

**MINERALIZATION OF PAHs AND OTHER CONTAMINANTS IN
FILTREXX SOXX**

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Table of Contents

Abstract	5
Background	7
- Filtrexx Filter Products	7
- PAHs and Herbicides in the Environment.....	8
- Bioremediation.....	13
- Project Goals.....	15
Materials and Methods	17
- Macrocosm Description for Uncontaminated System	17
- Microcosm Set-up for Uncontaminated System	18
- Macrocosm Description for Contaminated Systems.....	20
▪ Motor Oil	20
▪ Simazine.....	22
▪ Phenanthrene.....	26
- Microcosm Set-up for Contaminated Systems	29
▪ Glu. Min. with Oil, Sim, or PAH.....	29
▪ Simazine Mineralization	30
▪ Phenanthrene Mineralization	31
- Percent Moisture Determinations	31
- Ash Free Dry Weight.....	32
- Statistical Analysis of Data.....	32
Results	33
- Percent Moisture Graphs.....	37
- Glucose/Contaminate Mineralization Graphs.....	41
Discussion	47
Literature Review	53

List of Figures

Figure 1: Installation of Filtrexx SOXX at the Chattanooga Parking Facilities	16
Figure 2: Two weeks after installation	16
Figure 3: Diagram of Glucose Mineralization in Uncontaminated System.....	19
Figure 4: Diagram of Glucose Mineralization in Motor Oil Contaminated System.....	21
Figure 5: Simazine and Glucose Mineralization Diagram in Contaminated System.....	24
Figure 6: Diagram of Glucose Mineralization with Simazine Present and with Methanol Control.....	25
Figure 7: Phenanthrene and Glucose Mineralization Diagram in Contaminated System	28
Figure 8: Percent Moisture Change in Uncontaminated Systems	37
Figure 9: Average Percent Moisture in Simazine Contaminated Systems.....	38
Figure 10: Average Percent Moisture in Phenanthrene Contaminated Systems.....	39
Figure 11: Percent Moisture in All Contaminated Systems	40
Figure 12: Glucose Mineralization Rates in Uncontaminated Systems	41
Figure 13: Mineralization Rates for Contaminated Systems	42
Figure 14: Glucose Mineralization Rates in Simazine Contaminated Systems	43
Figure 15: Glucose v. Simazine Mineralization Rates for Simazine Contaminated Systems.....	44
Figure 16: Glucose v. Phenanthrene Mineralization Rates for PAH Contaminated Systems.....	45
Figure 17: Glucose Mineralization Rates for All Contaminated Systems	46
Appendix A: Excel Table Illustrating Percent Moisture Computations	56
Appendix B: T-Test Values	57

Abstract

Rates of mineralization of certain contaminants by microbes found in a compost/mulch mixture designed to mimic the material found in a commercial absorbent “SOXX” were studied in detail using lab based mesocosms. Subsets of the compost/mulch were individually exposed to used motor oil, Phenanthrene, and Simazine-the three contaminants that might be found at a site where the SOXX system would be installed. A second set of mesocosms remained unpolluted to measure the bacterial activity without contaminants. Soil/water slurries were then prepared for the mesocosms. For all of the mesocosms, the growth and activity of the soil bacteria could be measured by monitoring the amount of glucose added to the slurries compared to how much radioactive carbon dioxide was released. For the motor oil, ^{14}C glucose was added to the slurries, with $^{14}\text{CO}_2$ trapped in 0.1 NaOH in the microcosm. By measuring the amount of radioactivity in the glucose and the carbon dioxide in NaOH, the glucose mineralization rate with motor oil present was determined (0.1860 mg/g Wet/d). For Phenanthrene, two different experiments were conducted to evaluate the effects of the toxin in the compost. First, we added ^{14}C glucose to slurries, and trapped $^{14}\text{CO}_2$ in NaOH, to determine the heterotrophic activity of the compost. The second experiment used ^{14}C Phenanthrene to study a subset of bacteria capable of mineralizing Phenanthrene into CO_2 and H_2O . The same tools were used to study Simazine, with ^{14}C Simazine added to the system, instead of Phenanthrene. Lastly, soil/water slurries were set up monthly to measure the microbial activity in the uncontaminated microcosms, by adding ^{14}C glucose and

trapping the $^{14}\text{CO}_2$ in the microcosms. The Phenanthrene contaminated system showed an increased rate of phenanthrene mineralization (increased by 32.26%, 0.37 mg/g Wet/d, vs. 0.28 mg/g Wet/d for the control soils, T-test, $p=0.93$). The Simazine contaminated system showed a small increase with Simazine mineralization (increased by 1.5%, 0.26 mg/g Wet/d, vs. 0.25 g/g Wet/d, T-test, $p=0.03$). In the contaminate experiments, Simazine microcosms demonstrated the lowest rate of mineralization over the other contaminated systems. Furthermore, the uncontaminated systems showed a decline in the rate of ^{14}C glucose mineralization over time (decreased by 40%, July 0.34 mg/g Wet/d vs. November 0.14 mg/g Wet/d, T-test, $p=0.6$). This research shows the effects that three contaminants have on the microbes endogenously found in compost similar to mulch/compost mix in Filtrexx products, and the overall activity of the microbes over time with no contaminate. Further studies may determine if certain microbes should be added to Filtrexx products to help mineralize other contaminants that could potentially enter this system.

Background

Filtrexx Filter Products

Wastes and other non-degradable substances are often times the byproducts of human interaction within an environment. The construction of homes or businesses can create contaminants harmful to the water supply and can have adverse effects on human health (McFarland and Clark, 2004). Therefore, various techniques to help clean up these wastes have become the norm at many sites. One such is to try to remove potentially toxic organic compounds from water as it washes off impervious surfaces, such as parking lots, before it reaches local streams (Brady and Weil, 1996). Industrial companies, like Filtrexx, address this issue by developing products to filter water for contaminants and to help with erosion in certain geographical areas (Filtrexx). Ideally, this type of filtration product might also help mineralize or otherwise completely remove the contaminants both from the water and then later from the filter medium (Civilini, 1995 and Castaldi, 1996).

By analyzing the wastes that are present within an area, techniques to eliminate them can be developed and implemented to help protect the local environment. A current method utilized to help prevent contamination near construction sites is the use of the Filtrexx FilterMedia products. These are sediment-trapping devices that filter the water passing through the compost, allowing water to form a pond, and settling of particular filterable solids (Demars, et. al, 2000). This compost material can be analyzed to see what compounds are present in this

environment, and how active bacteria in the compost are mineralizing certain toxic compounds (US EPA, 1998).

The City of Chattanooga Parking Facilities has installed Filtrexx Water SOXX to help eliminate oils, tars, herbicides, road salts and other contaminants that are washed from trucks usually parked at that site (figures 1 and 2). By using the same compost/mulch mix used in the Filtrexx SOXX, a study can be conducted on how motor oil and other contaminants might affect the function and efficiency of this filtration technique.

PAHs and Herbicides in the Environment

The accumulation of contaminants in an environment is due to highly eroded soils, including soil loss from agricultural and industrial sites (Brady and Weil, 1996). Soil erosion occurs often in nature, but human disturbance can significantly increase the amount of erosion or sedimentation that would normally happen. According to the Georgia Soil and Water Conservation Committee, forests lose approximately 0.36 metric tons/ha (1 ton/acre) per year, compared to agriculture sites, which lose an average of 5.5 metric tons/ha (15 tons/acre) per year. The most noticeable effect of human interference is at construction sites, where almost 73.3 metric tons/ha (200 tons/acre) per year are lost to erosion (GA SWCC, 2002). The main issue with soil erosion is that contaminants used at the agricultural or industrial sites, leave with the soil and end up in ground or river water (Risse & Faucette, 2001). Contaminants also bind to the soil and can remove essential elements and organic matter from the soil,

which is needed for maintaining plant life. As a result, organic matter and other chemical elements can be over five times the normal values in the eroded topsoil (Brady and Weil, 1996).

One class of the destructive wastes that accumulate in the environment due to construction are polycyclic aromatic hydrocarbons, or PAHs, which have been shown to cause harmful effects on marine life and are carcinogenic to mammals (Nielsen, et al, 1999). PAHs are part of a chemical compound that is often found in petroleum, coal, and oil. Polycyclic hydrocarbons (PAHs) and herbicides have entered the environment from a variety of industrial and agricultural sources. PAHs usually enter the marine environment through oil spills, but can also be released into the environment from incineration of coal through factories or vehicle exhaust (Neff, 1985). In water, some PAHs can dissolve, but a majority of them bind to organic matter, such as soil, small organisms, and aquatic animals. PAHs are not very soluble in water, and are aromatic and lipophilic, which leads to their ability to be readily absorbed into the fat and tissues of many marine animals. Because PAHs are relatively resistant to degradation by bacteria, they have an accumulation effect in the environment, leading to the persistence and longevity of these contaminants in an environment (McElroy, et al, 1989).

PAHs are not typically found free floating in water, so to find the contaminate water sediment studies and studies on marine life must be conducted; however, quantifying the amount of PAHs in an environment proves to be very difficult. Several organisms can survive and exist in an environment exposed to PAHs, but the

predator that feeds on the organism will face adverse consequences as a result of the exposure. Again, this leads to an accumulation effect (known as biomagnification) in larger mammals or marine organisms, where markable physiological changes or death can result from this exposure (Nielsen, et al, 1999). Therefore, quantifying PAH residues and taking a defensive action against the contamination proves to be very difficult, since a majority of the PAHs are found within aquatic living organisms.

Phenanthrene (PHE), a type of PAH, has an adverse effect on the photosynthetic capabilities of *Photobacterium phosphoreum* and the aquatic plant *Lemna gibba* (duckweed) in contaminated sites. The organisms cannot survive because the toxicity of PHE increases significantly with exposure to sunlight, and these organisms produce photosynthetic products with PHE in the chemical make-up. A study by McConkey in 1996 and associates showed PHE itself did not have a harmful effect on the organisms, but the photoproduct was subsequently created was extremely toxic. Their study showed that exposure to PHE did have an effect on the photosynthesizing abilities of the organisms; however, when the photoproduct of PHE was exposed to the plant or bacteria, the organisms would immediately die. In both scenarios, solar radiation significantly decreased the fitness of the organisms (McConkey, 1996).

PHE contamination can lead not only to shorter life spans for the aquatic life, but also change to the physiological structures of the organisms. Studies have shown PAHs and herbicides found in aquatic environments, in active or in a degraded form, cause an estrogenic effect in fish and other amphibians (Gagne, 1999, and Filipov,

2005). Many of these chemicals are released from factories and some sewage treatment plants, and the combination of these chemicals leads to a “feminization” of the fish (Cooper, et al, 2000). As a result, the sexual reproduction and dynamics of the aquatic life reduce the number of male partners for the fish to reproduce a sufficient amount of offspring for future generations. While the organisms can live in the environment, their chances of future generations surviving decrease with an abnormal ratio of females to males in the environment.

With Simazine and other herbicides, the effects can vary based on the chemical structure of the compound. Studies have shown a difference in the effect of Simazine, depending on whether the compound is in a chain or ring structure. The chain Simazine can be incorporated into lipids, proteins, and glycogen; however, the ring Simazine is less likely to incorporate into the cell tissues of fish or other marine animals (Kaufman, et al, 1965). Simazine, in the chain form, has been shown to be a growth stimulant in plants, by enhancing protein synthesis in the vascular tissues (Francois and Robinson, 1990).

Another problem with Simazine or other pesticides is the effect that they can have on immune systems of mammals. A study conducted on mice and atrazine, an herbicide similar to Simazine, showed that a change in T-cell and B-cell production occurred after repeated exposure to atrazine (Filipov, et al, 2005). Atrazine exposed mice showed substantial decrease in the amount of T-cells produced by the thymus, and cytotoxic T-cells and memory T-cells failed to respond to antigenic stimulus. Atrazine also affected the number of dendritic cells in the mice’s bodies. The effect

of atrazine remains long after the primary exposure, and the interference with T-cell distribution in the body could have a major impact on this mammal's immune system.

Polycyclic aromatic hydrocarbons and pesticides have adverse effects on many taxonomic levels of organisms. While the usual exposure of pesticides is through water into aquatic environments and PAHs through human interference, the effect of both toxins is widespread in organisms, affecting physiology, reproductive success, and sexual determination, depending on which species are contaminated. Therefore, since PAHs and pesticides have such a consequence on the environment, it is of utmost importance to expand research and development into methods of toxin removal before they can be passed up trophic levels in ecosystems.

Bioremediation

Bioremediation is nature's way of recycling wastes into a form that can easily be used by the environment. It refers in part to microbial communities using metabolic pathways to effectively remove pollutants that have entered into the environment, and are potentially threatening to other living organisms.

Microorganisms have been assimilated to remove organic material and contaminants from domestic and industrial settings for years. Complications arise because most bacteria are able to degrade organic compounds are heterotrophic, and require an organic compound as an energy or source. To keep the degrading bacteria in the system, it is often necessary to add glucose or another easily metabolized carbon source to assist in cell growth and maintenance (Crawford, 1996).

Some bacteria can metabolize or breakdown contaminants in an environment through specific microbial processes. Anaerobic processes are now being studied due to their ability to break down some of the more complex halogenated hydrocarbons. Research on anaerobic bioremediation shows that some bacteria can treat and breakdown pollutants either aerobically or anaerobically, and can be applied to nitro-substituted compounds, halogenated molecules, and even hydrocarbons (Crawford, 1996). By using existing methods implemented by nature, better techniques to help minimize the release of harmful chemicals to clean up a toxic spill can be utilized (Wroblewski, 2004). The problem with studying indigenous microorganisms is the characterization of what type of organisms are in the system, whether fungal, bacterial, or protozoan. This is crucial in determining what type of requirements the

organisms need to survive, such as temperature, moisture, or oxygen supply.

