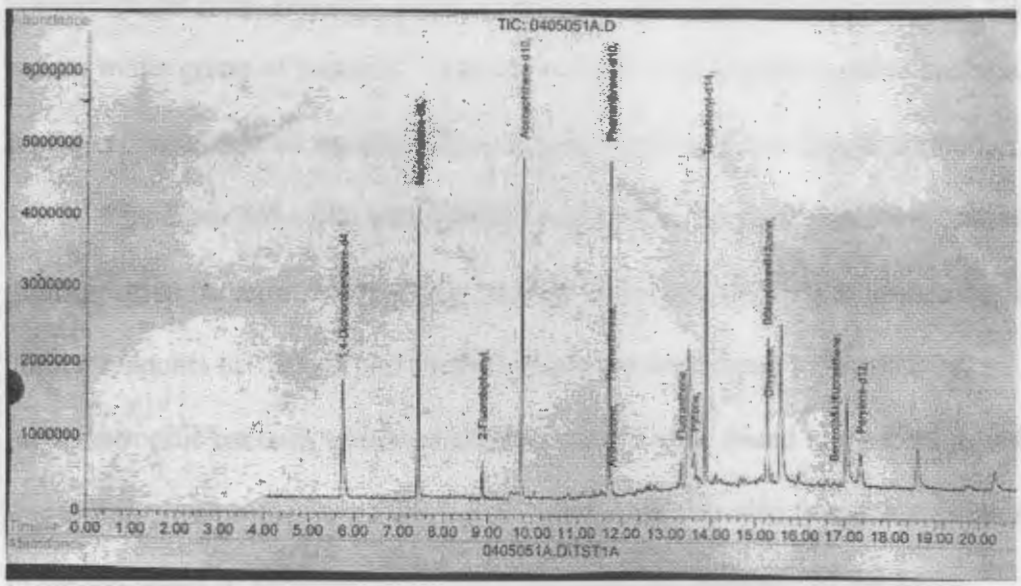


**Figure 7. Chromatogram of PAHs**

**3.2 Microbial Community Structure/FAME Results:**

Seventeen FAMEs were identified in the Leavittsburg and Lowellville top sediments (i15:0, a15:0, i16:0, 16:1w8, 16:1w7b, 16:1w6, 10Me16:0, br17:0b, br17:0a, i17:0, 17:0, 18:2w6, 18:1w9, 18:1w7c, 18:0, a19:0, and 19:0). Ten FAMEs were identified in the bottom sediments at Leavittsburg (i15:0, a15:0, i16:0, 16:1w7c, br17:0b, 18:2w6, 18:1w9, 18:1w7c, 18:0, and 19:0) and nine in Lowellville (i15:0, a15:0, i16:0, 16:1w7c, 16:1w6, 10Me16:0, br17:0b, br17:0a, i17:0, 17:0, 18:2w6, 18:1w9, 18:1w7c, 18:0, a19:0, and 19:0) (Table 4). Leavittsburg top contained the highest amounts of FAMEs and Leavittsburg bottom contained the lowest amount of FAMEs (Figures 8-11). Leavittsburg top sediments showed the most diverse FAME distribution while Leavittsburg bottom showed the least amount of diversity (Figure 12).

All samples had high concentrations of 16:0, 16:1w7, 18:1w9, and 18:1w7 which are general and non-specific microbial markers (Brigmon, 2001). In addition, 18:0, which are indicative of sulfate reducing and other anaerobic bacteria, also had high concentrations in all samples. Both i15 and a15 were detected in all samples which belong to the group of bacteria, Firmicutes, which are all gram-positive bacteria. 18:2w6 was also common in all samples but is usually mentioned as a fungal biomarker.

Mid-branched 17:0a was detected everywhere except Lowellville bottom and is a gram-positive bacteria. Surprisingly, the top layers of sediments at both locations had similar amounts of 16:1w6 and 16:1w8 which are considered biomarkers of methanotrophic bacteria which would be expected to be found in the bottom sediments due to their anaerobic nature. The FAME 10Me16:0 was also found in smaller amounts in the top layers of sediment which indicates possible presence of the genus

Desulfobacter, i.e. anaerobic bacteria reducing sulfates. FAMEs i17, 17:0, 19:0 and a19 were all also found generally in the top layer of sediments and are characteristic of general bacteria (Table 5).

Overall, both bottom locations had 84-86% of their community biomarkers were explained by green algae, higher plants, heterotrophic microeukaryotes, fungi, aerobic bacteria, sulfate reducing and other anaerobic bacteria. Within that percentage, 27-37% was sulfate reducing and other anaerobic bacteria. In both top locations, 64-68% of their community biomarkers explained by aerobic bacteria, microeukaryotes, sulfate reducing and other anaerobic bacteria. Within that percentage, 27-28% was aerobic bacteria and microeukaryotes (Table 6).

**Table 4. Average Weight Percents, Standard Deviation, and Relative Standard Deviation of FAMEs in Leavittsburg (LVT) and Lowellville (LWV) top and bottom sediments.**

| | Top Sediments | | | Bottom Sediments | | |
|---|---|---|---|---|---|---|
| **Leavittsburg** | **AVG** | **STDEV** | **RSD** | **AVG** | **STDEV** | **RSD** |
| i15 | 1.31 | 0.26 | 20 | 0.25 | 0.36 | 144 |
| a15 | 1.20 | 0.22 | 18 | 0.46 | 0.52 | 113 |
| 16:1w8 | 0.36 | 0.34 | 95 | 0.00 | 0.00 | |
| 16:1w7c | 2.02 | 0.24 | 12 | 0.53 | 0.58 | 110 |
| 16:1w6 | 1.47 | 0.13 | 9 | 0.00 | 0.00 | |
| 16:0 | 2.42 | 0.14 | 6 | 1.95 | 0.85 | 44 |
| 10Me16:0 | 1.04 | 0.48 | 46 | 0.00 | 0.00 | |
| br17:0b | 1.86 | 0.55 | 29 | 0.40 | 0.44 | 110 |
| br17:0a | 0.03 | 0.06 | 218 | 0.00 | 0.00 | |
| i17 | 1.04 | 0.21 | 20 | 0.00 | 0.00 | |
| 17:0 | 1.01 | 0.60 | 60 | 0.00 | 0.00 | |
| 18:2w6 | 1.55 | 0.25 | 16 | 1.46 | 0.69 | 47 |
| 18:1w9 | 2.11 | 0.10 | 5 | 1.50 | 0.95 | 63 |
| 18:1w7c | 2.45 | 0.14 | 6 | 0.73 | 0.81 | 110 |
| 18:0 | 1.36 | 0.05 | 3 | 1.24 | 0.51 | 41 |
| a19:0 | 0.53 | 0.35 | 66 | 0.00 | 0.00 | |
| 19:0 | 1.94 | 0.48 | 25 | 0.06 | 0.16 | 283 |

| | Top Sediments | | | Bottom Sediments | | |
|---|---|---|---|---|---|---|
| **Lowellville** | **AVG** | **St. Dev** | **RSD** | **AVG** | **St. Dev** | **RSD** |
| i15 | 0.98 | 0.44 | 45 | 0.27 | 0.43 | 159 |
| a15 | 0.78 | 0.48 | 62 | 0.40 | 0.54 | 133 |
| 16:1w8 | 0.19 | 0.36 | 186 | 0.00 | 0.00 | |
| 16:1w7c | 1.80 | 0.75 | 42 | 1.01 | 0.78 | 78 |
| 16:1w6 | 1.32 | 0.57 | 43 | 0.09 | 0.26 | 300 |
| 16:0 | 2.31 | 0.17 | 7 | 2.20 | 0.37 | 17 |
| 10Me16:0 | 0.95 | 0.44 | 46 | 0.00 | 0.00 | |
| br17:0b | 1.43 | 0.61 | 42 | 0.00 | 0.00 | |
| br17:0a | 0.00 | 0.00 | | 0.00 | 0.00 | |
| i17 | 0.59 | 0.64 | 109 | 0.00 | 0.00 | |
| 17:0 | 1.15 | 0.47 | 41 | 0.00 | 0.00 | |
| 18:2w6 | 0.82 | 0.71 | 86 | 0.93 | 0.73 | 78 |
| 18:1w9 | 1.88 | 0.13 | 7 | 1.66 | 0.68 | 41 |
| 18:1w7c | 2.11 | 0.36 | 17 | 1.26 | 0.74 | 59 |
| 18:0 | 1.37 | 0.26 | 19 | 1.51 | 0.14 | 9 |
| a19:0 | 0.08 | 0.22 | 283 | 0.00 | 0.00 | |
| 19:0 | 1.26 | 0.54 | 42 | 0.00 | 0.00 | |

**Table 5. FAME biomarkers located in the Mahoning River**

| Group | Fatty Acids |
|---|---|
| Green Algae & higher plants: | 18:1w9, 18:2w6 |
| Heterotrophic microeukaryotes: | 18:1w9, 18:2w6 |
| Fungi: | 18:1w9, 18:2w6 |
| Aerobic bacteria and microeukaryotes: | 16:1w7, 18:1w7, 18:1w9, 18:2w6 |
| Bacteria: | i15:0, a15:0, i17:0, 17:0, a19:0 |
| Aerobic bacteria: | 16:1w7, 18:1w7 |
| Gram-positive bacteria: | mid-branched fatty acids |
| Gram-positive bacteria and some gram-negative anaerobic bacteria: | a15:0, i15:0 |
| Methanotrophs: | Type I: 16:1w8c, 16:1w6 |
| Sulfate reducing bacteria and other anaerobic bacteria: | 16:0, 10Me16:0, i17:0, 17:0, 18:0 |

**Table 6. FAME biomarker percents**

| Group | LVT Top | LVT Bottom | LWV Top | LWV Bottom |
|---|---|---|---|---|
| Green algae, higher plants, Heterotrophic microeukaryotes, & fungi: | 11% | 27% | 10% | 17% |
| Aerobic bacteria and microeukaryotes: | 28% | 32% | 27% | 30% |
| Bacteria: | 10% | 2% | 9% | 2% |
| Aerobic bacteria: | 17% | 9% | 17% | 12% |
| Gram-positive bacteria: | 6% | 1% | 4% | 0% |
| Gram-positive bacteria and some gram-negative anaerobic bacteria: | 5% | 2% | 4% | 2% |
| Methanotrophs: | 4% | 0% | 4% | 0% |
| Sulfate reducing bacteria and other anaerobic bacteria: | 19% | 27% | 24% | 37% |

