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Christopher Vincent Ruhs

SOIL-MICROBE-VOLATILE ORGANIC COMPOUND (SMVOC) ANALYSIS AND AUTHENTIC SCIENCE INQUIRY INTO GAS CHROMATOGRAPHY FOR A GENERAL CHEMISTRY LABORATORY CLASS

By

Christopher Vincent Ruhs

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Approved:

Karen McNeal Assistant Professor of Geosciences (Director of Thesis and Major Professor)	Darrel Schmitz Professor and Department Head of Geosciences (Committee Member)
David Lang Associate Professor of Plant & Soils Sciences (Committee Member)	Christopher Dewey Associate Professor of Geosciences (Graduate Coordinator)
Gary Myers Professor of Arts & Sciences (Dean of the College of Arts & Sciences)	

Name: Christopher Vincent Ruhs

Date of Degree: August 6, 2011

Institution: Mississippi State University

Major Field: Geosciences

Major Professor: Dr. Karen S. McNeal

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Candidate for Degree of Master of Science

Sound research and effective teaching are both essential to the progress of science. This thesis encompasses two studies to address the two needs: a multi-scale soil study designed to validate a novel soil biological characterization method; and a pilot pedagogical study designed to test the efficacy of authentic science inquiry into gas chromatography.

The soil study relies on a comparison of six soils taken from the Bahamas and Michigan. The novel method, using soil-derived VOCs analyzed via gas chromatography-mass spectrometry (GC-MS), proved effective for resolving soils, as hypothesized, and may prove useful for analyzing soil biology rapidly and non-destructively in future studies.

The pilot pedagogical study compares traditional recipe-style instruction with authentic science inquiry in an undergraduate chemistry laboratory class. Pre- and post-assessments of students' conceptual understanding, retention of terms, and attitude

revealed the hypothesized superior efficacy of authentic science inquiry over traditional recipe-style instruction.

Key words: SMVOC, BIOLOG, FAME, GC-MS, soil, microorganisms, biological characterization method, authentic science inquiry, attitude, retention, pedagogy.

DEDICATION

I would like to dedicate this work to my wife and best friend, Anna Ruhs.

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The author expresses his sincere gratitude to the many people without whose selfless assistance this thesis could not have materialized. First of all, sincere thanks are due to my major professor, Dr. Karen McNeal, for her constant support and motivation, and to the members of my committee, Dr. Darrel Schmitz and Dr. David Lang, for their feedback and guidance. Sincere thanks to Stephen Holman for his help on the education project, to Janet Dewey for her help with the C/N analysis, and to Dr. Mark Williams and Shankar Shanmugam for their assistance with the Michigan trip. Sincere thanks to Sarah Redencic and Curry Templeton for their encouragement and help in the department and in the laboratory. Sincere thanks to Dr. Brenda Kirkland, Dr. Leo Lynch, and Dr. Christopher Dewey who were all responsible for convincing me to apply to the Geoscience Department in the first place. Finally, a special thanks to my friends and family, especially to my wife and her parents, who constantly encouraged and supported my efforts.

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NOMENCLATURE

ANOVA	Analysis of Variance
ATD	Automatic Thermal Desorber
BGERG	Biogeochemistry and Geoscience Education Research Group
C/N or CN	Carbon/Nitrogen or Carbon to Nitrogen Ratio
CLSU	Community Level Substrate Utilization
CSUP	Carbon Substrate Utilization Profile
DNA	Deoxyribonucleic Acid
FAME	Fatty Acid Methyl Ester
GC-MS	Gas Chromatography Mass Spectrometry
IRB	International Review Board
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
PLFA	Phospholipid Fatty Acid
PTR-MS	Proton Transfer Reaction Mass Spectrometry
SMVOC	Soil-Microbe-Volatile Organic Compound
SPME	Solid Phase Microextraction
STEM	Science Technology Engineering and Mathematics
TOC	Total Organic Carbon
VOC	Volatile Organic Compound

CHAPTER I

INTRODUCTION

Our understanding of the environment hinges on the effective communication of sound environmental research through peer-reviewed publications, scientific presentations, and high-quality instruction. Sound environmental research includes the analysis of results obtained through careful laboratory and field experiments which have been designed from previous scientific findings. However, if sound environmental research is not effectively communicated and explained it cannot contribute to our understanding of the environment, and its value will remain broadly unknown and un-actionable. This is poignant for educators, who are responsible for passing on scientific knowledge to the next generation of students, without whom future scientific research would stop. The current lop-sided priorities of today's American universities need to be re-adjusted to place as much emphasis on undergraduate education as is placed on research (Savkar and Lokere, 2010). One without the other is insufficient. Therefore, educators and researchers alike need to identify those elements of experimental design and scientific communication, especially in science education, which can be improved upon and do so.

Environmental research relies heavily upon investigation and evaluation of environmental parameters. This approach is used by researchers to conduct studies on weather, climate, land utilization/restoration, eutrophication, hazardous waste disposal, oil spills, global warming, carbon sequestration, environmental and economic

sustainability, protection of forests, wildlife, and fresh water resources, farming techniques, pollution, chemical fate, and biogeochemistry, just to name a few, all of which are currently at the forefront of environmental research. A vast array of tools and methods are used to investigate and evaluate environmental parameters for these studies, and the quality of data obtained relies upon the efficacy of those tools and methods used.

The tools and methods of interest in the first study of this thesis are specifically designed to characterize soils biologically. When measuring soil microbiology, there is often a trade-off between accuracy and precision. On the one hand, there are techniques, such as PCR (polymerase chain reaction) and DNA (deoxyribonucleic acid) sequencing, that allow researchers to identify the exact species of microorganisms in their samples repeatably. This usually entails sample preparation from laboratory-cultured microorganisms. Unfortunately, since the vast majority of microorganisms cannot be cultured in the laboratory, these techniques are limited. It has become painfully clear to researchers that many of the soil biological characterization methods in use today, while useful, do not represent the full complexity of microorganism communities in natural soils (Wintzingerode et al., 1997; Widmer, et al., 2001; Postier et al., 2008). On the other hand, there are techniques (CLSU/CSUP, FAME/PLFA) that allow researchers to obtain a community-level microorganism fingerprint from a soil sample. While requiring the collection, transportation, storage and processing of soil samples, and while not as fine-tuned as their laboratory-cultured counterparts, microbial data obtained from these techniques represent the complexity of the microbial communities present in soil samples more fully.

This study has been designed and conducted to validate a novel method for community-level soil biological characterization that represents the full complexity of

soil microbiology, without relying on laboratory-cultured microorganisms, and without requiring soil samples to be collected, transported, stored, or processed, saving time, resources, and cost. The efficacy of the novel method was compared with that of two other soil biological characterization methods, namely FAME and Biolog^{TM}, all three of which produce community-level soil microbial data via chemical proxies. Six soil locations were studied, three from the Bahamas and three from Michigan. Analysis of soil-derived VOCs using gas chromatography-mass spectrometry (GC-MS) can resolve soils with different biological characteristics and may provide other useful information about the biological nature of soils, ex. biomass, community composition, and spatial/temporal community composition shifts. Ideally, this work will establish the biological characterization method as useful, providing a novel means for capturing complex environmental soil data quickly and cheaply, and without the need to physically collect soil samples.

As mentioned previously, the progress of environmental science hinges not only on sound research, but also on communicating that research effectively, especially in the classroom. Teaching the next generation how to think about science, how to do scientific work, and how to use current tools and methods is paramount. Since the novel method mentioned in the first study relies on GC-MS, it is fitting that the second study should focus on undergraduate students' comprehension of GC-MS related concepts, and how effectively they are taught. To do so, the second study compares authentic science inquiry (experimental group) with the traditional recipe-style approach (control groups) in an undergraduate chemistry laboratory class.

Authentic science inquiry is a pedagogical approach that allows students to participate in the scientific process by letting them determine how an experiment should

be designed to answer a useful research question. Inquiry gives students a measure of autonomy by allowing them to choose their own problem or topic (or for more teacher-guided inquiry, students at least choose their own scenario from a teacher-curated selection), and it exposes them to current scientific methods and tools (Chinn and Malhotra, 2002). This pedagogical approach has been shown to be more effective than classical recipe-style instruction at transferring conceptual knowledge and experience to students in laboratory classes (McNeal et al., 2008; Sell et al., 2006; Wallace et al., 2003).

The pedagogical study found herein contains the results of pre- and post-assessments of students' conceptual understanding of chromatography, memory of the chromatography laboratory, and general attitude toward chromatography across six separate sections, which were placed into three groups (2 control and 1 experimental treatments) from the Mississippi State University Survey of Chemistry laboratory class (Course Number CH 1051). The study is preliminary in nature due to its small sample size and arose from an opportunity to collaborate with the Department of Chemistry at Mississippi State University: they had planned to make several changes to the introductory chemistry course curriculum the semester after the study was implemented. The author took advantage of the opportunity in an effort to study and promote worthwhile changes, not only to the curriculum, but also to the pedagogical approaches used.

CHAPTER II

SOIL-MICROBE-VOLATILE ORGANIC COMPOUND ANALYSIS

Introduction

Complexity of Soils

Soils are the anisotropic results of complex interactions between geological and biochemical systems, where mineralogical parent material, aggregated through natural weathering and transport processes, plays host to a dynamic and ever evolving collection of organic material and biota (Jenny, 1994). Specifically, soils contain an inorganic matrix, living microorganisms, organic material, pore water, and pore gases which evolve over time, stratify vertically, and vary by climate and topography.

Because natural soils are in a constant state of flux, continually equilibrating with the surrounding environment, it is difficult to quantitatively and repeatably capture the complex physical, biological and chemical interactions that occur. Further complicating matters, bacterial communities living in soils can change rapidly over time and in response to changes in various environmental parameters, including temperature, pressure, moisture levels, pH, oxygen levels, micronutrient availability, root presence, etc. (Castro et al., 2010; Buyer and Drinkwater, 1997; Arsensio et al, 2007), making it difficult to characterize soils biologically. As microbial communities change in a given soil, VOCs found in those soils are also likely to change, since they are known microbial metabolites (Leff, 2008). Many soil methods (CSUP/CLSU, FAME/PLFA) in use today require the collection, transportation, processing and storage of soil samples, which can

all impact the integrity of a soil sample if care is not taken to control community composition shift (Stenberg 1998, Petersen, 1994). Other methods (PCR, DNA sequencing) allow researchers to know the exact species of microorganisms in their samples repeatably, but can only do so with a limited number of species, as they rely on the small fraction of microorganisms that can be cultured in the laboratory (Wintzingerode et al., 1997; Widmer, et al., 2001; Postier et al., 2008). As such, soil biological characterization methods which rely heavily on laboratory processing and handling are subject to limitations in their ability to represent actual conditions in the environment. This is not to say that these methods are invalid, only that they are limited. Ultimately, researchers are interested in measuring the biological nature of complex soils through direct, quick, reliable, and cost-effective methods without having to resort to too much laboratory processing. In essence, increased efficacy and efficiency is desired.

Established Soil Biological Characterization Methods, FAME and CSUP

Two commonly used methods for biological characterization of soils, both used for comparison with the proposed SMVOC (Soil-Microbial-Volatile-Organic-Compound) method described in this work, are the fatty acid methyl ester (FAME) analysis and the carbon substrate utilization profiles (CSUP) method.

The FAME method collects community-level biological information from soils by chemical extraction of lipids from the cell walls of soil microorganisms. Different lipids produce unique fatty acids upon esterification, which are subsequently used to identify microorganisms present in the sample (Cavigelli, 1995). FAME analysis is an effective method for resolving soils with various microbial communities at the species level (Klug and Tiedje, 1993)

The CSUP method collects biological information from soils by exposing soil microorganisms to an array of carbon sources and measuring which carbon sources are utilized by the microorganisms. As carbon sources are utilized, microorganisms respire and produce CO_2. CO_2 production lowers the pH and initiates a color change through the use of a pH indicator. The pattern or "profile" of utilized carbon substrates reveals community level information (Zak, 1994). Both of these methods are useful, but require extensive sample preparation, are invasive to the ecosystem being studied, destroy the sample, and are incapable of collecting fine temporal data.

Volatile Organic Compounds (VOCs) and Soil Bacteria

Because of the low boiling points associated with covalently bonded molecules, myriad organic compounds have a vapor pressure high enough under atmospheric conditions to partition into the atmosphere in measurable quantities. Such organic compounds are termed "volatile organic compounds" (VOCs). The term encompasses a broad range of chemical species, including alkanes, alkenes, alcohols, aldehydes, esters, ethers, nitriles, organic acids, and terpenes. It has been shown that VOCs derived from soils are largely produced by various plant material (e.g. seeds, roots, leaves, wood) and more importantly for this work, from microorganisms, especially bacteria (Stotzky and Schenck, 1973; Moore-Landecker, 1988; Stahl and Parkin,1996). Once VOCs are produced by their sources, they diffuse into proximal soils, pore spaces, and pore water. Adsorption of VOCs onto soils and dissolution into soil water is offset by diffusion and advection into surrounding soil, water, and atmosphere. Wet soils tend to retain VOCs more than dry soils, especially for polar compounds, with diffusion taking place according to Henry's Law (Washington, 1996).

The method presented here utilizes all VOCs found in natural soils that can adsorb onto Airtoxics© (Perkin Elmer, Inc.) adsorbent, though only those VOCs that correlate with bacterial communities are of interest for this work. Previous studies have shown strong correlations between the metabolism of specific microbial communities and the production of specific VOCs (Grametbauer et al., 1988; Wood et al., 1993; Fierer et al., 2003), which indicates that VOCs can be useful as proxy indicators of carbon utilization and microbial activity. The literature has also shown that microbes play a significant role in the nitrogen, phosphorus, and sulfur cycles in soils (Naeem, 1997), and therefore affect soil health (Zelles, 1999; Dahlhoff, 2004; Gil-Sotres et al., 2005). Both assertions have led many researchers to test the hypothesis that VOCs could be used to indirectly measure biological soil characteristics and ultimately soil health. To date, the majority of biological characterization studies using VOCs, have been conducted in controlled laboratory settings with uniform and limited microbial communities (Linton and Wright, 1993; Stahl and Parkin 1994 and 1996; McNeal and Herbert, 2009). These experiments, while useful, do not fully capture the complexity of natural soils. By comparison, this study is designed to test whether VOCs can resolve between natural soils as well as Biolog^{TM} or FAME.

Other researchers (Bunge et al., 2008) who are interested in fast, dependable, and cheap methods of capturing the complex nature of soil biology have successfully characterized microbial VOCs using Proton Transfer Reaction-Mass Spectrometry (PTR-MS). PTR-MS has proven capable of 100 millisecond response times, and single digit parts-per-trillion by volume detection limits—a very accurate instrument. However, all PTR-MS experiments involving the characterization of VOCs have so far relied on headspaces generated by microorganisms cultured in growth media and do not represent

the complexity of natural soils. Furthermore, PTR-MS uses a proton transfer ionization method, making resolution of individual isomers through mass spectrometry impossible, since all isomers would have the same mass and be indistinguishable; and, only VOCs with a proton affinity higher than the H_30^+ (hydronium ion) are able to be ionized and therefore detected by mass spectrometry. However, the PTR-MS and similar research which seeks to understand the dynamic between soil microorganisms and VOC metabolites contributes important findings to our understanding of natural soil ecosystems.

Several methods for collecting VOCs (head space, purge and trap thermal desorption, and solid phase microextraction) exist, some of which have been compared for efficacy (Elmore, J.S., 1997). One of the best suited methods to field sampling is the solid phase microextraction (SPME) method developed in 1999 by a research team from Waterloo, Canada, wherein VOCs adsorb onto coated fibers, and later desorb onto a GC-MS for compound separation and identification (Koziel, J. et al., 1999). The method was a breakthrough since it captured VOCs without needing to collect soil samples like the headspace and purge and trap methods. It was also much cheaper, since it only required a glass tube, special fibers, and a syringe.

The study presented here contributes to the ongoing search for good VOC collection methods by validating the novel soil-microbe-VOC (SMVOC) analysis. Like the SPME method, the SMVOC method is useful for studying air samples from natural environments, such as subsurface air and soil pore gas samples, quickly and cheaply. The SMVOC method negates the need for soil sample transportation and storage and employs reusable Perkin Elmer Thermal Desorption Tubes, which can be filled with a variety of

adsorbents, can be coupled to a vacuum pump, and can be heated or cooled without damage to the hardware.

Research Goals

This study specifically addresses the hypothesis that soil-derived VOCs are useful indicators of soil microbial activity and community composition over a broad range of ecosystems and have better spatial resolution as compared to other biological characterization methods. The SMVOC method is designed to capture the complexity of soils without needing to destructively remove soil from sample sites; naturally produced soil VOCs are collected and used as proxies for biological characterization. It is desirable to compare this in-situ field method protocol to other soil biological characterization methods in order to determine if the novel method is as effective in spatially resolving soils. The work provides a SMVOC analysis method to the larger community of environmental scientists that allows for characterization of VOC fingerprinting, potentially indicative of both microbial activity and community composition through the non-invasive capture of VOCs, which are known microbial metabolites. The new method fits into the emerging field of metabolomics--a field that describes how metabolites are indicative of the metabolic pathways being expressed by different microorganisms.