Procedures for identifying the bacterial response to contaminants include:

introducing nutrients and waste products to an environment, measuring CO₂ production, and observing the disappearance of the contaminant (Crawford, 1996).

Bioremediation is also becoming the technology of choice for remediation of some contaminated sites, particularly sites with petroleum hydrocarbons. According to the National Pollution Discharge Elimination system, contaminated soils that erode into water are one of the largest suppliers of chemicals and contaminants into the environment (US EPA, 1997). Therefore, efforts to remove contaminants from the water must be implemented, like Filtrex products. One potential way to improve the Filtrex FilterMedia filtration system is by adding bacteria found in soil-based environments which naturally degrade oil, biphenyls, and other complex chemicals (Soulas and Martin-Laurent, 2006). Since Filtrex designs water filtration products to eliminate contaminants and wastes that are found draining off of parking lots, the addition of biodegrading bacteria to the products would greatly reduce the contaminants that would eventually accumulate in the SOXX. Contaminants would be broken down into environmentally benign products, such as carbon dioxide and water.

Project Goals

The main purpose of this research was to use the compost from Filtrex FilterMedia to study the effect on bacterial metabolism of several types of contaminants often associated with highly contaminated sites. For this experiment, the contaminants used were motor oil, Phenanthrene, and the herbicide Simazine. The effect on microbial activity within the compost in the system could be measured by setting up miniature macrocosms with a specific contaminate, and then performing studies to determine the effect they have on natural bacterial processes present in the compost/mulch. Some bacteria are capable of mineralizing these types of compounds and are already naturally found in the compost (Civilini, 1996); therefore, by monitoring the breakdown of ^{14}C labeled glucose into ^{14}C labeled carbon dioxide, the activity of these bacteria can be monitored over the course of several days. Also, the rate of glucose mineralization without contaminants could show the naturally occurring changes in the heterotrophic bacteria over a prolonged time.

By using this approach, it could be determined if the Filtrex FilterMedia products has a role in the treatment of certain types of contaminants, and perhaps not as adapted for other contaminants. Also, since the SOXX are placed outside and are composed of leaf litter and tree bark, natural biodegradation of the compost will occur which could ultimately have an effect on the ability for the naturally occurring bacteria to break down the contaminants.



Figure 1: Installation of Filtrexx SOXX at the Chattanooga Parking Facilities



Figure 2: Two weeks after installation

Materials and Methods

Macrocosm Description for Uncontaminated System

Three ten-gallon aquaria were set up containing a 50:50 mix of leaf litter and tree bark compost at twenty centimeters deep to mimic conditions of the SOXX. An additional aquarium was set up as a back-up macrocosm if needed. The aquaria were placed beside the windows in the Microbiology lab, so sunlight could reach them to also mimic environmental conditions where the SOXX would be found. Next, the equivalent of 1 inch of rainfall per week was computed and added to the aquariums as distilled H₂O. For this research, macrocosms are defined as the uncontaminated aquariums set up in the laboratory. The macrocosms were divided into 18 sections, so specific regions of the compost could be sampled.

The macrocosms were sampled randomly over a six-month period. By using a rigid, fiberglass pipe as a coring device, a column of compost could be removed from specific sections of the aquaria, with minimal damage to the remaining compost in the aquarium. The samples were then separated by depth at 0-2 centimeters and 10-12 centimeters to study the bacterial activity at the two depths in the macrocosm. The compost was then taken to the research lab to prepare for glucose mineralization in microcosms.

Microcosm Set-up and Sampling for Uncontaminated System

Once compost samples were removed from the mesocosms, the compost was divided into two samples sizes by depth. Microcosms were then created by adding approximately 3 cubic centimeters of this compost and 20 ml of water into 200 mL beakers. Next, 0.1 ml of ^{12}C glucose, then 0.1ml ^{14}C glucose was added to the compost-water slurry mix. For each sampling from the three aquariums at 0-2 cm and 10-12 cm, aerobic and anaerobic conditions were created in the microcosms (figure 3). To create anaerobic conditions, 3 ml sterile mineral oil was added immediately after the ^{14}C glucose to prevent oxygen from diffusing into the slurry mix. Stoppers were placed on the beakers to prevent any loss of carbon dioxide to the atmosphere. These stoppers have injection sites designed to allow the addition of 2ml of 0.1N sodium hydroxide through sterile syringes. NaOH was used to trap the ^{14}C carbon dioxide that is released from the bacterial breakdown of ^{14}C glucose. The microcosms were placed on shaker set at 70 rpm, to ensure even mixing. The microcosms were sampled approximately every 24 hours by removing the NaOH in the syringes, and replacing it with new NaOH for a seven day period. Next, 1 ml of the removed NaOH was added to vials with 4 ml of scintillation cocktail, and the remainder NaOH was discarded. The vials were placed in the scintillation counter to measure the radioactivity emitted from the ^{14}C -labeled CO_2 in the NaOH.

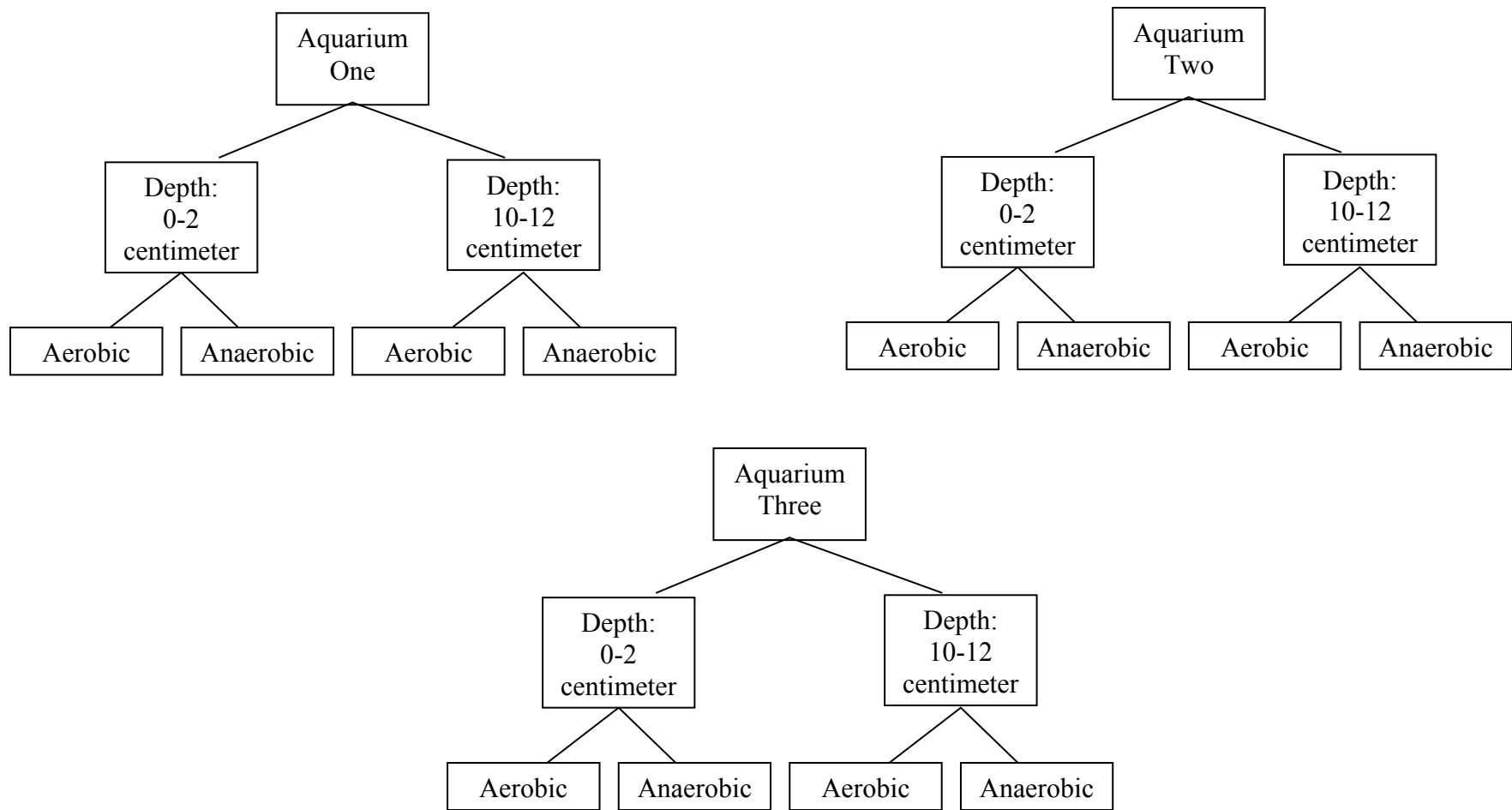


Figure 3: Diagram of Glucose Mineralization in Uncontaminated System (Time Study)

Macrocosm Set-up for Contaminated Systems

Motor Oil

The effect of motor oil or any other contaminate on the compost was measured by creating a smaller macrocosm than using a 10-gallon aquarium. For the used motor oil, three 400 ml beakers were filled to approximately the 200 ml mark, roughly 4-5 centimeters deep, of 50:50 leaf litter: tree bark compost, which was the same compost used in the aquarium set-up. Next, the equivalent of 1 inch of rainfall was computed for the compost in the beaker and added to the compost. The beakers sat for three days to allow the moisture to absorb into the compost. After three days, 5 ml of motor oil was pipetted over the top surface of the compost. The compost with motor oil remained undisturbed for three days to allow the motor oil to flow through the entire compost before glucose mineralization experiments could be performed on the macrocosm. The compost was then removed from each beaker and separated into two portions, top and bottom. For the beakers, since the compost was only 4-5 centimeters deep, the top was labeled α and the bottom β (figure 4). The six compost samples were then taken to the research lab to prepare for glucose mineralization experiments with microcosms.

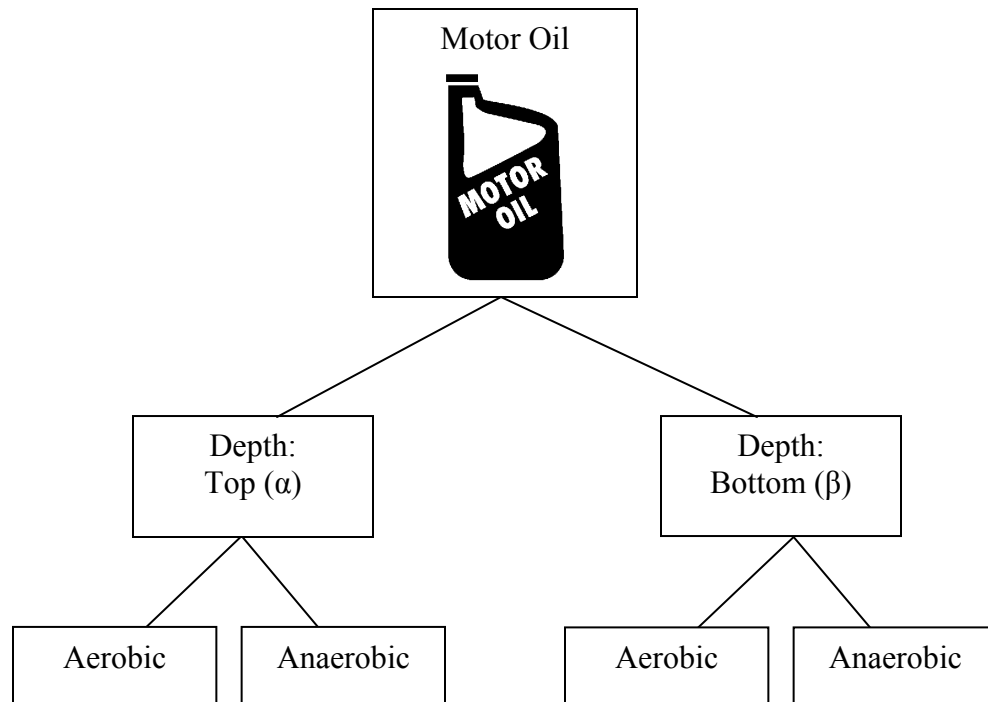


Figure 4: Diagram of Glucose Mineralization in Motor Oil Contaminated System

Macrocosm Set-up for Contaminated Systems (cont)

Simazine

For the Simazine experiments, two types of trials were run to see the effect of the contaminate (figure 7). The first experiment measured bacterial activity via glucose mineralization with Simazine contaminating the compost in the beakers. The second experiment measured the actual amount of Simazine mineralization that occurred in the compost. The methodology is described separately for clarity. For each compost, uniformly labeled Simazine 97.0% from the Novartis Corporation was used. The amount added was based on the application rate found in contaminated agriculture sites from other studies is 50 ppm (Turner, 2003). We calculated the same rate of application and added this amount of Simazine to each beaker (0.017 g Sim/5mL methanol).