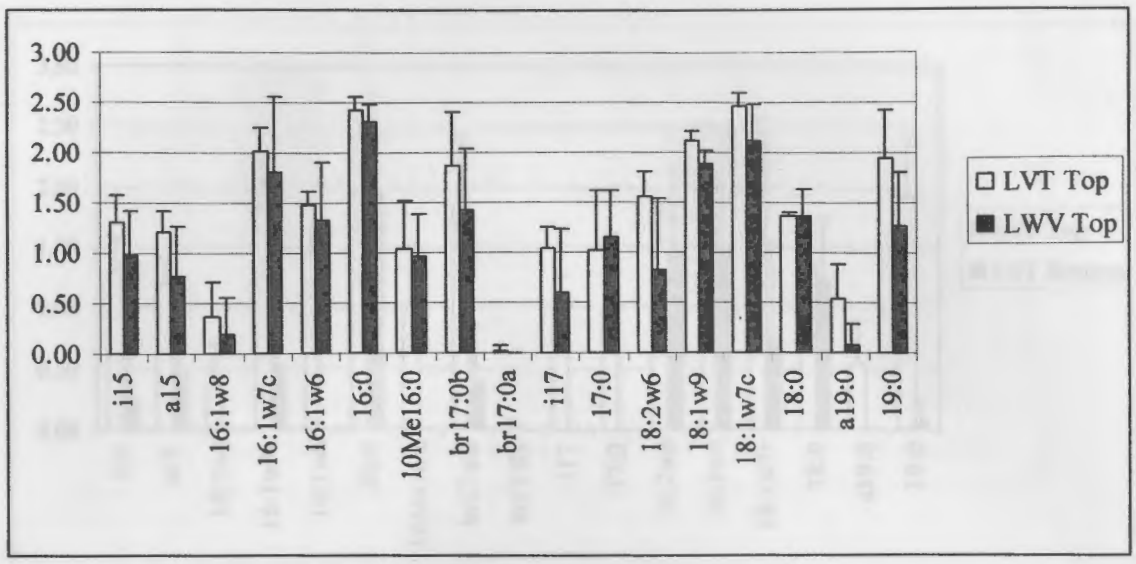


**Figure 8. Weight percent distribution of FAMEs by top sediments in Leavittsburg (LVT) and Lowellville (LWV).**

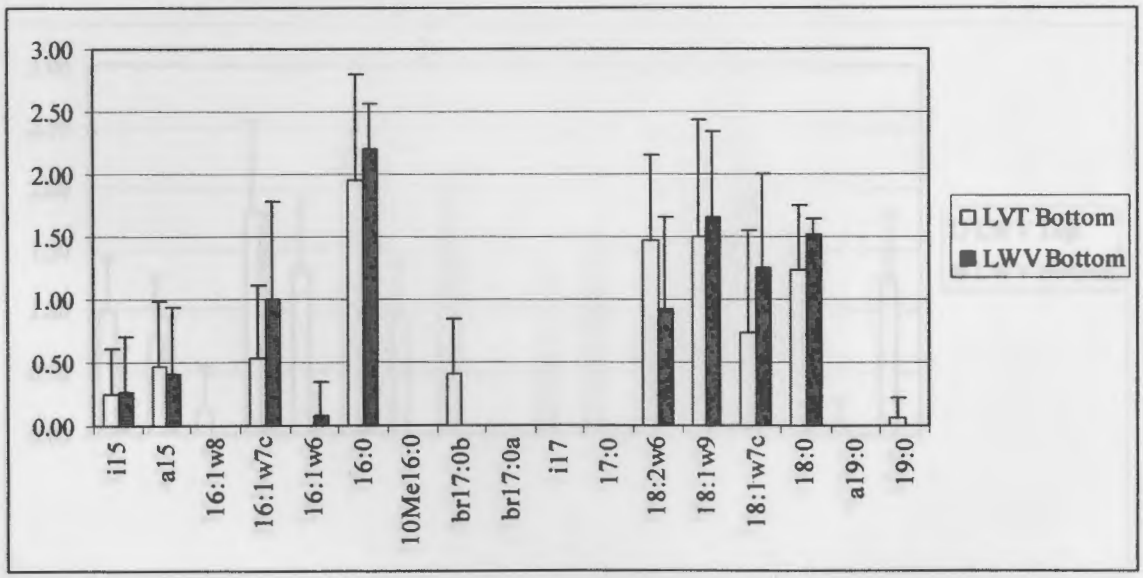


**Figure 9. Weight percent distribution of FAMEs by bottom sediments in Leavittsburg (LVT) and Lowellville (LWV).**

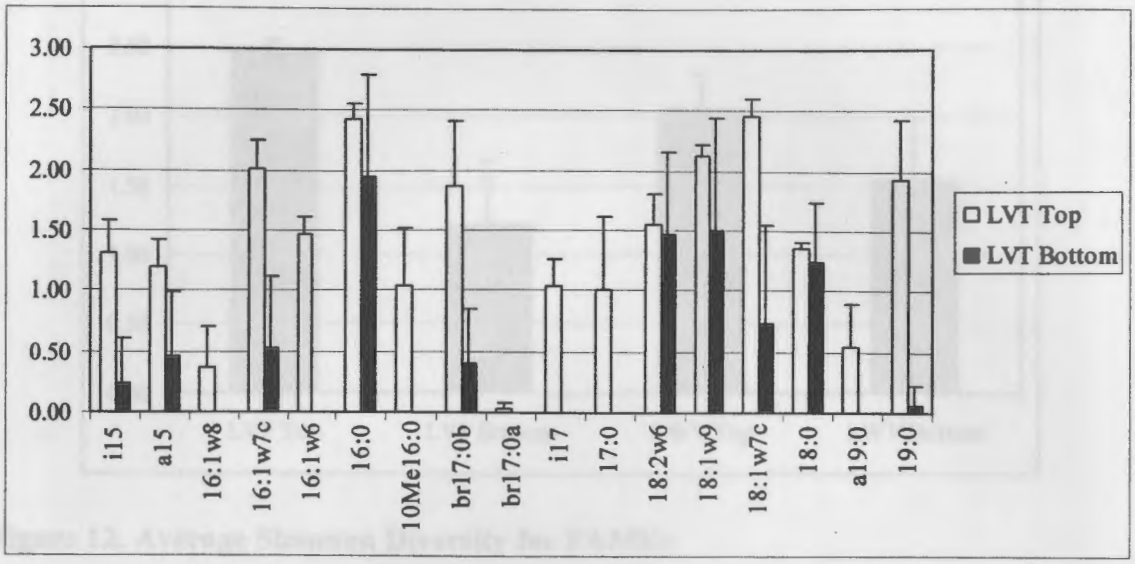


**Figure 10. Weight percent distribution of FAMEs in Leavittsburg sediments.**

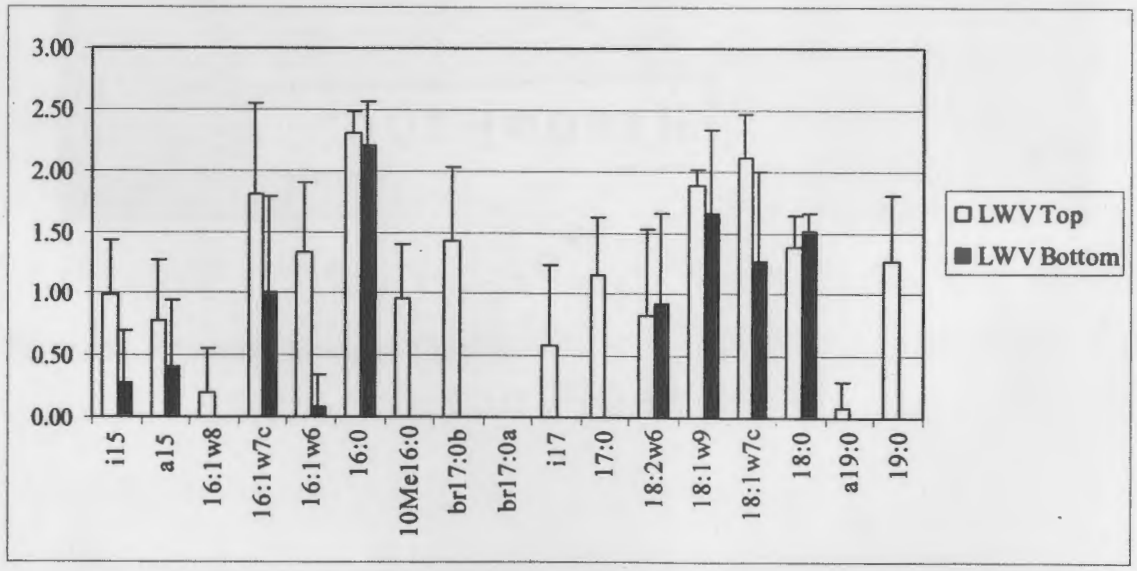


**Figure 11. Weight percent distribution of FAMEs in Lowellville sediments.**

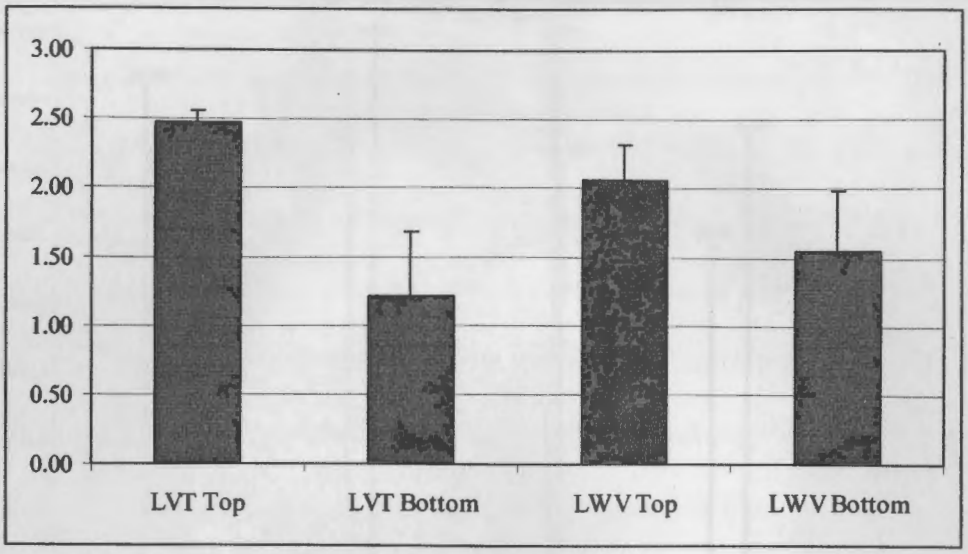


**Figure 12. Average Shannon Diversity for FAMEs**

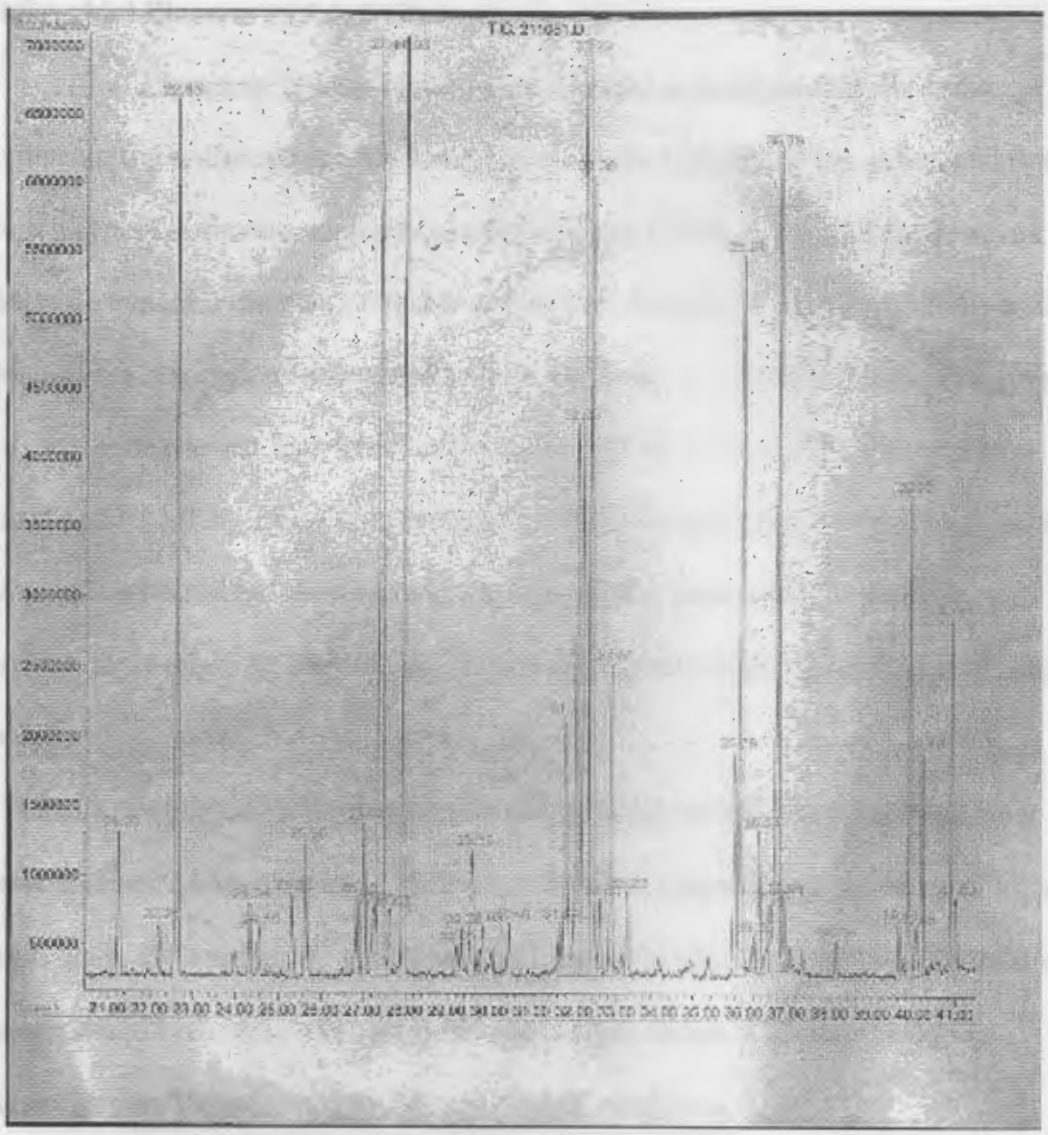

**Figure 13. Chromatogram of FAMEs**

### 3.3 Microbial Biomass and Activity:

Phosphate biomass levels were detected at the highest levels in the Leavittsburg top sediments and the lowest levels in the Lowellville top sediments. Both bottom layers of sediments contained similar amounts (Table 7, Figure 14). However, Lowellville bottom sediments contained the highest amounts of microbial activity and the lowest activity readings were detected in both top layers of sediments (Table 7, Figure 15). Lowellville showed high levels of microbial activity, but very low amounts of biomass and FAMEs. Leavittsburg sediments were exposed to the same protocol and the bottom sediments did not show as much activity as the contaminated sediments. Therefore, there might be potential for bioremediation due to the high amounts of activity recorded.