Methods

Study Sites

In order to test the relative resolution of the proposed SMVOC method compared with the two other soil biological characterization methods mentioned above, two climatically diverse regions have been chosen, and three sites within each region were

studied in triplicate (18 samples). This allows for the comparison of each method's resolution of soils at the regional and local levels.

Three study sites from San Salvador Bahamas were studied: two hypersaline pond locations, Salt Pond (N24° 01' 24.1" W74° 27' 02.3") and Triangle Pond (N24* 06' 25.2" W74* 31' 08.0") which were previously observed to support microbial mat environments (Pearl, 2000), and an archaeological dig site (N24° 00' 39.6" W74° 27' 35.7"). Nutrient-starved, but well-developed microbial mats are common in the hypersaline ponds that populate San Salvador, and the hypersalinity of these ponds exerts strong selective pressure for cyanobacterial dominance (Pinckney et al., 1995). Archaeological dig sites are also common, many of which are part of the ongoing archaeological study of the Lucayan Indian populations that once lived on the island (Berman and Pearsall, 2000).

Similarly, three study sites along a dune transect in Wilderness State Park, located on the North Western tip of the lower peninsula of Michigan, USA, were studied: a 15 year old dune (45° 43' 37.2" N 84° 56' 27.5" W) , a 95 year old dune (45° 43' 38.2" N 84° 56' 26.7" W), and a 145 year old dune (45° 43' 38.676" N 84° 56' 24.612" W), which lay along the Eastern edge of Lake Michigan and are thought to vary significantly in regards to microbial activity and community composition (Lichter, J., 1998). These sites are reported to be glacial sediments covered by a well-developed paleosol buried by eolian sands over the last 5.5 thousand years, forming a system of parallel dunes (Arbogast, et. al, 2002). The dunes are reported to be dominated by Actinobacteria, Proteobacteria and Acidobacteria (Shanmugam, et. al, 2011).

These six locations offer a variety of microbial community conditions, allowing for a robust comparison of the SMVOC method against the other soil biological

characterization methods. They are also part of on-going research efforts with our collaborators, making them ideal choices for further study.

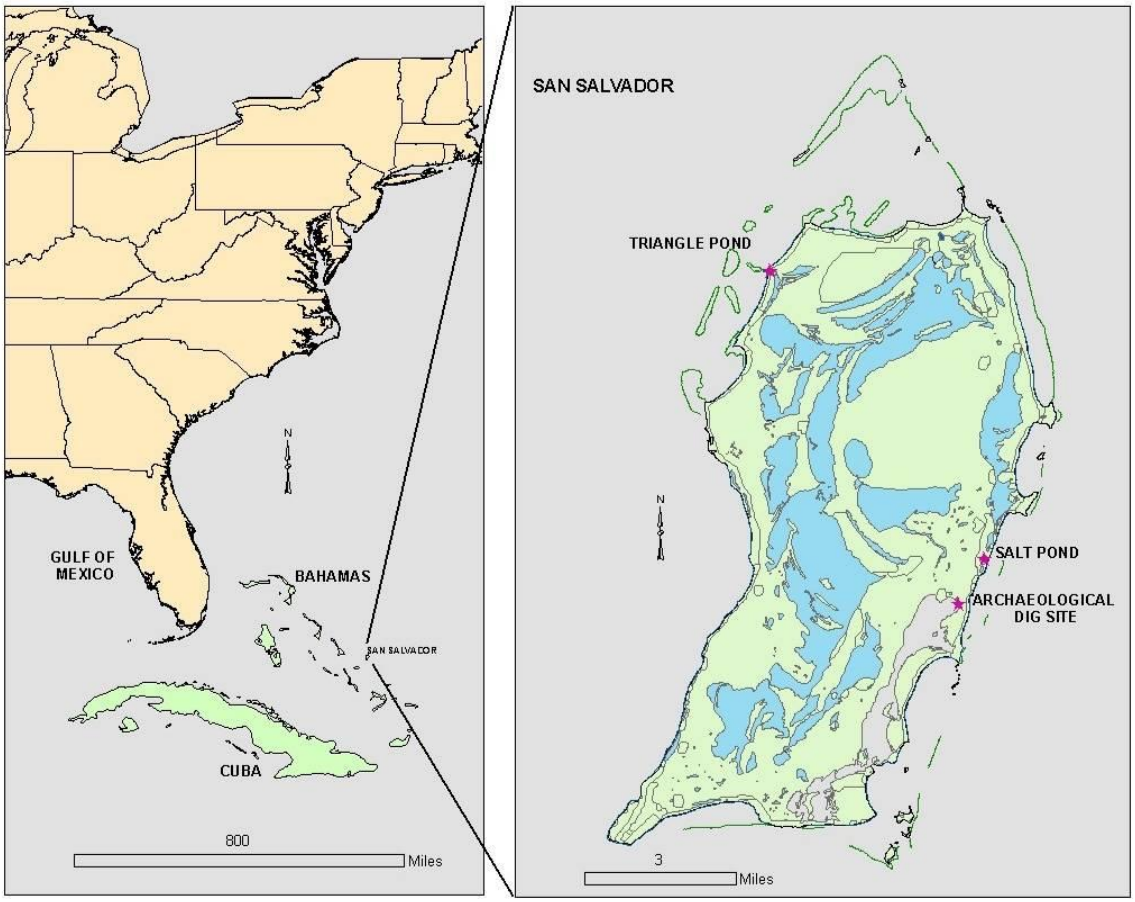


Figure 1 San Salvador, Bahamas: three study sites, including two hypersaline ponds and an archaeological dig site.

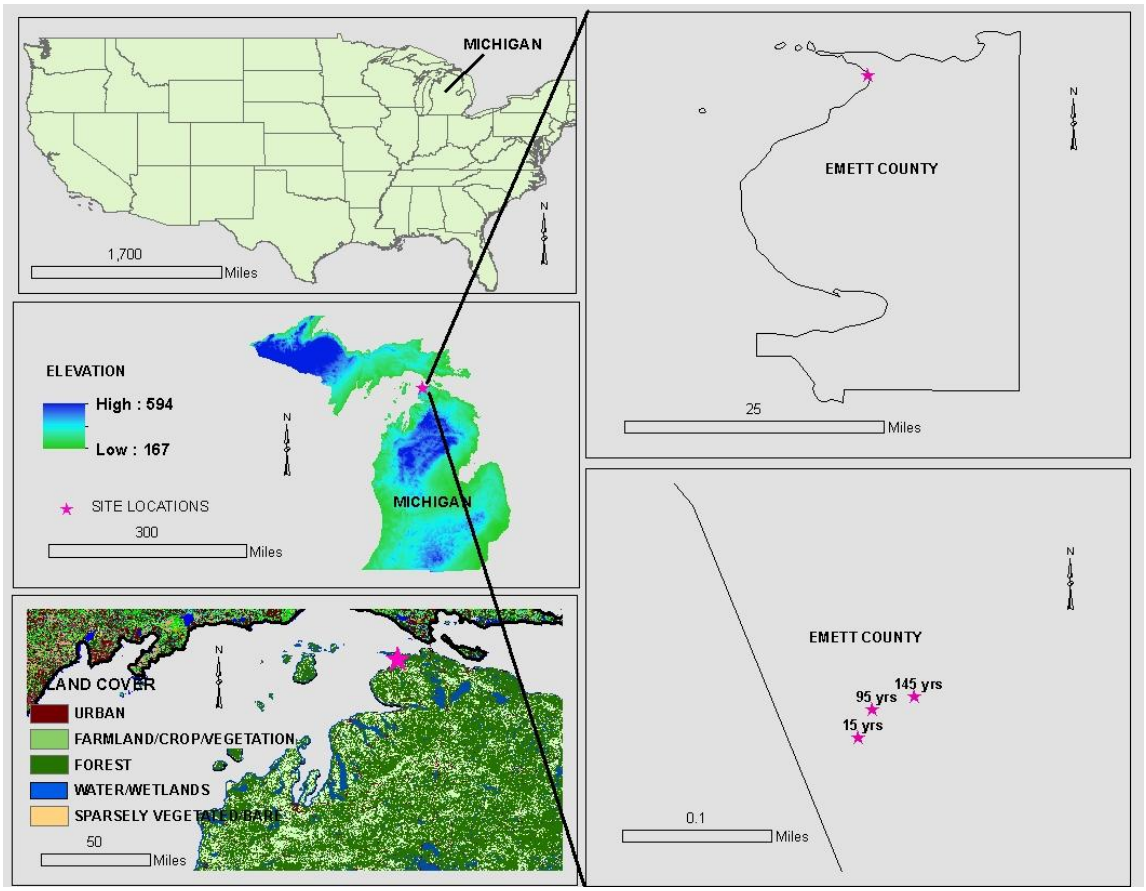


Figure 2 Emett County, Michigan: three study sites along a dune transect.

The three soil biological characterization methods were then compared to one another within each of six sites addressed. It was not necessary to control for the environmental conditions of the study sites; each site was treated as an individual and separate comparison of the three methods. However, sampling at each site was conducted on the same day for the three methods, allowing us to control for environmental conditions within each of the six sites over a reasonably small time period, making the comparisons individually valid.

Field Methods

In the field, soil moisture readings were collected using a Delta-T field soil moisture probe and soil pH was measured using a Spectrum Technologies, Inc. IQ 150 pH Meter. Collection of VOCs was accomplished by vacuuming soil-derived VOCs at 2mL/minute onto pre-conditioned adsorption tubes, filled with Perkin Elmer's proprietary Airtoxics© adsorbent, using an SKC, Inc. vacuum pump connected to a hollow soil probe (1/2" in diameter, 15" in length) with a plastic hose (see diagram 1). After field blanks were taken for the purpose of background subtraction, the probe was inserted into the soil at 1 inch/minute for 6 minutes. Triplicate gas samples were taken 1m apart. The adsorption tubes are designed to trap any volatile organic compounds exposed to it. Gas sample tubes were immediately capped, stored at room temperature, and brought back to MSU for further analysis.

After subsurface gas sampling, 200 grams of soil was collected from the hole made by the soil probe from 0-6 inches deep, and stored at -4° C until further analysis.

Lab Methods

For the VOC analysis, 1µL of a 40ppm toluene-D8 internal standard was injected into each of the adsorption tubes containing sample gases, then immediately recapped. The tubes were placed onto a Perkin Elmer TubroMatrix 350 Thermal Desorber with automatic carousel (Figure 3), which is designed to automatically heat the tubes until all the collected gases desorb from the Airtoxics© adsorbent within them.

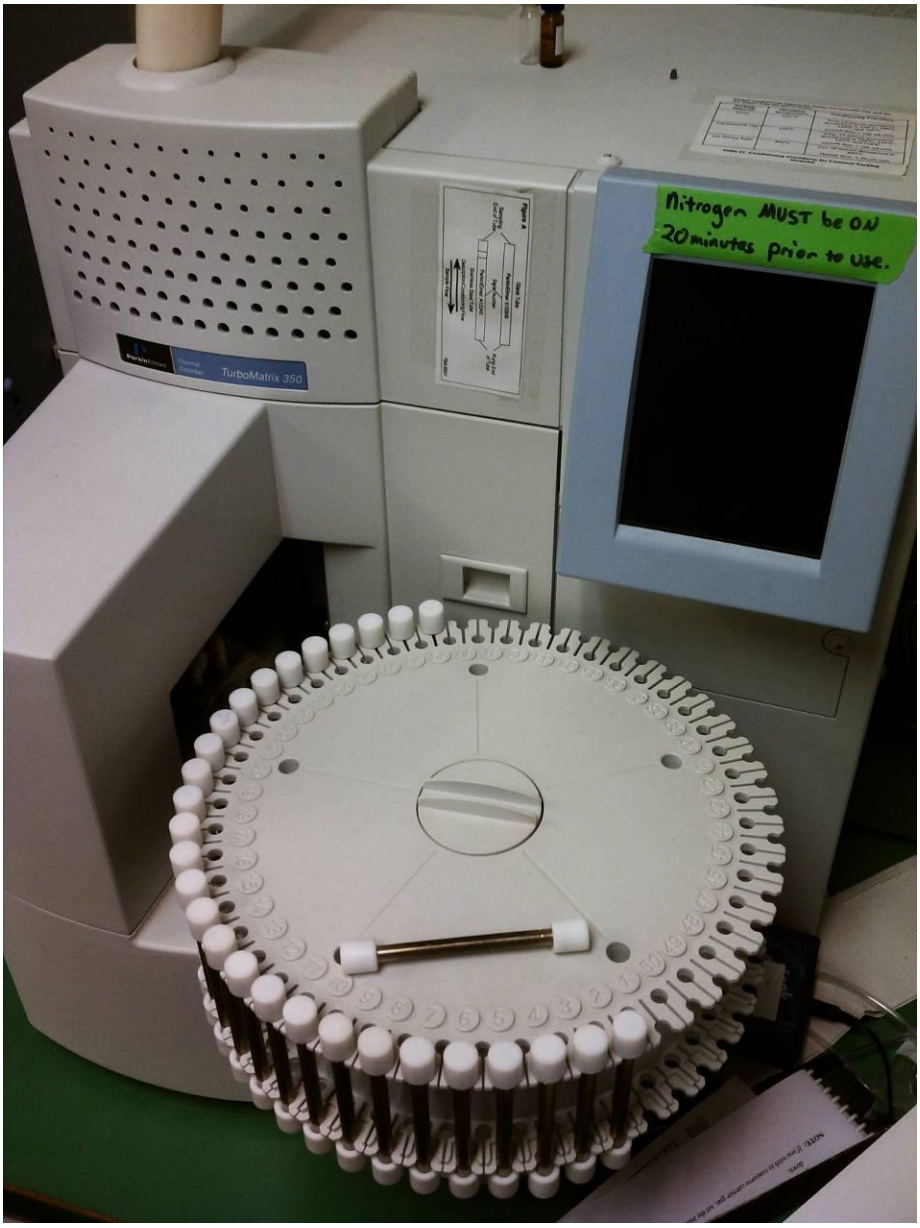


Figure 3 Perkin Elmer Automatic Thermal Desorption Tube and Carousel.

Tubes were heated for 10 minutes at 250° C with 50mL/min He_2 gas, split 25:1, collected onto an internal cold trap at -30° C for 2 minutes, then automatically injected at 220° C onto a 30m, 0.32mmID Perkin Elmer Elite-624 fused silica GC capillary column, coated with a 1.8µm 6% cyanopropylphenyl/94% dimethyl polysiloxane stationary phase with a 2mL/min He_2 gas mobile phase. The GC column housed within a Perkin Elmer

Clarus 600 gas chromatograph was held at an initial temperature of 40° C for 3.00 minutes, then ramped by 10.0 degrees/minute to 80° C, then ramped by 4.0 degrees/minute to 160° C, and finally held for 3.00 minutes: a total run time of 30.00 minutes. The mobile phase was 2.00 mL/minute He_2, and the sampling rate was 1.56350 points/second. After a 2 minute solvent delay, eluted VOCs were electrically ionized and scanned from 30 to 500 m/z by a calibrated Perkin Elmer Clarus 600 S mass spectrometer.

For the Carbon and Nitrogen (CN) analysis, 10g of the collected soil samples were baked in an oven at 200° C, ground into a fine powder and analyzed for carbon/nitrogen content using a Finnegan CNS analyzer (Beverly, MA).

For the grain size analysis, 10g of collected soil samples were treated with H_20_2 to remove organics, dried, and wet sieved (Van Reeuwijk, 1992).

For the Biolog analysis, 10 g of collected soil samples were diluted to $1x10^{-7}$ with pure (18.4 MΩ-cm) water and used to cultivate bacteria on Ecoplates© (Biolog™; Garland and Mills, 1994). Biolog™ absorbance readings were taken once every 24 hours for 3 days with a Fisher Scientific Multiscan MCC at ambient room temperature using a 340nm filter.

For the FAME analysis, 10g frozen (-4° C) soil samples were thawed, placed in centrifuge tubes, treated with 15mL 0.2M KOH (in methanol), vortexed for 20 seconds, set in a 37° C bath for ten minutes and vortexed for 10 seconds every 10 minutes for 1 hour, then neutralized with 3mL of 1M acetic acid. 10mL hexane was added to each tube to extract the fatty acids, vortexed for 20 minutes, and centrifuged at 1000g for 20 minutes at 4° C. The top 2/3 of the supernatant were collected, the solvent was evaporated, and the residual solute was dissolved in methylene chloride for fatty acid

methyl ester (FAME; Eder, 1995) analysis using GC-MS. 2µL samples were injected manually at 250° C onto a 30m, 0.32mmID Perkin Elmer Elite-225 fused silica GC capillary column, coated with a 1.8µm 50% cyanopropylphenyl/50% phenylmethyl polysiloxane stationary phase with a 1.5 mL/min He_2 gas mobile phase. The GC column housed within a Perkin Elmer Clarus 600 gas chromatograph was held at an initial temperature of 140° C for 3.00 minutes, then ramped by 4.0 degrees/minute to 240° C, and held for 15.00 minutes: a total run time of 43.00 minutes. The sampling rate was 1.56350 points/second. After a 5 minute solvent delay, eluted FAMEs were electrically ionized and scanned from 50 to 500 m/z by a calibrated Perkin Elmer Clarus 600 S mass spectrometer.