Glucose Mineralization set-up (for Simazine Contaminate)

Only a small amount of Simazine is necessary to contaminate the compost, and show an effect on the bacterial activity; therefore, 400 ml beakers were used to set up the macrocosms for the Simazine contaminated system, instead of aquariums. Three beakers were filled with 50:50 compost, and filled to approximately the 200 ml mark. Because Simazine must be dissolved in methanol, three additional control beakers were set up with 50:50 compost at the 200 ml mark (figures 5 and 6). An equivalent of 1 inch of rainfall was added to all the beakers and allowed to rest for three days. For the first three beakers, 5 ml of Simazine was added to the top of the

compost, followed by 2 ml of water to ensure diffusion through the compost. The last three beakers had only 5 ml of 50:50 methanol: water mix added to the top of the compost, followed by 2 ml of water. The compost then sat for three days to allow the Simazine or methanol to diffuse through all of the compost before further tests were performed. The compost for each beaker was then removed and separated into two portions, top α and bottom β . The twelve compost samples were then taken to the research lab to prepare for glucose mineralization experiments in microcosms.

Simazine Mineralization set-up

Three 400 ml beakers were set up with 50:50 compost filled to approximately the 200 ml mark on the beakers. The compost had an equivalent of 1 inch of distilled water added and allowed to sit for three days. Then, 5 ml of Simazine was added to the compost, followed by 2 ml of water to ensure proper absorption of Simazine throughout the compost. The compost contaminated with Simazine sat for three additional days. The compost for each beaker was separated into two piles, top and bottom, and prepared for Simazine mineralization experiments within the microcosms.

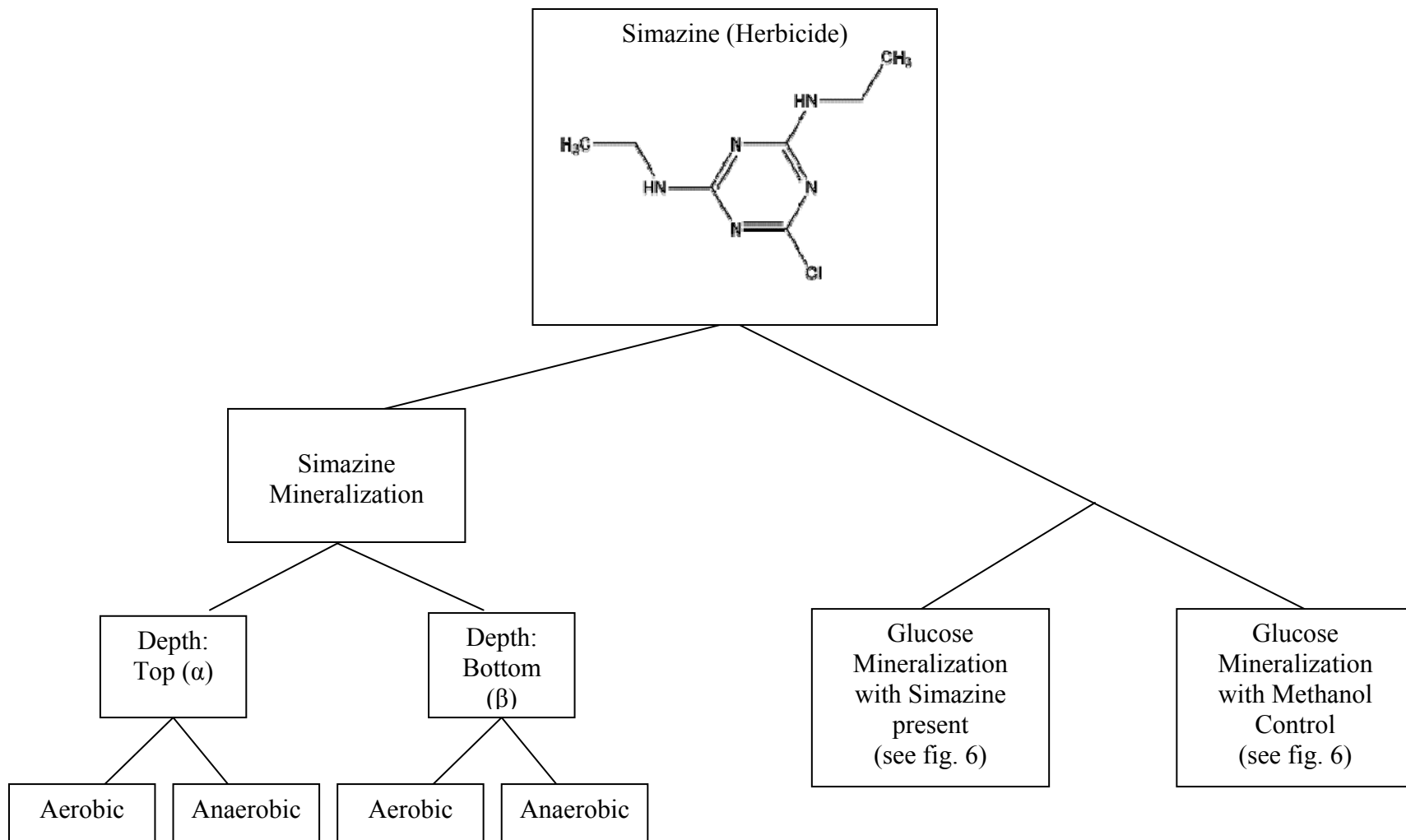


Figure 5: Simazine and Glucose Mineralization Diagram in Contaminated System

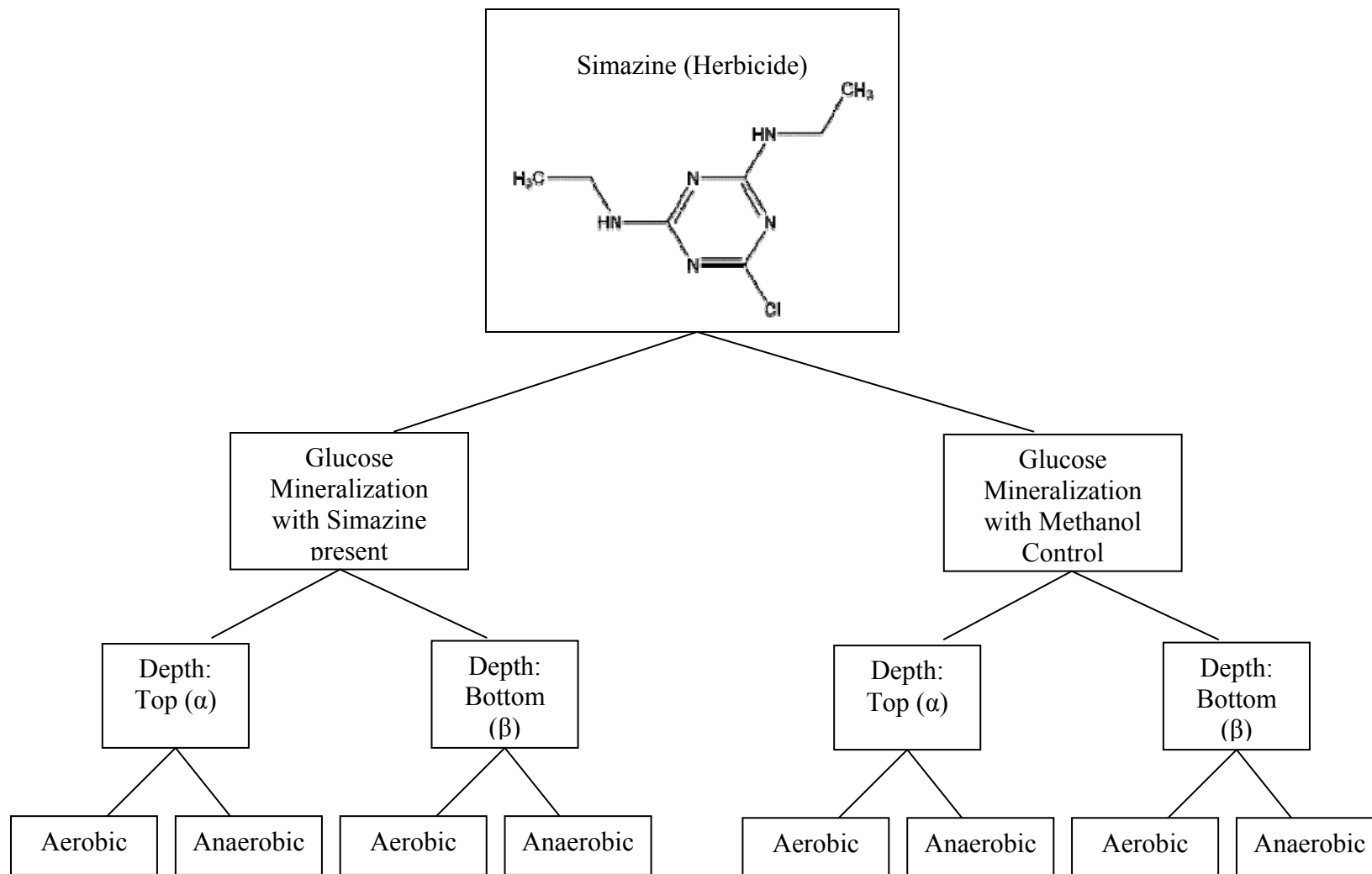


Figure 6: Diagram of Glucose Mineralization with Simazine Present and with Methanol Control

Macrocosm Set-up for Contaminated Systems (cont)

Phenanthrene

For the Phenanthrene experiments, two types of trials were run to see the effect of the contaminate, similar to the Simazine contaminant experiments (figure 7). The first experiment measured the amount of Phenanthrene mineralization that occurred in the compost, while the second experiment measured the amount of glucose mineralization with the presence of Phenanthrene in the beakers. As with Simazine, a small amount of contaminate can be used to determine the effect of the compost, so smaller beakers were used instead of aquariums for the macrocosms. The methodology for both experiments is also described separately for clarity.

Phenanthrene Mineralization set-up

Three 400 ml beakers were set up with 50:50 compost filled to approximately the 200 ml mark on the beakers. The compost had an equivalent of 1 inch of distilled water added and allowed to sit for three days. Then, 5 ml of Phenanthrene was added to the compost, followed by 2 ml of water to ensure proper absorption of Phenanthrene throughout the compost. The compost contaminated with Phenanthrene sat for three additional days. The compost for each beaker was separated into two piles, top and bottom, and prepared for Phenanthrene mineralization experiments within the microcosms.

Glucose Mineralization set-up (for Phenanthrene Contaminate)

Three 400 ml beakers were filled with 50:50 compost, and filled to approximately the 200 ml mark. An equivalent of 1 inch of rainfall was added to the beakers and allowed to rest for three days. Five ml of Phenanthrene was added to the top of the compost, followed by 2 ml of water to ensure diffusion through the compost. The compost then sat for three days to allow the Phenanthrene to absorb evenly through the compost. The compost for each beaker was then removed and separated into two portions, top α and bottom β . The six compost samples were then taken to the research lab to prepare for glucose mineralization experiments in microcosms.

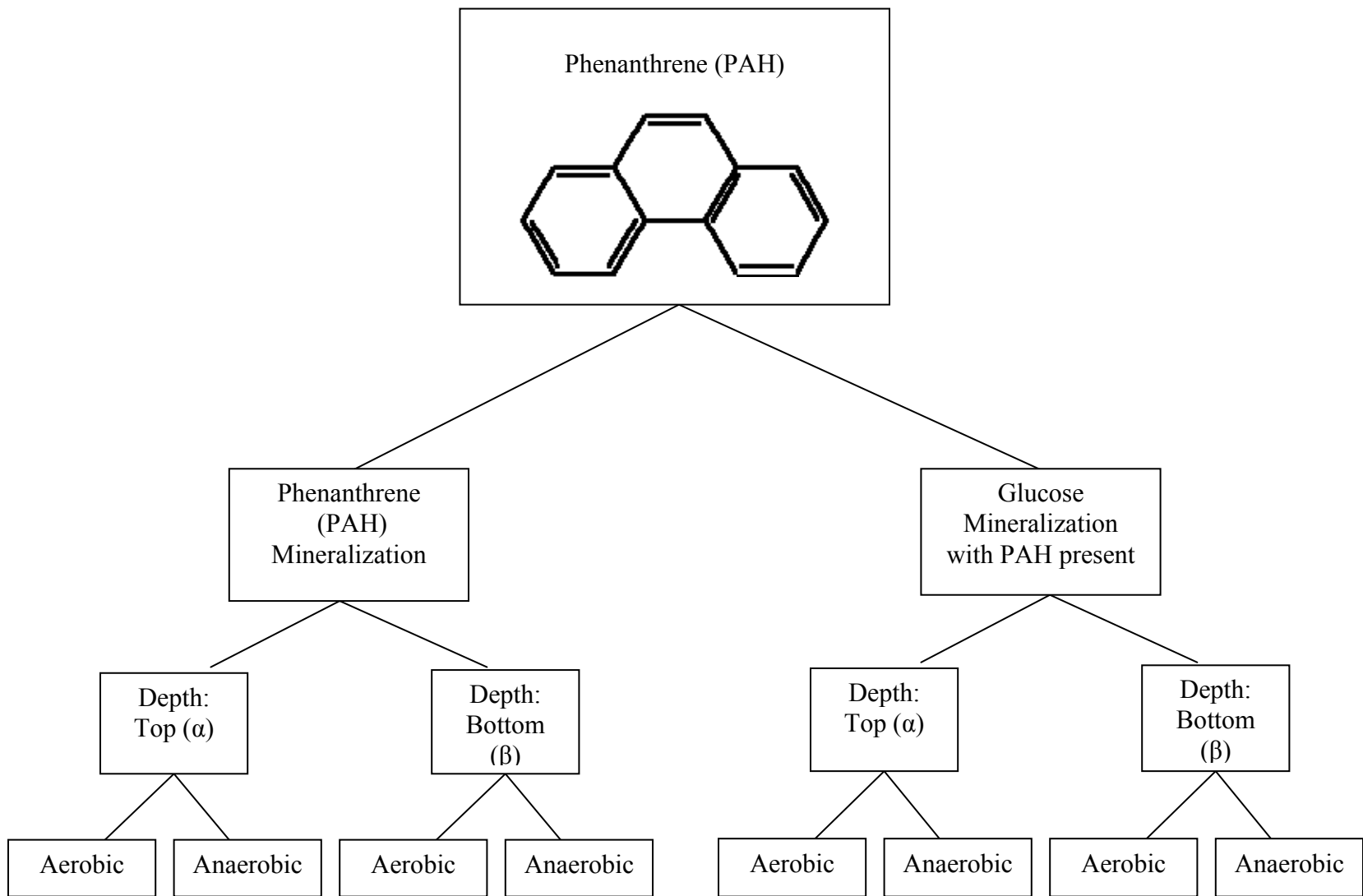


Figure 7: Phenanthrene and Glucose Mineralization Diagram in Contaminated System

Microcosm Set-up for Contaminated Systems

For each contaminate, motor oil, Simazine, and Phenanthrene, microcosms were set up to measure either glucose mineralization or contaminate mineralization. The methodology for glucose mineralization for all three contaminants followed the same procedure, and are described together. The microcosm set-up for the mineralization of Simazine and Phenanthrene are described separately due to the differences in preparation.