Results of microbial activity followed similar trends to those found in Mahoning River sediments. Mosher detected 13 nmol $g^{-1}$ INTF in Leavittsburg and 38 nmol $g^{-1}$ in Lowellville in-river sediment. This shows an increase in activity at the Lowellville site, however, not as high of an increase as the current data shows. This may be due to different specific location sites and environmental conditions.

However, Mosher's data shows contrasting results in microbial biomass in in-river Mahoning River sediment. Rather than increasing in concentration in Leavittsburg, Mosher detected 25.2 nmol phosphate $g^{-1}$ in Leavittsburg and 90.8 nmol $g^{-1}$ in Lowellville (Mosher, 2002).

The sediment was mixed and exposed to oxygen. Increased activity was likely stimulated by oxygen and mixing which allowed metabolism of carbon already present in the system. No organic carbon was added.

**Table 7: Average (AVG), standard deviation (STDEV), and relative standard deviation (RSD) of microbial biomass (nmol $PO_4$ $g^{-1}$) and microbial activity (nmol INTF $g^{-1}$) in Leavittsburg (LVT) and Lowellville (LWV) top and bottom sediments.**

| | Microbial Biomass | | | Microbial Activity | | |
|---|---|---|---|---|---|---|
| | AVG | STDEV | RSD | AVG | STDEV | RSD |
| LVT Top | 349 | 111 | 32 | 10 | 15 | 153 |
| LVT Bottom | 146 | 59 | 40 | 65 | 23 | 36 |
| LWV Top | 89 | 43 | 48 | 2 | 6 | 276 |
| LWV Bottom | 131 | 44 | 33 | 195 | 21 | 11 |