Statistical Analyses

ANOVAs were used to distinguish between sample means for the six sites across the three soil biological characterization methods. Significant differences were interpreted as a method's ability to resolve between soil sample means. *The null hypothesis is that no statistically significant differences exist amongst soil sample means from the six study sites.* The null hypothesis was rejected if statistically significant ($\rho < 0.05$) differences existed amongst the data obtained from the soil samples.

Similarly, principle component analysis (PCA) was applied to VOC, FAME, and Biolog™ results; PCA loading plots were interpreted to determine each method's ability to resolve soils. *The null hypothesis is that no differences exist amongst the PCA loading plots.* The null hypothesis was rejected if the visual comparison of the PCA loading plots reveals that better resolution was achieved by any of the three soil biological characterization methods.

Results

Soil Characteristics

Physical soil characteristics, obtained by measuring carbon/nitrogen ratios, total organic carbon (TOC) percentages, percent moisture, and pH, from each of the six study sites are found in Table 1. On average, the Bahamian sites had higher carbon to nitrogen ratios, TOC, percent moisture, and pH than the Michigan sites. Interestingly, the Archaeological Dig Site and the 145 Year Dune both showed lower carbon/nitrogen ratios than their counterparts within their respective sites. Table 2 tabulates the grain size analysis, which was conducted via wet sieving. Bahamian soil samples tended to have larger grain sizes than Michigan soil samples. These parameters were measured to provide insight into the inorganic matrix which plays host to various biota, and can be used as a reference to the soil biological characterization methods.

Table 3 delineates the biological characterization scores obtained from the three biological characterization methods, followed by a sample SMVOC chromatograph (Figure 3) as an example of the kind of data, specifically the type of compounds, obtained by the novel method. The biological characterization scores from the three methods are the main focus of this work; they are tabulated below and statistically analyzed by three one-way ANOVAs and three PCAs. Table 24 in Appendix A, Table 25 in Appendix B, and Table 26 in Appendix C list the specific nutrients and compounds analyzed in the Biolog^{TM}, FAME, and SMVOC analyses, respectively.

Table 1 Values for all measured parameters from the six study sites

	San Salvador, Bahamas			Mackinaw City, Michigan		
Site	**Triangle Pond**	**Salt Pond**	**Archaeological Dig Site**	**15 Yr. Old Dune**	**95 Yr. Old Dune**	**145 Yr. Old Dune**
Ratio of Carbon to Nitrogen	270.3	209.7	66.94	64.82	66.63	28.87
		Average: 123.9			**Average: 80.00**	
Total Organic Carbon (%)	5.5638	6.4313	6.7345	0.0650	0.0826	0.4384
		Average: 6.2432			**Average: 0.1953**	
Percent Moisture (m^3/m^3)	0.026	0.361	0.302	0.011	0.026	0.017
		Average: 0.230			**Average: 0.018**	
pH	8.06	8.10	7.51	7.92	8.22	7.02
		Average: 7.89			**Average: 7.72**	

Table 2 Sand fractions from the six study sites, obtained from wet sieving

Site	Sand Fractions					
	1000um	**500um**	**250um**	**88um**	**< 88um**	**Total**
Triangle Pond	4.00%	50.43%	41.08%	1.56%	2.94%	100.00%
Salt Pond	0.13%	12.52%	66.91%	17.11%	3.33%	100.00%
Archaeological Dig Site	1.59%	10.14%	49.14%	26.64%	12.49%	100.00%
Average for Bahamas	**1.91%**	**24.36%**	**52.38%**	**15.10%**	**6.25%**	
15 Year Dune	0.01%	5.59%	42.15%	50.89%	1.37%	100.00%
95 Year Dune	0.00%	8.80%	36.90%	53.14%	1.16%	100.00%
145 Year Dune	0.19%	3.87%	13.90%	80.28%	1.76%	100.00%
Average for Michigan	**0.07%**	**6.09%**	**30.98%**	**61.44%**	**1.43%**	

Table 3 Average, normalized biological characterization scores from the six study sites

	San Salvador, Bahamas			Mackinaw City, Michigan		
Site	**Triangle Pond**	**Salt Pond**	**Archaeological Dig Site**	**15 Year Dune**	**95 Year Dune**	**145 Year Dune**
Average Normalized Biolog™ Absorbance	0.4170	0.5419	0.8972	0.4631	0.4745	0.5774
		Average: 0.6187			**Average: 0.5050**	
Average Normalized Peak Area for FAME	21,298,053	12,072,139	22,653,578	14,896,144	12,792,499	6,682,981
		Average: 18,674,590			**Average: 11,457,208**	
Average Normalized Peak Area for SMVOC Analysis	2,302,850	389338	16,423,059	12,692,852	197,078	1,089,954
		Average: 6,371,749			**Average: 4,659,961**	

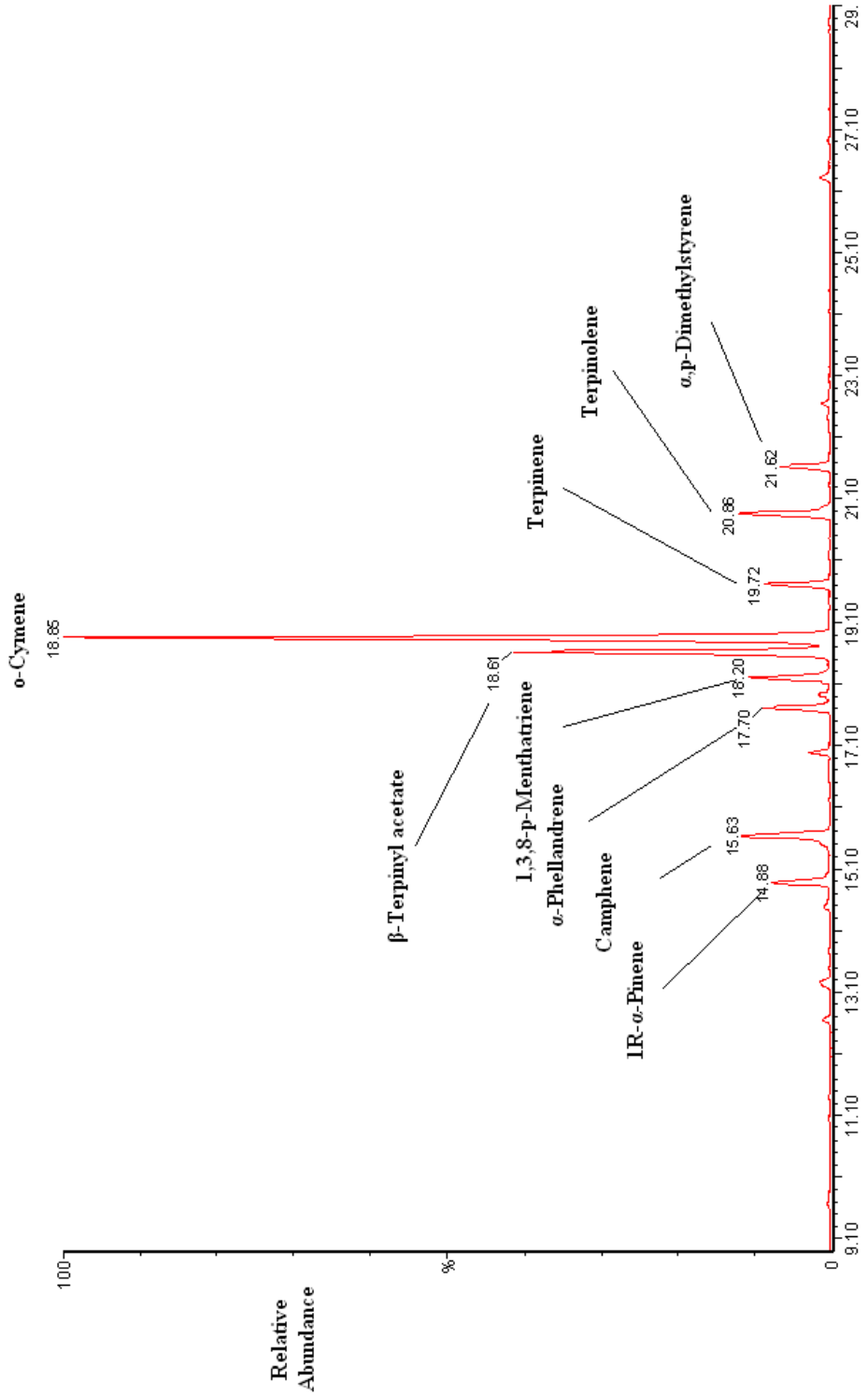


Figure 4 Sample chromatograph of VOCs collected from Triangle Pond, San Salvador, Bahamas.

Biolog™ Results

Biolog™ absorbance scores (Table 4) were obtained via the Biolog™ analysis.

Table 4 Average, normalized raw data obtained from the BiologTM analysis.

Nutrient	Salt Pond	Triangle Pond	Archaegological Dig Site	15 Year Dune	95 Year Dune	145 Year Dune
1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
2	0.0000	0.0000	0.1033	0.0367	0.0067	0.1267
3	0.5567	0.2733	1.2467	0.3533	0.4533	0.4967
4	0.0733	0.0300	0.3033	0.0367	0.4867	0.2333
5	1.6600	1.4800	2.1567	1.4067	0.9467	1.2433
6	0.0067	0.0067	0.0000	0.0167	0.0100	0.0167
7	1.9000	1.9133	1.7700	1.9900	1.7700	2.0433
8	1.9300	1.7567	1.9900	1.8300	1.4933	1.7067
9	1.2400	1.0533	1.7133	1.0667	1.1233	1.1600
10	0.1133	0.0333	0.2100	0.0333	0.0467	0.1367
11	0.3700	0.3300	0.3133	0.3200	0.2800	0.2800
12	0.7867	0.6233	1.6900	0.6500	0.6533	0.9067
13	0.9500	0.7267	1.3900	0.9067	0.7133	1.1933
14	0.6200	0.4967	0.9133	0.6267	0.5267	0.7800
15	1.1433	0.8767	1.7600	0.8833	0.8033	1.0967
16	0.2467	0.0000	0.6400	0.2800	0.5733	0.4367
17	0.2767	0.1200	1.1133	0.0767	0.1733	0.3567
18	0.3667	0.1167	0.4267	0.2400	0.1900	0.4367
19	0.7600	0.2300	1.4633	0.3233	0.2767	0.7433
20	0.7200	0.3933	1.4600	0.6133	0.4567	0.7367
21	0.4333	0.4433	1.2333	0.3633	0.4600	0.5300
22	0.1067	0.0767	0.4000	0.1267	0.3733	0.4400
23	1.9100	1.7800	2.1233	1.7867	1.6933	1.6833
24	0.2133	0.1267	0.4100	0.0833	0.2300	0.1633
25	0.1300	0.0367	0.8000	0.0200	0.2467	0.2733
26	0.0000	0.0000	0.1000	0.0000	0.0467	0.0967
27	0.1900	0.1733	0.5000	0.1967	0.2567	0.2667
28	0.0000	0.0000	0.0000	0.0000	0.0767	0.0867
29	0.0367	0.0233	0.7800	0.0433	0.0733	0.0833
30	0.0333	0.0133	0.1400	0.0267	0.0467	0.0567
31	0.3700	0.2000	0.7733	0.4167	0.5200	0.4500
32	0.1967	0.0100	0.7867	0.0667	0.1767	0.2167

One-Way ANOVA

Independent Biolog™ observations (N=192) from each of the six sites were obtained and analyzed using a one-way ANOVA. Checks for normality on the Biolog™ observations via the Kolmogorov-Smirnov test revealed a violation of the assumption of normality. Therefore, the data were transformed as follows:

Transformed Absorbance Data = Square Root (Absorbance Data) (1)

Tests for normality via the Kolmogorov-Smirnov test and for homogeneity of variances via Levene's test were then performed on the transformed data and yielded no evidence of problems with either assumption, $p > 0.05$.

Because the one-way ANOVA revealed a statistically significant difference among study sites, $F(5, 186) = 2.993$, $MSE = 0.163$, $p = 0.013$, the null hypothesis that *"no statistically significant differences exist amongst BIOLOG™ absorbance means from the six study sites"* is rejected.

Table 5 One-way ANOVA results for the BiologTM absorbance data obtained from the six study sites

	Sum of Squares	df	Mean Square	F	Significance
Between Sites	2.444	5	0.489	2.993	0.013
Within Sites	30.376	186	0.163		
Total	32.820	191			

A Tukey's HSD post-hoc test at alpha = 0.05 indicated that the Archaeological Dig Site ($M = 0.8436$, $SD = 0.43764$, $n = 32$) had significantly higher Biolog™ absorbance scores than both the 15 Year Dune ($M = 0.5443$, $SD = 0.41498$, $n = 32$) and the Triangle Pond ($M = 0.4881$, $SD = 0.42951$, $n = 32$). No other pair-wise differences were statistically significant.

Table 6 Statistically significant groups as indicated by Tukey's HSD post-hoc test

Site	Group 1	Group 2
Archaeological Dig Site	0.8436	
145 Year Dune	0.6723	0.6723
Salt Pond	0.6041	0.6041
95 Year Dune	0.5989	0.5989
15 Year Dune		0.5443
Triangle Pond		0.4881

FAME Results

Peak area FAME scores (Table 7) were obtained via the FAME analysis.

Table 7 Average, normalized raw data obtained from the FAME analysis

FAME	Salt Pond	Triangle Pond	Archaegological Dig Site	15 Year Dune	95 Year Dune	145 Year Dune
1	1,598,107	5,453,535	7,294,672	4,593,706	4,097,547	2,247,848
2	9,103,361	18,307,870	20,843,388	30,095,577	22,765,053	12,670,127
3	1,776,422	4,512,225	5,344,314	10,099,747	7,480,841	3,786,530
4	459,667	2,158,802	3,275,904	3,410,152	2,238,280	1,085,056
5	4,879,963	11,030,834	15,844,402	14,460,314	9,044,994	6,152,922
6	91,203,800	160,000,000	163,000,000	88,904,863	91,231,672	43,808,864
7	42,471,494	43,763,744	45,841,337	18,285,450	15,750,258	9,596,771
8	35,877,282	60,854,120	36,219,318	19,017,306	29,348,075	11,075,753
9	20,563,769	10,584,353	9,740,511	14,476,497	11,124,575	6,358,617
10	0	0	1,465,622	0	0	0
11	3,737,523	7,777,512	9,674,836	10,602,723	6,970,403	3,881,951
12	0	0	3,045,785	8,862,327	6,661,028	2,136,396
13	3,062,958	7,152,165	5,851,216	2,528,680	2,862,890	1,950,537
14	1,919,569	2,863,567	4,075,960	3,532,056	2,618,528	1,195,163
15	494,460	2,407,887	2,490,104	3,001,358	2,485,877	1,104,267
16	0	1,082,215	2,183,510	3,446,650	3,498,535	2,585,831
17	1,392,632	3,876,408	4,481,948	4,372,290	2,823,617	1,467,900
18	12,135,768	20,467,414	24,849,189	17,341,771	15,636,601	9,278,304
19	36,823,349	89,803,132	127,000,000	41,742,366	39,051,236	28,861,651
20	21,846,158	30,333,914	29,435,265	18,353,910	16,912,716	9,564,977
21	3,386,122	7,847,594	12,266,560	8,399,208	6,961,416	3,105,301
22	14,297,246	23,181,631	40,617,277	17,811,351	18,316,301	10,484,092
23	7,248,703	14,497,476	16,805,961	16,069,408	11,720,026	6,405,514
24	9,252,719	18,181,536	17,680,386	10,558,152	8,261,627	3,527,404
25	2,981,098	1,578,796	2,295,226	1,258,921	1,933,003	2,392,879
26	4,083,817	2,421,794	3,955,520	2,421,733	2,606,864	2,042,504
27	4,935,204	7,142,842	5,559,881	5,029,703	4,125,376	3,444,928
28	11,287,648	23,770,918	20,535,623	23,287,185	13,813,395	4,604,234
29	4,178,424	11,649,489	12,160,763	13,288,744	4,232,402	1,858,083
30	4,059,968	5,110,916	4,641,309	2,889,549	3,412,208	897,138
31	19,179,073	62,426,944	43,785,133	43,638,782	28,582,131	9,600,883

One-Way ANOVA

Independent FAME observations (N=192) were obtained from the six study sites and analyzed using a one-way ANOVA. Checks for normality and homogeneity of variances on the FAME observations via the Kolmogorov-Smirnov test and Levene's test

respectively revealed violations of both assumptions. Therefore, data were transformed as follows:

$$\text{Transformed Peak Area} = \text{Fourth Root (Peak Area)} \quad (2)$$

Tests for normality via the Kolmogorov-Smirnov and for homogeneity of variances via Levene's test were then performed on the transformed data and yielded no evidence of problems with either assumption, $p > 0.05$. Because the one-way ANOVA revealed a statistically significant difference among study sites, $F(5, 180) = 2.548$, $MSE = 380.229$, $p = 0.030$, the null hypothesis that "*no significant differences exist amongst FAME peak area means from the six study sites"* is rejected.

Table 8 One-way ANOVA results for the FAME peak area data obtained from the six study sites

	Sum of Squares	df	Mean Square	F	Significance
Between Sites	48844.974	5	968.995	2.548	0.030
Within Sites	68441.273	180	380.229		
Total	73286.247	185			

A Tukey's HSD post-hoc test at alpha = 0.05 indicated that the Archaeological Dig Site ($M = 59.9069$, $SD = 19.13163$, $n = 32$) had significantly higher FAME peak area scores than the 145 Year Dune ($M = 45.0451$, $SD = 14.48144$, $n = 32$). No other pair-wise differences were statistically significant.

Table 9 Statistically significant groups as indicated by Tukey's HSD post-hoc test

Site	Group 1	Group 2
Archaeological Dig Site	59.9069	
Triangle Pond	56.0002	56.0002
15 Year Dune	55.4521	55.4521
95 Year Dune	52.8835	52.8835
Salt Pond	47.4745	47.4745
145 Year Dune		45.0451

SMVOC Results

Peak area SMVOC scores (Table 10) were obtained via the SMVOC analysis.

Table 10 Average, normalized raw data obtained from the SMVOC analysis

Compound	Salt Pond	Triangle Pond	Archaegological Dig Site	15 Year Dune	95 Year Dune	145 Year Dune
1	66,791		1,261,954			
2	50,903					
3	164,305		2,370,217			
4	293,721		325,631	935,458		
5	1,646,870					
6	514,765	1,200,569	506,175	1,403,193	405,256	
7	1,480,233	1,556,852	824,304	1,406,976		
8	143,288	66,915				
9	130,047					
10	486,497	721,815	22,453			
11	329,519		23,310			
12	346,240				25,721	
13	101,218		320,141		10,569	
14	37,938					
15	47,740		4,052,591		6,982	
16		3,023,674			194,018	37,476
17		444,428		7,885,342	95,146	
18		907,539	130,000,000	13,135,874	5,795	20,508
19		3,952,280	132,000,000	22,748,828		330,985
20		1,332,302				
21		4,790,100			1,703,499	
22		5,156,853	60,666,667			2,629,419
23		18,063,071		3,021,056		5,930,463
24		401,401				
25		136,000				
26		286,507	673,172	23,705,365		79,305
27						
28		1,583,026				
29		384,564				
30		1,209,204	14,189,945	13,311,930		450,336
31		1,192,949		823,756	82,355	
32		747,185	14,377,804	2,914,939		741,807
33		771,095	21,668,546			
34		2,734,367		333,184		40,018
35			57,282			
36			59,009			
37			4,950,146			
38			4,071,655			1,090,025
39			2,091,679		7,189	

Table 10 (continued)

40		9,102,755	535,252	33,250
41		110,000,000	38,125,514	2,910,888
42		9,178,979	9,413,038	271,667
43		12,724,935	5,824,305	174,058
44		172,201		
45		337,381		
46		158,573		
47		404,570		
48		241,234		
49		1,696,394		
50		755,296		
51		2,675,932		
52			20,800,272	56,233
53			2,956,781	29,232
54			4,929,785	2,728,358
55			10,828,961	
56			104,000,000	
57			2,489,921	127,871
58			405,856	
59				9,878
60				65,465
61				19,353
62				139,456
63				8,156,211
64				56,628
65				157,952
66				24,551
67				13,467
68				56,601

One-Way ANOVA

Independent SMVOC observations (N=131) were obtained from the six study sites and analyzed using a one-way ANOVA. Checks for normality and homogeneity of variances on the FAME observations via the Kolmogorov-Smirnov test and Levene's test respectively revealed violations of both assumptions. Therefore, data were transformed as follows:

$$\text{Transformed Peak Area} = \text{LOG}_{10}\,(\text{Peak Area}) \qquad (3)$$

Tests for normality via the Kolmogorov-Smirnov and for homogeneity of variances via Levene's test were then performed on the transformed data and yielded no evidence of problems with normality, $p > 0.05$, but did show a continued violation of the assumption of homogeneity of variances. Since ANOVA is fairly robust against such violations, and since running a non-parametric test would make comparison between the biological characterization methods less valid, the decision to use a one-way ANOVA was reserved and coupled with the decision to use a more conservative, non-parametric post-hoc test. Because the one-way ANOVA revealed a statistically significant difference among study sites, $F(5, 125) = 16.665$, $MSE = 0.627$, $p < 0.001$, the null hypothesis that "*no statistically significant differences exist amongst SMVOC peak area means from the six study sites"* is rejected.

Table 11 One-way ANOVA results for the SMVOC peak area data obtained from the six study sites

	Sum of Squares	df	Mean Square	F	Significance
Between Sites	52.204	5	10.441	16.665	0.000
Within Sites	78.313	125	0.627		
Total	130.517	130			

A Tamhane's T2 post-hoc test at alpha = 0.05 indicated that the 15 Year Dune ($M = 6.6569$, $SD = 0.66921$, $n = 23$) had significantly higher SMVOC peak area scores than the Triangle Pond ($M = 6.0340$, $SD = 0.54957$, $n = 22$), the Salt Pond ($M = 5.361$, $SD = 0.51284$, $n = 15$), the 145 Year Dune ($M = 5.3022$, $SD = 0.84227$, $n = 24$), and the 95 Year Dune ($M = 4.6517$, $SD = 0.75017$, $n = 14$); the Archaeological Dig Site ($M = 6.2115$, $SD = 1.04289$, $n = 33$) and the Triangle Pond had significantly higher SMVOC

peak area scores than the Salt Pond, the 145 Year Dune, and the 95 Year Dune. No other pair-wise differences were statistically significant.

Table 12 Statistically significant groups as indicated by Tamhane's post-hoc test

Site	Group 1	Group 2	Group 3
15 Year Dune	6.6569		
Archaeological Dig Site	6.2115	6.2115	
Triangle Pond		6.0340	
Salt Pond			5.361
145 Year Dune			5.3022
95 Year Dune			4.6517

PCAs for the Three Biological Characterization Methods

PCAs of the data obtained from the three biological characterization methods are shown in Figures 4, 5, and 6 below. The Biolog™ PCA shows three defined groups, separating Archaeological Dig Site samples and 95 Year Dune samples from the other four sites. The extraction sums of squared loadings for the first three factors are 16.38, 0.735, and 0.398, accounting for 90.709%, 4.085%, and 2.210% of the variance respectively. The FAME PCA is somewhat less clear, showing a tight grouping of the Archaeological Dig Site with the 145 Year Dune, a Salt Pond Group, and then a scattered collection of the other three sites. One 15 Year Dune triplicate seems to lie outside the main core. The extraction sums of squared loadings for the first three factors are 16.670, 0.454, and 0.356, accounting for 92.609%, 2.522%, and 1.977% of the variance respectively. The SMVOC PCA shows four distinct groups: 15 Year Dune, 95 Year Dune, Triangle Pond, and then the other three. These results reveal that the SMVOC Analysis provides better spatial resolution than both the Biolog™ and the FAME methods, leading to the rejection of the null hypothesis, which states that *"no differences exist amongst the PCA loading plots."* The extraction sums of squared loadings for the

first three factors are 3.149, 2.893, and 2.334, accounting for 17.493%, 16.073%, and 12.964% of the variance respectively.

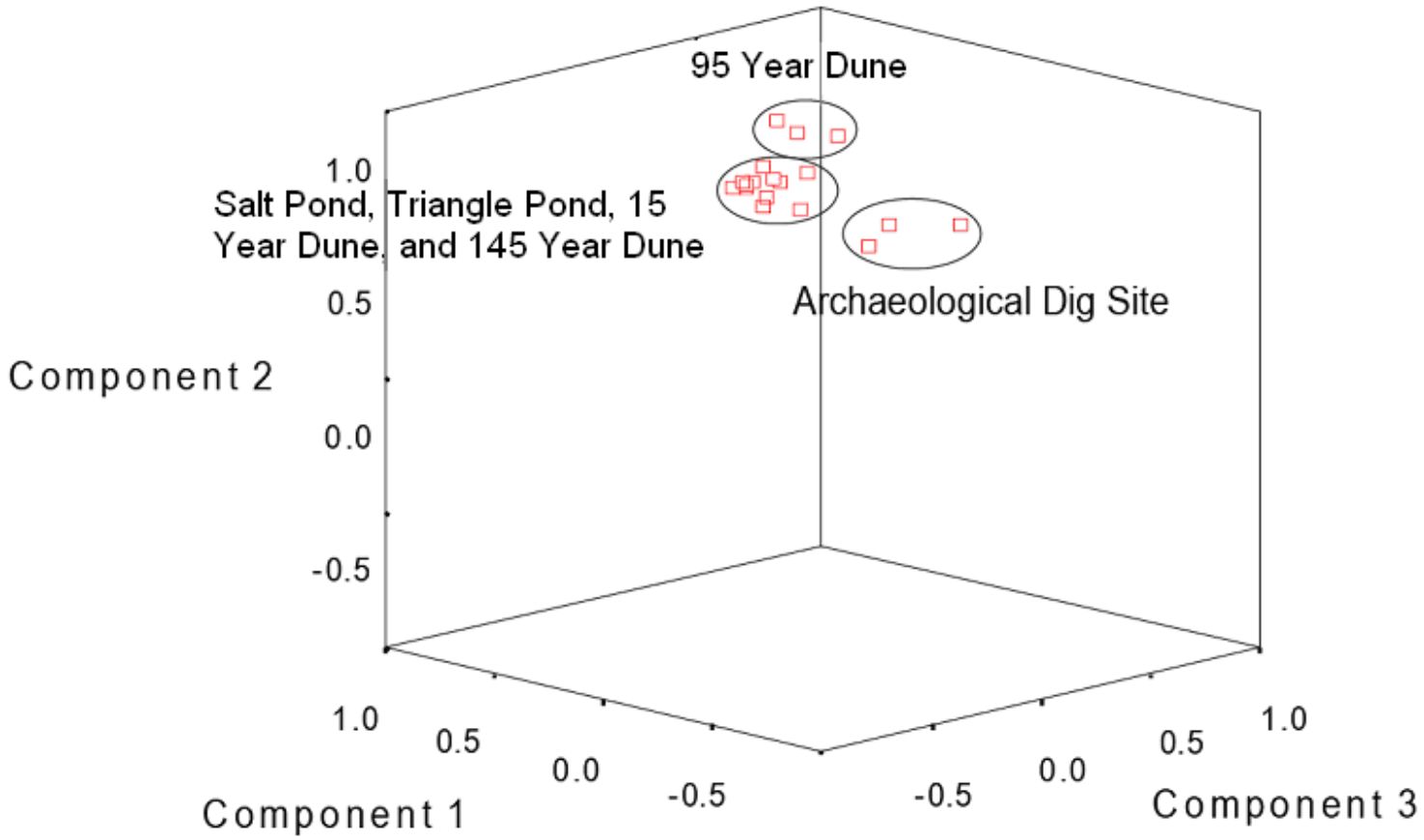


Figure 5 PCA showing resolution of the six study sites using the Biolog™ analysis

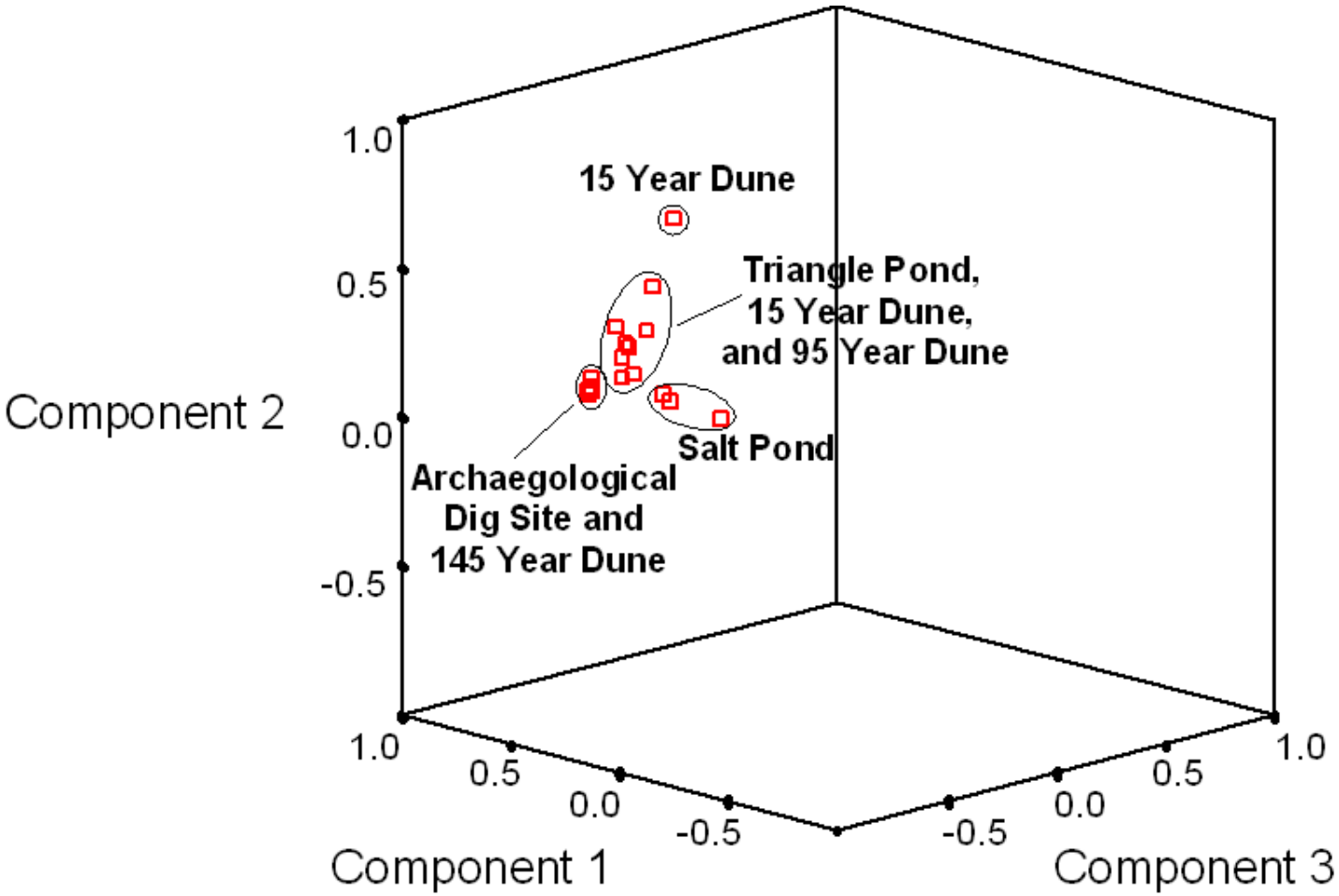


Figure 6 PCA showing resolution of the six sites using the FAME method

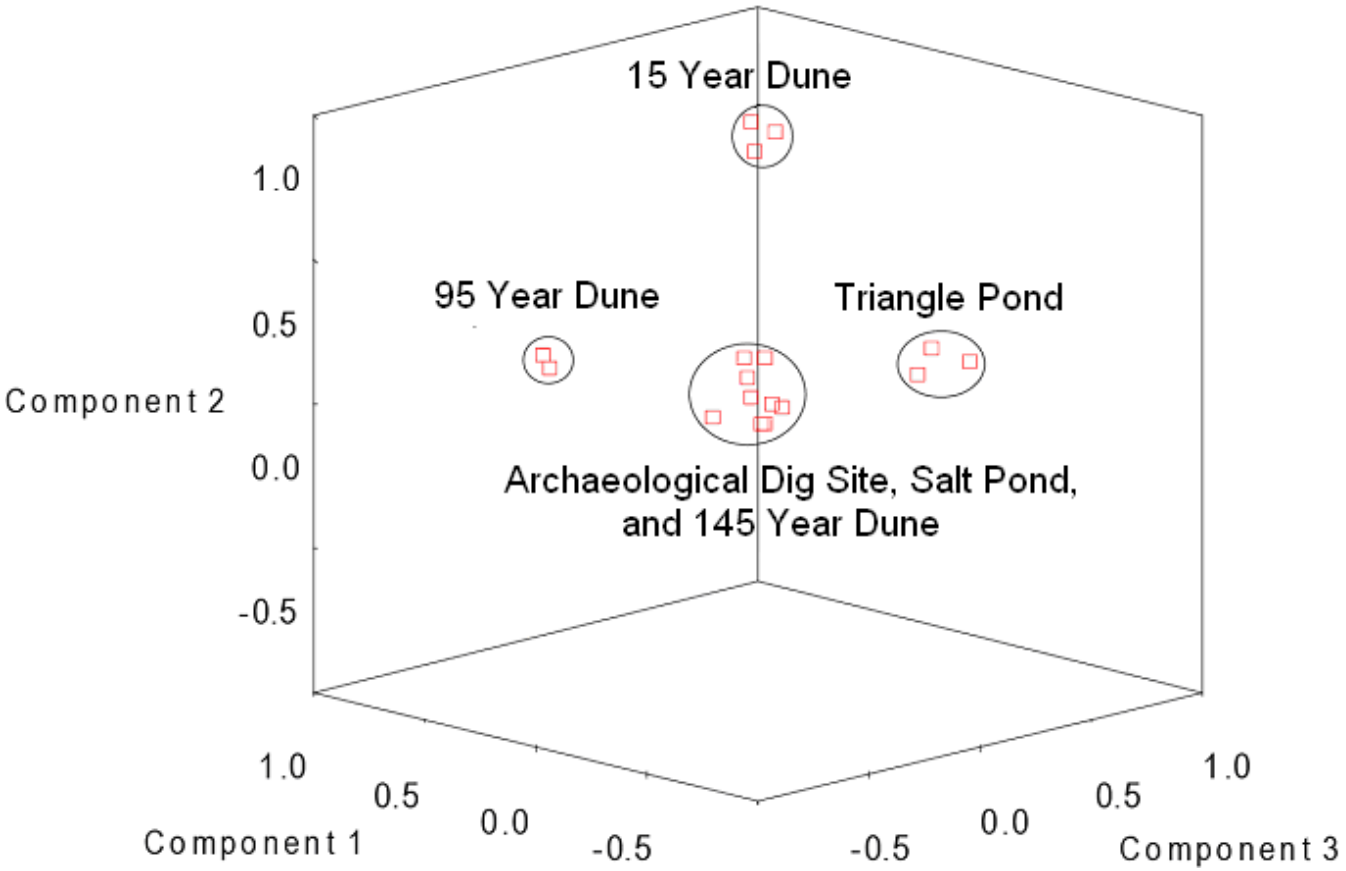


Figure 7 PCA showing resolution of the six sites using the SMVOC method

Discussion

Sites from San Salvador, Bahamas had higher carbon to nitrogen ratios, percent carbon, percent moisture, pH, Biolog™ absorbance scores, FAME peak areas, and SMVOC peak areas than sites from Mackinaw City, Michigan on average. Higher carbon to nitrogen ratio averages, percent carbon averages, Biolog™ absorbance scores, FAME peak areas, and SMVOC peak areas indicate that San Salvador soils have more extensive microbial community activity, a result which is consistent with reports that microorganisms flourish in extreme environments such as those found in the hyper-saline ponds of San Salvador, Bahamas (Chafetz and Buczynski, 1992; Pinckney, et al., 1995; Paerl et al. 2000).

According to one-way ANOVA and post-hoc test results, there is very weak agreement among the three methods as to which sites have the highest microbial activity (see Table 13), a finding which suggests that a single biological characterization method is not sufficient to understand the full complexity of soil microbiology; and since different soil biological characterization methods measure different levels of diversity, a multiple-method approach is needed (Rondon et al., 1999). The results obtained from the Biolog™ method indicate that the Archaeological Dig Site and the 145 Year Dune have the highest microbial activity; the results obtained from the FAME method indicate that the Archaeological Dig Site and the Triangle Pond have the highest microbial activity; and the results obtained from the SMVOC analysis indicate that the 15 Year Dune and the Archaeological Dig Site have the highest microbial activity (see Table 9).

Table 13 Comparison of how the three biological characterization methods rank the six study sites by microbial activity, from highest to lowest

Biolog™	**FAME**	**SMVOC**
Archaeological Dig Site	Archaeological Dig Site	15 Year Dune
145 Year Dune	Triangle Pond	Archaeological Dig Site
Salt Pond	15 Year Dune	Triangle Pond
95 Year Dune	95 Year Dune	Salt Pond
15 Year Dune	Salt Pond	145 Year Dune
Triangle Pond	145 Year Dune	95 Year Dune

According to one-way ANOVA and post-hoc results, the Biolog™ and FAME methods were only able to group the six study sites into two separate groups (see Tables 4 and 6), while the SMVOC Analysis was able to group the six study sites into three separate groups (see Table 8). Similarly, PCA analyses showed that the SMVOC Analysis is able to spatially resolve the six study sites better than the other two methods. However, PCA reveals very little agreement as to how the six sites are grouped by the three biological characterization methods, similar to the findings of Fang et al. (2001). Again, since the three biological characterization methods are complementary and not redundant, a multiple-method approach is recommended (Rondon et al., 1999).

Even though the results of this study display very little agreement in how the six study sites are grouped, it establishes that SMVOCs are useful for resolving between complex, natural soils non-destructively. This study does not, however, provide much insight into *how* the SMVOC Analysis is achieving the resolution, but it is strongly believed to be biologically related (Linton and Wright, 1993; Stahl and Parkin, 1994; Stahl and Parkin, 1996). Further work is needed to determine what exactly the SMVOCs are revealing. Future research questions might include the following: (1) Although previous work has shown that *some* SMVOCs are produced by soil microorganisms

(Stotzky and Schenck, 1973; Moore-Landecker, 1988; Stahl and Parkin,1996), do SMVOCs correlate *mostly* with microbial communities, or do they also correlate with chemical and/or physical parameters? (2) If SMVOCs correlate well with microbial communities, which specific SMVOCs correlate with which specific microorganisms? (3) How do SMVOCs change over time within one study site? (4) How vulnerable are SMVOCs to organic decay within a Perkin Elmer desorption tube? (5) Are there any problems associated with running messy environmental samples through a high-precision GC-MS? (6) How well does the SMVOC analysis compare to other biological characterization methods in repeatability? (7) Can SMVOCs provide insight into the metabolic pathways of microbial communities?

If specific correlations exist between SMVOCs and microbial communities, if SMVOCs show high quality spatial and temporal resolution repeatably, and if SMVOCs are not prone to rapid organic decay within the Perkin Elmer desorption tubes, it is this and other (McNeal and Herbert, 2009) authors' opinion that two lines of research ought to be pursued: (1) spatial and temporal field studies should be conducted across soils from many diverse ecosystems in order to build a comprehensive library of how SMVOCs relate to those diverse soils and the microbial communities they contain—this is a "top down" approach which would rely heavily on statistical analyses to tease out the complex relationships between specific SMVOCs and specific microbial communities; and (2) laboratory studies should be conducted, exposing Perkin Elmer desorption tubes to individual bacterial and fungal cultures, so that specific microbial species can be directly correlated to specific SMVOCs—this is the complementary "bottom up" approach, which, while limited by the small number of microorganisms researchers are able to culture in the laboratory, would provide species-specific correlations. Together,

these two lines of research would produce a comprehensive library by which researchers could rapidly and non-destructively characterize the microbial communities within soils. While this method would probably not replace other soil biological characterization methods, it would be a powerful complement to them.

Conclusion

This study was specifically designed to address the hypothesis that SMVOCs are useful indicators of soil microbial activity and community composition over a diverse range of ecosystems, and that the SMVOC Analysis provides better spatial resolution of those ecosystems compared to other biological characterization methods. We conclude here that the hypothesis is sound. Therefore, this work, in conjunction with previous work by McNeal and Herbert (2009), provides a novel SMVOC Analysis to the scientific community, currently useful for community-level SMVOC fingerprinting.

A comprehensive library cataloguing relationships between SMVOC fingerprints and microbial communities would be needed in order to fully establish a reliable, rapid, and non-destructive soil biological characterization method that is applicable to a variety of soil ecosystems and capable of capturing the complex spatial and temporal dynamics of these systems. If possible, such a method would gain wide acceptance amongst biogeochemical and environmental researchers, and complement other methods in multiple-method studies.

CHAPTER III

AUTHENTIC SCIENCE INQUIRY INTO GAS CHROMATOGRAPHY FOR A GENERAL CHEMISTRY LABORATORY CLASS

Introduction

Recipe-style Instruction

Chemistry courses offered in today's colleges and universities employ a traditional three hour lecture class with a supplemental laboratory class. Students are commonly asked to purchase laboratory manuals, which include instructions for laboratory exercises to be completed throughout the course. These "recipe-style" laboratory manuals are used to give valuable hands-on experiences to students, which should in theory relate directly to topics discussed in the lecture, allowing the student to see how empirical exploration leads to the creation of scientific knowledge. However, many students do not make the intuitive connections between the classroom lectures, the laboratory exercises, and the real world (Roth, 1994; McGarvey, 2004; Reid and Shah, 2006). The traditional recipe-style approach often does not give students the opportunity to grapple with real scientific problems, therefore, they frequently do not learn to think like scientists, and would be confused or overwhelmed if asked to conduct actual research.

A review conducted by Hofstein and Lunetta (2004) concluded that as of 2002, there are still several problems with the current recipe-style approach to science laboratory classes prevalent in colleges and universities today. They include the fact that

recipe-style instruction requires little or no thought on the part of the student as to why a specific method or concept is useful in the real world, or how they would go about investigating a research problem if no "recipe" were handed them. Another problem addressed is the lack of rich assessment, leaving students with the impression that laboratory work is non-essential. Finally, Holfstein and Lunetta lament educators' lack of resources and ignorance of current research, both of which are essential to successful authentic science inquiry based laboratory classes.

Authentic Science Inquiry

Educators must be prepared to teach environmental science concepts effectively to college level students, especially in laboratory classes, which focus on the application of content knowledge. Authentic science inquiry is a pedagogical approach that allows students to conduct actual science using modern laboratory instrumentation, current methodologies and protocols, and complex data analysis (Chinn and Malhotra, 2002). Many educators are adverse to employing authentic science inquiry, describing the pedagogy as problematic, time consuming, expensive, confusing to themselves and their students, and misaligned with their understanding of the purpose of science instruction (Welch et al., 1981). However, it has clearly been shown to be more effective in laboratory classes than the traditional recipe-style approach at transferring conceptual knowledge and experience to students (McNeal et al., 2008; Sell et al., 2006; Wallace et al., 2003). Authentic science inquiry allows the student to address a scientific question of his or her own choosing. By choosing a scientific question, the student invests himself or herself into the direction the investigation takes and into the scientific outcome. This investment acts as motivation for the student to pay close attention to the methods, to the

results, and to the rationale underpinning them. Simply, scientific inquiry can engage a student effectively in a scientific problem, and if educators wish to produce actual scientists, it would be to their benefit to use the pedagogy.

It would be desirable for all educators at every level to use engaging pedagogies such as authentic science inquiry wherever possible; however, this is not always an economically viable option. It would be impractical to require every high school, community college, and university to purchase and maintain expensive laboratory instrumentation and computational software in order to conduct highly specialized experiments for the sake of instruction; however, educators do a disservice to college students who are taught methods and concepts unrelated to or far removed from the modern, real-world science currently being conducted at the universities themselves. A balance between economic feasibility and high-quality instruction is ideal.

Specifically, in the case at Mississippi State University's Chemistry Department, the concepts of chromatography have traditionally been explored by laboratory students through a simple laboratory exercise involving adsorbent paper and dots of ink. This exercise is used to teach chemistry students that chromatography is about separating chemicals, and that chemical mixtures, such as dyes, can be separated and visually resolved on chromatography paper. It is hoped that students gain an understanding of elution, partitioning, and retention time.

However, chromatography may seem for many of these students nothing more than a novelty. The students may remain completely unaware of the real-world use of chromatography for the separation of various aqueous, organic, gaseous, and volatile compounds for preparatory or analytical purposes. Furthermore, students may remain

oblivious to the immense number of research and real-world situations in which this technique is employed and would therefore have no concept of when or why to use it.

This is not to suggest that the traditional laboratory exercise is useless, but is simply limited as it only models chromatography without requiring higher cognition to complete. Again, it would be impractical for every educational institution to fund, operate, and maintain its own gas or liquid chromatography laboratory for the sake of instruction; however, it would be desirable to employ authentic science inquiry for those universities with department laboratories that are already set up for modern chromatography. Generally, students would benefit from participation in authentic science inquiry whenever economically practical and educationally valuable for any STEM (science, technology, engineering, and mathematics) subject, at the discretion of the individual university, college department, or educator, so that the next generation of scientists is familiar with the best tools in use today.

Attitude and Self-efficacy

Students' performance in science classes is significantly affected by self-efficacy and attitude (Shibeci, 1983; Oliver and Simpson, 1988; Butler 1999; George, 2000). Therefore, it is essential for science education researchers to measure self-efficacy and attitude as it relates to performance outcomes. However, because no single accepted theory exists to explain the relationships between student attitudes, self-efficacy, and performance, more work is needed to identify the relationships amongst these factors, which will hopefully lead to reliable prediction and modeling of student performance as well as standard, effective attitude and self-efficacy measurement tools; a comprehensive theory is sought (Bandura, 1977; Butler, 1999).

Self-efficacy can be defined as an individual's belief in his or her own ability to do something well; this affects students' motivation, behavioral choices, productivity, and coping skills, and is itself affected by students' previous experiences, by watching others, by self-image, and by social expectations (Bandura, 1977; Bandura, 1991; Hampton & Mason 2003; Multon et al., 1991; Pajares & Miller 1994; Shell et al., 1995).

Attitude is "a bipolar evaluative judgment indicating the amount of affect for or against an object or behavior—that is, an attitude is a positive or negative feeling about a particular object or behavior" (Butler, 1999). Attitudes persist over time, are learned, and are related to behavior (Koballa, 1988) and should therefore be studied alongside student performance. It has been shown that attitudes are influenced by students' demographic factors (race, ethnicity, gender, income level, etc.), perceptions (social, environmental, ethnic, etc.), and previous experiences (Lawrenz, 1976; Schibeci and Riley, 1986; Greenfield, 1996). There remains some debate as to whether positive attitudes toward science cause higher academic achievement in science, or whether higher academic achievement causes positive attitudes. Regardless of the direction of causality, attitude and achievement are inextricably linked (Freedman, 1997). Research specifically shows that hands-on, activity-based laboratory instruction enhances students' attitude toward science (Johnson et al., 1974; Fraser, 1980), and therefore, should be measured in studies involving instructional or pedagogical efficacy.

Conceptual Understanding

The authors of *How People Learn: Brain, Mind, Experience and School* (Bransford et al., 2000) point out that experts of a given subject organize ideas into a complete conceptual model, indicative of meaningful relationships, which allows for

efficient recall and lateral application, whereas novices tend toward a linear and superficial understanding of concepts. Meaningful content understanding is based not on a person's ability to acquire knowledge, but on a person's ability to first recognize underlying concepts and mechanisms and then to organize those concepts and mechanism in a meaningful way. When a student creates an effective conceptual model of a given subject, new information on the subject can rapidly be integrated into proper place and context within the conceptual model while misconceptions are quickly identified by their inability to be integrated into the conceptual model; the relationships within the conceptual model can be applied laterally or creatively to new situations.

Research Goals

Because Mississippi State University has functioning chromatography laboratories at the disposal of educators, and in order to investigate the potential benefits of employing authentic science inquiry, a pilot study was developed to assess the chromatography module from the Mississippi State University introductory chemistry laboratory class (course number CH 1051). This module was designed to include the use of readily available, modern laboratory instrumentation and current methods and protocols, thus forming an authentic science inquiry pedagogical approach. The experimental module was compared with a control module that used the traditional recipe-style instruction of the laboratory exercise, which relied on bench-scale simulation of chromatographic concepts using ink and chromatography paper.

The goal of this work is to promote continued improvement in current laboratory pedagogies at Mississippi State University (MSU). This research represents direct added value for the MSU chemistry department and can easily be reproduced for any subject in

the STEM fields. *This study specifically addresses the hypothesis that authentic science inquiry-based activities are more effective than traditional laboratory activities for the development of student conceptual understanding, content retention, and positive attitudes.*

The study is a pilot study that is designed to explore whether exposure to authentic science inquiry is more effective in familiarizing students with chromatography and the concepts pertaining to chromatography than is a typical laboratory class which uses procedural instruction and non-authentic, bench-scale proxies. This study contributes to the broader question, "How can educators motivate students to learn?" An assessment was used to measure how students' attitudes toward and retention and conceptual understanding of chromatography changes when they are allowed to investigate a scientific scenario of their own choosing using real-world tools instead of bench-scale mimics (authentic science inquiry).

Methods

Participants and Context

Mississippi State University offers a once-weekly, three hour Overview of Chemistry (CH 1051) laboratory class to undergraduate non-science majors each semester. It is taught in conjunction with an Overview of Chemistry (1043) three hour lecture class. Together, the classes cover a broad variety of chemistry-related subjects and practices, including properties of matter, classifications of matter, the metric system, components of atoms, atomic models, electron configuration, ionic bonding, covalent bonding, Lewis-dot diagrams, intermolecular forces, the mole, molar mass, stoichiometry, gas laws, molarity, acids and bases, pH, red-ox reactions, and organic

compounds. Each week, the laboratory classes meet to conduct laboratory experiments related to topics covered in the lectures. The single laboratory class dedicated to chromatography was the class of interest for this study. Students from six different sections (N=81) were involved in the study during the spring 2009 semester.

Previous experience from the six sections was determined from responses collected in the educational survey (Figure 8).

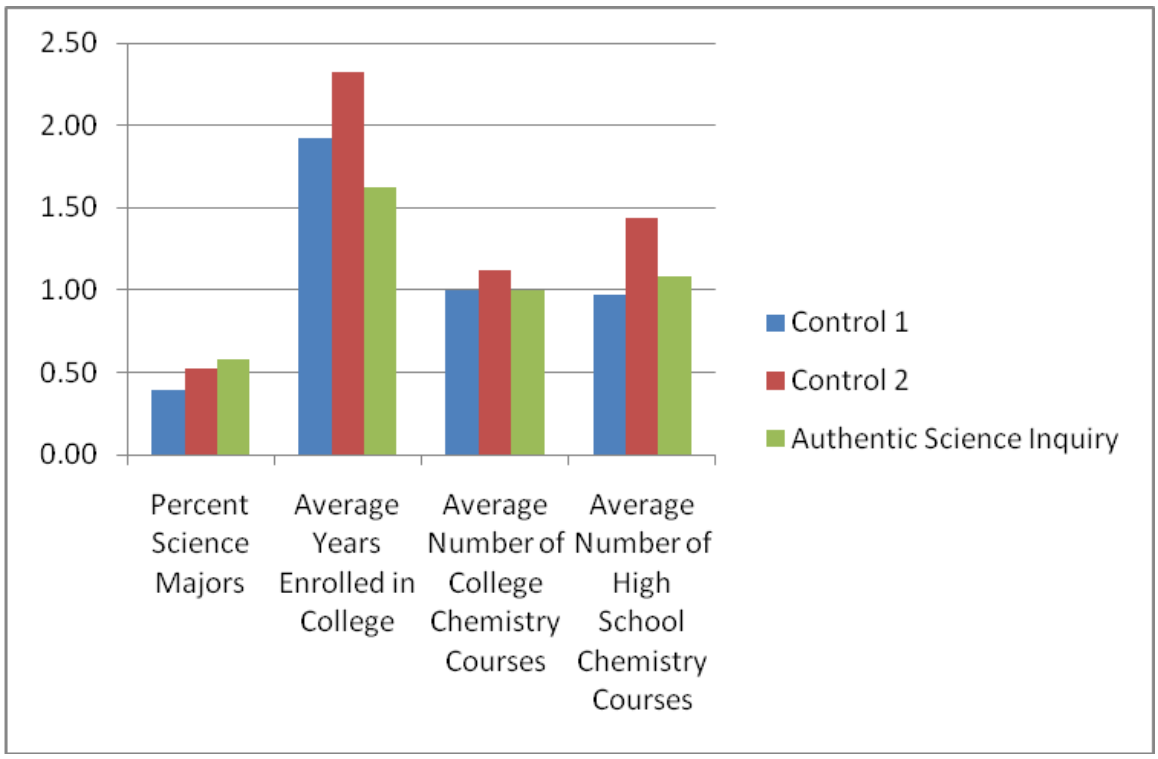


Figure 8 Previous experience of participants as measured by the percentage of science majors, number of years enrolled, number of college chemistry courses taken, and number of high school chemistry courses taken

Control Group 1

Control Group 1 had an average age of 20.24 years. Demographic information collected from the surveys is shown below (Figures 9 and 10).

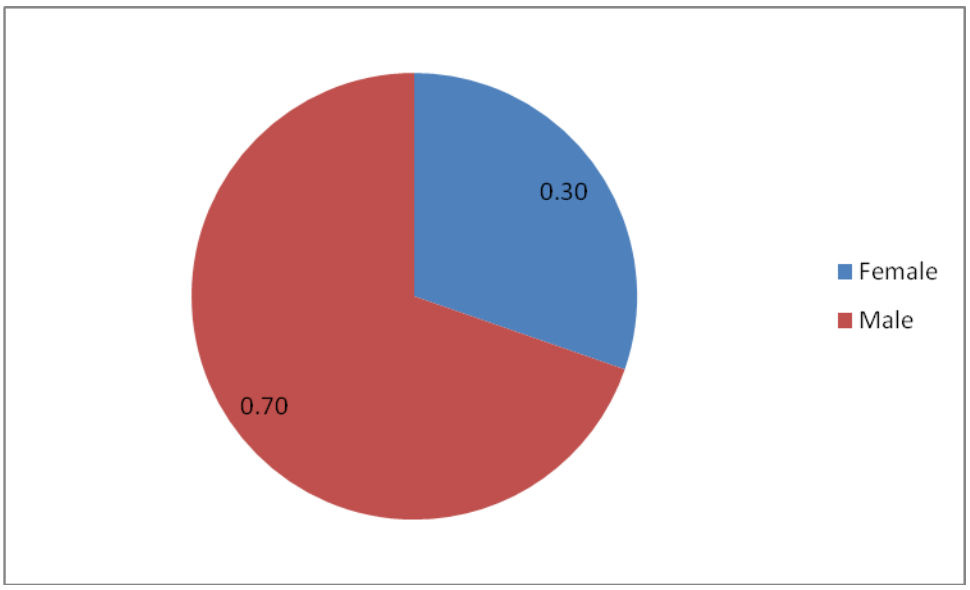


Figure 9 Gender distribution for Control Group 1

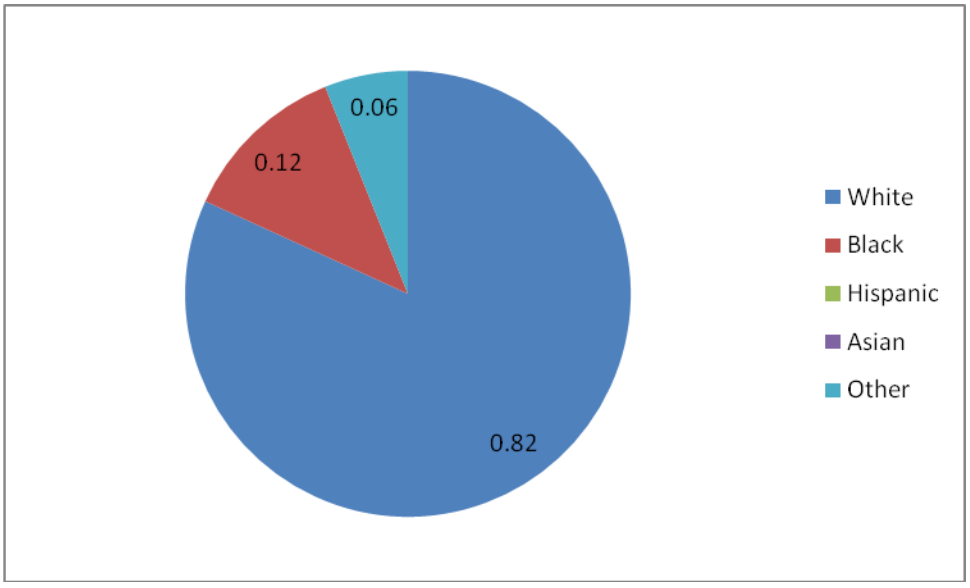


Figure 10 Ethnic distribution for Control Group 1

Control Group 2

Control Group 2 had an average age of 20.72 years. Demographic information collected from the surveys is shown below (Figures 11 and 12).

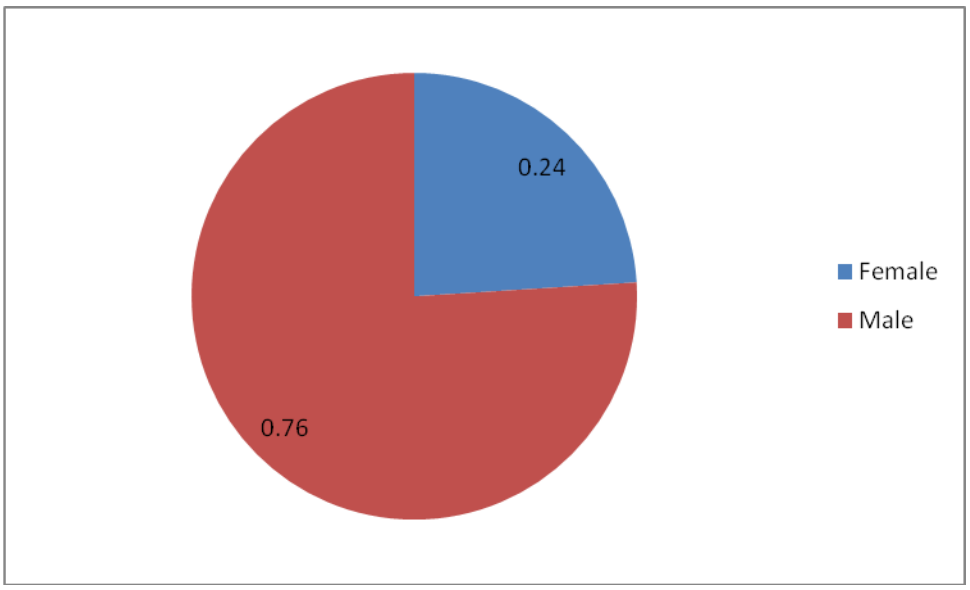


Figure 11 Gender distribution for Control Group 2

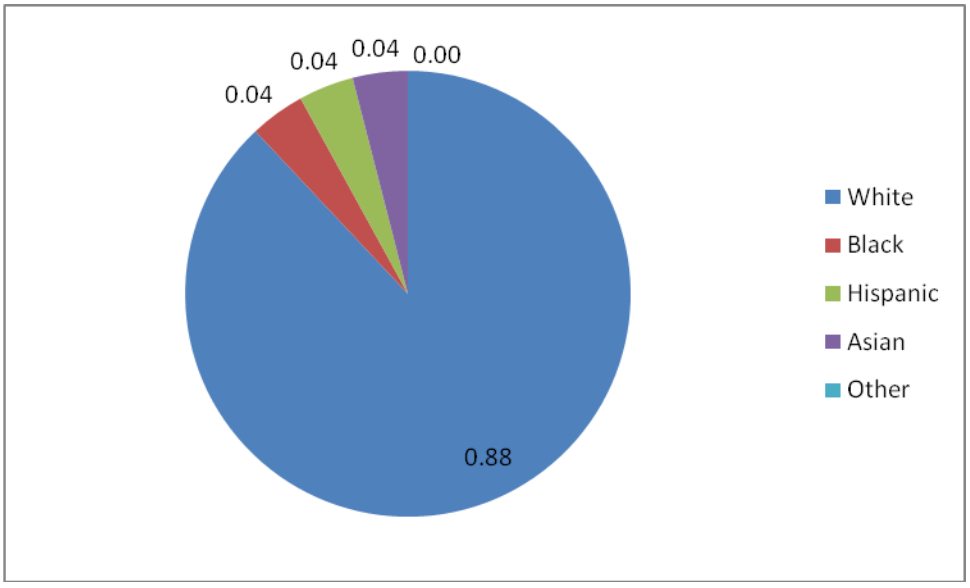


Figure 12 Ethnic distribution for Control Group 2

Authentic Science Inquiry

Control Group 3 had an average age of 19.63 years. Demographic information collected from the surveys is shown below (Figures 13 and 14).

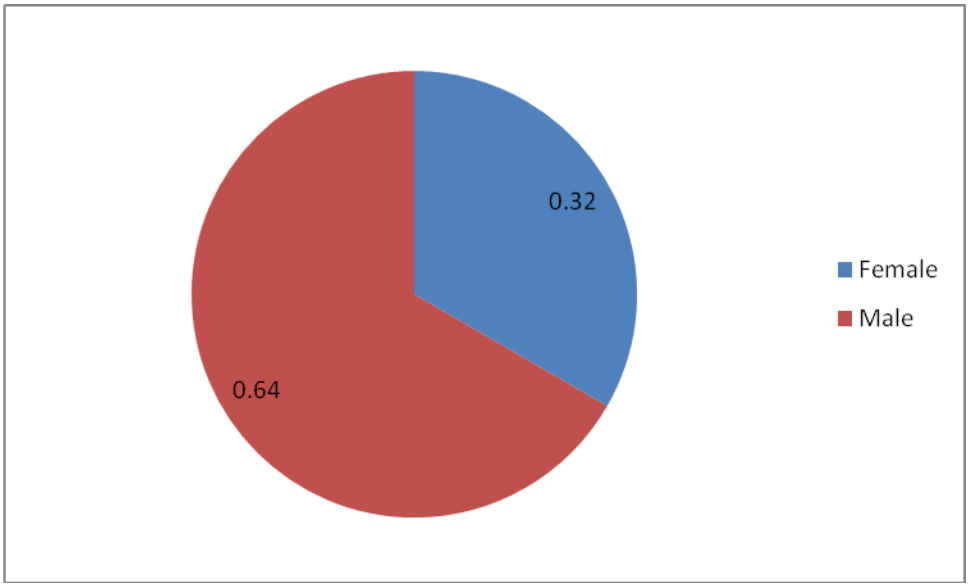


Figure 13 Gender distribution for authentic science inquiry

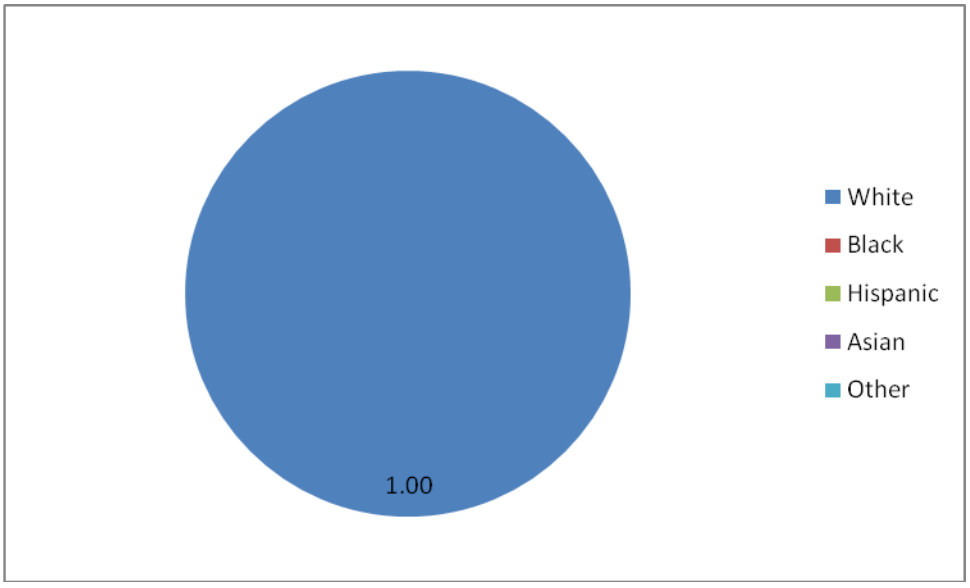


Figure 14 Ethnic distribution for authentic science inquiry

Instructional Sequence

Students were given a brief 15 minute introduction into chromatography, beginning with the invention and naming of chromatography by Mikhail Semyonovich

Tsvet. Solubility and adsorption (partitioning) was discussed as they relate to molecules moving through a chromatographic column. The terms “mobile phase”, “stationary phase”, “components to be separated”, and “elution” were defined and used. An analogy describing chromatography as a swarm of bees and wasps flying over a field of flowers was used in order to give students a meso-scale visualization to associate with the process of chromatography, since the process cannot be observed directly in a modern chromatographic column. In this analogy, the wind (mobile phase) blows the group of insects across a field of flowers (stationary phase). The bees (component 1) move across the field (elute) more slowly than the wasps do, since bees stop at each flower (partition into the stationary phase); the wasps (component 2) move across the field more quickly than the bees, because flowers do not attract wasps.

Following the 15 minute introduction, students were separated into two groups, each group was handed a Perkin Elmer desorption tube and asked to choose from three scenarios to address using chromatography. A small sample of volatile organic compounds (VOC’s) was associated with each one of the three scenario options. After each group chose their scenario to address, they extracted 5uL of the associated VOC sample and injected it into their PerkinElmer desorption tube. The scenarios offered were as follows:

Scenario 1

Unknown chemicals have been spilled at the Dow chemical plant in Midland, Michigan. No one seems to know how or when this spill occurred. A sample of the spill has been collected and may be hazardous. Should Dow employees treat the spill as an environmental hazard, or can the spill simply be mopped up and discarded?

Scenario 2

Jared Marley, 45, was found dead in his home last night. The autopsy report shows clear signs of nervous system depression and poisoning. A glass of clear fluid was found on the table next to his body and is suspected to be the cause of his death. A sample of the fluid was collected for you to decide whether it is likely to be the cause of poisoning or not.

Scenario 3

Recently, a graduate student at Mississippi State University synthesized a volatile organic compound. She would like to further analyze this compound, but needs to know if it is pure. A sample of her compound has been collected to determine its purity.

After the Perkin Elmer thermal desorption tubes were injected with the appropriate VOC samples, students were taken to the Mississippi State University Biogeochemistry and Geoscience Education Research Group's (BGERG) Laboratory to analyze each sample on a Perkin Elmer Atomatic Thermal Desorber tandem Gas Chromatography-Mass Spectrometer (ATD GC-MS) (Figure 16). Students recorded their observations with instructor guidance and discussion.

The instructional sequence for the control classes was the same as the instructional sequence described above, except that the normal chromatography laboratory exercise using absorbent paper, pen ink, and eluting solution (Figure 15) was followed in place of using three scenarios, desorption tubes, and GC-MS.

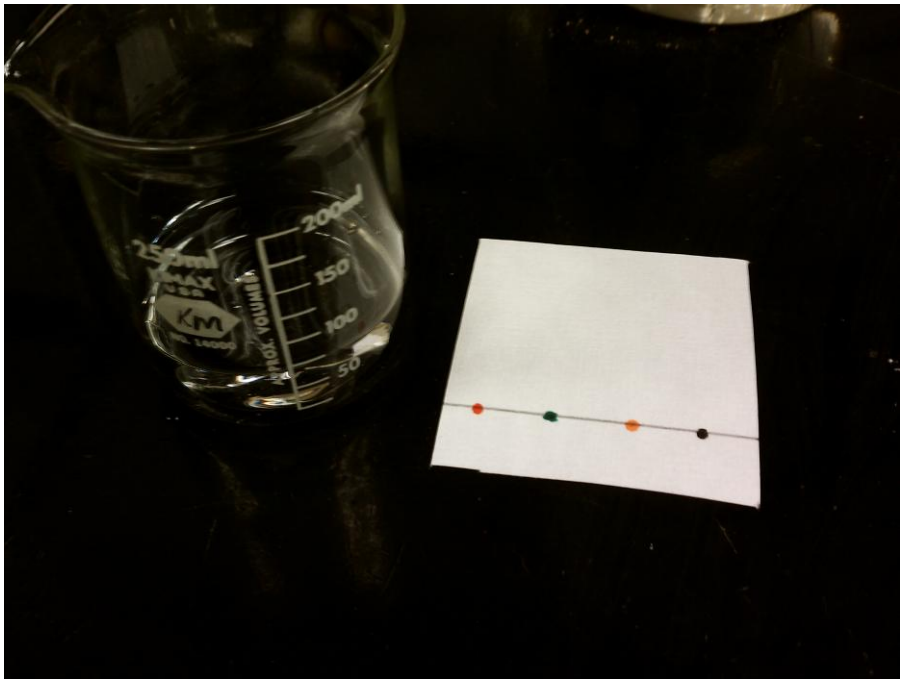


Figure 15 Setup for recipe-style laboratory exercise, using adsorbent paper, pen ink, and eluting solution

Figure 16 Setup for authentic science inquiry, using ATD GC-MS

Data Collection

IRB approval for existing data was sought and granted by IRB Compliance Administrator, Christine Williams on January 25, 2010: IRB #10-014 (see Appendix D).

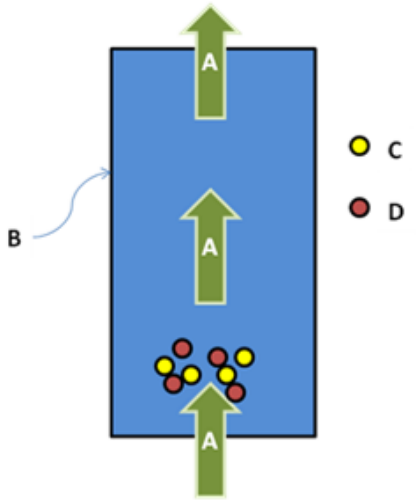
Pre-assessments were given to students one week before the chromatography lab class meets to determine demographics, prior knowledge, and attitude before exposure. For the chromatography laboratory class itself, two sections (N=22) were led through an authentic science inquiry-based class, while the other four sections (N=59) were led through the traditional recipe-style based class. Post-assessments were then given to students one week after the chromatography laboratory class met to determine the students' memory of the laboratory class, improvement in conceptual understanding of chromatography, and attitude toward the subject prior to exposure.

The demographic questions collected in the pre-assessment addressed age, gender, major, number of years enrolled in college or university, number of chemistry courses completed in college or university, number of high school chemistry courses, and ethnicity.

A single question regarding content retention was used in the post-assessment: "*List as many separate components of the chromatography lab as you can remember. Use an itemized list format.*"

Conceptual understanding of chromatography, used in both the pre- and the post-assessments, was determined using a diagram with related questions (see figure 2).

Diagram 1



Give labels for A, B, C, D in diagram 1.

Explain how chromatography works using complete sentences.

Refer to diagram 1. If "A" is non-polar, "B" is polar, "C" is polar, and "D" is non-polar, predict the outcome. Why? Use complete sentences.

Refer to Diagram 1. If "A" is polar, "B" is non-polar, "C" is polar, and "D" is non-polar, predict the outcome. Why? Use complete sentences.

Refer to Diagram 1. If "A" is neutral, "B" is covered in 20mm sized holes, "C" measures 10mm across, and "D" measures 30mm across, predict the outcome. Why? Use complete sentences.

Figure 17 Conceptual Understanding Questions for pre-post assessments

A rubric was used to assess the student responses of the above conceptual questions (see Table 10). Finally, a Likert-scale attitudinal questionnaire was given in the post-assessment. Students were asked to place an X after each statement, indicating their level of agreement with the statement (e.g., "strongly disagree", "disagree", "unsure", "agree", or "strongly agree"). The statements provided are shown in Table 2.

Table 14 Rubric used for conceptual questions and content retention

1. Concept Questions
    a. Label the parts of the diagram above.
        3 pts: Correctly identified "A", "B", "C and D" (together) using the terms "mobile phase", "stationary phase", "particles to be separated" or appropriate analogs to these terms.
        2 pts: Correctly identified two of the three.
        1 pt: Correctly identified one of the three.
        0 pts: Correctly identified none of the three.

    b. Explain how chromatography works.
        3 pts: (1) Correctly describes and uses the terms "mobile phase" and "stationary phase". (2) Correctly explains that some particles are more attracted to one phase over the other. (3) Correctly explains that all particles move forward, but particles that are more attracted to the stationary phase move slower than particles that are more attracted to the mobile phase, therefore causing a separation between the particles.
        2 pts: Gave any two of the above
        1 pt: Gave any one of the above
        0 pts: Gave none of the above

    c. If "A" is non-polar, "B" is polar, "C" is polar, and "D" is non-polar, predict the outcome. Why?
        3 pts: Correctly predicted the outcome of "D" particles eluting first and "C" particles eluting subsequently with no misconceptions in their explanation.
        2pts: Correctly predicted the outcome of "D" particles eluting first and "C" particles eluting subsequently with minor misconceptions or inaccuracies in their explanation.
        1pt: Correctly predicted the outcome of "D" particles eluting first and "C" particles eluting subsequently**,** but had major misconceptions or inaccuracies in their explanation.
        0 pts: Did not make a correct prediction and had misconceptions in their explanation, or did not give an explanation.

    d. If "A" is polar, "B" is non-polar, "C" is polar, and "D" is non-polar, predict the outcome. Why?
        3 pts: Correctly predicted the outcome of "C" particles eluting first and "D" particles eluting subsequently with no misconceptions in their explanation.
        2pts: Correctly predicted the outcome of "C" particles eluting first and "D" particles eluting subsequently, but had minor misconceptions or inaccuracies in their explanation.
        1pt: Correctly predicted the outcome of "C" particles eluting first and "D" particles eluting subsequently**,** but had major misconceptions or inaccuracies in their explanation.
        0 pts: Did not make a correct prediction and had misconceptions in their explanation, or did not give an explanation

Table 14 (continued)

e. If "A" is neutral, "B" is covered in 20nm sized holes, "C" measures 10nm across, and "D" measures 30nm across, predict the outcome. Why?
3 pts: Correctly predicted the outcome of "D" particles eluting first and "C" particles eluting subsequently with no misconceptions in their explanation.
2pts: Correctly predicted the outcome of "D" particles eluting first and "C" particles eluting subsequently, but had minor misconceptions or inaccuracies in their explanation.
1pt: Correctly predicted the outcome of "D" particles eluting first and "C" particles eluting subsequently**,** but had major misconceptions or inaccuracies in their explanation.
0 pts: Did not make a correct prediction and had misconceptions in their explanation, or did not give an explanation.

f. What is chromatography used for? Give two examples.
3 pts: Identified chromatography as useful for making separations between chemicals and gave two correct examples.
2pts: Identified chromatography as useful for making separations between chemicals, but only gave one correct example.
1pt: Identified chromatography as useful for making separations between chemicals, but gave no examples~OR~gave two correct examples, but did not identify the use for chromatography.
0 pts: Could not identify a use for chromatography or identified chromatography as useful for something it is not, and gave at least one incorrect example.

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2. Memory/Retention

a. A numerical value will be assigned equal to the number of items on their list after a thematic analysis of the responses is conducted.

Table 15 Likert scale questionnaire for post-assessment

Please indicate how strongly you agree or disagree with the following statements:
0 1 2 3 4 **Strongly Disagree-----Disagree-----Not Sure-----Agree-----Strongly Agree**
I enjoyed learning about chromatography.
I could explain the theory of chromatography to a friend or colleague.
Chromatography is useful in real life.
After lab class, I further investigated chromatography for my own curiosity.
I have a clear idea of how chromatography works.
There are many chemists who use chromatography in real life.
4 3 2 1 0 **Strongly Disagree-----Disagree-----Not Sure-----Agree-----Strongly Agree**
I am not interested in learning more about chromatography.
Chromatography is difficult to understand.
I cannot think of a scenario where chromatography would be needed.
Chromatography is a boring subject.
I would do poorly on an exam covering the theory of chromatography.
Chromatography is not a useful tool.

Qualitative and Quantitative Data Analysis

The validity and reliability of the rubric and the pre- post-assessments in this pilot study will be addressed in-depth before they are used in future work. However, for this initial exploratory study, only a 10% internal reliability assessment was performed for the rubric to justify its use. Questions used in the pre- post-assessments were derived from conceptual questions traditionally asked in previous years by graduate teaching assistants from the Mississippi State University Department of Chemistry. The Likert-scale questionnaire used in this study was developed to assess students' self-efficacy and

confidence in regards to chromatography, and includes both positive and negative statements.

Conceptual understanding scores from the control group and the test group were obtained using a rubric (see Table 10) and compared using a Mann-Whitney non-parametric test. *The null hypothesis is that the two pedagogies will produce no statistically significant difference among mean conceptual understanding scores from the six sections.* The null hypothesis will be rejected if statistically significant differences exist between the control and test groups.

Attitudinal responses from the control group and test group were obtained using a Likert-scale questionnaire (see Table 11) and compared using a one-way ANOVA. *The null hypothesis is that the two pedagogies will produce no statistically significant difference among mean attitude scores from the six sections.* The null hypothesis will be rejected if statistically significant differences exist between the control and test groups.

Retention scores from the control group and the test group were obtained using a rubric (see the bottom of Table 10) and compared using a one-way ANOVA. *The null hypothesis is that the two pedagogies will produce no statistically significant difference among mean retention scores from the six sections.* The null hypothesis will be rejected if statistically significant differences exist between the control and test groups.

Reliability and Validity

Table 16 Validity and reliability criteria important for qualitative survey and rubric design

Criteria	Description and Approaches	Educational Survey & Rubric
Construct Validity	A measure of whether or not strong support for the content of items exists. This can be estimated through both convergence and divergence of theory and reality. We expect concepts that should be related, such as expertise in chromatography and overall understanding of chromatography, to actually relate when measured by the instrument and scoring rubric. Similarly, concepts that need not be related, such as chromatography understanding and attitude towards lab work, should not show significant correlation.	Construct validity was not addressed. The survey and rubric used in this study are in the initial stages of development and will require further analysis to validate their use.
Content Validity	A measure of whether or not items actually measure the latent trait that they are intended to measure (also called "face" validity). This is often evaluated through expert review of items and revision in response to expert opinion.	Rubric and survey questions were developed in discussion with the laboratory coordinator, a faculty member who understands and uses GC-MS, and myself.
Criterion Validity	The degree to which a measure correlates with other measures of the same latent trait (also called "concurrent" validity). Generally, qualitative measures are used to establish criterion validity for quantitative instruments, although quantitative or alternative qualitative measures (i.e., interviews) can be used to validate survey instruments.	Criterion validity was not addressed. The survey and rubric used in this study are in the initial stages of development and will require further analysis to validate their use.
External Validity.	A measure of the extent to which results can be generalized to populations outside of the study sample. This is a difficult validation to achieve, although the power of survey research lies in its ability to sample many populations, and hence generate measures of external validity. For qualitative work, this might also be called transferability.	External validity was not addressed. The survey and rubric used in this study are in the initial stages of development and will require further analysis to validate their use.

Table 16 (continued)

Internal Validity	Internal validity is most commonly considered when an attempt is made to determine a causal relationship between variables. In general, a researcher needs to ensure that they are not biasing study findings through personal expectations, their own actions, or failure to consider study limitations. For qualitative work, this can be called credibility.	Since each of the three instructors were directed to followed the same basic outline in their instructional sequences by the laboratory coordinator, the laboratory classes should have provided consistency across the three groups.
Communication Validity	Researchers develop surveys in order to generate an understanding of a study population. While researchers often assume that participants will interpret questions as intended, explicitly considering this aspect of instrument validity can generate important insights.	Communication validity was not addressed directly; however, they type of responses given by students were consistent across the three groups in their relation to the question or statement at hand.
Cultural Validity	A measure of the extent to which culture impacts participant interpretation of survey questions.	Cultural validity was not addressed. The survey and rubric used in this study are in the initial stages of development and will require further analysis to validate their use.
Internal Consistency Reliability	Although most often considered for quantitative instruments, internal consistency can provide a sense of the reliability of a qualitative survey. The stability of test results across samples of similar populations, consistency in test results over time, and generation of similar results using slightly different forms all provide evidence that a survey is generating reproducible findings.	Internal Consistency Reliability was not addressed. The survey and rubric used in this study are in the initial stages of development and will require further analysis to validate their use.
Inter-rater Reliability	In qualitative design, inter-rater reliability can ensure that findings are reproducible. Often, this is established through an iterative process whereby multiple researchers code identical data and establish consistency in analytical results. For the Plate Tectonics Survey, we utilized the inter-rater technique multiple times.	A 10% internal reliability check was performed on the rubric used in this study. The rubric used in this study was explained to an external evaluator. Ten pre-tests and ten post-tests were randomly drawn from the pool of completed surveys and given to the external evaluator, who graded the surveys according to his understanding of the rubric. The results showed a 7.14% error.

Results

Conceptual Understanding

Conceptual understanding scores (Table 17) were obtained from the conceptual understanding portion of the surveys.

Table 17 Percentage changes from pre- to post-test scores, obtained from the conceptual understanding portion of the survey

Control 1	Control 2	Authentic Science Inquiry
13.33	13.33	40
26.67	13.33	20
20	13.33	80
26.67	13.33	60
20	13.33	6.67
26.67	20	13.33
26.67	20	66.67
0	6.67	-13.33
26.67	13.33	0
0	13.33	13.33
20	0	20
20	20	13.33
20	0	6.67
20	13.33	0
0	13.33	53.33
13.33	20	13.33
0	13.33	26.67
6.67	0	33.33
26.67	6.67	0
6.67	13.33	93.33
0	6.67	66.67
33.33	0	53.33
13.33	20	
0	13.33	
20	0	
13.33	13.33	
20		
0		
20		
0		
20		
-6.67		
13.33		

Mann-Whitney

Independent conceptual understanding observations (N=81) from each of the six sections were obtained and grouped by instructor (3 groups). Checks for normality via the Kolmogorov-Smirnov and for homogeneity of variances via Levene's test revealed irreconcilable violations of both assumptions. Therefore, a non-parametric Mann-Whitney test was used. The pair-wise Mann-Whitney tests revealed no statistically significant difference between Control Group 1(M = 14.141, SD = 10.899, n = 33) and Control Group 2 (M = 11.281, SD = 6.737, n = 26): U(58) = 335.500, Z = -1.470, p = 0.142, with a Cohen's d = 0.346 and a Pearson's correlation = 0.170, both of which are interpreted as a small effect size; no statistically significant difference between Control Group 1 and Authentic Science Inquiry (M = 30.303, SD = 29.509, n = 22): U(54) = 269.500, Z = -1.627, p = 0.104, with a Cohen's d = 0.714 and a Pearson's correlation = 0.336, both of which are interpreted as a medium effect size; and a statistically significant difference between Control Group 2 and Authentic Science Inquiry: U(47) = 186.500, Z = -2.116, p = 0.034, with a Cohen's d = 0.889 and a Pearson's correlation = 0.406, both of which are interpreted as a large effect size. The null hypothesis that "*the two pedagogies will produce no statistically significant difference among mean conceptual understanding scores from the six sections"* is rejected.

Table 18 Statistically significant groups as indicated by Mann-Whitney pair-wise tests

Group 1	**Group 2**
Control Group 1	Control Group 1
Control Group 2	
	Authentic Science Inquiry

Attitude Results

Conceptual understanding scores (Table 19) were obtained from the Likert-scale attitude portion of the surveys.

Table 19 Changes from pre- to post-test scores, obtained from the Likert-scale attitude portion of the survey

Control 1	Control 2	Authentic Science Inquiry
-0.2	0.05	0.48
0.77	1.15	0.28
-0.05	0.07	-0.18
0.82	-0.35	1.33
0.13	0.3	0.85
0.65	0.85	1.23
0.32	1	-0.6
-0.12	-0.37	1.5
-0.1	-0.15	1.02
0	0.12	1.93
0.33	0.97	0.95
1.23	0.18	2.5
0.43	-0.12	3.25
1.25	1.02	0.95
0.55	0.03	0.22
0.32	-0.22	1.32
0.2	0.4	0.05
0.23	0.17	0.73
-0.67	-0.28	1.05
0.63	0.05	0.3
0.2	-0.5	2.58
-0.22	-1.23	0.15
-0.13	0.63	
-0.4	-1.48	
0.6	0.2	
1	0.05	
-0.47		
0.03		
-0.45		
0.25		
-0.35		
0.08		
-1.03		

One-Way ANOVA

Independent attitude observations (N=81) from each of the six sections were obtained, grouped by instructor (3 groups), and analyzed using a one-way ANOVA.

Checks for normality via the Kolmogorov-Smirnov and for homogeneity of variances via Levene's test were performed on the data and yielded no evidence of problems with either assumption, $p > 0.05$.

The one-way ANOVA revealed a statistically significant difference amongst the three groups, $F(2, 78) = 12.210$, $MSE = 0.481$, $p < 0.001$. The null hypothesis is that *"the two pedagogies will produce no statistically significant difference among mean attitude scores from the six sections"* is rejected.

Table 20 One-way ANOVA results for the attitude data obtained from the six sections

	Sum of Squares	df	Mean Square	F	Significance
Between Groups	11.754	2	5.877	12.210	0.000
Within Groups	37.545	78	0.481		
Total	49.299	80			

A Tukey's HSD post-hoc test at alpha = 0.05 indicated that the Authentic Science Inquiry Treatment group ($M = 0.9950$, $SD = 0.94505$, $n = 22$) had significantly higher attitude scores than both Control Group 1 ($M = 0.1767$, $SD = 0.52615$, $n = 33$) with a Cohen's d = 1.070 and a Pearson's correlation = 0.526, both of which are interpreted as a large effect size, and Control Group 2 ($M = 0.0977$, $SD = 0.63025$, $n = 26$) with a Cohen's d = 1.117 and a Pearson's correlation = 0.630, both of which are interpreted as a large effect size. Control Group 1 was not statistically significantly different than Control Group 2, with a Cohen's d = 0.136 and a Pearson's correlation = 0.068, both of which are interpreted as a small effect size.

Table 21 Statistically significant groups as indicated by Tukey's HSD post-hoc test

Site	Group 1	Group 2
Authentic Science Inquiry	0.9950	
Control Group 1		0.1767
Control Group 2		0.0977

Retention Results

Retention scores (Table 22) were obtained from the retention portion of the surveys.

Table 22 Number of terms retained by students, obtained from the retention portion of the survey

Control 1	Control 2	Authentic Science Inquiry
5	0	3
3	5	6
10	3	4
5	3	4
7	7	3
5	6	0
0	2	5
3	9	5
0	5	3
2	9	4
7	4	2
4	5	2
6	2	6
0	8	1
7	4	5
2	3	2
0	0	0
2	7	3
5	3	3
4	4	4
4	3	2
8	3	4
5	7	
6	0	
6	0	
6	4	
5		
4		
5		
4		
6		
5		
6		

One-Way ANOVA

Independent retention observations (N = 81) from each of the six sections were obtained, grouped by instructor, and analyzed using a one-way ANOVA. Checks for normality on the retention observations via the Kolmogorov-Smirnov test revealed a

violation of the assumption of normality. Therefore, the data were transformed as follows:

$$\text{Transformed Retention Data} = (\text{Retention Data})^{1.2} \quad (4)$$

Tests for normality via the Kolmogorov-Smirnov and for homogeneity of variance via Levene's test were then performed on the transformed data and yielded no evidence of problems with either assumption, $p > 0.05$.

The one-way ANOVA revealed no statistically significant differences amongst the three groups, $F(2, 78) = 2.142$, $MSE = 12.697$, $p = 0.124$. The null hypothesis "*that the two pedagogies will produce no statistically significant difference among mean retention scores from the six sections"* cannot be rejected.

Table 23 One-way ANOVA results for the retention data obtained from the six sections

	Sum of Squares	df	Mean Square	F	Significance
Between Groups	54.401	2	27.201	2.142	0.124
Within Groups	990.330	78	12.697		
Total	1044.732	80			

Discussion

The results of this pilot study show that students who were given an authentic science inquiry approach to the topic of chromatography showed significantly better conceptual understanding of chromatography than Control Group 2, and showed significantly more positive attitudes toward chromatography than both Control Group 1 and Control Group 2, a result which is consistent with other literature pertaining to the use of authentic science inquiry (Roth, 1994; Chinn and Malhotra, 2002; Wallace et al., 2003;McGarvey, 2004; Hofstein and Lunetta, 2004; Reid and Shah, 2006; Sell et al.,

2006; McNeal et al., 2008). The retention scores for these students, however, were lower than the scores from the control group, though this difference was not statistically significant. It may be important to note that the retained terms were generally observed to be of higher quality in the authentic science inquiry group, even though quality of terms was not studied.

This pilot study shows clear preliminary evidence supporting the use of authentic science inquiry in the laboratory classroom with exposure to modern instrumentation as opposed to the traditional recipe-style approach, using bench-scale proxies. Even with such positive preliminary evidence, however, it is impossible to make any strong conclusions from this work, as further studies on a larger scale are needed to validate it. However, this study could provide a platform on which to build a larger efficacy assessment of all the modules taught in the Overview of Chemistry (CH 1051) laboratory class. It may prove worthwhile to re-write the introductory chemistry laboratory curriculum in order to address the inefficacy of traditional recipe-style laboratory classes and to emphasize authentic science inquiry.

Conclusion

This study reveals that there is room for improvement in the laboratory science curriculum in use at Mississippi State University's Department of Chemistry. If educators in this or any other science department wish to produce capable and productive scientists to meet the challenges of our modern world, then a high-quality instruction and researched-based best practices must constantly be pursued. A re-assessment of pedagogical approaches employed is needed in the very least, though a research-based overhaul of the curriculum is probably required.

CHAPTER IV

CONCLUSION

There is a need for scientists, researchers, and educators to improve upon previously established scientific methods in order to achieve higher efficacy and efficiency and to improve upon previously established pedagogies so that the next generation of scientists is able to continue achieving a high rate of scientific progress, especially in addressing the environmental concerns at the forefront of scientific research. The benefits for investing in these two areas are clear: better research leads to better scientific understanding of the world around us, and better science instruction leads to better scientists who are able to conduct high quality research. Both are needed, not only to sustain the progress of science, but also to help mitigate the many economical and environmental problems facing us today. One without the other is insufficient, a fact which should be taken seriously and acted upon by university faculty. The two studies presented in this thesis address the two needs.

The soil study provides a new tool for soil microbiologists, biogeochemists, and environmental scientists for characterizing soils rapidly and non-destructively via chemical fingerprinting. While this method is described here as useful for soil biological characterization, it can also be applied more generally to any situation which calls for the collection of air-born volatile organic compounds, such as measurement of pollution, chemical fate, and analysis of air quality. Since volatile organic compounds are produced by microorganism communities, organic decay, humic material, root systems, flora, and

fauna, the method could be thought of generally as an environmental fingerprinting method, rather than as solely a soil biological characterization method. Indeed, the method may prove quite useful in a variety of applications—a line of research which should be explored in future environmental studies.

Each successive generation since industrialization has needed to deal with rising global population, pollution, sea-level, energy consumption, and energy costs. If educators are not producing capable, productive, and informed citizens, especially scientists, on a national and global scale, then addressing these concerns will become exponentially more difficult for future generations. The pedagogical pilot study in this thesis is designed to assess the efficacy of current instructional approaches in comparison with authentic science inquiry at Mississippi State University. The study shows promising preliminary results supporting the use of authentic science inquiry, and further studies may support its necessity. Pedagogical practices should be assessed regularly across all scientific curricula at Mississippi State University and other universities in order to determine where improvements can be made. Educators can deepen their own understanding of best practices by habitually familiarizing themselves with current science and education literature.

Since our understanding of weather, climate, land utilization/restoration, eutrophication, hazardous waste disposal, oil spills, global warming, carbon sequestration, environmental and economic sustainability, protection of forests, wildlife, and fresh water resources, farming techniques, pollution, chemical fate, biogeochemistry, and many other areas of interest depend upon the effective communication of high quality research, both research methods and pedagogical approaches should be equally emphasized.

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APPENDIX A

TABLE OF BIOLOG™ NUTRIENTS

Table 24 Names of the nutrients used in Biolog^{TM}

Nutrient	Name of Nutrient
1	Water
2	β-Methyl-D-Glucose
3	D-Galactonic Acid γ-Lactone
4	L-Arginine
5	Pyruvic Acid Methyl Ester
6	D-Xylose
7	D-Galacturonic Acid
8	L-Asparagine
9	Tween 40
10	l-Erythritol
11	2-Hydroxy Benzoic Acid
12	L-Phenylalanine
13	Tween 80
14	D-Mannitol
15	4-Hydroxy Benzoic Acid
16	L-Serine
17	α-Cyclodextrin
18	N-Acetyl-D-Glucosamine
19	γ Hydroxybutyric Acid
20	L-Threonine
21	Glycogen
22	D-Glucosaminic Acid
23	Itaconic Acid
24	Glycyl-L-Glutamic Acid
25	D-Cellobiose
26	Glucose-1-Phosphate
27	α-Ketyobutyric Acid
28	Phenylethylamine
29	α-D-Lactose
30	D,L-α-Glycerol Phosphate
31	D-Malic Acid
32	Putrescine

APPENDIX B

TABLE OF FATTY ACID METHYL ESTER COMPOUNDS

Table 25 Names of the fatty acid methyl esters analyzed

FAME	Name of FAMEs
1	Methyl tetradecanoate
2	Pentadecanoic acid, methyl ester
3	Tetradecanoic acid, 12-methyl-, methyl ester
4	Octadecanoic acid, 8-methyl-, methyl ester
5	Pentadecanoic acid, methyl ester
6	Pentadecanoic acid, 14-methyl-, methyl ester
7	Hexadecanoic acid, methyl ester
8	9-Hexadecenoic acid, methyl ester, (Z)-
9	Hexadecanoic acid, 14-methyl-, methyl ester
10	Diethyl Phthalate
11	Heptadecanoic acid, methyl ester
12	Heptadecanoic acid, 10-methyl-, methyl ester
13	Hexadecanoic acid, 14-methyl-, methyl ester
14	Octadecanoic acid, methyl ester
15	8-Octadecenoic acid (Z)-, methyl ester
16	9-Octadecenoic acid (Z)-, methyl ester
17	10-Octadecenoic acid, methyl ester
18	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
19	6,9,12-Octadecatrienoic acid, methyl ester
20	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester
21	Eicosanoic acid, methyl ester
22	11-Eicosenoic acid, methyl ester
23	7,10,13-Eicosatrienoic acid, methyl ester
24	5,8,11,14-Eicosatetraenoic acid, ethyl ester, (all-Z)-
25	Oleic acid, 3-(octadecyloxy)propyl ester
26	Hexadecanedioic acid
27	Docosanoic acid, methyl ester
28	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester
29	9-Octadecenamide, (Z)-
30	Hexacosanoic acid, methyl ester
31	Methyl tetradecanoate

APPENDIX C

TABLE OF SOIL-MICROBE-VOLATILE ORGANIC COMPOUNDS

Table 26 Names of the soil-microbe-volatile organic compounds analyzed

SMVOC	Name of SMVOC
1	Hexanal
2	1-Methoxy-2-propyl acetate
3	Heptanal
4	5-Hepten-2-one, 6-methyl-
5	Octanal
6	1-Hexanol, 2-ethyl-
7	Nonanal
8	Hexane
9	Heptane, 3-methylene-
10	Cyclohexene, 3,3,5-trimethyl-
11	Cyclohexene, 3,5,5-trimethyl-
12	o-Xylene
13	Hexanal, 2-ethyl-
14	2-Hexenal, 2-ethyl-
15	p-Xylene
16	1,3-Octadiene
17	1,3-trans,5-cis-octatriene
18	1R-α-Pinene
19	Camphene
20	1-Decen-3-one
21	3-Octanone
22	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, acetate
23	Benzene, 1-methyl-3-(1-methylethyl)-
24	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1α,4α,7α)]-
25	Ethanol 2,2'-oxybis-
26	Limonene
27	1H-3a,7-Methanoazulene, octahydro-1,9,9-trimethyl-4-methylene-, (1α,3aα,7α,8aα)-
28	(+)-Cycloisosativene
29	β-Pinene
30	1-Octen-3-one
31	Benzene, 1-methyl-4-(1-methylethenyl)-
32	Naphthalene
33	Benzene, 2-methoxy-4-met yl-1-(1-methylethyl)-
34	2-Hexene, 3,5-dimethyl-
35	Benzene, chloro-
36	Benzaldehyde
37	1,3,8-p-Menthatriene
38	Ethylbenzene
39	Tricycloheptane, 1,7,7-trimethyl-
40	Benzene, 1-methyl-2-(1-methylethyl)-
41	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-

Table 26 (continued)

42	Cyclohexene, 1-methyl-4-(1-methylethylidene)-
43	2-Hexanone
44	2-Heptanone
45	2-Heptanone, 6-methyl-
46	Benzene, 1,3,5-trimethyl-
47	Benzaldehyde, 3-methyl-
48	Acetophenone
49	Bornyl chloride
50	δ-Selinene
51	2,6-Dimethyl-1,3,6-heptatriene
52	β-Myrcene
53	α-Phellandrene
54	Limonene
55	Tetracyclodecane, 4,4-dimethyl-
56	1-Hepten-3-one
57	1,2-Dimethyl-1,4-cyclohexadiene
58	Cyclohexanol
59	Cyclohexanone
60	Propane, 1,2,3-trichloro-
61	Dec ne
62	Undecane
63	Undecane, 5-methyl-
64	Undecane, 3-methyl-
65	Dodecane
66	4-Dodecene, (E)-
67	Hexadecane

APPENDIX D

INTERNATIONAL REVIEW BOARD LETTER OF APPROVAL

MISSISSIPPI STATE UNIVERSITY

Compliance Division
Administrative Offices
Animal Care and Use (IACUC)
Human Research Protection Program (IRB)
1207 Hwy 182 West
Starkville, MS 39759
(662) 325-3496 - fax

Safety Division
Biosafety (IBC)
Radiation Safety
Hazardous Waste
Chemical & Lab Safety
Fire & Life Safety
70 Morgan Avenue
Mississippi State, MS 39762
(662) 325-8776 - fax

http://www.orc.msstate.edu
compliance@research.msstate.edu
(662) 325-3294

January 25, 2010

Chris Ruhs
405 Carver Dr.
Apt C
Starkville, MS 39759

RE: IRB Study #10-014: Authentic Inquiry into Gas Chromatography for a General Chemistry Laboratory Class

Dear Mr. Ruhs:

The above referenced project was reviewed and approved via administrative review on 1/25/2010 in accordance with 45 CFR 46.101(b)(4). Continuing review is not necessary for this project. However, any modification to the project must be reviewed and approved by the IRB prior to implementation. Any failure to adhere to the approved protocol could result in suspension or termination of your project. The IRB reserves the right, at anytime during the project period, to observe you and the additional researchers on this project.

Please note that the MSU IRB is in the process of seeking accreditation for our human subjects protection program. As a result of these efforts, you will likely notice many changes in the IRB's policies and procedures in the coming months. These changes will be posted online at http://www.orc.msstate.edu/human/aahrpp.php.

Please refer to your IRB number (#10-014) when contacting our office regarding this application.

Thank you for your cooperation and good luck to you in conducting this research project. If you have questions or concerns, please contact me at cwilliams@research.msstate.edu or call 662-325-5220.

Sincerely,

[For use with electronic submissions]

Christine Williams
IRB Compliance Administrator

cc: Karen McNeal (Advisor)