Glucose Mineralization with Motor Oil, Simazine, or Phenanthrene

After the contaminated compost samples were removed from the macrocosms (beakers), the compost was divided by top and bottom. Microcosms were set up exactly as the microcosms with the uncontaminated compost, with 3 cubic centimeters of contaminated compost, 20 ml of water, 0.1ml of ^{12}C glucose, and 0.1 ml ^{14}C glucose. For each sampling from the top and bottom depths of the beakers, aerobic and anaerobic conditions were created within the microcosms (figure 4, 5, 7). Anaerobic conditions were created with 3 ml sterile mineral oil added immediately after the ^{14}C glucose. Stoppers were placed on the beakers, and injected with 2ml of 0.1N sodium hydroxide through sterile syringes. The microcosms were placed on shaker set at 70 rpm, and sampled every 24 hours for seven days with the removal of NaOH and injection of new NaOH. Next, 1 ml of the removed NaOH was added to vials with 4 ml of scintillation cocktail, and the remainder NaOH was discarded. The

vials were placed in the scintillation counter to measure the radioactivity emitted from the ^{14}C -labeled CO_2 in the NaOH for each contaminate.

Simazine Mineralization

After the Simazine contaminated compost was removed from the macrocosms, it was separated into two portions, top α and bottom β . Approximately 3 cubic centimeters of compost contaminated with Simazine and 20 ml of water was added to 200 ml beakers to create a slurry mix. Next, 100 μl of ^{12}C Simazine, and 100 ml ^{14}C Simazine was added to each microcosm. Aerobic and anaerobic microcosms were created for each beaker at α and β depths, with anaerobic microcosms created using 3 ml of sterile mineral oil to the slurry mix. Stoppers were placed on the beakers preventing loss of carbon dioxide to the environment, and 2 ml of sodium hydroxide was injected into the microcosms. The microcosms were placed on shaker set at 70 rpm, and sampled every 24 hours for seven days with the removal of NaOH and injection of new NaOH. One milliliter of the removed NaOH from each microcosm was placed into scintillation vials and prepared for the scintillation counter.

Microcosm Set-up for Contaminated Systems (cont)

Phenanthrene Mineralization

The Phenanthrene contaminated compost from each macrocosm was separated into two portions, top α and bottom β . Three cubic centimeters from each pile was placed into a 200 ml beaker, with 20 ml of water. Next, 0.1 ml ^{12}C glucose and 0.1 ml ^{14}C was injected into every microcosm. Five ml of mineral oil was injected into half of the microcosms, creating anaerobic conditions for each macrocosm at top and bottom depths. The microcosms were sampled every 24 hours by removal of 0.1 N NaOH, and new NaOH was injected into the microcosms. The removed NaOH with trapped ^{14}C carbon dioxide was placed into scintillation counter vials and prepared for measurement in the scintillation counter.

Percent Moisture Determinations

For every experiment, whether contaminated or uncontaminated, tests were performed to determine the amount of moisture within the compost. When the compost was removed from the macrocosms, either the beaker macrocosms or the aquariums, it was separated into two portions, top and bottom depth. For the aquariums, the percent moisture was determined at 0-2 centimeters and 10-12 centimeters. Since the beaker macrocosms were not as deep, the compost was separated by top and bottom. For each portion of compost, a sample of approximately 8-10 grams was removed and placed into aluminum pans and weighed. The wet compost was then placed into the dryer for several days until all of the

moisture in the compost was evaporated. The compost within the aluminum pans was then weighed to determine the amount of weight loss due to the evaporation of the water. This comparison for wet weight compost versus dry weight compost could be used to calculate the percentage of water found in the compost (Appendix A).

Ash Free Dry Weight

After the compost was dried and measured for water loss, the compost was then ground up into small particles and weighed in a crucible. The compost was then placed into an oven at 500°C to burn all organic material in the compost. The remainder compost was then weighed to determine the percent of inorganic compounds present in the compost.

Statistical Analysis of Data

T-Tests for paired two samples means were performed on the data through Excel. The mean values between contaminated and control systems were compared to assess if the data was statistically significantly different. Tables with this information are listed in Appendix B.

Results

This experiment found that the amount of moisture in the uncontaminated aquarium mesocosms increased over time nearly 40%. Compost from 0-2 cm generally had 15-20% decrease in moisture compared to the lower depths (10-12 cm), but the relative amount of moisture at both depths increased as the experiment progressed. The amount of moisture in the mesocosms was the highest in October, increasing moisture for both depths 30-35%. The greatest amount of moisture was found at the 10-12 centimeter depth of the compost (approximately 75-80% moisture, Figure 8). After October, the amount of water added to the compost was decreased; as a result, the relative percent moisture decreased 15% throughout November (Figure 8).

The first experiment with the used motor oil showed 37.34% at the higher depths, and 54.61% for the lower. Compared to the control moisture in the aquariums in August, at 46.23% upper and 47.3% lower depths, the amount of moisture was significantly different (T-test, $P=0.67$).

Three macrocosm experiments were performed with the presence of Simazine. The percent moisture for all three experiments were compared, with the higher depths for all three experiments within 5-7% of each other with no significant difference. The lower depths for all three experiments were found to be within 3.5-5% difference. The relative amount of moisture was slightly higher at the lower depth than the higher, with the higher depths around 48-53% moisture and the lower depths with 55-59% moisture (Figure 9).

For the two different Phenanthrene experiments, the percent moisture for both experiments at higher depth is within 2-3% of each other, 47-49.5% and the lower depth is close to 1-1.5% difference 55-56%; therefore, there is no real difference in the amount of moisture that was added to each macrocosm in the Phenanthrene experiments (Figure 10). Again, the amount of moisture was higher in the deeper depths of the mesocosms.

Finally, the percent moisture rates of all the contaminated mesocosms were compared to ensure that the differences in mineralization rates cannot be attributed to differences in the amount of water present in the mesocosms. There is more range in the amounts of relative moisture for all the contaminated systems (Figure 11). A t-test was performed on all the variables, and showed no statistical, significant differences between the moisture amounts in the different contaminated mesocosms (Appendix B).

Glucose mineralization rates for the uncontaminated mesocosms were measured every month to determine the rate of glucose mineralization (Figure 12). The rate of glucose mineralization significantly dropped over time from 0.35 mg/g Wet/day, and then leveled off around 0.13 mg/g Wet/day. The rate of glucose mineralization at 10-12 centimeters deep was lower throughout the experiment compared to the higher depth at 0-2 centimeters. On average, the lower depths had a lesser mineralization rate of almost 0.4 mg/G Wet/day; however, the t-test showed no significant difference between the beginning of the experiment (July) compared to the end (November).

Glucose mineralization rates for the contaminated motor oil mesocosm showed a higher rate of glucose degradation in the upper portion of the compost than the lower (Upper=0.20 g/g Wet/ day v. Lower=0.15 g/g Wet/day). There was a significant difference when comparing the aerobic to anaerobic conditions (t-test, $p=0.87$).

Glucose mineralization rates were obtained from mesocosms that contained Simazine (SC1) and mesocosms containing methanol (SC3) (Figure 14). The rate of glucose degradation was approximately the same with aerobic or anaerobic conditions at any depth for both experiments. Glucose degradation was higher in the mesocosms exposed to Simazine (0.25g/g Wet/day), than the mesocosms with the 50:50 methanol: water mix (0.15 g/g Wet/day).

The rate of glucose mineralization in the presence of Simazine (SC1) and the rate of Simazine break-down (SC2) were measured in separated experiments (figure 15). The rates were compared to show the differences in the mineralization. The breakdown of glucose was significantly higher (0.25g/g Wet/day) than the rate of Simazine degradation, which had very little breakdown (>0.02 g/g Wet/day). When comparing oxygen versus no oxygen, the T-test did show a significant difference, $P=0.92$, between Simazine and glucose mineralization.

The rate of Phenanthrene break-down (PC1) and rate of glucose mineralization in the presence of Phenanthrene (PC2) were also measured separately (Figure 16). Some phenanthrene was broken down (0.09 g/g Wet/day), but the rate of

glucose degradation (0.27 g/g Wet/day) is almost three times higher than phenanthrene degradation.

Finally, the glucose mineralization rates for all contaminated systems were measured and compared (Figure 17). Generally, the higher depths for any contaminate had a higher rate of glucose metabolism than the lower depths, roughly 10% higher. Overall, most glucose mineralization rates fell within the 0.15-0.25 mg/g Wet/day range.

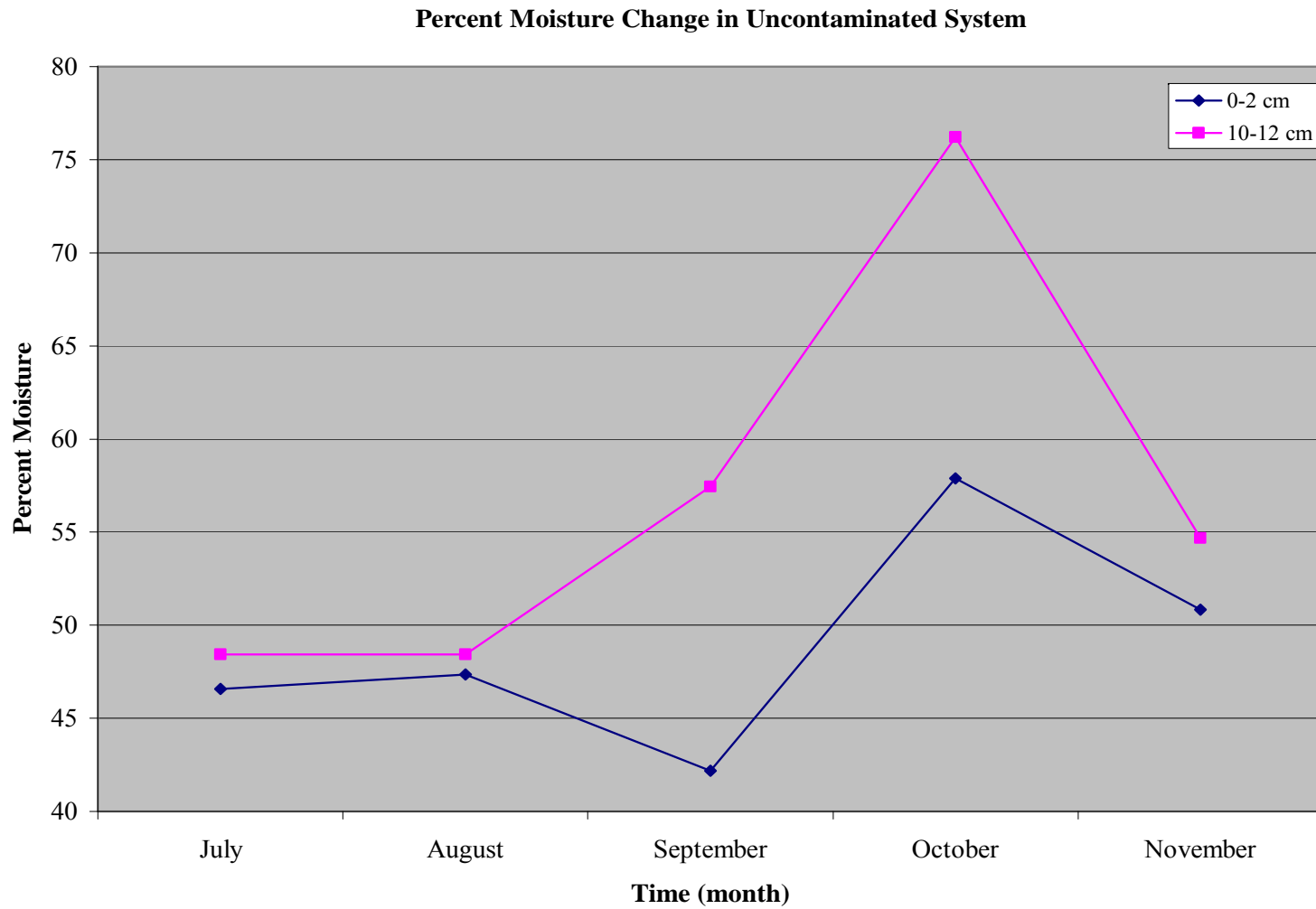
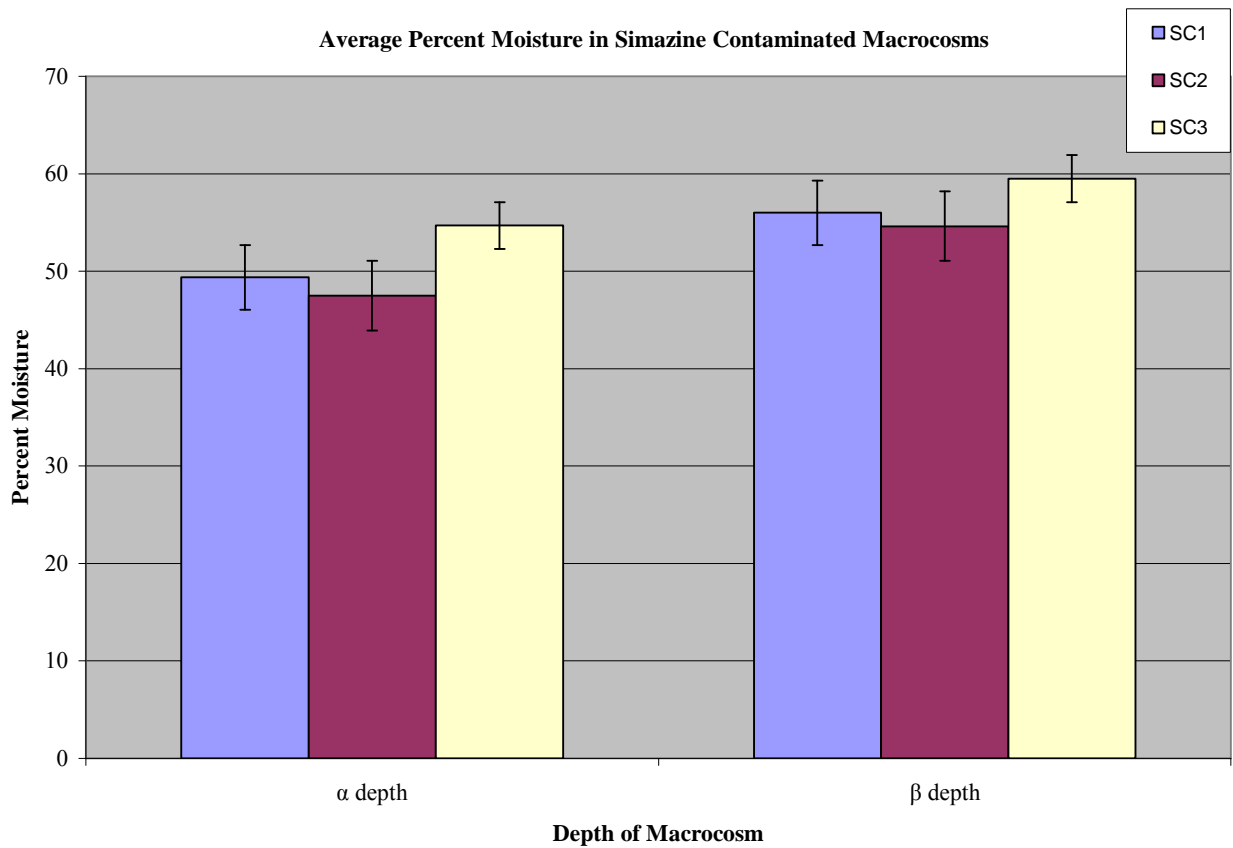