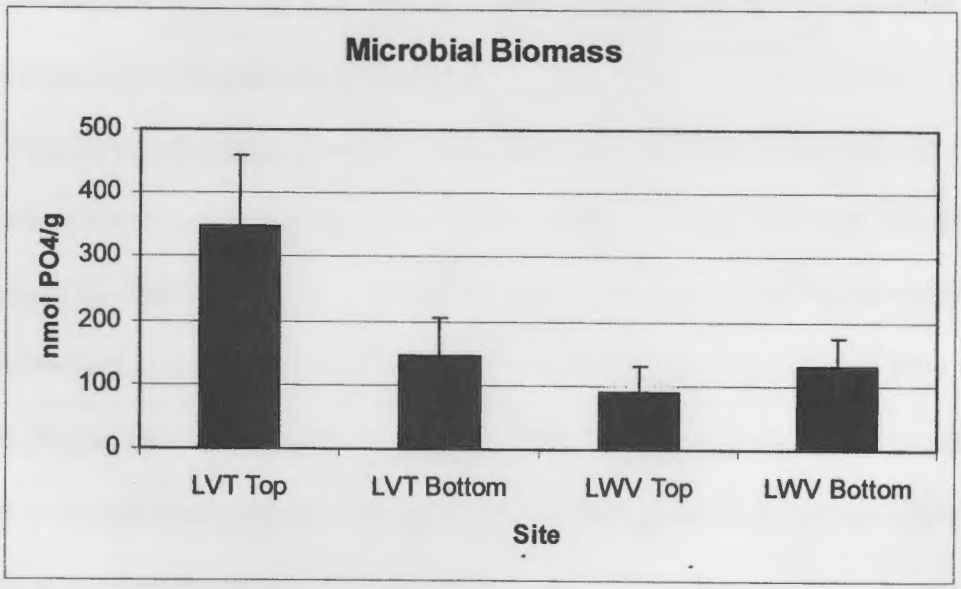


**Figure 14. Comparison of microbial biomass at Leavittsburg (LVT) and Lowellville (LWV) top and bottom sediments.**

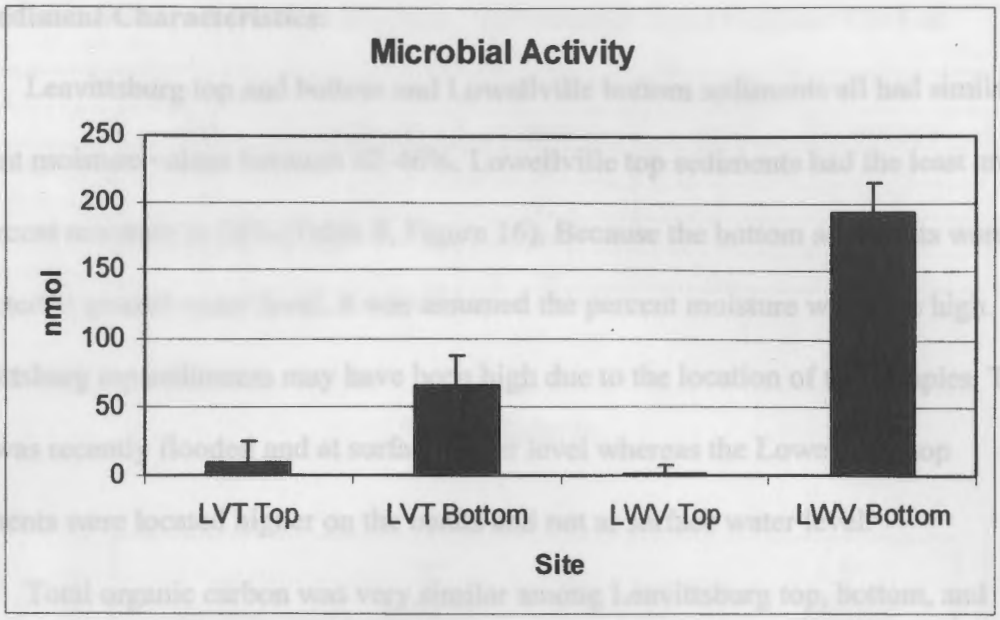


**Figure 15. Comparison of microbial activity at Leavittsburg (LVT) and Lowellville (LWV) top and bottom sediments.**

### 3.4 Sediment Characteristics:

Leavittsburg top and bottom and Lowellville bottom sediments all had similar percent moisture values between 42-46%. Lowellville top sediments had the least amount of percent moisture at 24% (Table 8, Figure 16). Because the bottom sediments were collected at ground water level, it was assumed the percent moisture would be high. The Leavittsburg top sediments may have been high due to the location of the samples. The area was recently flooded and at surface water level whereas the Lowellville top sediments were located higher on the banks and not at surface water level.

Total organic carbon was very similar among Leavittsburg top, bottom, and Lowellville top sediments, between 5-6% TOC. Lowellville bottom sediments contained the highest amount of percent TOC at 11% (Table 8, Figure 17). This is due to the high concentrations of PAHs detected in that area.

Particle size distribution was relatively similar among sand, clay, and silt distribution in the Leavittsburg top, bottom, and Lowellville top sediments. Values ranged from 25-30% sand, 3-5% clay, and 66-70% silt in these locations. However, Lowellville bottom showed contrasting results with 63% sand, 6% clay, and 32% silt (Table 9, Figure 18). This cannot be correct as the sediment is industrial sludge and contains no visible sand particles. The method does not appear to be reliable when the sediment is an industrial sludge.

**Table 8. Summary of Percent Moisture and Percent Total Organic Carbon**

| | % Moisture | | | % TOC | | |
|---|---|---|---|---|---|---|
| | AVG | STDEV | RSD | AVG | STDEV | RSD |
| LVT Top | 42 | 7 | 17 | 5 | 2 | 40 |
| LVT Bottom | 44 | 9 | 21 | 6 | 3 | 44 |
| LWV Top | 24 | 4 | 16 | 5 | 1 | 23 |
| LWV Bottom | 46 | 6 | 14 | 11 | 2 | 21 |

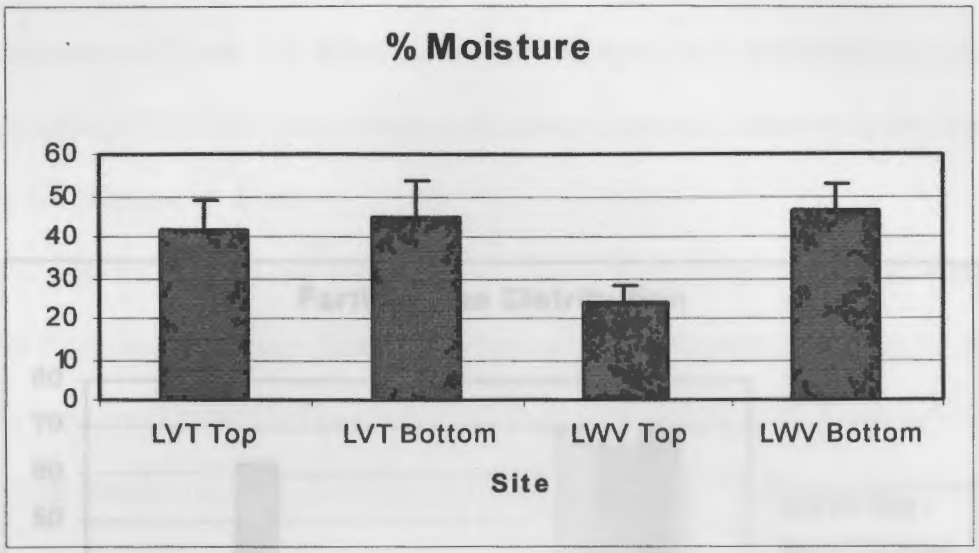


**Figure 16. Comparison of percent moisture at Leavittsburg (LVT) and Lowellville (LWV) top and bottom sediments.**

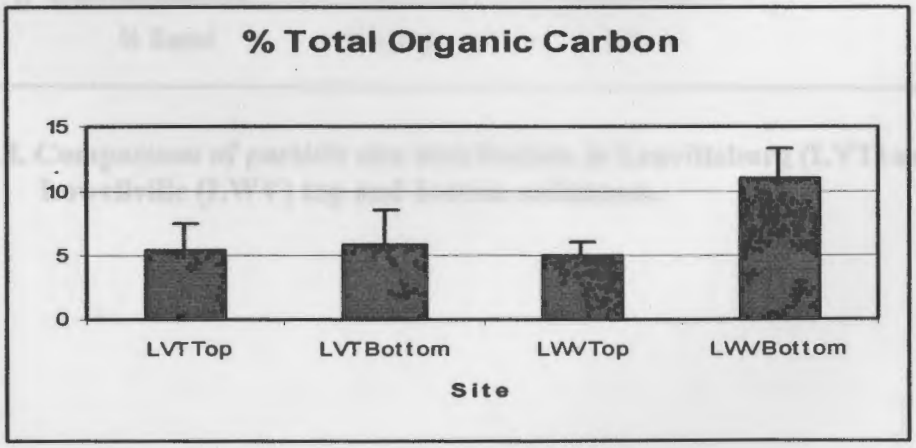


**Figure 17. Percent Total Organic Carbon at Leavittsburg (LVT) and Lowellville (LWV) top and bottom sediments.**

**Table 9. Summary of Particle Size Distribution of Sediments**

| | % Sand | | | % Clay | | | % Silt | | |
|---|---|---|---|---|---|---|---|---|---|
| | AVG | STDEV | RSD | AVG | STDEV | RSD | AVG | STDEV | RSD |
| LVT Top | 25 | 8 | 30 | 5 | 3 | 50 | 70 | 10 | 14 |
| LVT Bottom | 30 | 13 | 43 | 4 | 3 | 69 | 66 | 16 | 24 |
| LWV Top | 27 | 3 | 11 | 3 | 1 | 43 | 70 | 4 | 6 |
| LWV Bottom | 63 | 9 | 14 | 6 | 1 | 25 | 32 | 8 | 24 |

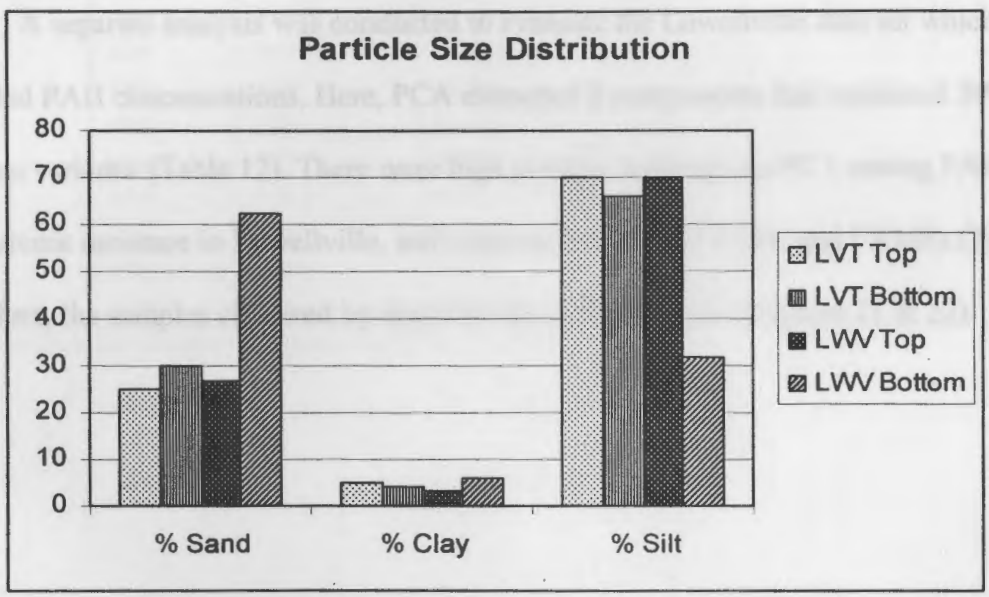


**Figure 18. Comparison of particle size distribution in Leavittsburg (LVT) and Lowellville (LWV) top and bottom sediments.**

**3.5 SPSS Results:**

Principal component analysis (PCA) was used to analyze the research variables. Due to the limit of quantification of PAHs in Leavittsburg, all research variables except PAHs were used to ordinate the data for Leavittsburg and Lowellville. PCA extracted 2 components that explained 64% of the data variance for both locations with no PAHs (Table 10). High positive loadings on PC 1 among FAMEs and biomass were found within the data set (Table 11). When the sediment samples were plotted on ordination, axes consisting of the first two principle components clustered primarily by depth and then by site (Figures 19 & 20).

A separate analysis was conducted to evaluate the Lowellville data set which included PAH concentrations. Here, PCA extracted 2 components that explained 59% of the data variance (Table 12). There were high positive loadings on PC 1 among FAMEs and percent moisture in Lowellville, and opposite loadings of PAHs and FAMEs (Table 13). Here, the samples clustered by depth on the ordination plot (Figures 21 & 22).