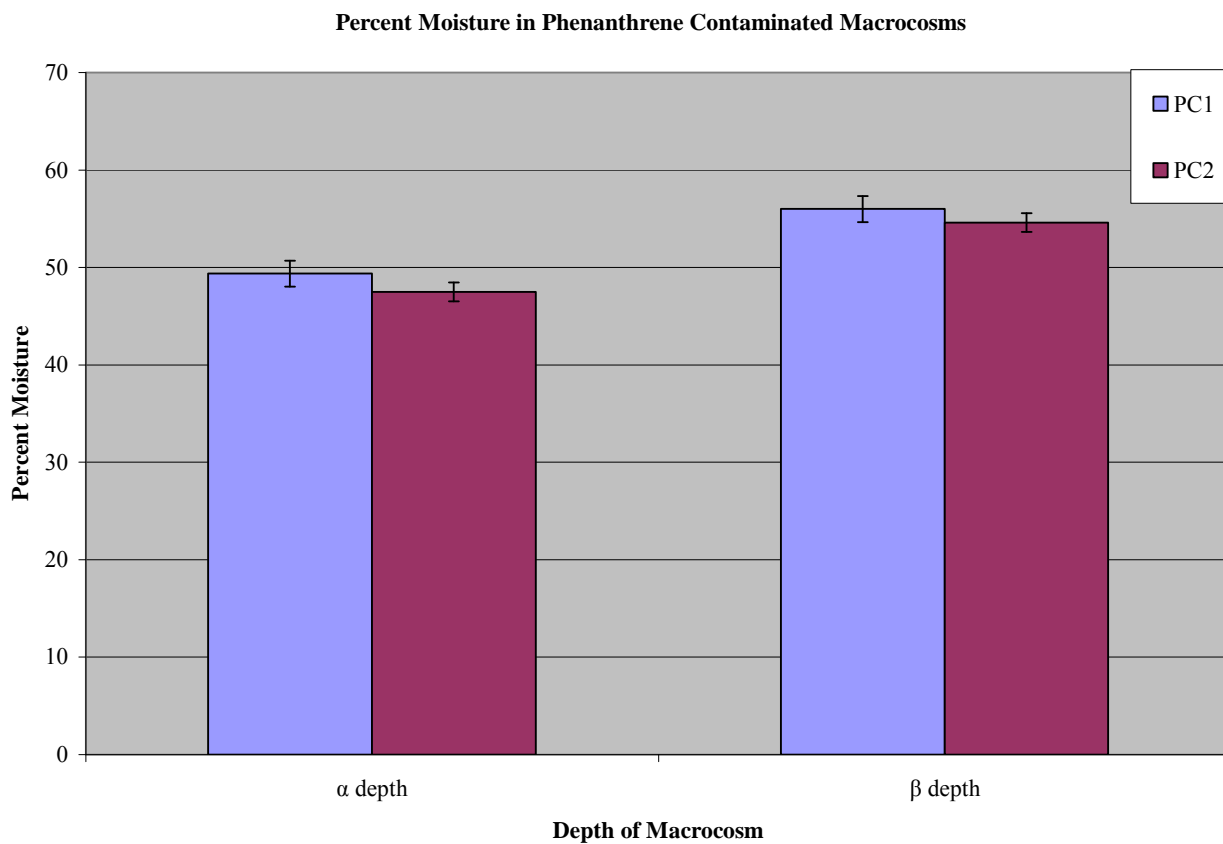
Figure 8: Graph showing the change in percent moisture of the aquariums over time.



Key:

- SC1 Experiment-Glucose Mineralization with the Presence of Simazine
- SC2 Experiment-Simazine Mineralization
- SC3 Experiment-Glucose Mineralization with Methanol Control

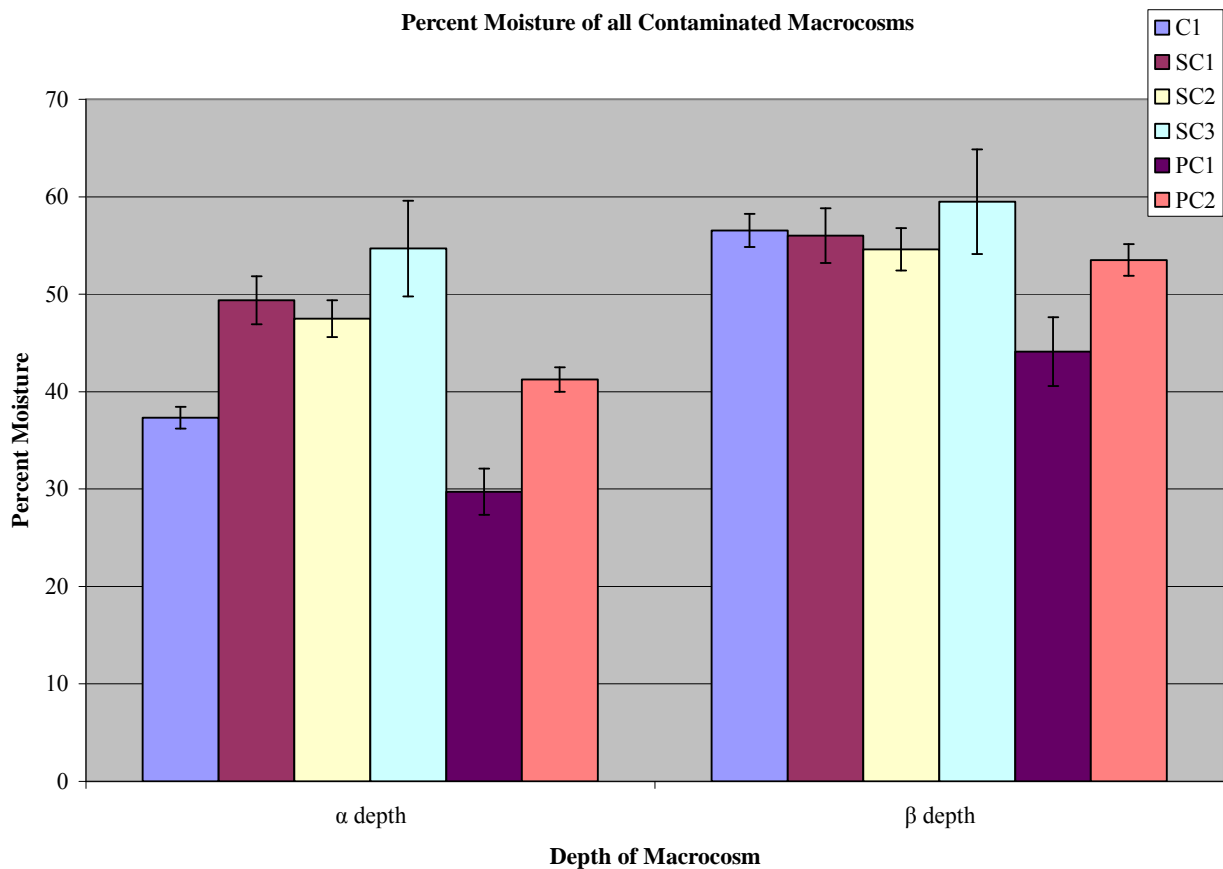
Figure 9: Average percent moisture for each Simazine experiment. Each bar represents an average of the three mesocosms used in that experiment.



Key:

- PC1 Experiment-Phenanthrene Mineralization
- PC2 Experiment-Glucose Mineralization with the presence of Phenanthrene in the Macrocosm

Figure 10: Average percent moisture of Phenanthrene contaminated macrocosms. (Each bar represents an average of the three mesocosms used in that experiment.)









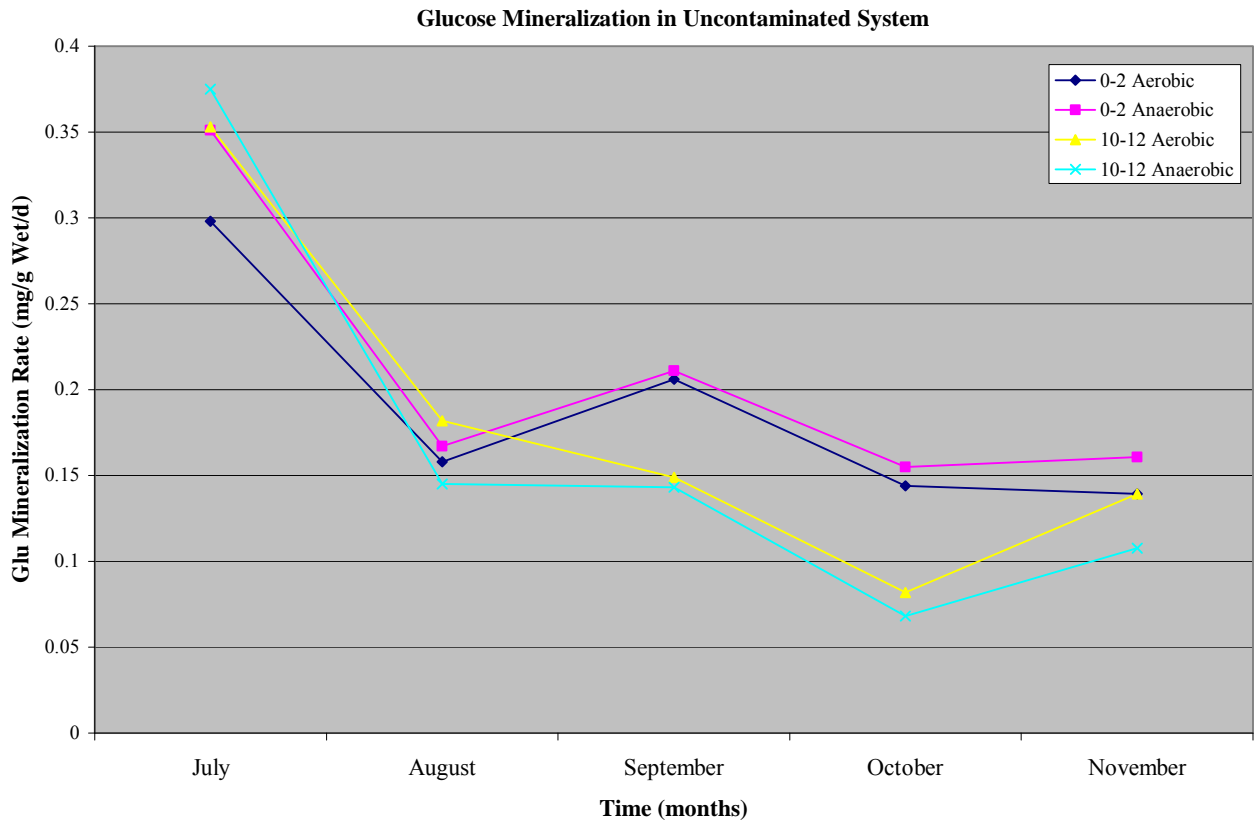
 C1 Experiment-Glucose Mineralization with the Presence of Motor Oil	 SC3 Experiment-Glucose Mineralization with Methanol Control
 SC1 Experiment-Glucose Mineralization with the Presence of Simazine	 PC1 Experiment-Phenanthrene Mineralization
 SC2 Experiment-Simazine Mineralization	 PC2 Experiment-Glucose Mineralization with the Presence of PAH

Figure 11: Comparison of percent moisture in all contaminated macrocosms.



<u>Time:</u> (Month)	<u>Glu Min Rate:</u> (mg/g Wet/ d)			
	<u>0-2 Aerobic</u>	<u>0-2 Anaerobic</u>	<u>10-12 Aerobic</u>	<u>10-12 Anaerobic</u>
July	0.298	0.351	0.353	0.375
August	0.158	0.167	0.182	0.145
September	0.206	0.211	0.149	0.143
October	0.144	0.155	0.082	0.068
November	0.139	0.161	0.139	0.108

Figure 12: Glucose Mineralization Rates in Uncontaminated System

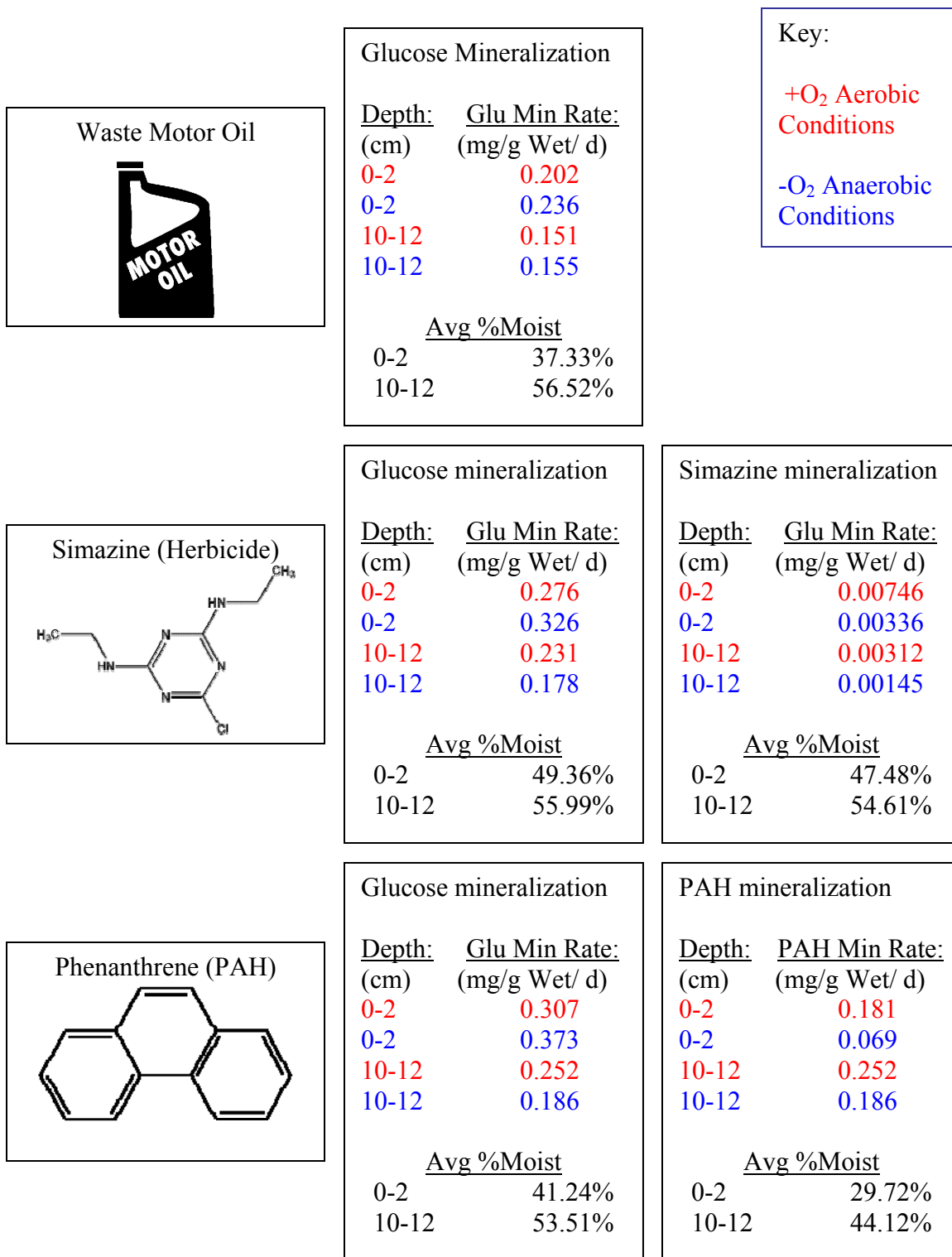


Figure 13: An overview of the data for each contaminated macrocosm, showing the different rates of mineralization for either glucose or contaminate, and the relative percent moistures in the contaminated macrocosms.

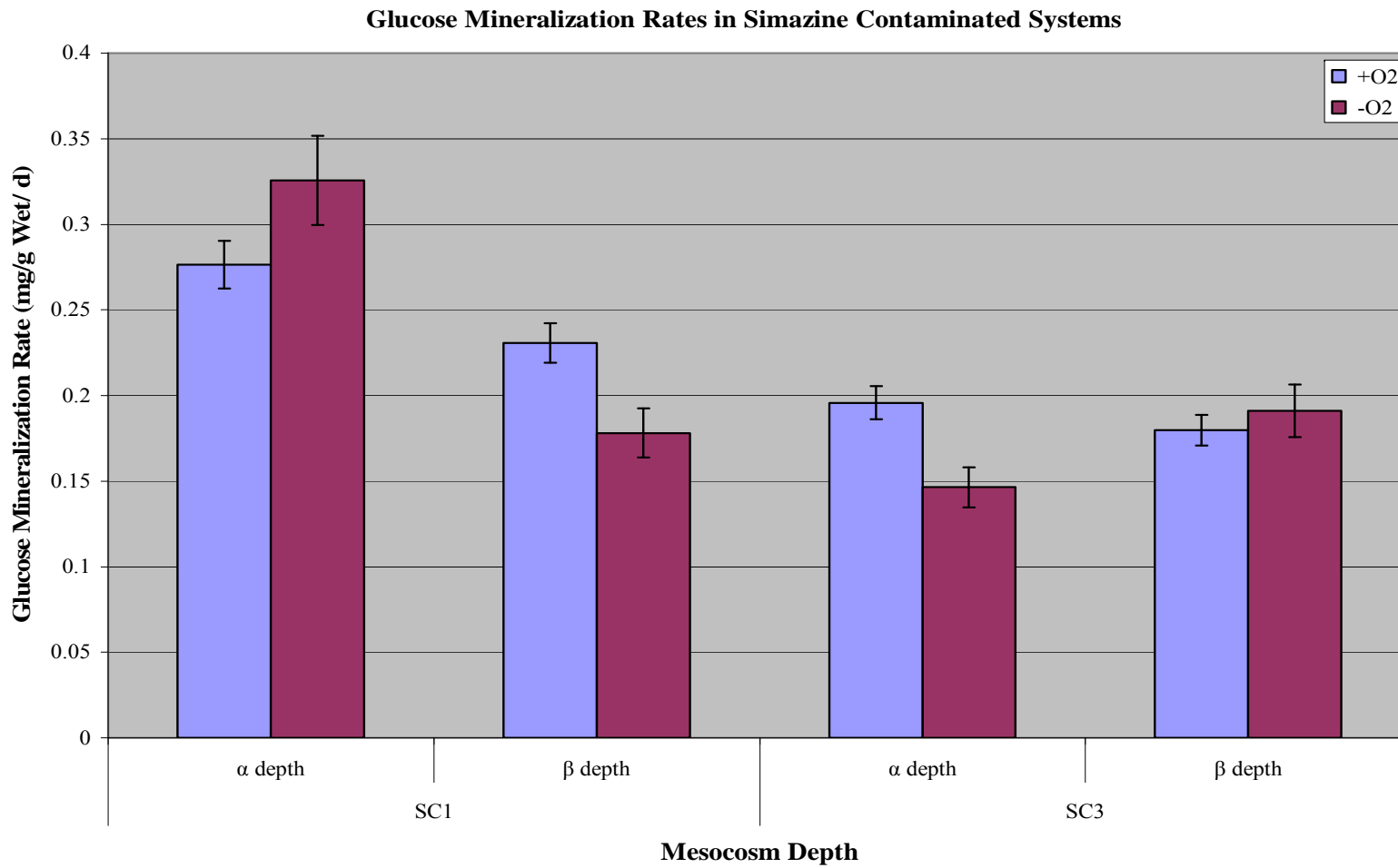


Figure 14: Comparison of Glucose mineralization rates for Simazine Experiments

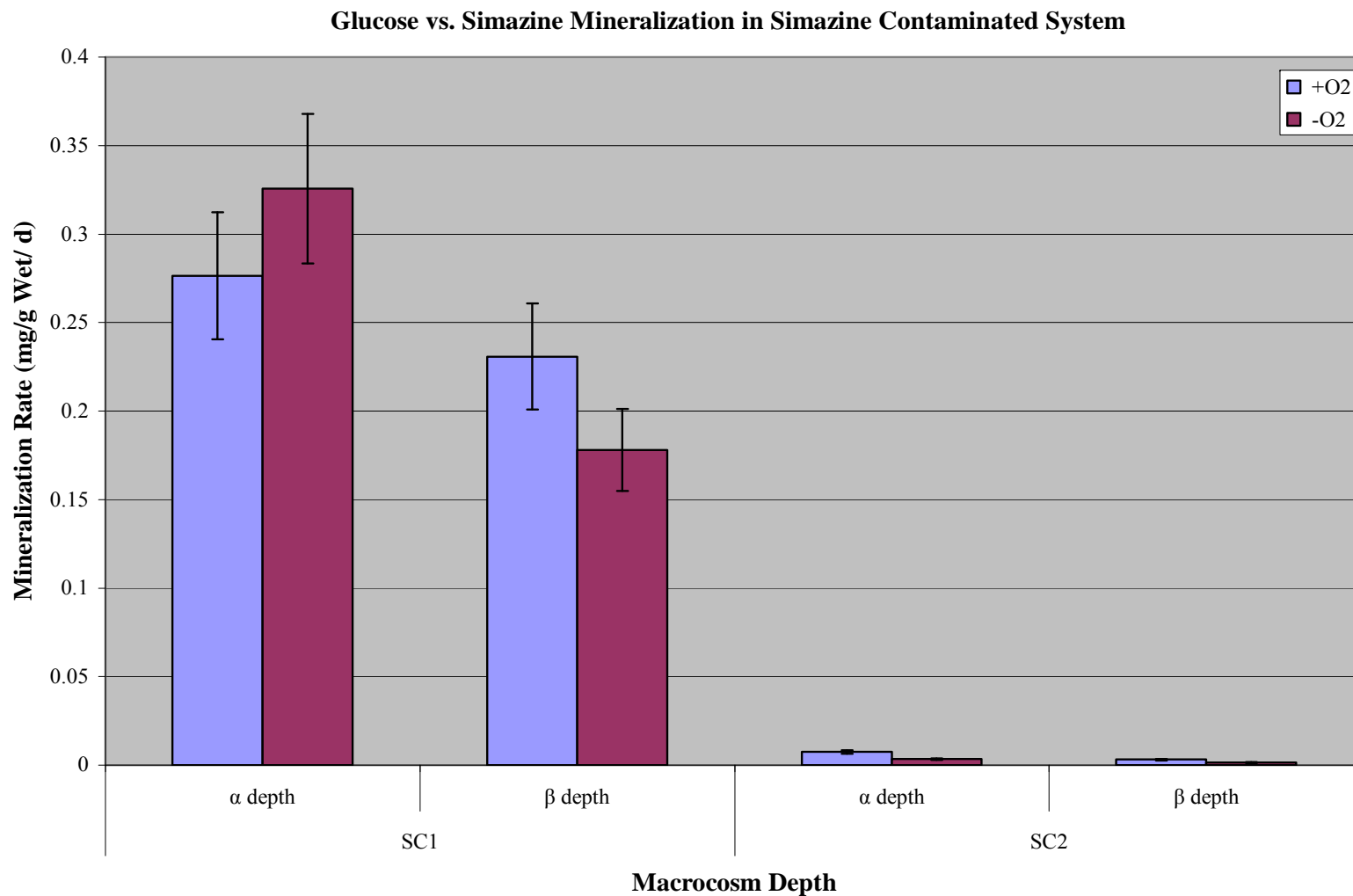


Figure 15: Glucose vs. Simazine Mineralization rates in Simazine Contaminated Systems

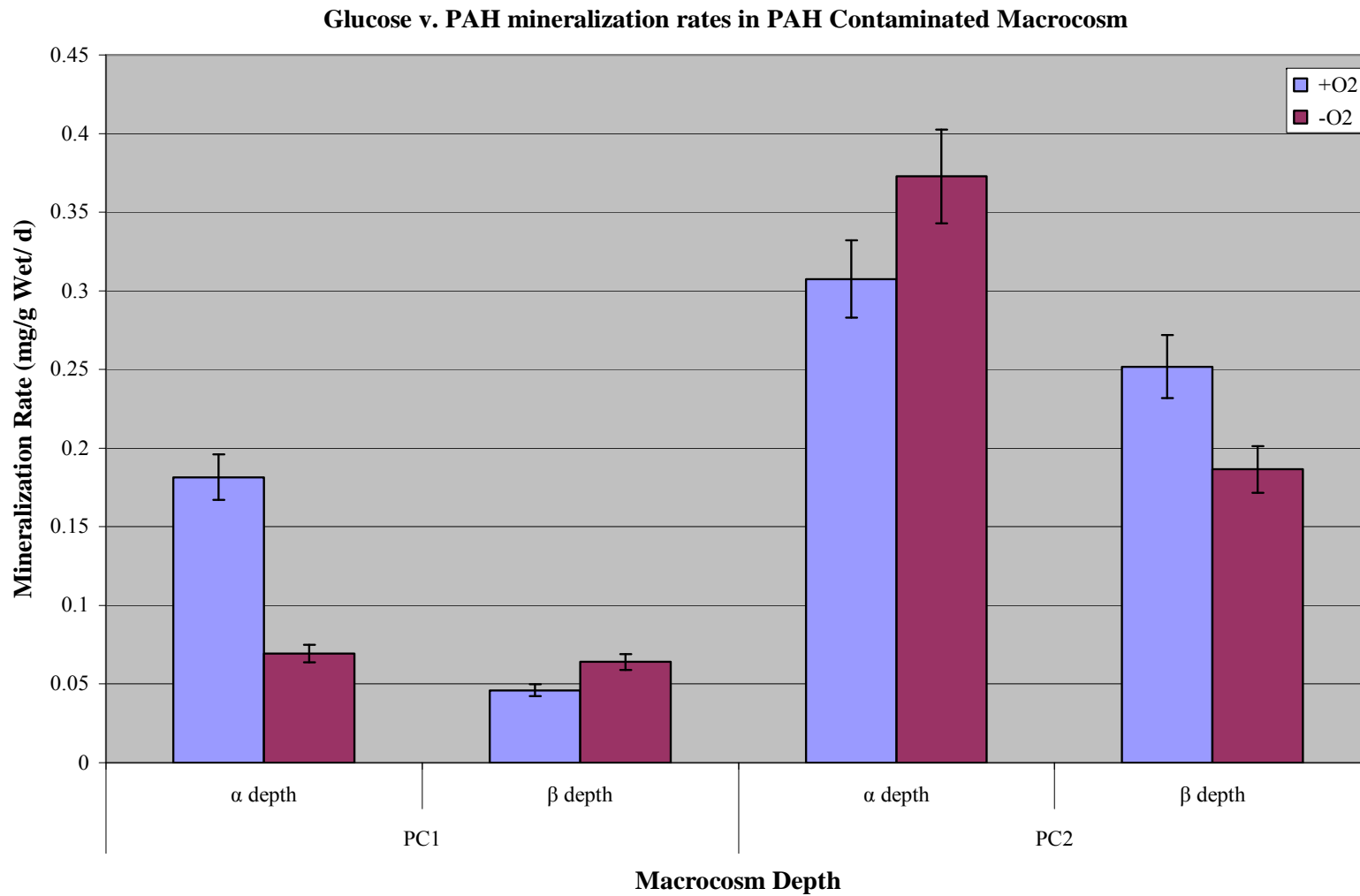


Figure 16: Glucose v. PAH Mineralization rates in PAH Contaminated Macrocosms

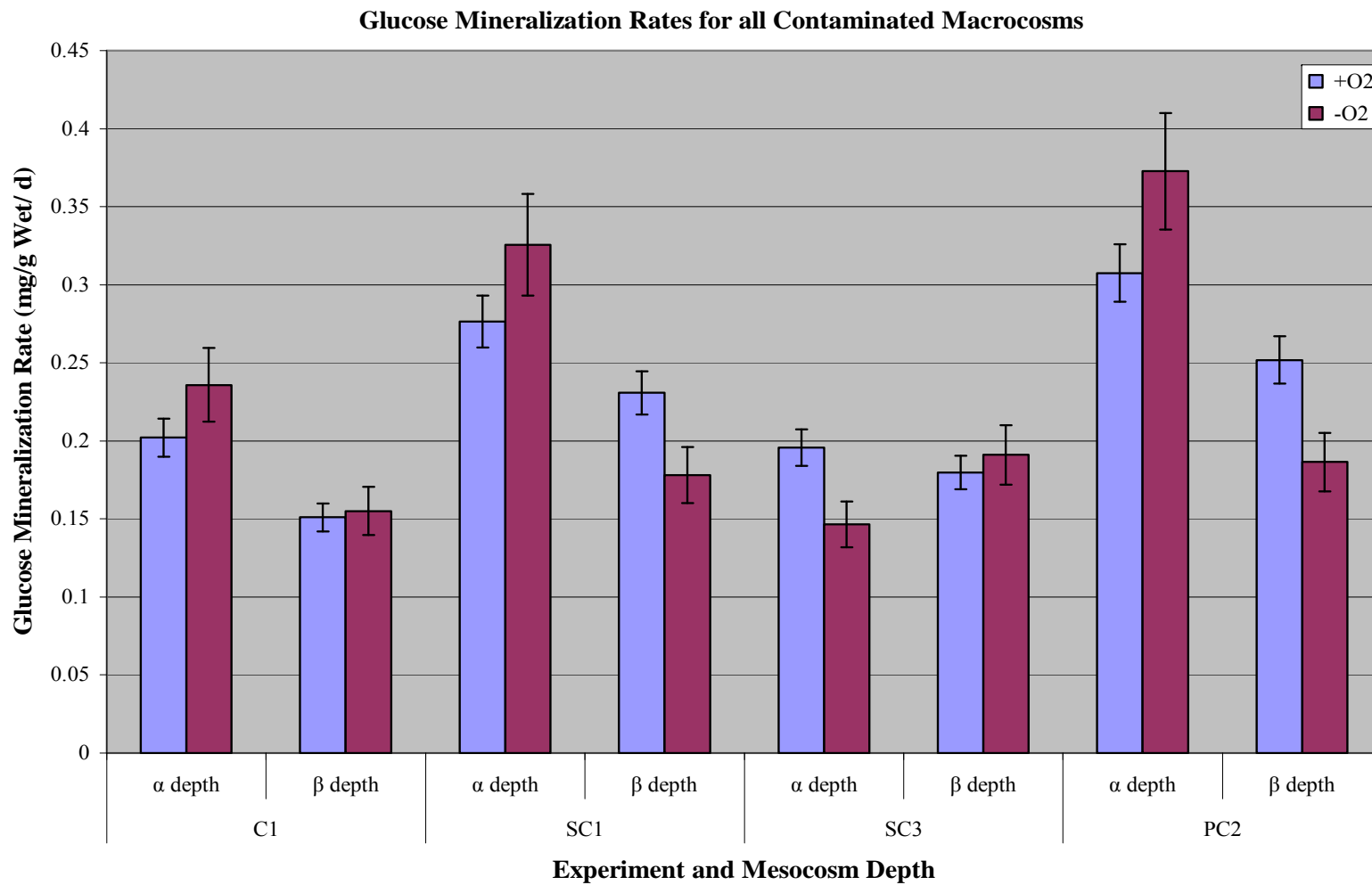


Figure 17: Glucose Mineralization Rates for all Contaminated Macrocosms

Discussion

This study attempted to duplicate conditions that might be found in Filtrexx FilterMedia products that would be installed at industrial settings. The macrocosms were separately contaminated with used motor oil, Simazine, and Phenanthrene to determine the effects of these three most common contaminants on the SOXX. This experiment also measured the microbial activity in a non-contaminated system over time, to determine if the bacteria change as time progresses. The microbial activity was monitored by measuring the amount of ^{14}C glucose or contaminate and the amount of radioactive CO_2 gas released by the microcosm.

Throughout the experiment, moisture played a key role in the amount of glucose metabolized in the microcosms. With the uncontaminated systems, the amount of water increased in the higher and lower depths of the macrocosm aquariums through October until the amount of water added was changed. The outside temperature did decrease as the experiment progressed, making the amount of water normally evaporate quickly during the warmer, summer months, decrease as the temperature began to cool. As a result, more water could not leave the aquariums, and a higher percentage of water was found at the lower aquarium depths. Also, fungal hyphae began to grow around 5-6 centimeters deep in the aquariums, which would prevent water from escaping the lower levels of the aquariums. Tunnels were created in the aquariums around the edges of the compost to allow the lower depth of the macrocosms to aerate and release excess water. By decreasing the amount of water added weekly to the macrocosms and ventilating the aquariums, the percent

moisture decreased back to a lower level. Overall, the highest amount of moisture was found in the lower depths of the macrocosms because of the accumulation of water and the inability for the compost to release the excess moisture.

The heterotrophic bacterial activity in the uncontaminated system can be directly compared to percent moisture of the compost. The highest rate of glucose mineralization occurred when the experiment first began in July. The compost had been in the aquarium for approximately one week, and then the soil was removed for microcosm experiments. The amount of moisture in the system was near ideal, and competition for the resources in the macrocosms had not begun to affect the microbial activity in the compost. Within the next month, the glucose mineralization rate reduced by almost 50%, and then leveled off around the 0.15-0.2 mg/g Wet/day. The rate of glucose breakdown did lower during October, where the percent moisture of the system was extremely high.

As the experiment progressed, the mineralization of glucose was also higher in the 0-2 centimeter range than what was found in the 10-12 centimeter range. The mineralization rate in the 10-12 centimeter depth of the aquariums was substantially lower, with more water preventing oxygen dependent bacteria from surviving. Even when moisture conditions are not a factor in the compost, the amount of oxygen that can diffuse through the compost at the lower depth is substantially less than the amount at the surface. In this experiment, the bacterial growth was monitored by the amount of glucose added to the system and the amount of carbon dioxide that was removed. With anaerobic conditions in the macrocosms, the bacteria that would

normally breakdown glucose through the Emden-Meyerhof pathway must adjust to the change in their metabolism pathway. Most organisms that breakdown glucose aerobically into carbon dioxide can breakdown glucose into lactate to still provide energy to the cell during anaerobic conditions. Some bacteria can easily adjust to the change in oxygen by living off the lactose, while other aerobic bacteria would die. The amounts of bacteria that are now collected when sampling the macrocosms have either adapted to the anaerobic conditions or are found at a higher depth to ensure aerobic conditions. Therefore, the amount of glucose metabolized into carbon dioxide is dependent on depth and the amount of moisture in the compost.

This experiment studied the effect of adding used motor oil to the compost to see how the heterotrophic activity would change. When comparing the mesocosms the upper depths had less moisture than the lower depths; additionally, the rate of glucose mineralization was higher for the upper depth than the lower, but the difference was not significant. When comparing the aerobic to anaerobic conditions, there was a significant difference. This would lead to the probability that the lower depths with increased moisture and combined with anaerobic conditions, would have an affect on the rate of glucose mineralization. Also, the rate of glucose mineralization was significantly different than the uncontaminated system, showing that the presence of motor oil did have an influence of the bacterial activity.

This experiment also tested for the effect of exposing the compost to Simazine, a common herbicide. The relative amount of water in all the macrocosms was similar, so the rate of glucose or Simazine breakdown could not be attributed to

water conditions. The moisture in the lower portion of the beakers was approximately 5-7% higher than the upper level of the compost; however, this did not seem to impact mineralization rates. The macrocosm contaminated with Simazine showed a higher rate of glucose metabolism over the macrocosm exposed only to 50:50 methanol: water, with the highest amount of mineralization at the α , or higher, depth. Also, aerobic or anaerobic conditions do not seem to significantly affect the amount of glucose that the bacteria metabolized.

In contrast, the rate of Simazine mineralization was very small in comparison to both glucose studies with Simazine, yet there was a significant difference with Simazine breakdown, indicating that some mineralization did occur, even if a small amount. Simazine is an aromatic benzene ring with three nitrogens as bases, and this compound would be difficult to metabolize because the nitrogen bases would form a lone pair of electrons during breakdown. The extra electrons could interfere with the ATP production, by increasing the negativity inside the cell. Unless bacteria can overcome the challenges of extra electron pairs, then mineralization of Simazine is not beneficial. More research should be performed to determine the complete effect of Simazine on this system.

As with Simazine, the amount of moisture in the macrocosms was not significantly different between the glucose or phenanthrene mineralization experiments; however, there was a higher percentage of moisture at the lower depths, β , for both experiments. The amount of glucose and phenanthrene mineralization was higher at α depth than β depth, again correlating moisture to mineralization rates.

Considering that glucose is a preferred food source for many bacteria, it is not surprising that glucose mineralization was significantly higher than phenanthrene. Phenanthrene's structure is composed of three aromatic benzene rings, which would be difficult for any cell to metabolize, contributing to reduced mineralization rates.

The glucose mineralization rates for all contaminated systems were measured and compared. Generally, there is not a noticeable difference in anaerobic versus aerobic microcosms. The α depths in the macrocosms for any contaminate had a higher rate of glucose metabolism than the β depths, with most glucose mineralization rates falling within the 0.15-0.25 mg/g Wet/day range. Therefore, the higher portions of the compost typically had an increase in the amount of glucose mineralization, due to more oxygen and evaporation of water that is available.

With the majority of the experiments in this research, there was not a significant difference in aerobic versus anaerobic conditions in the microcosms. The amount of mineral oil that was added to the compost may not have provided a thick enough barrier to prevent oxygen from diffusing into the slurry compost mix. While the quantity of oxygen available to the compost was hindered by the mineral oil, it did not provide a significant effect on the results. More mineral oil might be necessary to ensure that anaerobic conditions exist in the microcosms. Future research could study the amount of dissolved oxygen, to further enforce that the microcosms truly turned anaerobic.

In conclusion, Filtrexx products are effective in trapping any contaminants that are exposed to the compost; however, this research does not show that the

endogenous bacteria found in the compost are capable of metabolizing these chemicals at a high volume. The endogenous bacteria in Filtrexx compost are capable of mineralizing glucose and growing with the presence of motor oil, phenanthrene, or Simazine. Further studies into adding exogenous bacteria capable of break down contaminants would also enhance bioremediation of the SOXX. If not, herbicides, motor oil, or other contaminants that flow into the filters through water could accumulate in the compost and would eventually need to be removed. By adding specific types of bacteria to the compost that could breakdown some of the most common contaminants, then the system could continually filter and effectively remove them from the environment. Additionally, the bacterial activity is dependent upon the amount of moisture that is present. The lower depths showed less heterotrophic activity, probably due to the increased moisture contributing to anaerobic conditions. The ability for the bacteria to survive desiccation or extreme humidity would influence how the compost filters the water. When freshly installed, the bacterial activity is high, but decreases until leveling off at a certain rate of metabolism. Therefore, the compost does change over time, which causes problems for supplementing additional bacteria into the compost to break down contaminants. Since drought or other weather scenarios could affect conditions in the SOXX, further research at an installation site would be necessary for characterizing the ideal moisture content for optimal toxic mineralization.

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Appendix A: Percent Moisture Calculations

1. To calculate percent moisture, the weight of the aluminum pan (A) to hold the compost is first measured.
2. The compost is added, and the total weight is measured (B).
3. The wet weight of the compost (X) is calculated by subtracting the Dish Base (A) from the Dish Base+Wet Soil Weight (B).
4. After the compost has been dried, the weight of the dried compost in the pan is weighed (C).
5. The dry weight of the compost (Y) is calculated by subtracting the Dish Base (A) from the Dish Base+Dry Soil Weight (C).
6. The percent moisture is calculated by subtracting dry weight of the compost (Y) from the wet weight of the compost (X), divided by the wet weight of the compost (X). This decimal is multiplied by 100 to get the Percentage of moisture in the compost. Figure 18 is an excerpt from Excel that was used to calculate the percent moisture for the Simazine Contaminated System.

Figure 18: Excel Table Illustrating Percent Moisture Computations

Sample	Dish Base Dry Wt.	Dish Base + Wet Soil Wt.	Dish Base + Dry Soil Wt.	Wet Wt. (X)	Dry Wt. (Y)	Percent Moisture
MM-cosm N	A	B	C	B-A	C-A	$\frac{(X-Y)}{X} * 100$
MM-cosm #1 α	1.872	10.505	5.95	8.633	4.078	52.763
MM-cosm #1 β	1.857	14.168	7.046	12.311	5.189	57.851

Appendix B: T-Test Values

All Contaminate % Moisture Comparison

SC1, SC2, SC3		
	α depth	β Depth
Mean	50.512675	56.70271
Variance	13.9940181	6.34385
Observations	3	3
t Stat	-8.7570736	
P(T<=t) two-tail	0.01279048	
t Critical two-tail	4.30265273	

PC1, PC2		
	α depth	β Depth
Mean	35.4801069	48.81429
Variance	66.4267902	44.15997
Observations	2	2
t Stat	-12.530104	
P(T<=t) two-tail	0.05069976	
t Critical two-tail	12.7062047	

All Contaminants		
	α Depth	β Depth
Mean	43.3060747	54.04319
Variance	81.6654095	27.77921
Observations	6	6
t Stat	-4.7679906	
P(T<=t) two-tail	0.00502374	
t Critical two-tail	2.57058183	

sc1 and sc2		
	α depth	β Depth
Mean	52.6781442	51.0482
Variance	21.9959261	25.42915
Observations	2	2
t Stat	6.53458244	
P(T<=t) two-tail	0.09667318	
t Critical two-tail	12.7062047	

C1 v UC-AUG		
	α depth	β Depth
Mean	46.9303	50.58248
Variance	184.017	19.44525
Observations	2	2
t Stat	-0.5641	
P(T<=t) two-tail	0.67302	
t Critical two-tail	12.7062	

PC1 v UC-SEPT		
	α depth	β Depth
Mean	49.539	36.91618
Variance	94.8289	103.6564
Observations	2	2
t Stat	40.2814	
P(T<=t) two-tail	0.0158	
t Critical two-tail	12.7062	

PC2 v UC-NOV		
	α depth	β Depth
Mean	47.3782	53.10895
Variance	75.2766	50.77673
Observations	2	2
t Stat	-5.2272	
P(T<=t) two-tail	0.12034	
t Critical two-tail	12.7062	

SC1 v UC-OCT		
	α depth	β Depth
Mean	52.6781	69.94056
Variance	21.9959	91.7236
Observations	2	2
t Stat	-4.9952	
P(T<=t) two-tail	0.12578	
t Critical two-tail	12.7062	

SC1 and SC3		
	α depth	β Depth
Mean	51.0482043	57.09673
Variance	25.4291548	11.54863
Observations	2	2
t Stat	-5.2018208	
P(T<=t) two-tail	0.12090897	
t Critical two-tail	12.7062047	

SC3 v UC-OCT		
	α depth	β Depth
Mean	57.0967	69.94056
Variance	11.5486	91.7236
Observations	2	2
t Stat	-2.9397	
P(T<=t) two-tail	0.20874	
t Critical two-tail	12.7062	

PC1 v PC2		
	α depth	β Depth
Mean	36.9161806	47.37822
Variance	103.656388	75.27664
Observations	2	2
t Stat	-9.831154	
P(T<=t) two-tail	0.06453339	
t Critical two-tail	12.7062047	

SC2 v UC-NOV		
	α depth	β Depth
Mean	51.0482	53.10895
Variance	25.4292	50.77673
Observations	2	2
t Stat	-1.3991	
P(T<=t) two-tail	0.39506	
t Critical two-tail	12.7062	

All Uncontaminated		
	0-2 CM	10-12 CM
Mean	48.96486	57.03489
Variance	39.14653	122.8166
Observations	15	15
t Stat	-3.69779	
P(T<=t) two-tail	0.002388	
t Critical two-tail	2.144787	

Mineralization Rates

SC1, SC2, SC3		
	α depth	β Depth
Mean	0.15919094	0.130698
Variance	0.01805422	0.010257
Observations	6	6
t Stat	1.07005663	
P(T<=t) two-tail	0.33350644	
t Critical two-tail	2.57058183	

SC1, SC2, SC3		
	+ Oxygen	- Oxygen
Mean	0.14889	0.141003
Variance	0.01348	0.015285
Observations	6	6
t Stat	0.50178	
P(T<=t) two-tail	0.63713	
t Critical two-tail	2.57058	

PC1, PC2		
	α depth	β Depth
Mean	0.23277018	0.137086
Variance	0.01816499	0.009745
Observations	4	4
t Stat	2.372169	
P(T<=t) two-tail	0.09831095	
t Critical two-tail	3.1824463	

PC1, PC2		
	+ Oxygen	- Oxygen
Mean	0.1967	0.173155
Variance	0.01275	0.020895
Observations	4	4
t Stat	0.58911	
P(T<=t) two-tail	0.59721	
t Critical two-tail	3.18245	

All Contaminates		
	α depth	β Depth
Mean	0.19367897	0.136544
Variance	0.01453295	0.007389
Observations	12	12
t Stat	2.87159328	
P(T<=t) two-tail	0.01519814	
t Critical two-tail	2.20098516	

SC1 and SC2		
	SC1	SC2
Mean	0.15323809	0.103349
Variance	0.02954227	0.014082
Observations	4	4
t Stat	1.46414856	
P(T<=t) two-tail	0.23936795	
t Critical two-tail	3.1824463	

SC1 and SC3		
	SC1	SC3
Mean	0.23608046	0.194907
Variance	0.00643958	0.000604
Observations	4	4
t Stat	1.02568979	
P(T<=t) two-tail	0.38051543	
t Critical two-tail	3.1824463	

PC1 v PC2		
	PC1	PC2
Mean	0.23277018	0.137086
Variance	0.01816499	0.009745
Observations	4	4
t Stat	2.372169	
P(T<=t) two-tail	0.09831095	
t Critical two-tail	3.1824463	

All Contaminates		
	+ Oxygen	- Oxygen
Mean	0.16943	0.160793
Variance	0.01023	0.01343
Observations	12	12
t Stat	0.57845	
P(T<=t) two-tail	0.57461	
t Critical two-tail	2.20099	

SC1 and SC2		
	Oxygen + or -	
	+ Oxygen	- Oxygen
Mean	0.12945	0.127135
Variance	0.02091	0.024372
Observations	4	4
t Stat	0.11149	
P(T<=t) two-tail	0.91827	
t Critical two-tail	3.18245	

SC1 and SC3		
	+ Oxygen	- Oxygen
Mean	0.22068	0.210302
Variance	0.00184	0.006264
Observations	4	4
t Stat	0.42024	
P(T<=t) two-tail	0.7026	
t Critical two-tail	3.18245	

PC1 v PC2		
	+ Oxygen	- Oxygen
Mean	0.1967	0.173155
Variance	0.01275	0.020895
Observations	4	4
t Stat	0.58911	
P(T<=t) two-tail	0.59721	
t Critical two-tail	3.18245	

Glucose Min Rates

C1 v UC-AUG		
	α depth	β Depth
Mean	0.19073032	0.158248
Variance	0.00126579	0.000268
Observations	4	4
t Stat	1.45492783	
P(T<=t) two-tail	0.24168579	
t Critical two-tail	3.1824463	

PC2 v UC-NOV		
	α depth	β Depth
Mean	0.24503948	0.17129
Variance	0.01284002	0.003934
Observations	4	4
t Stat	1.8603198	
P(T<=t) two-tail	0.15977926	
t Critical two-tail	3.1824463	

SC1 v UC-OCT		
	α depth	β Depth
Mean	0.22528214	0.139708
Variance	0.00808011	0.006078
Observations	4	4
t Stat	3.83130409	
P(T<=t) two-tail	0.03133285	
t Critical two-tail	3.1824463	

SC3 v UC-OCT		
	α depth	β Depth
Mean	0.16029832	0.130199
Variance	0.00058199	0.004117
Observations	4	4
t Stat	1.04049672	
P(T<=t) two-tail	0.37459367	
t Critical two-tail	3.1824463	

C1 v UC-AUG		
	+ Oxygen	- Oxygen
Mean	0.17326	0.17572
Variance	0.00055	0.001687
Observations	4	4
t Stat	-0.1678	
P(T<=t) two-tail	0.87742	
t Critical two-tail	3.18245	

PC2 v UC-NOV		
	+ Oxygen	- Oxygen
Mean	0.20948	0.206853
Variance	0.00709	0.013309
Observations	4	4
t Stat	0.09102	
P(T<=t) two-tail	0.93321	
t Critical two-tail	3.18245	

SC1 v UC-OCT		
	+ Oxygen	- Oxygen
Mean	0.18331	0.181682
Variance	0.00758	0.011458
Observations	4	4
t Stat	0.07603	
P(T<=t) two-tail	0.94418	
t Critical two-tail	3.18245	

SC3 v UC-OCT		
	+ Oxygen	- Oxygen
Mean	0.15038	0.140121
Variance	0.00255	0.002686
Observations	4	4
t Stat	0.71582	
P(T<=t) two-tail	0.52579	
t Critical two-tail	3.18245	

PC1 v UC-SEPT		
	α depth	β Depth
Mean	0.16695822	0.100511
Variance	0.00439469	0.002819
Observations	4	4
t Stat	2.48773806	
P(T<=t) two-tail	0.08866109	
t Critical two-tail	3.1824463	

PC1 v UC-SEPT		
	+ Oxygen	- Oxygen
Mean	0.14561	0.121856
Variance	0.00495	0.00483
Observations	4	4
t Stat	0.79702	
P(T<=t) two-tail	0.48369	
t Critical two-tail	3.18245	

SC2 v UC-NOV		
	α depth	β Depth
Mean	0.07768347	0.062856
Variance	0.00704334	0.00506
Observations	4	4
t Stat	1.15965112	
P(T<=t) two-tail	0.33010177	
t Critical two-tail	3.1824463	

SC2 v UC-NOV		
	+ Oxygen	- Oxygen
Mean	0.07228	0.068256
Variance	0.00599	0.006252
Observations	4	4
t Stat	0.37015	
P(T<=t) two-tail	0.73586	
t Critical two-tail	3.18245	