**Table 10. Results of PCA for Leavittsburg and Lowellville locations without PAHs**

| Component | Initial Eigenvalues | | | Extraction Sums of Squared Loadings | | |
|---|---|---|---|---|---|---|
| | Total | % of Variance | Cumulative % | Total | % of Variance | Cumulative % |
| 1 | 10.092 | 48.059 | 48.059 | 10.092 | 48.059 | 48.059 |
| 2 | 3.309 | 15.758 | 63.817 | 3.309 | 15.758 | 63.817 |
| 3 | 1.871 | 8.909 | 72.726 | | | |
| 4 | 1.126 | 5.364 | 78.090 | | | |
| 5 | 1.101 | 5.241 | 83.331 | | | |
| 6 | .879 | 4.187 | 87.518 | | | |
| 7 | .679 | 3.236 | 90.754 | | | |
| 8 | .458 | 2.180 | 92.934 | | | |
| 9 | .385 | 1.834 | 94.768 | | | |
| 10 | .283 | 1.349 | 96.117 | | | |
| 11 | .211 | 1.004 | 97.120 | | | |
| 12 | .177 | .845 | 97.965 | | | |
| 13 | .101 | .479 | 98.444 | | | |
| 14 | .095 | .451 | 98.895 | | | |
| 15 | .082 | .391 | 99.286 | | | |
| 16 | .045 | .212 | 99.498 | | | |
| 17 | .037 | .176 | 99.675 | | | |
| 18 | .029 | .136 | 99.811 | | | |
| 19 | .023 | .110 | 99.921 | | | |
| 20 | .012 | .055 | 99.976 | | | |
| 21 | .005 | .024 | 100.000 | | | |

Extraction Method: Principal Component Analysis.

**Table 11. Component matrix for Leavittsburg and Lowellville locations without PAHs**

| | Component | |
|---|---|---|
| | 1 | 2 |
| Biomass | .627 | .302 |
| Activity | -.712 | .474 |
| Moisture | .194 | -.735 |
| TOC | -.314 | .760 |
| i15 | .926 | .071 |
| a15 | .790 | .300 |
| 16:1wb | .606 | -.081 |
| 16:1w7c | .811 | .264 |
| 16:1w6 | .926 | -.187 |
| 16:0 | .485 | .706 |
| 10Me16:0 | .872 | -.223 |
| br17:0b | .941 | -.120 |
| br17:0a | .356 | -.039 |
| i17 | .725 | -.124 |
| 17:0 | .818 | -.264 |
| 18:2w6 | .204 | .249 |
| 18:1w9 | .545 | .648 |
| 18:1w7c | .845 | .294 |
| 18:0 | .014 | .657 |
| a19:0 | .729 | -.008 |
| 19:0 | .951 | -.144 |

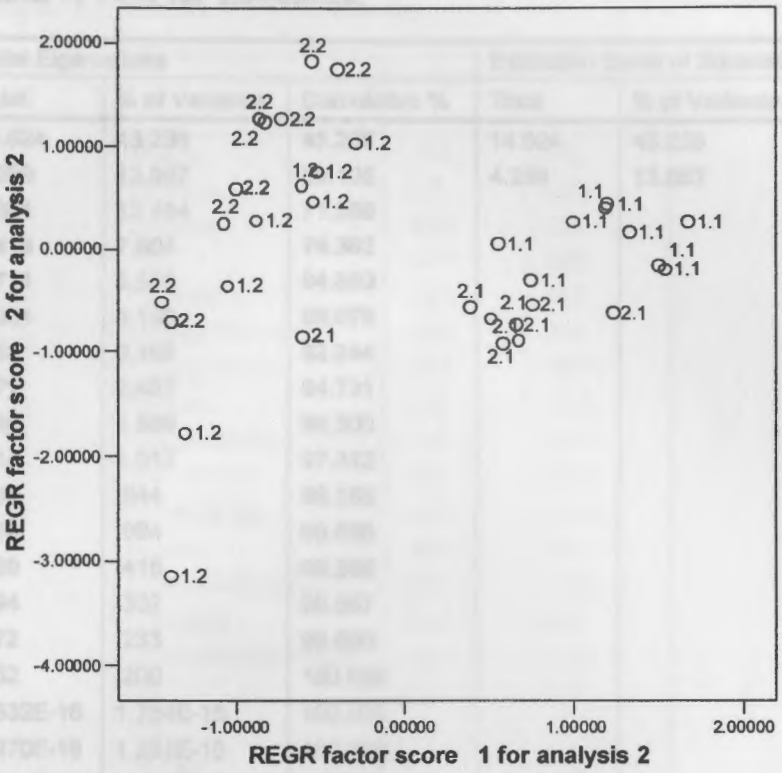


**Figure 19. Ordination for Leavittsburg and Lowellville locations (without PAH parameter). Numbers indicate site and depth; 1.1 represents Leavittsburg top sediments, 1.2 represents Leavittsburg bottom sediments, 2.1 represents Lowellville top sediments, and 2.2 represents Lowellville bottom sediments.**

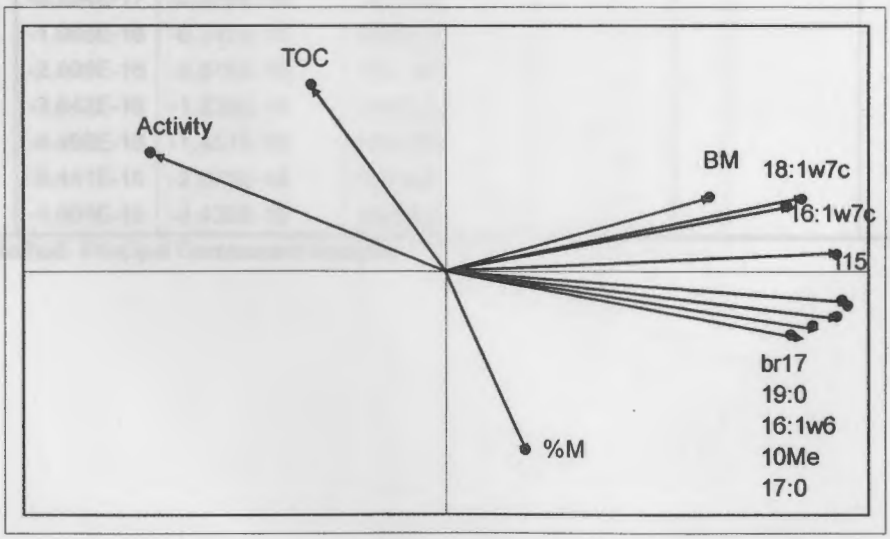


**Figure 20. Principal component loadings for Leavittsburg and Lowellville sediments comparing biomass (BM), activity, TOC, % moisture (%M), and FAMEs.**

**Table 12. Results of PCA for Lowellville**

| Component | Initial Eigenvalues | | | Extraction Sums of Squared Loadings | | |
|---|---|---|---|---|---|---|
| | Total | % of Variance | Cumulative % | Total | % of Variance | Cumulative % |
| 1 | 14.024 | 45.238 | 45.238 | 14.024 | 45.238 | 45.238 |
| 2 | 4.299 | 13.867 | 59.105 | 4.299 | 13.867 | 59.105 |
| 3 | 3.861 | 12.454 | 71.559 | | | |
| 4 | 2.419 | 7.804 | 79.362 | | | |
| 5 | 1.711 | 5.518 | 84.880 | | | |
| 6 | 1.301 | 4.198 | 89.078 | | | |
| 7 | .981 | 3.166 | 92.244 | | | |
| 8 | .771 | 2.487 | 94.731 | | | |
| 9 | .486 | 1.569 | 96.300 | | | |
| 10 | .314 | 1.012 | 97.312 | | | |
| 11 | .261 | .844 | 98.155 | | | |
| 12 | .215 | .694 | 98.850 | | | |
| 13 | .129 | .415 | 99.265 | | | |
| 14 | .094 | .302 | 99.567 | | | |
| 15 | .072 | .233 | 99.800 | | | |
| 16 | .062 | .200 | 100.000 | | | |
| 17 | 5.532E-16 | 1.784E-15 | 100.000 | | | |
| 18 | 3.970E-16 | 1.281E-15 | 100.000 | | | |
| 19 | 3.563E-16 | 1.149E-15 | 100.000 | | | |
| 20 | 1.992E-16 | 6.426E-16 | 100.000 | | | |
| 21 | 1.364E-16 | 4.399E-16 | 100.000 | | | |
| 22 | 7.682E-17 | 2.478E-16 | 100.000 | | | |
| 23 | 2.967E-17 | 9.570E-17 | 100.000 | | | |
| 24 | -5.003E-17 | -1.614E-16 | 100.000 | | | |
| 25 | -8.294E-17 | -2.676E-16 | 100.000 | | | |
| 26 | -1.966E-16 | -6.342E-16 | 100.000 | | | |
| 27 | -2.999E-16 | -9.676E-16 | 100.000 | | | |
| 28 | -3.842E-16 | -1.239E-15 | 100.000 | | | |
| 29 | -4.499E-16 | -1.451E-15 | 100.000 | | | |
| 30 | -6.441E-16 | -2.078E-15 | 100.000 | | | |
| 31 | -1.064E-15 | -3.432E-15 | 100.000 | | | |

Extraction Method: Principal Component Analysis.

**Table 13. Component matrix for Lowellville**

| | Component | |
|---|---|---|
| | 1 | 2 |
| Activity | -.846 | .352 |
| Moisture | .767 | -.471 |
| Biomass | -.282 | .538 |
| TOC | -.672 | .600 |
| Napthalene | -.331 | .185 |
| Acenaphthylene | -.366 | .293 |
| Acenaphthene | -.612 | -.027 |
| Fluorene | -.839 | -.343 |
| Phenanthrene | -.719 | -.510 |
| Anthrancene | -.741 | -.434 |
| Fluoranthene | -.853 | -.114 |
| Pyrene | -.861 | .015 |
| Benzoaanthracene | -.763 | -.102 |
| Chyrsene | -.834 | -.217 |
| Benzobkfluoranthene | -.374 | -.142 |
| i15 | .845 | .101 |
| a15 | .621 | .350 |
| 16:1wb | .486 | -.165 |
| 16:1w7c | .653 | .344 |
| 16:1w6 | .879 | -.202 |
| 16:0 | .479 | .667 |
| 10Me16:0 | .839 | -.310 |
| br17:0b | .861 | -.316 |
| i17 | .488 | -.106 |
| 17:0 | .868 | -.319 |
| 18:2w6 | .106 | .682 |
| 18:1w9 | .426 | .697 |
| 18:1w7c | .758 | .435 |
| 18:0 | -.286 | .449 |
| a19:0 | .387 | -.071 |
| 19:0 | .844 | -.329 |

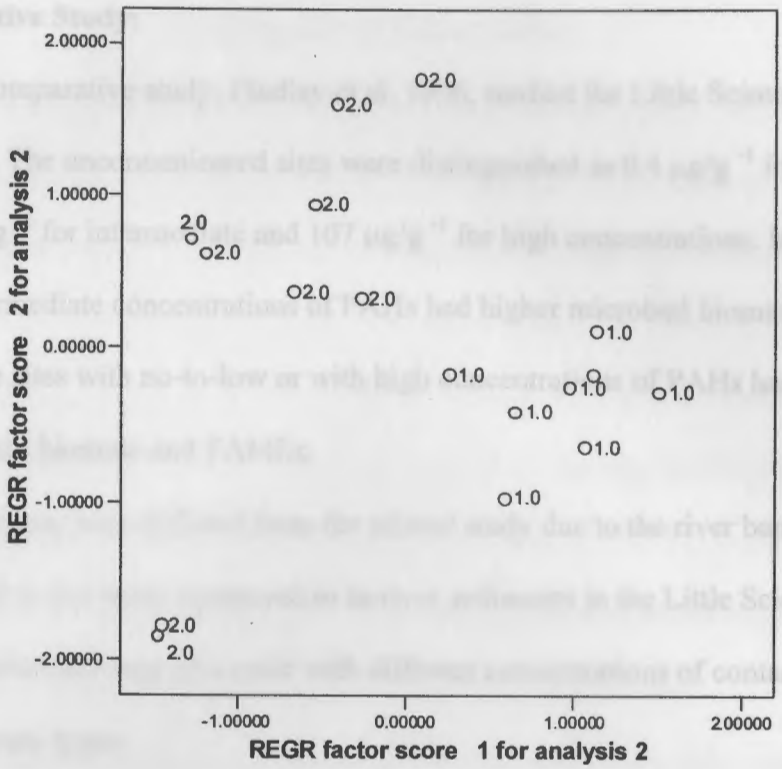


**Figure 21 . Ordination for Lowellville using all parameters. Numbers indicate depth; 1 represents top sediments and 2 represents bottom sediments.**

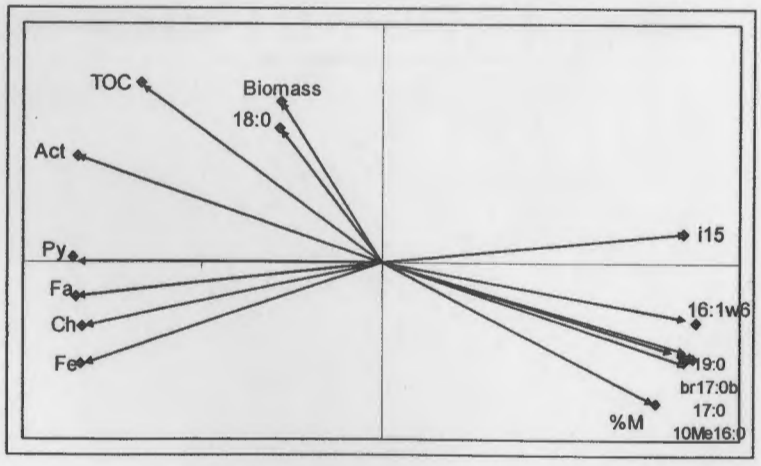


**Figure 22. Principal component loadings of biomass, total organic carbon (TOC), activity (Act), FAMEs, percent moisture (%M) and PAHs (Py = Pyrene, Fa = Fluoranthene, Ch = Chrysene, Fe = Fluorene) in Lowellville sediments.**

### 3.6 Comparative Study:

In a comparative study, Findlay et al. 1996, studied the Little Scioto River in Marion, Ohio. The uncontaminated sites were distinguished as 0.4 $\mu g/g^{-1}$ for no-to-low PAHs, 27 $\mu g/g^{-1}$ for intermediate and 107 $\mu g/g^{-1}$ for high concentrations. In general, sites with intermediate concentrations of PAHs had higher microbial biomass and FAMEs, while sites with no-to-low or with high concentrations of PAHs had significantly less biomass and FAMEs.

Results may have differed from the related study due to the river bank sediments being analyzed in this study compared to in-river sediments in the Little Scioto River. Different communities may also exist with different concentrations of contaminants and different substrate types.

# Chapter 4: Conclusion

There is a significant positive correlation of microbial activity with PAH concentrations, which indicates promise for bioremediation. In contrast, concentration of PAHs had little influence on biomass and FAMEs. Multivariate ordination (Principal Components Analysis) indicated opposite loadings (negative vs. positive) of PAHs and FAMEs. In addition, there was more variation in community structure found between the two depths than by between the two sites. Using PCA (excluding the PAH data), the sediment samples clustered primarily by depth, more than by site. PCA extracted 2 components that explained 64% of the data variance for both locations with no PAHs. PCA extracted 2 components that explained 59% of the data variance.

There were a higher percentage of anaerobes in the bottom core samples than in the top core samples as would be expected. However, there was a higher total abundance of anaerobes located in the upper sediment.

Consistent results were found during each of several separate extractions, indicating that fatty acid analyses were very reliable and reproducible.

# Chapter 5: Recommendations

More research is needed to evaluate the relationships among PAHs and microbial ecology in the Mahoning River sediments. In previous studies, it was found that the total FAMEs and biomass had increased at intermediate levels of PAHs, but decreased at high levels of PAHs or no PAHs (Langworthy et. al., 2002). To see if this would follow the same trend in the Mahoning River, an intermediate site would need to be selected. Lowellville is known for high concentrations of PAHs, therefore FAMEs and biomass may have been decreased because of these higher concentrations.

Other recommendations include exploring seasonal changes among winter, spring, summer and fall. Instead of river bank sediments, water from the river and in-river sediments could be analyzed before and after dredging to investigate changes in community structure during the restoration project. Also, different substrate types can be evaluated to see if the community changes by substrate type. Use of molecular methods could also be used to determine community structure at the species level.

Analyzing FAMEs provides a potentially powerful monitoring and research tool for complex microbial communities. This tool can be applied to the microbial degradation of environmental pollutants such as PAHs in contaminated sediment and water (Brigmon, 2001).

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# Appendices:

## Appendix 1: Solutions for the simultaneous extraction of PAHs and lipids and the determination of the biomass

Optima Grade Chloroform (Fisher): preserved with 0.75% ethanol.

50 mM Phosphate buffer: add 8.7 g of $K_2HPO_4$ (Sigma) to approximately 950 ml of Millipore water. Adjusted pH to 7.4 with 1N hydrochloric acid (HCl). Adjust to 1000 ml final volume in 1L volumetric flask with Millipore water.

0.2 N KOH: One pellet potassium hydroxide pellet (Fisher). Add 5 ml optima grade methanol and multiply weight of pellet by 89.29. That is total amount of methanol needed.

0.5 µM Glycerol phosphate: Add 0.2101g glycerol phosphate (Sigma) to 100 ml volumetric flask and filled to 100 ml with Milli-Q water. Pipette 1.0 ml of this solution in 100 ml volumetric flask and fill to 100 ml with Milli-Q water. Store in refrigerator until use.

Saturated potassium persulfate solution: Add 10 g of $K_2S_2O_8$ (Sigma) and 2 ml of conc. sulfuric acid (Fisher) to 200 ml volumetric flask and fill to 200 ml with Milli-Q water. This mixture is light sensitive and must be stored in the refrigerator until use. Before use, it must be warmed up to room temperature.

2.5 % Ammonium molybdate solution: Add 2.5 g of $(NH_4)_6Mo_7O_{24}$ (Sigma) in 84 ml Milli-Q water plus 15.89 ml conc. sulfuric acid in 100 ml volumetric flask. Dilute with Milli-Q water to 100 ml. Store in amber glass bottle because this solution is light sensitive.

Malachite green: Add 1.11 g of polyvinyl alcohol (Sigma) to 800 ml of Milli-Q water in 1000 ml beaker. Heat to 80°C while stirring. Cool and dilute to 1000 ml with Milli-Q water in volumetric flask. Add 0.11 g malachite green (Sigma) and stir.

Sodium sulfate ($Na_2SO_4$) columns: Add 1 g of dry $Na_2SO_4$ (Fisher) to clean 6 ml glass column. The columns were then packed with 2 ml of DCM without letting the packing go dry.

Solvent exchange: samples in DCM were concentrated to 100 µl using a nitrogen evaporator. Hexane in the amount of 1 ml was added and the samples again concentrated to 100 µl. This was repeated two more times.

Unisil (100 – 200 mesh) activated silicic columns (Clarkson Chromatography): 0.5 g of unisil were placed into 10 ml tubes and heated at 100°C for 2 hours to activate. The activated unisil was dissolved in 2 ml of chloroform and was transferred to the glass column. The tube was rinsed 4 times with 1 ml of chloroform and the solution transferred

to the column. The chloroform was pulled through at 1 drop/second without the unisil go dry. The sides of the column were rinsed with two 1 ml aliquots of chloroform and 2 ml of hexane. Copper filings (cleaned in 2 rinses of 1 N HCl, methanol, DCM, and hexane and dried under nitrogen) were added to the column. The columns were then ready to use.

Aminopropyl ($NH_2$) columns (VWR): 1 ml of optima grade chloroform, then another 2 ml were added to the column before pressurizing it and letting it drip. Hexane in the amount of 2 ml was added and pulled through at 1 drop per second, without letting the packing go dry.

JT Baker C-18 columns (VWR): 1 g of C-18 was added to a glass column and washed with 2 ml of DI water and pulled to dryness with vacuum. The column was then rinsed with methanol to completely dry column. Two milliliters of methanol were added and pulled through at 1 drop/ second, without letting the column go dry. The column was then closed and 1.5 ml of chloroform was added. The suspension was stirred to release bubbles and valves were opened to pull the chloroform through. The column was then washed with 1 ml of chloroform, two 1 ml aliquots of acetonitrile (ACN), and 2 ml of ACN:$H_2O$ (1:1). The flow was stopped such that a small amount of ACN:$H_20$ remained over the packing.

**Appendix 2: Solutions for Microbial Activity**

1.08 mM iodonitrotetrazolium (INT) chloride solution: Add 0.03 g of INT (Sigma) to 0.100 ml of N,N-dimethylformadine (Sargent-Welch) in 50 ml volumetric flask and mix well with a glass rod. Bring to volume with Milli-Q water in the 50 ml volumetric flask and sonicate for 20 minutes.

**Appendix 3: Standards for PAHs and Lipids**

0.1 mg/ml Arachidic Acid Ethyl Ester Standard (Sigma): Add 10 mg to 5 ml of Optima grade chloroform and quantitatively transfer to 100 ml volumetric flask. Bring volume up to approximately 97 ml with chloroform and let stand for 2 hours. Bring to volume with chloroform and freeze in test tubes.

Surrogate Solution: Restek B/N surrogate mix

2-fluorobiphenyl
nitrobenzene-d5
*p*-terphenyl-d14

1,000 µg/ml each in methylene chloride, 1ml/ampul

Calibration Mix: Restek SV Calibration Mix #5 / 610 PAH Mix

acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(ghi)perylene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorine, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, pyrene

2,000 µg/ml each in methylene chloride, 1ml/ampul

Internal Standards: Restek SV Internal Standard Mixes

acenaphthene-d10, chrysene-d12, 1,4-dichlorobenzene-d4, naphthalene-d8, perylene-d12, phenanthrene-d10

2,000 µg/ml each in methylene chloride, 1ml/ampul

PUFA No. 1: Supelco

C14:0, C16:0, C16:1 ω7, C18:1 ω7, C18:1 ω9, C18:2 ω6, C18:4 ω3, C20:1 ω9, C20:2 ω6, C20:5 ω3, C22:1ω9, C22:1 ω11, C22:5 ω3, C22:6 ω3

PUFA No. 2: Supelco

C14:0, C16:0, C16:1 ω7, C18:0, C18:1 ω7, C18:1 ω9, C18:2 ω6, C18:3 ω3, C18:3 ω6, C20:3 ω6, C20:4ω6, C20:5 ω3, C22:4 ω6. C22:5 ω3, C22:6 ω3

PUFA No. 3: Supelco

C14:0, C16:0, C16:1 ω7, C16:2 ω4, C16:4 ω1, C18:0, C18:1 ω7, C18:1 ω9, C18:2 ω4, C18:2 ω6, C18:3ω3, C18:3 ω4, C18:4 ω3, C20:1 ω9, C20:4 ω3, C20:4 ω6, C20:5 ω3, C22:5 ω3, C22:6 ω3

BAME Standards: Supelco

**Appendix 4: Correlated Internal Standards with PAHs and Surrogates**

| Internal Standards: | Correlating PAHs and Surrogates |
|---|---|
| Napthalene-d8: | Nitrobenzene-d5 (surrogate), Napthalene |
| Acenaphthene-d10: | 2-fluorobiphenyl (surrogate), Acenaphthylene, Acenaphthene, Fluorene |
| Phenanthrene-d10: | Phenanthrene, Anthracene, Fluoranthene, Pyrene, |
| Chrysene-d12: | Terephenyl-d14 (surrogate), Benzo(a)anthracene, Chrysene, Benzo(b,k)fluoranthene, Benzo(a)pyrene |
| Perylene-d12: | Dibenz(ah)anthracene, Ideno(1,2,3-cd)pyrene, Benzo(ghi)perylene |

**Appendix 5. Limit of Detect (LOD) and Limit of Quantitation (LOQ) of PAHs in ppm (µg/ml)**

| Compound | SIM (m/z) | LOD (S/N ≥ 3) | LOQ (S/N ≥ 10) |
|---|---|---|---|
| Nitrobenzene-d5 | 82 | < 0.2 | < 0.2 |
| Naphthalene | 128 | < 0.2 | < 0.2 |
| Acenaphtylene | 152 | < 0.2 | < 0.2 |
| Acenaphtene | 153 | < 0.2 | < 0.2 |
| Fluorene | 166 | < 0.2 | < 0.2 |
| Phenanthrene | 178 | < 0.2 | 0.2 |
| Anthracene | 178 | < 0.2 | 0.2 |
| Fluoranthene | 202 | < 0.2 | 0.2 |
| Pyrene | 202 | < 0.2 | 0.2 |
| Benzo(a)anthracene | 228 | 0.2 | 0.4 |
| Chrysene | 228 | 0.2 | 0.4 |
| Benzo(b&k)fluoranthene | 276 | 0.4 | 2.0 |
| Benzo(a)pyrene | 252 | 1.0 | 5.0 |
| Dibenz(ah)anthracene | 276 | 2.0 | 5.0 |
| Indeno(1,2,3-cd)pyrene | 278 | 2.0 | 5.0 |
| Benzo(ghi)perylene | 276 | 2.0 | 5.0 |

**Appendix 6: Standard Curve for PAHs**

A standard curve was performed using 0.2, 0.4, 1.0, 2.0, 5.0 and 10.0 µg/ml concentrations of the calibration mix, 20 µl of internal standard, and 50 µl of surrogate solution were added and the volume adjusted to 1.0 ml with hexane. Two ml autosampler vials were used.

Ex. Anthracene Standard Curve

$y = 43.777x + 1.9573$
$R^2 = 0.9999$

| Conc. (µg/ml) | Response* | Calc. Conc. |
|---|---|---|
| 0.2 | 10 | 0.18 |
| 0.4 | 20.5 | 0.42 |
| 1 | 47 | 1.03 |
| 2 | 87 | 1.94 |
| 5 | 222 | 5.03 |
| 10 | 439.5 | 9.99 |

* Response was calculated by dividing actual GC/MS response by $10^4$

**Appendix 7: Standard Curve for Biomass**

A standard curve was performed by adding 0, 15, 30, 60, 100, and 150 µl of 0.5 µM glycerol phosphate to ampules to give concentrations of 0, 1.5, 3, 6, 10, and 15 nmol per ampule. The ampules were rinsed with methanol and dried under nitrogen at 37°C. 450 µl of saturated potassium persulfate was added, the ampule flame sealed and let stand overnight at 105°C. The next day the ampules were cooled to room temperature, opened and 100 µl 2.5 % ammonium molybdate (B2) was added and let stand for 10 minutes. 450 µl of malachite green was added and let stand for 20 minutes. The mixture was then read in a spectrophotometer at 610 nm. All samples and concentrations were run in duplicate.

To calculate the phosphate biomass, the duplicate absorbances were averaged. The linear equation created from the standard curve was used to calculate nmol of phosphate per ampule. To get nmol of phosphate per gram of dry weight, the following equation was used:

$$\frac{\text{Total amount of DCM (15 ml)}}{\text{Total amount of DCM recovered (13.5 ml)}} \times \frac{\text{Amount of chloroform added (1 ml)}}{\text{Amount taken out (0.1 ml)}} \times \text{nmol phosphate/ampule}$$

This number was then divided by the dry weight of the corresponding sample to get nmol of phosphate per gram of dry weight.

$y = 0.074x + 0.1139$
$R^2 = 0.9997$

| Conc. nmol | ABS |
|---|---|
| 0 | 0.106 |
| 1.5 | 0.234 |
| 3 | 0.329 |
| 6 | 0.568 |
| 10 | 0.851 |
| 15 | 1.222 |

**Appendix 8: Standard Curve for Microbial Activity**

A standard curve for microbial activity was performed using iodonitrotetrazolium formazan (INTF) (Sigma) with the following weights.

$y = 0.0036x + 0.0096$
$R^2 = 0.9981$

| Conc. nmol | INTF (mg) | ABS |
|---|---|---|
| 0 | 0.0 | 0 |
| 21 | 0.1020 | 0.0775 |
| 45 | 0.2110 | 0.1840 |
| 89 | 0.4270 | 0.3405 |
| 175 | 0.8274 | 0.6180 |
| 265 | 1.2450 | 0.9795 |

10.0 µl of N,N-dimethylformamide were added to the INTF along with 49.9 ml of ACN. This solution was sonicated for 20 minutes and covered with foil since they are light sensitive. The solutions were read in the spectrophotometer at 490 nm. A standard curve was created and the linear equation was used for the calculations.

**Appendix 9: Equations for Particle Size Distribution**

$$\text{Sand \%} = (R_{40s} - R_{blank}) \times \frac{100}{\text{Dry sediment (g)}}$$

$$\text{Clay \%} = R_7 - R_{blank}) \times \frac{100}{\text{Dry sediment (g)}}$$

Silt % = 100 – (Sand % - Clay %)

R = specific gravity

**Appendix 10: Calculations for determining FAMEs**

The FAMEs were quantified according to the procedures in the Handbook of Methods in Aquatic Microbial Ecology, Chapter 32 Quantitative Description of Microbial Communities using Lipid Analysis by Findlay and Dobbs.

## Appendix 11: Data Counts

**Microbial Biomass and Activity**

| Leavittsburg | Microbial Biomass | Microbial Activity |
|---|---|---|
| | (nmol PO4 gdw) | (nmol INTF gdw) |
| T1 | 300 | 0 |
| T2 | 344 | 0 |
| T3 | 510 | 33 |
| T4 | 385 | 1 |
| T5 | 352 | 0 |
| T6 | 330 | 0 |
| T7 | 197 | 33 |
| T8 | 211 | 0 |
| T9 | 510 | 19 |
| B1 | 259 | 96 |
| B2 | 184 | 89 |
| B3 | 76 | 80 |
| B4 | 191 | 35 |
| B5 | 110 | 49 |
| B6 | 87 | 66 |
| B7 | 120 | 78 |
| B8 | 171 | 32 |
| B9 | 119 | 57 |

| Lowellville | Microbial Biomass | Microbial Activity |
|---|---|---|
| | (nmol PO4 gdw) | (nmol) |
| T1 | 52 | 0 |
| T2 | 61 | 0 |
| T3 | 30 | 1 |
| T4 | 136 | 1 |
| T5 | 111 | 0 |
| T6 | 140 | 0 |
| T7 | 49 | 1 |
| T8 | 132 | 18 |
| T9 | 93 | 0 |
| B1 | 118 | 162 |
| B2 | 103 | 177 |
| B3 | 137 | 233 |
| B4 | 83 | 203 |
| B5 | 81 | 199 |
| B6 | 199 | 198 |
| B7 | 194 | 215 |
| B8 | 151 | 181 |
| B9 | 110 | 183 |

**Sediment Characteristics**

| Leavittsburg | % Moisture | % TOC | % Sand | % Clay | % Silt |
|---|---|---|---|---|---|
| T1 | 41.1 | 5.1 | 17.5 | 2.5 | 80 |
| T2 | 43.0 | 4.0 | 32.5 | 7.5 | 60 |
| T3 | 43.6 | 7.5 | 25 | 5 | 70 |
| T4 | 42.1 | 5.9 | | | |
| T5 | 38.4 | 5.8 | | | |
| T6 | 53.0 | 7.8 | | | |
| T7 | 35.6 | 7.2 | | | |
| T8 | 29.7 | 3.2 | | | |
| T9 | 49.7 | 1.4 | | | |
| B1 | 54.5 | 7.8 | 22.5 | 2.5 | 75 |
| B2 | 44.6 | 5.9 | 22.5 | 2.5 | 75 |
| B3 | 30.5 | 2.2 | 45 | 7.5 | 47.5 |
| B4 | 55.4 | 9.4 | | | |
| B5 | 43.0 | 4.4 | | | |
| B6 | 29.8 | 1.8 | | | |
| B7 | 51.5 | 7.0 | | | |
| B8 | 45.7 | 7.6 | | | |
| B9 | 44.9 | 7.1 | | | |

| Lowellville | % Moisture | % TOC | % Sand | % Clay | % Silt |
|---|---|---|---|---|---|
| T1 | 23.7 | 5.6 | 25 | 2.5 | 72.5 |
| T2 | 27.6 | 6.1 | 30 | 5 | 65 |
| T3 | 21.6 | 4.6 | 25 | 2.5 | 72.5 |
| T4 | 30.3 | 5.3 | | | |
| T5 | 23.4 | 4.4 | | | |
| T6 | 26.7 | 6.0 | | | |
| T7 | 19.9 | 2.4 | | | |
| T8 | 22.9 | 5.4 | | | |
| T9 | 18.5 | 4.4 | | | |
| B1 | 47.2 | 13.1 | 70 | 5 | 25 |
| B2 | 40.5 | 13.8 | 52.5 | 7.5 | 40 |
| B3 | 53.0 | 12.2 | 65 | 5 | 30 |
| B4 | 43.8 | 8.2 | | | |
| B5 | 39.3 | 8.3 | | | |
| B6 | 54.7 | 11.9 | | | |
| B7 | 54.7 | 13.4 | | | |
| B8 | 39.4 | 8.2 | | | |
| B9 | 42.6 | 10.8 | | | |

**WeightPercent Distribution of FAMEs**

| Leavittsburg | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 |
|---|---|---|---|---|---|---|---|---|---|
| i15 | 1.46 | 1.47 | 1.44 | 1.43 | 1.39 | 1.41 | 0.82 | 0.91 | 1.49 |
| a15 | 1.38 | 1.28 | 1.39 | 1.16 | 1.16 | 1.39 | 0.84 | 0.86 | 1.36 |
| 16:1w8 | 0.39 | 0.00 | 0.89 | 0.00 | 0.88 | 0.00 | 0.36 | 0.37 | 0.37 |
| 16:1w7c | 1.83 | 1.85 | 1.94 | 1.97 | 2.01 | 1.68 | 2.37 | 2.36 | 2.14 |
| 16:1w6 | 1.47 | 1.58 | 1.64 | 1.60 | 1.60 | 1.31 | 1.41 | 1.36 | 1.30 |
| 16:0 | 2.35 | 2.31 | 2.32 | 2.43 | 2.42 | 2.34 | 2.48 | 2.37 | 2.75 |
| 10Me16:0 | 1.32 | 1.17 | 1.48 | 1.37 | 1.30 | 1.30 | 0.73 | 0.69 | 0.00 |
| br17:0b | 2.14 | 2.08 | 2.35 | 2.18 | 2.16 | 2.34 | 1.06 | 0.94 | 1.53 |
| br17:0a | 0.07 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| i17 | 1.21 | 1.13 | 1.18 | 1.09 | 1.08 | 1.25 | 0.74 | 0.63 | 1.08 |
| 17:0 | 1.57 | 1.33 | 1.55 | 0.00 | 0.00 | 1.30 | 1.01 | 1.06 | 1.31 |
| 18:2w6 | 1.63 | 1.58 | 1.14 | 1.69 | 1.66 | 1.23 | 1.62 | 1.42 | 1.97 |
| 18:1w9 | 2.08 | 2.03 | 2.19 | 2.13 | 2.31 | 2.05 | 1.97 | 2.18 | 2.10 |
| 18:1w7c | 2.45 | 2.38 | 2.71 | 2.49 | 2.61 | 2.47 | 2.21 | 2.37 | 2.41 |
| 18:0 | 1.31 | 1.38 | 1.38 | 1.34 | 1.35 | 1.45 | 1.30 | 1.38 | 1.37 |
| a19:0 | 0.94 | 0.91 | 0.84 | 0.00 | 0.60 | 0.60 | 0.00 | 0.51 | 0.42 |
| 19:0 | 2.18 | 2.18 | 2.22 | 2.16 | 2.20 | 2.09 | 1.15 | 1.05 | 2.22 |

| B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 |
|---|---|---|---|---|---|---|---|---|
| 0.79 | 0.75 | 0.00 | | 0.00 | 0.00 | 0.44 | 0.00 | 0.00 |
| 1.18 | 1.09 | 0.00 | | 0.00 | 0.00 | 0.67 | 0.00 | 0.75 |
| 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.35 | 0.91 | 0.00 | | 0.00 | 0.00 | 0.91 | 0.00 | 1.07 |
| 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2.43 | 2.44 | 1.54 | | 1.93 | 0.00 | 2.41 | 2.38 | 2.44 |
| 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.93 | 0.90 | 0.00 | | 0.00 | 0.00 | 0.61 | 0.00 | 0.79 |
| 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.45 | 1.49 | 0.00 | | 2.31 | 2.10 | 1.41 | 1.31 | 1.64 |
| 1.97 | 2.05 | 0.00 | | 1.54 | 0.00 | 2.04 | 2.03 | 2.34 |
| 1.63 | 0.00 | 0.00 | | 0.00 | 0.00 | 1.30 | 1.16 | 1.78 |
| 1.42 | 1.42 | 1.28 | | 1.47 | 0.00 | 1.39 | 1.36 | 1.56 |
| 0.00 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.46 | 0.00 | 0.00 | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

| Lowellville | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 |
|---|---|---|---|---|---|---|---|---|---|
| i15 | | 1.31 | 1.13 | 1.19 | 1.17 | 0.65 | 0.00 | 1.22 | 1.18 |
| a15 | | 1.03 | 0.98 | 0.98 | 0.97 | 0.00 | 0.00 | 1.05 | 1.20 |
| 16:1w8 | | 0.83 | 0.72 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 16:1w7c | | 2.16 | 2.06 | 2.00 | 2.11 | 1.91 | 0.00 | 2.38 | 1.76 |
| 16:1w6 | | 1.82 | 1.39 | 1.47 | 1.49 | 1.66 | 0.00 | 1.62 | 1.14 |
| 16:0 | | 2.43 | 2.34 | 2.36 | 2.37 | 2.39 | 1.94 | 2.44 | 2.19 |
| 10Me16:0 | | 1.39 | 0.96 | 1.08 | 0.81 | 1.40 | 0.00 | 1.04 | 0.94 |
| br17:0b | | 1.78 | 1.55 | 1.80 | 1.38 | 1.41 | 0.00 | 1.61 | 1.90 |
| br17:0a | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| i17 | | 1.04 | 0.38 | 0.00 | 0.00 | 0.00 | 1.82 | 0.66 | 0.81 |
| 17:0 | | 1.49 | 1.36 | 1.35 | 1.32 | 1.16 | 0.00 | 1.28 | 1.21 |
| 18:2w6 | | 1.48 | 0.89 | 0.00 | 0.00 | 0.00 | 1.48 | 1.43 | 1.32 |
| 18:1w9 | | 2.07 | 1.64 | 1.82 | 1.84 | 2.00 | 1.90 | 1.92 | 1.90 |
| 18:1w7c | | 2.38 | 2.08 | 2.03 | 2.39 | 2.35 | 1.36 | 2.41 | 1.91 |
| 18:0 | | 1.24 | 1.33 | 0.91 | 1.41 | 1.56 | 1.76 | 1.21 | 1.53 |
| a19:0 | | 0.62 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 19:0 | | 1.47 | 1.35 | 1.68 | 1.25 | 1.48 | 0.00 | 1.24 | 1.65 |

| B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 |
|---|---|---|---|---|---|---|---|---|
| 0.00 | 0.53 | 1.11 | 0.00 | 0.00 | 0.00 | 0.78 | 0.00 | 0.00 |
| 0.37 | 0.82 | 1.26 | 0.00 | 0.00 | 0.00 | 1.16 | 0.00 | 0.00 |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.36 | 1.48 | 1.92 | 0.00 | 0.00 | 1.48 | 1.66 | 0.00 | 1.14 |
| 0.00 | 0.00 | 0.77 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2.32 | 2.29 | 2.66 | 1.97 | 1.38 | 2.36 | 2.47 | 2.26 | 2.08 |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1.31 | 1.16 | 1.75 | 0.00 | 0.00 | 1.61 | 1.46 | 0.00 | 1.04 |
| 2.11 | 1.97 | 2.10 | 0.00 | 1.22 | 1.94 | 2.10 | 1.73 | 1.77 |
| 1.73 | 1.51 | 1.65 | 0.00 | 0.00 | 1.81 | 1.94 | 1.32 | 1.35 |
| 1.60 | 1.70 | 1.52 | 1.53 | 1.21 | 1.40 | 1.58 | 1.57 | 1.48 |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |