BIOREMEDIATION OF SULFUR ORGANIC COMPOUNDS

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ABSTRACT

BIOREMEDATION OF SULFUR ORGANIC COMPOUNDS

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In nature microorganism have been converting organic compounds into inorganic compounds. This natural process is currently used in practical applications by man. One of these applications is the clean up of soils contaminated with organic compounds, such as petroleum hydrocarbons. The three petroleum hydrocarbons in this project are 1-octane sulfonic acid, 1-propane sulfonic acid, and 1-hexane sulfonic acid and they contain a sulfonic acid. Microorganism in the soil will convert theses harmful petroleum hydrocarbons into harmless waste byproducts such as carbon dioxide. Respiration is the process the microorganism use to convert the organic contaminates into carbon. The amount of carbon released by microorganism can be measured by the amount carbon dioxide released in the soil. The carbon dioxide is converted to carbon by evolution of carbon dioxide method (a simple chemical titration process). The respiration process can be dependant on the amount of organic matter in the soil, moisture, the soil texture the contamination takes place in, the rate of contamination, and the type of contaminant. However, the amount of organic matter can significantly effect the respiration in the different soil textures.

ACKNOWLEDGEMENTS

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INTRODUCTION

Microbes are becoming extremely important for remediating up contaminated soil and water. Respiration is a natural process of bioremediation for microorganisms and is an important part of the remediation of organic and inorganic contamination. Organic contaminants (such as octane, propane, and hexane) are used for their carbon source and in the process are decomposed into safer compounds of CO_2 and H_2O.

The main product of soil respiration is carbon dioxide. Carbon dioxide can be trapped by either standardized potassium hydroxide or sodium hydroxide. Once the carbon is absorbed, the carbonates are precipitated with barium chloride. Then the concentration of carbon is measured by titrating the basic compound with standardized hydrochloric acid. The chemical equations for this procedure are listed.

$$CO_2 + 2\,NaOH \longleftrightarrow Na_2CO_3 + 2\,H_2O$$
$$Na_2CO_3 + BaCl_2 \longrightarrow BaCO_3\,(s) + 2\,NaCl$$
$$NaOH + HCl \longrightarrow NaCl + H_2O$$
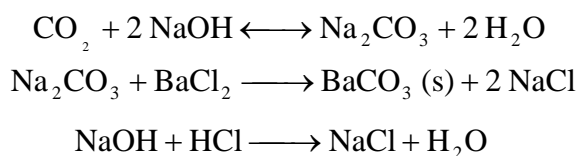
Soil texture is comprised of sand, silt, and clay. Sand particles are 2 to 0.05 mm in diameter, silt is 0.05 to 0.002 mm, and clay particles are less than 0.002 mm in diameter. Coarser soils contain a greater percent of sand while fine textured soils contain more clay. A soil with equal percentages of all three soil separates is called a loam. The percent of the soil separates in soil has a direct relationship on the amount of carbon dioxide respired. Soils with a higher percent sand contain more available oxygen for

microorganisms. However, sandy soils have a greater tendency to lose moisture faster. Clay soils have reduced available oxygen but can maintain a moderate amount of moisture.

Clay is the most interesting soil separate due to its high surface charge. Several types of clay exist and they are classified according to their crystalline structure. The types of clay particles are kaolinite, illite, vermiculite, montmorillonite, and chlorite. Kaolinitic clays have a low surface charge, while montmorillonite has a high surface charge. The higher surface charge causes organic particles to adhere strongly to the clay surfaces. This creates a problem for the soil microorganism to digest the contaminants and reduces the amount of carbon evolved during soil respiration.   Another chief concern of soils with too much highly surface charged clay is the possibility of a water saturation condition creating an anaerobic environment.

LITERATURE REVIEW

Bioremediation techniques are very promising, environmentally sound, less expensive, and have a wide range for the potential degradation of organic contaminants such as petroleum hydrocarbons. The major challenges of biological treatment are (1) heterogeneity of the contaminants, (2) extremity of the concentration of hydrocarbons, (3) site conditions, and (4) regulatory constraints (Balba et al., 1998). The questions for bioremediation applications are (1) what concentration of the contaminants can be obtained? (2) What is the fate of the contaminant(s)? (3) How much time is needed to obtain the set remediation goal? (4) What is the cost for this remediation (Jørgensen et al., 2000)?

Several methods are available to bioremediate a soil through ex situ or in situ techniques. Each technique changes the soil chemistry, physical structure, microorganisms, and cost (Table 1). Examples of ex situ methods are landfarming/solid phase, composting, biologically enhanced soil washing, and slurry bioreactors. *In situ* methods are done in place and consist of remediation of subsurface soil and groundwater (Balba et al., 1998). Biopiles are defined as the piling of the material to be biotreated by adding nutrients and air into piles or windrows usually to a height of 2 - 4 m. Landfarming is the older practice of treating oil wastes by adding oil sludge and nutrients to agricultural land and mixing by agricultural practices. Slurry-phase involves a water phase added to enhance the physical mixing. Treatment-bed occurs where usually only

nutrients are added and the bed (usually 0 - 1 m height) is agitated mechanically at intervals by a mixing device (Jørgensen et al., 2000).

Non- bioremediation methods can be costly, sometimes ineffective and consist of excavation and containment in landfills, vapor extraction, stabilization and solidification, soil flushing, soil washing, solvent extraction, thermal desorption, vitrification and incineration (Balba et al., 1998). The advantages of bioremediation over non-bioremediation methods are the soil remediation can be accomplished without excavating the site. It can be done below an existing building.

Sulfur

A large portion of the aquifers containing petroleum hydrocarbon contaminants contained sulfate. Microorganisms reducing sulfate often contribute to the degradation of petroleum hydrocarbons (Schroth et al., 2001). Sulfate reducers are strict anaerobes and produce biosurfactants improving the solubility of hydrophobic pollutants and thus their biodegradability (Grishchenkov et al., 2002). The presence of the acid catalyst *p*-toluene sulfonic acid caused an increase in the degradation rates of polystyrene (Karmore and Madras, 2001). Unfortunately, the interaction between sulfur compounds and bioremediation has not yet been fully explored and information on this subject is limited.

Table 1. Remediation methods and changes on soil characteristics and the estimated costs of treatment (adapted from Houghton, 1996)[†]

Treatment	soil chemistry change	physical structure change	Microorganisms altered	Approximate remediation cost
Removal to landfill	?	?	?	Up to 100 £/tonne
Solidification				
Cement and Pozzolan based	N‡	N	N	25 ± 175
Lime based	N	N	N	25 ± 50
Vitrification	N	N	N	50 ± 525
Physical processes				
Soil washing	Y	N	N	25 ± 150
Physical-chemical washing	Y	N	N	50 ± 175
Vapor extraction	Y	Y	Y	75
Chemical processes				
Solvent extraction	Y	N	?	50 ± 600
Chemical dehalogenation	Y	N	?	175 ± 450
In situ flushing	Y	Y	?	25 ± 80
Surface amendments	Y	Y	Y	10 ± 25
Thermal treatment				
Thermal desorption	Y	N	N	25 ± 225
Incineration	N	N	N	50 ± 1200
Biological treatments				
Windrow turning	Y	N	Y	10 ± 50
Land farming	Y	N	Y	10 ± 90
Bioventing	Y	Y	Y	15 ± 75
Bioslurry	Y	N	Y	50 ± 85
Biopiles	Y	N	Y	15 ± 35
In situ bioremediation	Y	Y	Y	175

[†] N indicates these factors will not generally survive in a particular treatment method. ‡ indicates they will generally survive. N indicates they will not survive. ? indicates the consequences are unclear. Source: Semple et al., 2001

Respiration

Soil respiration is the key parameter in the observation of soil micro flora. Microorganism activity is measured by the rate of CO_2 is released, or the O_2 consumption occurs. A maximum rate occurs at the beginning of contamination followed by decreased carbon dioxide production near the end of the treatment (Brohon et al., 2001); (Balba et al., 1998). Soil respiration provides a rapid time-course date for testing different biological treatment options including nutrient supplement and microbial inoculation (Balba et al., 1998). One of the advantages of respiration is it confirms active hydrogen degradation and can assess any possible inhibitory effects of organic and inorganic compounds and soil pH on soil microbial activities (Balba et al., 1998).

Coarser textures containing more sand have more active microbial populations and have higher soil respiration activity. Clay soils contain more soil organic carbon and tend to evolve more carbon through carbon dioxide during microbial respiration. The smaller soil particles (clay and silt) have higher surface activity and increase the protection from decomposition of soil organic matter physically and chemically (Franzluebbers, 1999).

Soils disturbed through human impact versus soils in their natural state will maintain the same rate of active microbial activity. One advantage of a disturbed soil is the overall sample distribution will have similar representation of the ecosystem. Less change of seasonal variation occurs due to soil dry or field moist conditions compared with disturbed soils indicating the initial water content is not needed for conducting a preliminary respiration assessment (Franzluebbers, 1999).

Currently, the two methods for soil respiration are the automated equipment and the simple respirometric flask. The automated equipment method measures the oxygen consumption using an U-tube based on barometric control reading the partial pressure of oxygen. The simple respirometric flask method utilizes a small amount of soil in a flask and measures the amount of evolved carbon dioxide trapped by sodium hydroxide (NaOH) or potassium hydroxide (KOH) after carbonates are precipitated with barium chloride (Balba et al., 1998).

Physical and Chemical Properties of Soil

The activity of microorganisms is heavily influenced by the physical and chemical properties of soil. Microorganisms have decreased activity if the moisture content is too dry or too wet, the pH is too acidic or too basic, the temperature is near freezing or freezing, the organic matter or nutrient content is low, low oxygen availability, and if the contaminant concentration is too high or too low. When the contaminant concentration is too low it will not provide enough sustenance for the microbial population to flourish. In addition, if the concentration is too high, a toxic reaction results and reduces or kills part of microbial population. Once a new carbon source is introduced into the soil by organic or inorganic contamination, the microbial population utilizes it as a new food source. An indicator of favorable microbial activity is an increased microbial count or increased soil respiration, especially for the first two weeks a contaminant has entered the environment (Margesin et al., 2000).

Texture plays an important role in microorganism activity. The type of soil particles (sand, silt, and clay) and the type of clay affect the degree of degradation. Sand

has a capacity to remove more organic contaminants compared to loam or clay soils. Soils containing moderate or high amounts of clay have decreased remediation effectiveness due to the clays highly charged nature. The clay particles bind the organic contaminates tightly decreasing the microorganism's ability to degrade the organic contaminants. As percent clay increases in the soil, the effectiveness of microorganisms to degrade contaminants and the remediation quality of the soil are decreased (Lee et al., 2002).

The percent of clay and the type of clay directly effect the activity of soil microorganisms. Soils with a high shrink-swell potential (such as montmorillonite) have a higher surface charge compared to soil of lower surface charge (such as kaolinite). The higher charge clay causes a stronger adherence of organic particles to the clay surfaces and inhibits the ability of soil microorganisms to digest the organic matter (Franzluebbers, 1999).

Hydrocarbons

Petroleum hydrocarbons enter the environment frequently and in large volumes through natural and human methods. Petroleum hydrocarbons are the product of crude oil refineries, auto exhaust, and the petrochemical industry (Mohan Rao et al., 1997). Marine environments may become contaminated by the seepage of natural petroleum deposits. The main contributor of contamination is human activities. Accidental spills result from production, transportation, and storage of petroleum. Another problem is the leakage of underground storage tanks at automobile service stations and petroleum pipelines (Balba et al., 1998). The main environmental concern is every 1.0 kilogram per

ton of crude oil corresponds to 11,500 tons of hydrocarbons being released into the atmosphere (Mohan Rao et al., 1997). One example of a major environmental catastrophe is the spillage of millions of gallons of crude oil during the Gulf War in 1991. The contamination spanned 49 square km and created 330 oil lakes (Balba et al., 1998).

Volatilization

Bioaccumulation

| Parent organic pollutant | Biological, chemical and physical processes | → | Degradation Product | → | Mineralization to CO_2 |

Intra-soil Processing

Leaching
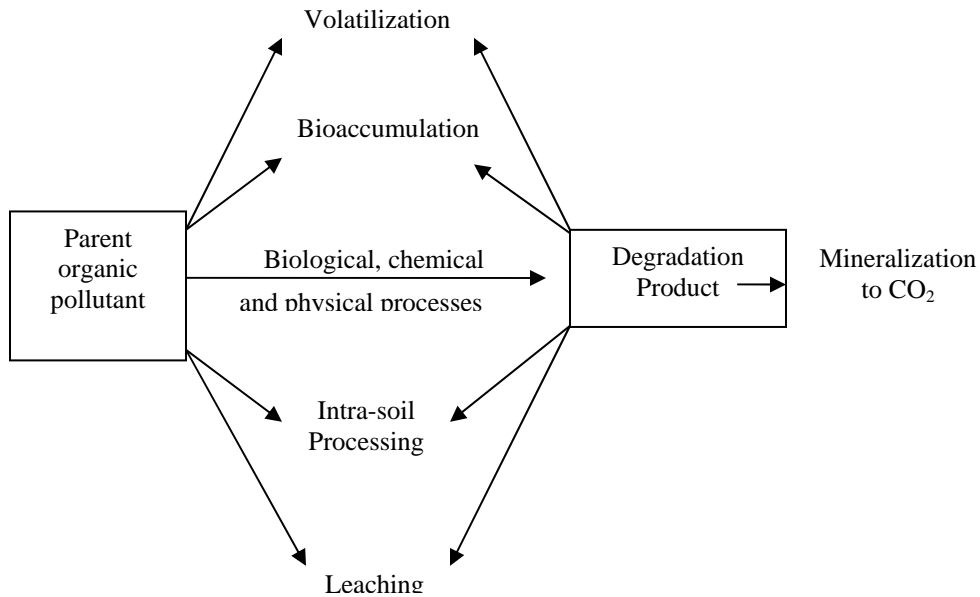
Fig. 1. Summary of the environmental fates of organic pollutants in soil (source: Semple et al., 2001).

In France, a contaminated agricultural soil contained a mixture of soil microorganism able to degrade hydrocarbons (Fig. 1). Bacterial counts increased after an oil treatment. The saturated alkane hydrocarbons were the most degraded while the linear and branched alkanes were partially degraded. The indigenous soil bacteria and fungi consisted of *Pseudomonas), Brevundimonas, Sphingomonas, Acinetobacter, Rhodococcus, Arthrobacter, Corynebacterium, Aspergillus niger, Pencillium restrictum, Brevundimonas Pseudomonas vesicularis, Trichoderma hazianum, T. koningii, Norcardia, Acinetobacter, Flavobacterium, Micrococcus, Corynebacterium,*

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Achromobacter, Alcaligenes, Mycobacterium, Bacillus, Aspergillus, Mucor, Fusarium, Penicillium, Rhodotorula, Candida, and *Sporobolomyce*s (Balba et al.,1998; Chaîneau et al., 1999).

Non-methane hydrocarbons are the precursors for ozone production at ground level (Stikkers, 2002). Some of the hydrocarbons are in the form of propane, *n*-octane, hexane and *n*-hexane. When hexane is inhaled in the form of *n*-hexane it causes numbness of the extremities, headaches, and fatigue (Senzolo et al., 2001). One method of introducing hydrocarbons into the atmosphere is by burning fossil fuels for gasoline by the emissions of vehicles. Octane and propane are chiefly found as contaminants in the atmosphere and propane was 40 % of the non-methane hydrocarbons released through vehicle exhaust. These pollutants are the main contributor to the creation of harmful ozone (Chang et al., 2001). The breakdown of crude oil to hydrocarbons during the consumption of gasoline is the primary cause of reduced air quality. "Gasoline is the most important product at most oil refineries. It consists of numerous compounds that can be broadly classified into hydrocarbon groups of paraffins, aromatics, and olefins. Production of gasoline consists of blending the output of several refining processes designed to break down crude oil into hydrocarbons from these broad groupings with the appropriate physical characteristics for use as gasoline" (Stikkers, 2002, 38).

Petroleum hydrocarbons are a mixture of saturates, aromatics, resins (nitrogen, sulfur, and oxygen), and asphaltenes. The saturates are comprised of straight alkane chains, branched alkanes, and cycloalkanes. The intermediate length alkanes (C_{10}-C_{20}) are the easiest to degrade. The shorter chain compounds (C_2-C_9) are harder to degrade

further and are more toxic to the environment. The longer alkane chains (C_{20}-C_{40}) are waxes causing them to be hydrophobic making them difficult to degrade. The aromatics consist of benzene, toluenes, xylems, thiophenes, dibenzothiopheres, and naphthenoaromatics (Balba et al., 1998). The intermediate length hydrocarbons (C_6-C_{10}) have higher concentrations in the atmosphere compared to the smaller length hydrocarbons (C_2-C_5) (Mohan Rao et al., 1997).

Heterogeneous mixtures

Substrate mixtures are commonly encountered in bioremediation and wastewater treatment. *P. putida* F1 can grow on benzene, toluene, phenol and their mixtures.   In mixture experiments, the rate of consumption of one substrate was influenced by the presence of the others, although the degree of influence varied widely. These substrates are catabolized. The same enzymatic pathway and the occurrence of contaminants in mixtures is an important problem due to the removal or degradation of one compound inhibited by another compound and because different conditions may be required to treat different compounds within the mixture. A mixture of homologous carbon and energy substrates on the biodegradation of a chemical can be positive or negative. Positive results include increased growth at low substrate concentrations and induction of required degradative enzymes. The negative concerns are decreased biodegradation rates, induced competitive inhibition, toxicity, and formation of toxic intermediates by non-specific enzymes (Reardon et al., 2000).

Mixed substrate competition and inhibition are recognized as potential problems in addressing the efficacy of biodegradation in nature. In a soil contaminated with

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toluene, benzene, and phenol, the toluene consumption began first and was depleted first. Benzene consumption began later, but degraded simultaneously with toluene. Phenol consumption did not occur until both the other substrates concentrations' were near zero. Toluene and benzene were better growth substrates than was phenol, resulting in faster growth on toluene and benzene. Toluene significantly inhibited the biodegradation rate of both benzene and phenol, and it slowed the consumption of phenol (but not benzene). Phenol did not impact the biodegradation of either toluene or benzene (Reardon et al., 2000).

Inoculation

Contaminated soils can be degraded through inoculation of bacteria containing biodegradative phenotypes. These biodegradative phenotypes can persist throughout treatment, compared to indigenous soil bacteria, leading to increased degradation of contaminants (Fava and Bertin, 1999). However, aged soils are more resistant to degradation even when biodegradative bacteria are introduced. Further study should be conducted to explore why this occurs.

One question can be posed for inoculating a contaminated environment for bioremediation. Does it matter if the inoculants should be indigenous or non-indigenous species? Normally, indigenous strains are superior to non-indigenous strains because they have already adapted to the natural environment. Both indigenous and non-indigenous inoculants maintained similar survival rates. Their growth rates were dependant upon the availability of the carbon source and the competition of nutrients with other microorganism (Blumenroth and Wagner-Döbler, 1998).

Preadapted Inocula

The number of microorganisms required to remediate an organic or carbon contaminated site needs to be known. While the physical-chemical conditions and the concentration of the substance to be tested can be controlled, the concentration of the bacterial inoculum is variable. The number of specific biodegraders should be tested. Adapting an inoculum to a specific biodegrading substance should be investigated. Inoculum density of a pure strain of *Pseudomonas putida* adapted to *para*-nitrophenol and its lag time were related. As the inoculum density increased, the lag time decreased, indicating the importance of allowing the bacteria to grow to a large enough number for detection of biodegradation. Comparing the results of *para*-nitrophenol degradation of a pure adapted strain of *P. putida* and a non-adapted mixed inocula (activated sludge and river waters) provided similar results. However, one concern of the raw inocula was introducing organic contamination to the substrate (Thouand et al., 1969).

GEMs

The introduction of pesticides and chemical waste products into the biosphere has led to a large accumulation of toxic chemicals. Currently, microbes have not had sufficient time to adapt to the proper mineralization or catabolic pathways to biodegrade these compounds. Designing more efficient bacteria for bioremediation is plausible due to their incredible metabolic and physiological versatility of using a variety of pollutants as a carbon sources or energy sources.

New research is leading the way in creating genetically engineered microorganisms (GEMs) including *Pseudomonas* sp. capable of degrading a single or a variety of substrates. GEMs need to survive in microcosms similar to contaminated groundwater, sediments, and sewage by using modified catabolic pathways for the degradation of a targeted substrate (Heuer et al., 1995). One example of GEM's failure to survive is *Pseudomonas putida* AC30(pMFB2) (a soil bacteria) introduced into an aquatic environment. Currently, several genetically modified Pseudomonads are being used for the degradation of toxic organic substances. The Pseudomonads' metabolic and catabolic pathways have been manipulated for biodegradation of contaminants (Heuer et al., 1995; Min et al., 1998). Examples of GEMs are *Pseudomonas putida* AC30(pMFB2) containing the rifampicin resistant gene and possessing the PCBs degrading gene (*bph*ABC)MFB2 on its plasmid and a chlorobenzoic acid degrading gene inserted into it's DNA. One drawback of a mixture of substrates for *P. putida* AC30(pMFB2) is it can not use biphenyl as its sole source of carbon and energy and needs an additional carbon and energy source to complete its metabolism of the toxic substrate (Min et al., 1998).

Some biological approaches to bioremediation problems include a) differences in carbon sources in wastes products, b) the concentration level of waste chemicals from high to low, c) the inhibition of mixtures on one another where toxic chemicals adversely hinder microbial processes or are poisonous to them, d) bioavailability where non-polar compounds rapidly adsorb onto particulate matter in soils, sediments, and water and thereby become less bioavailable to microbial systems, and e) slow degradation rates or slow rates of remediation exist. Various bacterial systems for expression of cloned genes have been developed and most are designed for lab research, not for use in the

environment. Most of these microbes have been cloned or produced for antibiotic resistance and should not be used as bioremediation tools to be released into the environment because they could cause antibiotic resistance (Timmis et al., 1994).

GEMs pose a potential problem if inoculated into the environment. Problems of their introduction could include a) a failure to accomplish their goal due to non-available elements limiting their survival and activity, b) if they survive, they may cause damage to the function and structure of the ecosystem, and c) their newly inserted metabolic and catabolic genes may be transferred to the indigenous bacteria with unknown consequences. It is important to predict the influence of a GEM on the natural ecosystem on a case by case basis (Min et al., 1998).

Conclusion

Bioremediation is an important process in the breakdown of organic contaminants (such as the petroleum hydrocarbons). Bioremediation is cost effective compared to industrial remediation methods and more environmentally sound. One advantage to bioremediation is it can be done in place or without the excavation of a site. Feasibility studies (using soil respiration methods) can be used to assess the impact of the contamination of the soil microorganisms. Microorganisms have great potential to convert contaminants to harmless compounds. However, they may have to be cultured to overcome the toxic effects of high concentrations of compounds. The effects of genetically modified microorganism can not yet be assessed.

MATERIALS AND METHODS

Determination of Organic Matter Colorimetric Method

A 2 *N* potassium dichromate, solution was prepared by dissolving 98.08 grams of $K_2Cr_2O_7$ in a 400 mL of deionized water. Using a hot plate, the salt brought to solution at medium room temperature with stirring. The solution was transferred to a 1 L volumetric flask and allowed to cool before diluting to volume with deionized water and mixing.

Each sample consisted of two grams of the soil ground with a mortar and pestle. Then, one gram of the ground soil was transferred into a 250 mL Erlenmeyer flask. Exactly five mL of 2 *N* potassium dichromate was added. Under the hood, ten mL of the concentrated sulfuric acid (not less than 96 percent H_2SO_4) was added and mixed for one minute. The solution stood for 30 minutes in the exhaust hood. When the solution has cooled, 195 mL of deionized water was added to the Erlenmeyer flask and mixed. Using a No. 1 Whatman filter paper filter, the solution was filtered. A cuvet tube was rinsed once with the solution to be tested and then refilled to measure the light transmittance at 625 nm.

Organic Matter Calculations:

$$\% \text{ OM} = \frac{(2.0 - \log \% \text{ T})}{0.048287}$$

$$\text{CO}_2 \text{ - C mg} = 200 \text{ g of soil x} \frac{1000 \text{ mg of soil}}{1 \text{ g of soil}} \text{ x} \frac{\% \text{ OM}}{100 \% \text{ Soil}} \text{ x} \frac{50 \% \text{ Carbon (C)}}{100 \% \text{ OM}}$$

Example Calculation for Carbon in Clay :

The percent transmittance was 82.4, inserting it into the equation yields:

$$\% \text{ OM} = \frac{(2.0 - \log 82.4)}{0.048287} = 1.74$$

$$200 \text{ g of soil x} \frac{1000 \text{ mg of soil}}{1 \text{ g of soil}} \text{ x} \frac{1.74 \% \text{ OM}}{100 \% \text{ Soil}} \text{ x} \frac{50 \% \text{ Carbon (C)}}{100 \% \text{ OM}} = 1740 \text{ mg CO}_2 \text{ - C}$$

Determination of Sulfate by turbidemetric and colorimetric method

The standard sulfate solution of 1000 ppm SO_4^{-2} S was made by dissolving 4.430 grams of anhydrous Na_2SO_4 with deionized water in a 1000 mL volumetric flask. Using the 1000 ppm SO_4^{-2} S, a 100 ppm solution was created with deionized water in a 100 mL volumetric flask. To create the calibration curve, the solution was diluted to two ppm, five ppm, 10 ppm, 20 ppm, 25 ppm, 30 ppm, 40 ppm, 50 ppm, 75 ppm, and 100 ppm of SO_4^{-2}-S were made. A blank was created and calibrated at 420 µm. Each of these, standards had exactly 10 mL transferred into a 50 mL beaker and the same amount of

barium chloride was added to each. The solutions were swirled until the crystals dissolved and allowed to stand for one minute before their % transmittance was measured.

Twenty grams of soil were added to 250 mL Erlenmeyer flask with 100 mL of deionized water and ten drops of 1:3 acetic acid. The flasks were shaken for five minutes and filtered with a Whatman No. 42 filter paper into a 250 mL beaker. Ten mL of the extract was pipetted into a 50 mL beaker and about 0.4 grams of barium chloride was added using the exact same amount for all samples. The beaker was swirled and the solution was allowed to stand one minute. The % transmittance was determined by comparing the solutions against a blank. If the transmittance was lower than 30 %, the sample was diluted and the new transmittance was recorded.

Determination of Sulfate by turbidemetric and colorimetric method:

% Transmittance = ppm of SO_4^{-2} extract

ppm SO_4^{-2} in dry soil = ppm of soil SO_4^{-2} extract x dilution x 5

Example calculation for clay with 1500 ppm 1-hexane sulfonic acid repetition A:

83.0 % T = 4.6 ppm of SO_4^{-2} extract

4.6 ppm of SO_4^{-2} extract x 10 dilution x 5 = 230 ppm SO_4^{-2} in dry soil

Evolution of Carbon dioxide

Two hundred grams of clay, clay loam, and a loam soils were each sieved and added to separate pint sized plastic containers for each texture. An additional amount of 0.5 grams of bacto-peptone was added to the soil to facilitate microbial growth. The experiment consisted of a control, low and high applications of each contaminant. The low rates of contaminant consisted of 0.3 grams (1500 ppm) of 1-octane sulfonic acid, 1-propane sulfonic acid, or 1-hexane sulfonic acid. The high contaminate rate consisted of 0.6 grams (3000 ppm) of 1-octane sulfonic acid, 1-propane sulfonic acid, or 1-hexane sulfonic acid. The soils were separated by textures (clay, clay loam, and loam). After all the materials were added and mixed into each soil, two glass vials were inserted into the soil, which supported each vertically. Deionized water was used to bring the soils to field capacity. Than 10 mL of 1 N NaOH was added to each vial using a burette. Cellophane and rubber bands were placed over the containers to allow for diffusion of oxygen into the container and reduction of moisture loss. The experiment was performed using three repetitions of each trial.

The following week, the NaOH was transferred into a 250 mL Erlenmeyer flask with the addition of 10 mL of barium chloride at pH 7. A few drops of the acid-base indicator phenolphthalein were added. The solution was titrated with HCl until it reached a neutral pH. The following weeks, the samples were weighed and brought to field capacity with deionized water. However, only one of the vials was filled with 10 mL of NaOH. The container was resealed with cellophane and a rubber band and allowed to continue the incubation.

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Calculation for Evolution of Carbon from Carbon Dioxide

$$\text{meq.}\,CO_2 = (\text{ml of NaOH} \times \text{N NaOH}) - (\text{ml of HCl} * \text{N HCl})$$

$$\text{mg}\,CO_2\text{-C evolved} = \text{meq.}\,CO_2\text{-C} \times \frac{6\,\text{mg carbon}}{1\,\text{meq.}\,CO_2\text{-C}}$$

* atomic weight of carbon dioxide is 44, but the atomic weight of carbon dioxide-
carbon is 12. One meq. of CO_2–C is 12 mg C $\div$ 2 = 6 mg of CO_2–C.

Example calculation for Clay containing 1500 Hexane (Week 1 – Rep. A):

$$(23.4\,\text{ml NaOH} \times 0.9671\,\text{N NaOH}) - (0.20\,\text{mL HCl} \times 0.5956\,\text{N HCl}) = 22.51\,\text{meq.}\,CO_2\text{-C}$$

$$22.51\,\text{meq of}\,CO_2 * \frac{6\,\text{mg of carbon}}{1\,\text{meq}\,CO_2\text{-C}} = 135.06\,\text{mg of}\,CO_2\text{-C}$$

Half Life Calculations:

Decay constant (k):

$$k = \frac{2.303}{\text{late time - early time}} \times \left[\log\left(\frac{\text{early concentration}}{\text{late concentration}} \right) \right]$$

$$\text{Concentration} = \text{mg of}\,CO_2\text{-C remaining in soil} / \text{mg}\,CO_2\text{-C from OM}$$

* The original early concentration (C_0) was determined by taking the amount of
CO_2-C from the humus and subtracting the amount of CO_2-C from the sample.
The late concentration ($C_{(0-x)}$) is determined by subtracting the early concentration
with amount of CO_2-C from the sample for that week. For the following
equations the late concentration is substituted for the early concentration.

half life (t ½)

$$t_{1/2} = \frac{0.693}{k}$$

Example calculation for loam control soil:

Week 0 $C_0 = 3030$ mg CO_2-C from OM/ 3030 mg CO_2-C from OM= 1.00

Week 1 $C_0 = 1$

         $C_{(0-x)}$ = 2884.94 mg CO_2-C evolved/ 3030 mg CO_2-C from OM

         = 0.95

$$k = \frac{2.303}{\text{Week 1 - Week 0}} \times \left[\log\left(\frac{1.00}{0.95}\right) \right] = 0.491$$

$$t_{1/2} = \frac{0.693}{0.491} = 14.1$$

OBJECTIVES OF RESEARCH

The objective of this research was to study the respiration rates of the petroleum hydrocarbons containing a sulfonic acid functional group. Questions were addressed. How did soil texture change the rate of soil respiration? What was the difference in respiration rates among the three petroleum hydrocarbons used in this study? Did the different rates of contamination induce or inhibit the rate of respiration? What was the relationship between the concentration of sulfur released by mineralization and the rate of respiration of the different textures, petroleum hydrocarbons, and rates of contamination? What differences existed in the carbon to sulfur ratio between the textures, petroleum hydrocarbons, and rates of contamination? What were half life values and their relationship with the soil texture, petroleum hydrocarbons, and the rate of concentration of the petroleum hydrocarbons?

Due to time constraints, further questions could not to be addressed. How much did the percent organic matter effect the outcome of the experiment? At what rate was the sulfonic acid functional group converted to sulfate? How did this rate compare to soil respiration rates over time and to the half life of each compound? What effect did the sulfonic acid portion of the petroleum hydrocarbons have on the rate of respiration?

RESULTS

The clay soil had 1.74 % organic matter (OM) or 0.87 % carbon (C) and 1740 mg of carbon (C). The clay soil had 0.27 % sulfur (S) or 0.53 mg S and a carbon to sulfur ratio (C/S) of 3283. The clay loam soil had 1.03 % OM, 0.52 % C, and contained 1030 mg of C. The sulfur percentage were 0.37 % S, 0.73 mg of S, and a 1411C/S. The loam soil had 3.03 % OM, 1.52 % C, and contained 3030 mg of C. The loam soil also contained 0.60 % S, 1.20 mg of S, and a 2525 C/S ratio (Table 2).

The three contaminates used in this project were 1-propane sulfonic acid, 1-hexane sulfonic acid, and 1-octane sulfonic acid. The 1-propane sulfonic acid contained 35.02 % C, 32.38 % S, and a 1.50 C/S. The 1-hexane sulfonic acid contained 43.09 % C, 19.17 % S, and a 2.25 C/S. The 1-octane sulfonic acid had 48.95 % C, 16.33 % S, and a 3.00 C/S (Table 2).

Evolution of Carbon dioxide

The three soils were allowed to respired carbon dioxide (CO_2) over a sixteen week period. The CO_2were converted to milligrams (mg) of carbon. Measurements were taken at 1 week intervals for the first five weeks, at week ten, and at week sixteen (Table 3).

Table 2. The % OM, mg C, mg S of the control soils, and the % C, % S, and C/S ratio of the control soils and the contaminants.

	OM	C	C	S	S	C/S	
	%	%	mg	%	mg		
Clay	1.74	0.87	1740	0.27	0.53	3283	:1
Clay Loam	1.03	0.52	1030	0.37	0.73	1411	:1
Loam	3.03	1.52	3030	0.60	1.20	2525	:1
1-propane sulfonic acid		35.02		23.38		1.50	:1
1-hexane sulfonic acid		43.09		19.17		2.25	:1
1-octane sulfonic acid		48.95		16.33		3.00	:1

During the first five weeks of the clay control soil it expired 127.91, 40.05, 12.16, 11.57, and 9.66 mg CO_2-C and at week ten and sixteen 71.70 and 55.60 mg of carbon from carbon dioxide (CO_2-C). The clay textured soil containing 1500 ppm of 1-propane sulfonic acid (Propane) expired 129.01, 14.79, 20.86, 12.52, 10.86, 32.85, and 46.50 mg of CO_2-C. The 3000 ppm of Propane expired 122.81, 18.48, 44.68, 26.46, 19.08, 37.23, and 46.24 mg CO_2-C. The 1500 ppm of 1-hexane sulfonic acid (Hexane) expired 129.29, 16.81, 30.39, 22.41, 12.17, 44.98, and 39.67 mg of CO_2-C. The 3000 ppm of Hexane expired 90.44, 16.33, 64.66, 56.12, 30.51, 53.74, and 37.99 mg of CO_2-C. The 1500 ppm of 1-octane sulfonic acid (Octane) expired 137.21, 6.69, 24.91, 21.10, 15.03, 36.73, and 43.63 mg of CO_2-C. The 3000 ppm of Octane expired 138.32, 2.28, 15.14, 30.39, 18.60, 53.40, and 42.79 mg of CO_2-C.

The clay loam control sample expired 134.44, 46.96, 20.98, 13.71, 10.86, 75.33, and 57.11 mg of CO_2-C. The 1500 ppm of Propane expired 130.23, 22.17, 42.18, 14.07, 12.05, 36.05, and 39.25 mg of CO_2-C. The 3000 ppm of Propane expired 115.52, 13.71,

60.24, 47.78, 19.79, 39.59, and 41.19 mg of CO_2-C. The 1500 ppm of Hexane expired 129.16, 4.54, 52.19, 32.89, 12.88, 46.83, and 30.41 mg of CO_2-C. The 3000 ppm of Hexane expired 94.97, 2.10, 62.43, 57.76, 47.78, 55.09, and 32.77 mg of CO_2-C. The 1500 ppm of Octane expired 122.64, 16.21, 40.75, 37.18, 35.39, 40.94, and 39.17 mg of CO_2-C. The 3000 ppm of Octane expired 134.25, 18.90, 41.94, 58.62, 46.00, 59.44, and 39.59 mg of CO_2-C.

The loam control respired 145.06, 56.37, 18.84, 19.31, 16.22, 100.47, and 86.76 mg of CO_2-C. The 1500 ppm of Propane expired 148.90, 13.71, 38.97, 21.69, 16.69, 66.61, and 57.45 mg of CO_2-C. The 3000 ppm of Propane expired 146.19, 11.57, 44.92, 47.19, 35.99, 56.27, and 57.45 mg of CO_2-C. The 1500 ppm of Hexane expired 162.75, 8.95, 33.61, 23.13, 17.05, 68.84, and 45.99 mg of CO_2-C. The 3000 ppm of Hexane expired 118.59, 78.47, 65.31, 30.75, 19.08, 67.37, and 48.10 mg of CO_2-C. The 1500 ppm of Octane expired 151.58, 17.29, 32.06, 26.22, 16.81, 55.59, and 57.28 mg of CO_2-C. The 3000 ppm of Octane expired 160.10, 15.46, 22.65, 38.97, 23.72, 64.59, and 55.66 mg of CO_2-C.

Half Life

Half life is the rate for the decomposition of organic matter over time. It indicates the decay rate of substance. The higher the half life rate the longer it takes for the substance to break down and the lower the half life value the quicker a substance will break down. Following is the half life values the clay (Table 4), clay loam (Table 5), and loam samples (Table 6).

The clay control half life over sixteen weeks ranged from 9.1, 27.5, 89.2, 93.1, and 110.7 over the first five weeks. At ten weeks the half life values were 72.6 and at sixteen weeks it were 107.6 weeks. The half life values for 1500 ppm of Propane were 9.0, 75.1, 52.7, 86.8, 99.4, 161.9, and 133.7 weeks. The 3000 ppm of Propane half life values were 9.5, 60.3, 24.4, 40.3, 55.1, 138.6, and 130.2 weeks. The 1500 ppm of Hexane half life values were 9.0, 66.0, 36.0, 48.0, 87.4, 116.0, and 154.4 weeks. The 3000 ppm of Hexane half life values were 13.0, 69.6, 17.2, 19.0, 34.0, 93.8, and 154.2 weeks. The 1500 ppm of Octane half life values were 8.4, 165.8, 44.0, 51.2, 71.1, 143.1, and 140.7 weeks. The 3000 ppm of Octane half life values were 8.4, 486.9, 72.8, 35.8, 57.5, 97.9, and 142.9 weeks.

The half life of the clay loam control for the first five weeks were 5.0, 12.9, 27.7, 41.5, and 51.6 weeks. At ten weeks the half life values was 35.2 and at sixteen weeks it was 50.9 weeks. The half life values for the clay loam 1500 ppm of Hexane were 5.1, 27.8, 14.1, 40.8, 46.9, 76.0, and 79.8 weeks. The 3000 ppm of Propane half life values were 5.8, 45.9, 10.0, 11.8, 27.4, 65.9, and 71.9 weeks. The 1500 ppm of Hexane half life values were 5.2, 137.1, 11.5, 17.4, 43.3, 57.3, and 100.6 weeks. The 3000 ppm of Hexane half life values were 7.2, 308.3, 10.0, 10.1, 11.4, 46.4, and 88.0 weeks. The 1500 ppm of Octane half life values were 5.5, 38.4, 14.8, 15.5, 15.6, 64.1, and 76.1 weeks. The 3000 ppm of Octane half life values were 5.0, 32.5, 14.1, 9.5, 11.3, 40.8, and 68.3 weeks.

Table 3. The amount of carbon evolved from carbon dioxide during microbial respiration of clay, clay loam, and loam soils and these soils contaminated with 1500 and 3000 ppm 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).

					Week			
Amendment		1	2	3	4	5	10	16
					CO_2-C			
					-------mg-------			
Clay	Control	127.91	40.05	12.16	11.57	9.66	71.70	55.60
	1500 ppm Propane	129.01	14.79	20.86	12.52	10.86	32.85	46.50
	3000 ppm Propane	122.81	18.48	44.68	26.46	19.08	37.23	46.24
	1500 ppm Hexane	129.29	16.81	30.39	22.41	12.17	44.98	39.67
	3000 ppm Hexane	90.44	16.33	64.66	56.12	30.51	53.74	37.99
	1500 ppm Octane	137.21	6.69	24.91	21.10	15.03	36.73	43.63
	3000 ppm Octane	138.32	2.28	15.14	30.39	18.60	53.40	42.79
Clay Loam	Control	134.44	46.96	20.98	13.71	10.86	75.33	57.11
	1500 ppm Propane	130.23	22.17	42.18	14.07	12.05	36.05	39.25
	3000 ppm Propane	115.52	13.71	60.24	47.78	19.79	39.59	41.19
	1500 ppm Hexane	129.16	4.54	52.19	32.89	12.88	46.83	30.41
	3000 ppm Hexane	94.97	2.10	62.43	57.76	47.78	55.09	32.77
	1500 ppm Octane	122.64	16.21	40.75	37.18	35.39	40.94	39.17
	3000 ppm Octane	134.25	18.90	41.94	58.62	46.00	59.44	39.59
Loam	Control	145.06	56.37	18.84	19.31	16.22	100.47	86.76
	1500 ppm Propane	148.90	13.71	38.97	21.69	16.69	66.61	57.45
	3000 ppm Propane	146.19	11.57	44.92	47.19	35.99	56.27	57.45
	1500 ppm Hexane	162.75	8.95	33.61	23.13	17.05	68.84	45.99
	3000 ppm Hexane	118.59	78.47	65.31	30.75	19.08	67.37	48.10
	1500 ppm Octane	151.58	17.29	32.06	26.22	16.81	55.59	57.28
	3000 ppm Octane	160.10	15.46	22.65	38.97	23.72	64.59	55.66

* 1-propane sulfonic acid (propane), 1-hexane sulfonic acid (Hexane),and 1-octane sulfonic acid (Octane)

The half life values of the loam control were 14.1, 35.1, 103.7, 100.5, 118.9, 93.9, and 126.0 weeks. The 1500 ppm of Propane half life values were 13.7, 145.2, 50.6, 90.0, 116.2, 143.4, and 195.0 weeks. The 3000 ppm of Propane half life values were 14.0, 172.4, 44.0, 41.2, 53.2, 167.2, and 192.4 weeks. The 1500 ppm of Hexane half life values were 12.5, 221.7, 58.6, 84.3, 113.5, 138.4, and 243.4 weeks. The 3000 ppm of Hexane half life values were 17.4, 27.1, 29.8, 62.1, 99.2, 138.3, and 227.4 weeks. The 1500 ppm of Octane half life values were 13.5, 115.0, 61.5, 74.4, 115.2, 171.9, and 196.1 weeks. The 3000 ppm of Octane half life values were 12.8, 85.4, 86.7, 49.9, 81.0, 146.4, and 199.3 weeks.

Table 4. The half life data for the clay soil and the clay soil contaminated with 1500 ppm and 3000 pm 1-proprane sulfonic acid (Propane),   1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).

	Week	c early	c late	k	$t_{1/2}$		Week	c early	c late	k	$t_{1/2}$
		--------------mg--------------		-----------weeks---------				----------------mg----------------		-------------weeks----------------	
Control	0		1740.00								
	1	1740.00	1612.09	0.0764	9.1						
	2	1612.09	1572.04	0.0252	27.5						
	3	1572.04	1559.87	0.0078	89.2						
	4	1559.87	1548.30	0.0074	93.1						
	5	1548.30	1538.64	0.0063	110.7						
	10	1538.64	1466.94	0.0095	72.6						
	16	1466.94	1411.34	0.0064	107.6						
1500 ppm Propane	0		1740.00			3000 ppm Propane	0		1740.00		
	1	1740.00	1610.99	0.0770	9.0		1	1740.00	1617.19	0.0732	9.5
	2	1610.99	1596.21	0.0092	75.1		2	1617.19	1598.71	0.0115	60.3
	3	1596.21	1575.35	0.0132	52.7		3	1598.71	1554.02	0.0284	24.4
	4	1575.35	1562.82	0.0080	86.8		4	1554.02	1527.56	0.0172	40.3
	5	1562.82	1551.97	0.0070	99.4		5	1527.56	1508.49	0.0126	55.1
	10	1551.97	1519.12	0.0043	161.9		10	1508.49	1471.26	0.0050	138.6
	16	1519.12	1472.62	0.0052	133.7		16	1471.26	1425.01	0.0053	130.2
1500 ppm Hexane	0		1740.00			3000 ppm Hexane	0		1740.00		
	1	1740.00	1610.71	0.0772	9.0		1	1740.00	1649.56	0.0534	13.0
	2	1610.71	1593.90	0.0105	66.0		2	1649.56	1633.22	0.0100	69.6
	3	1593.90	1563.51	0.0193	36.0		3	1633.22	1568.56	0.0404	17.2
	4	1563.51	1541.10	0.0144	48.0		4	1568.56	1512.44	0.0364	19.0
	5	1541.10	1528.93	0.0079	87.4		5	1512.44	1481.93	0.0204	34.0
	10	1528.93	1483.95	0.0060	116.0		10	1481.93	1428.19	0.0074	93.8
	16	1483.95	1444.28	0.0045	153.4		16	1428.19	1390.20	0.0045	154.2
1500 ppm Octane	0		1740.00			3000 ppm Octane	0		1740.00		
	1	1740.00	1602.79	0.0822	8.4		1	1740.00	1601.68	0.0828	8.4
	2	1602.79	1596.11	0.0042	165.8		2	1601.68	1599.41	0.0014	486.9
	3	1596.11	1571.20	0.0157	44.0		3	1599.41	1584.26	0.0095	72.8
	4	1571.20	1550.10	0.0135	51.2		4	1584.26	1553.87	0.0194	35.8
	5	1550.10	1535.07	0.0097	71.1		5	1553.87	1535.27	0.0120	57.5
	10	1535.07	1498.34	0.0048	143.1		10	1535.27	1481.87	0.0071	97.9
	16	1498.34	1454.71	0.0049	140.7		16	1481.87	1439.08	0.0049	141.9

Table 5. The half life data for the clay loam soil and the clay loam soil contaminated with 1500 ppm and 3000 pm 1-proprane sulfonic acid (Propane),   1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).

	Week	c early	c late	k	t $_{1/2}$		Week	c early	c late	k	t $_{1/2}$
		--------------mg---------------		----------weeks----------				----------------mg----------------		-------------weeks---------------	
Control	0	0.00	1030.00								
	1	1030.00	895.56	0.1399	5.0						
	2	895.56	848.60	0.0539	12.9						
	3	848.60	827.62	0.0250	27.7						
	4	827.62	813.90	0.0167	41.5						
	5	813.90	803.05	0.0134	51.6						
	10	803.05	727.72	0.0197	35.2						
	16	727.72	670.60	0.0136	50.9						
1500 ppm Propane	0	0.00	1030.00			3000 ppm Propane	0	0.00	1030.00		
	1	1030.00	899.77	0.1352	5.1		1	1030.00	914.48	0.1190	5.8
	2	899.77	877.60	0.0250	27.8		2	914.48	900.77	0.0151	45.9
	3	877.60	835.42	0.0493	14.1		3	900.77	840.53	0.0692	10.0
	4	835.42	821.34	0.0170	40.8		4	840.53	792.75	0.0585	11.8
	5	821.34	809.30	0.0148	46.9		5	792.75	772.96	0.0253	27.4
	10	809.30	773.24	0.0091	76.0		10	772.96	733.37	0.0105	65.9
	16	773.24	733.99	0.0087	79.8		16	733.37	692.18	0.0096	71.9
1500 ppm Hexane	0	0.00	1030.00			3000 ppm Hexane	0	0.00	1030.00		
	1	1030.00	900.84	0.1340	5.2		1	1030.00	935.03	0.0968	7.2
	2	900.84	896.30	0.0051	137.1		2	935.03	932.93	0.0022	308.3
	3	896.30	844.11	0.0600	11.5		3	932.93	870.51	0.0693	10.0
	4	844.11	811.22	0.0398	17.4		4	870.51	812.74	0.0687	10.1
	5	811.22	798.34	0.0160	43.3		5	812.74	764.96	0.0606	11.4
	10	798.34	751.50	0.0121	57.3		10	764.96	709.87	0.0150	46.4
	16	751.50	721.09	0.0069	100.6		16	709.87	677.10	0.0079	88.0
1500 ppm Octane	0	0.00	1030.00			3000 ppm Octane	0	0.00	1030.00		
	1	1030.00	907.36	0.1268	5.5		1	1030.00	895.75	0.1397	5.0
	2	907.36	891.14	0.0180	38.4		2	895.75	876.85	0.0213	32.5
	3	891.14	850.39	0.0468	14.8		3	876.85	834.91	0.0490	14.1
	4	850.39	813.21	0.0447	15.5		4	834.91	776.29	0.0728	9.5
	5	813.21	777.81	0.0445	15.6		5	776.29	730.29	0.0611	11.3
	10	777.81	736.88	0.0108	64.1		10	730.29	670.86	0.0170	40.8
	16	736.88	697.71	0.0091	76.1		16	670.86	631.27	0.0101	68.3

Table 6. The half life data for the loam soil and the loam soil contaminated with 1500 ppm and 3000 pm 1-proprane sulfonic acid (Propane),   1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).

	Week	c early	c late	k	$t_{1/2}$		Week	c early	c late	k	$t_{1/2}$
		--------------mg--------------		----------weeks----------				----------------mg---------------		------------weeks---------------	
Control	0		3030.00								
	1	3030.00	2884.94	0.0491	14.1						
	2	2884.94	2828.57	0.0197	35.1						
	3	2828.57	2809.73	0.0067	103.7						
	4	2809.73	2790.42	0.0069	100.5						
	5	2790.42	2774.20	0.0058	118.9						
	10	2774.20	2673.74	0.0074	93.9						
	16	2673.74	2586.97	0.0055	126.0						
1500 ppm Propane	0		3030.00			3000 ppm Propane	0		3030.00		
	1	3030.00	2881.10	0.0504	13.7		1	3030.00	2883.81	0.0495	14.0
	2	2881.10	2867.38	0.0048	145.2		2	2883.81	2872.24	0.0040	172.4
	3	2867.38	2828.42	0.0137	50.6		3	2872.24	2827.31	0.0158	44.0
	4	2828.42	2806.72	0.0077	90.0		4	2827.31	2780.13	0.0168	41.2
	5	2806.72	2790.03	0.0060	116.2		5	2780.13	2744.14	0.0130	53.2
	10	2790.03	2723.42	0.0048	143.4		10	2744.14	2687.87	0.0041	167.2
	16	2723.42	2665.97	0.0036	195.0		16	2687.87	2630.42	0.0036	192.4
1500 ppm Hexane	0		3030.00			3000 ppm Hexane	0		3030.00		
	1	3030.00	2867.25	0.0552	12.5		1	3030.00	2911.41	0.0399	17.4
	2	2867.25	2858.30	0.0031	221.7		2	2911.41	2837.83	0.0256	27.1
	3	2858.30	2824.69	0.0118	58.6		3	2837.83	2772.51	0.0233	29.8
	4	2824.69	2801.57	0.0082	84.3		4	2772.51	2741.76	0.0112	62.1
	5	2801.57	2784.52	0.0061	113.5		5	2741.76	2722.69	0.0070	99.2
	10	2784.52	2715.68	0.0050	138.4		10	2722.69	2655.32	0.0050	138.3
	16	2715.68	2669.69	0.0028	243.4		16	2655.32	2607.22	0.0030	227.4
1500 ppm Octane	0		3030.00			3000 ppm Octane	0		3030.00		
	1	3030.00	2878.42	0.0513	13.5		1	3030.00	2869.90	0.0543	12.8
	2	2878.42	2861.13	0.0060	115.0		2	2869.90	2846.71	0.0081	85.4
	3	2861.13	2829.07	0.0113	61.5		3	2846.71	2824.07	0.0080	86.7
	4	2829.07	2802.85	0.0093	74.4		4	2824.07	2785.10	0.0139	49.9
	5	2802.85	2786.04	0.0060	115.2		5	2785.10	2761.38	0.0086	81.0
	10	2786.04	2730.45	0.0040	171.9		10	2761.38	2696.79	0.0047	146.4
	16	2730.45	2673.17	0.0035	196.1		16	2696.79	2641.13	0.0035	199.3

DISCUSSION

Organic Matter

Organic matter played an important role in the respiration of the three different soil textures. When a soil has more organic matter it increases the amount of food or carbon for the microorganisms. The loam soil had the highest amount of organic matter, 3030 mg C, indicating the highest amount of carbon initially in the soil (Figure 2). The clay loam had the lowest percent organic matter and had the smallest amount of carbon initially in the soil. The more organic matter in the soil the respiration will occur.

The hydrocarbon petroleum contaminates were harder to separate out when comparing their carbon percentage. However, the different rates were clearly noticeable in each soil texture. The all samples with 1500 ppm of petroleum hydrocarbons had higher percent carbon remaining in the soil compared to all the samples with 3000 ppm of petroleum hydrocarbons.

The data indicated there was no correlation between the three different contaminants. In the loam soil Octane had the highest remaining carbon compared to the Hexane and Propane. However, the 1500 ppm of Hexane was higher than the 1500 ppm Propane and the 3000 ppm Propane was higher than the 3000 ppm Hexane (Figure 3).

The clay loam samples indicated the most noticeable trend compared to the loam and clay soil samples. Both the 1500 ppm and 3000 ppm of Propane had a higher % C

than the 1500 ppm and the 3000 ppm of Hexane and Octane. The 1500 ppm and 3000 ppm of Hexane more % C than the 1500 ppm and 3000 ppm of Octane (Figure 4).

Both rates of Hexane in the clay samples contained the least % C compared to the Propane and Octane samples. However, the 1500 ppm Propane contained more % C than the 1500 ppm Octane and the 3000 ppm Octane contained more % C than the 3000 ppm Propane (Figure 5).
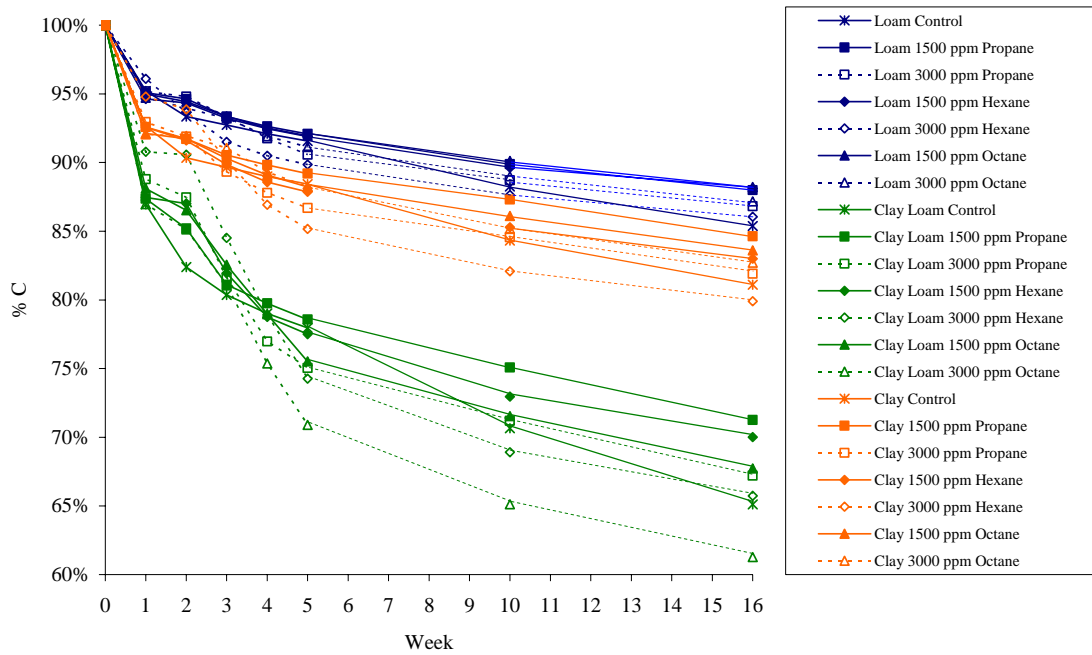

Figure 2. The percent carbon over 16 weeks of respiration for the clay, clay loam, and loam soilsand these soils contaminated with 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).
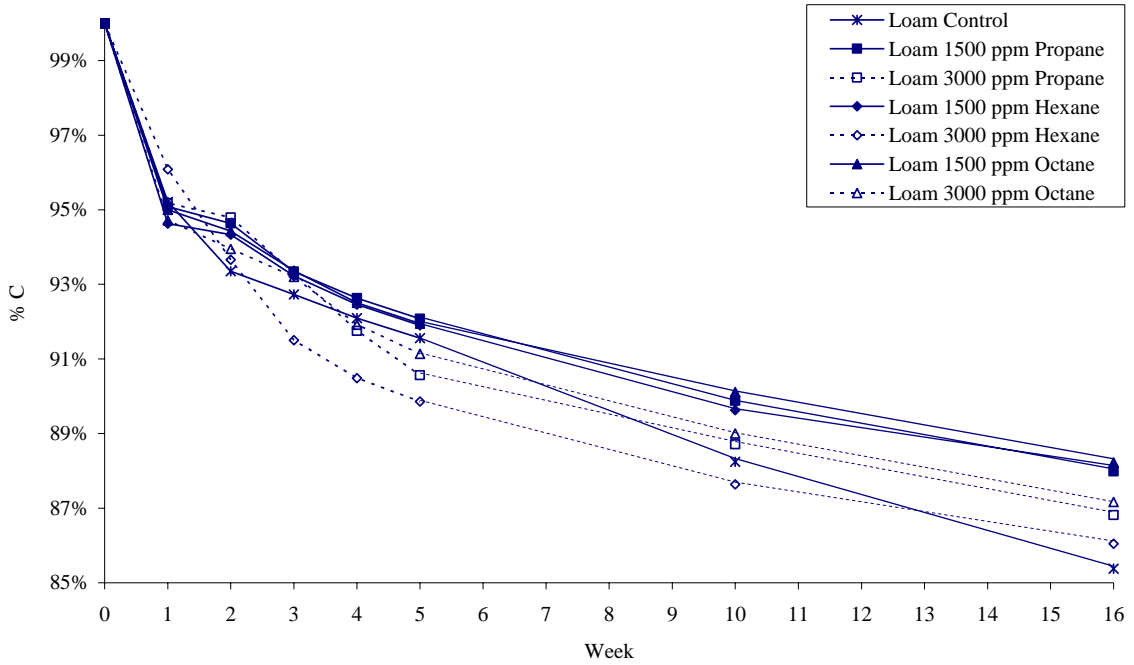
Figure 3. The percent carbon over 16 weeks of respiration for the loam soil and the loam soil contaminated with 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).
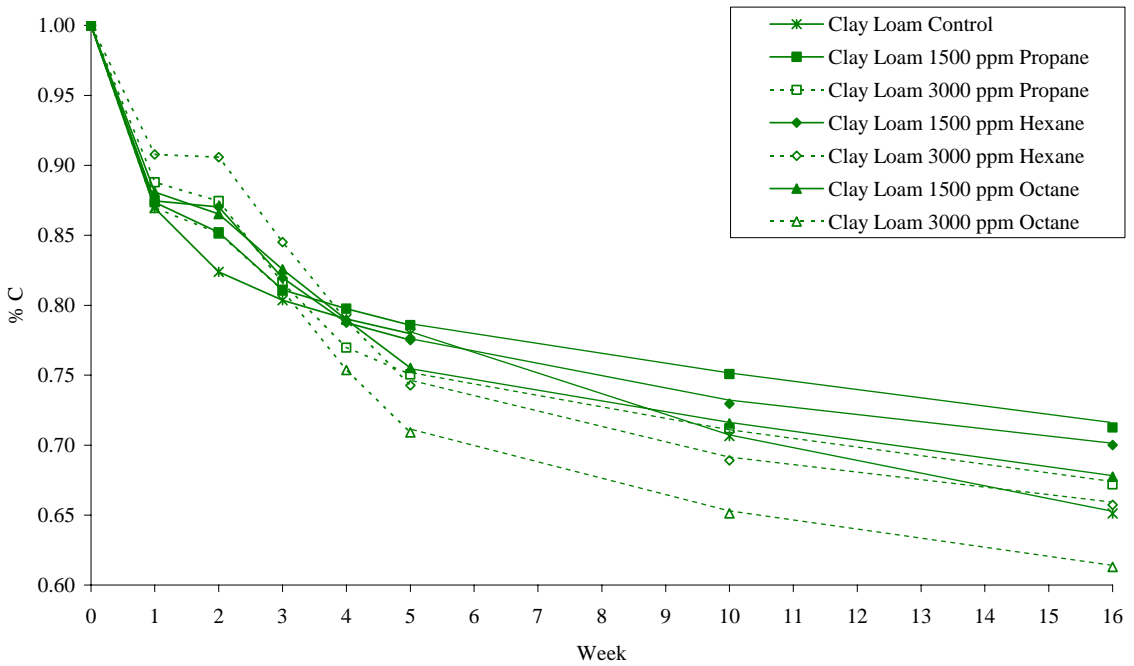

Figure 4. The percent carbon over 16 weeks of respiration for the clay loam soil and the clay loam soil contaminated with 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).
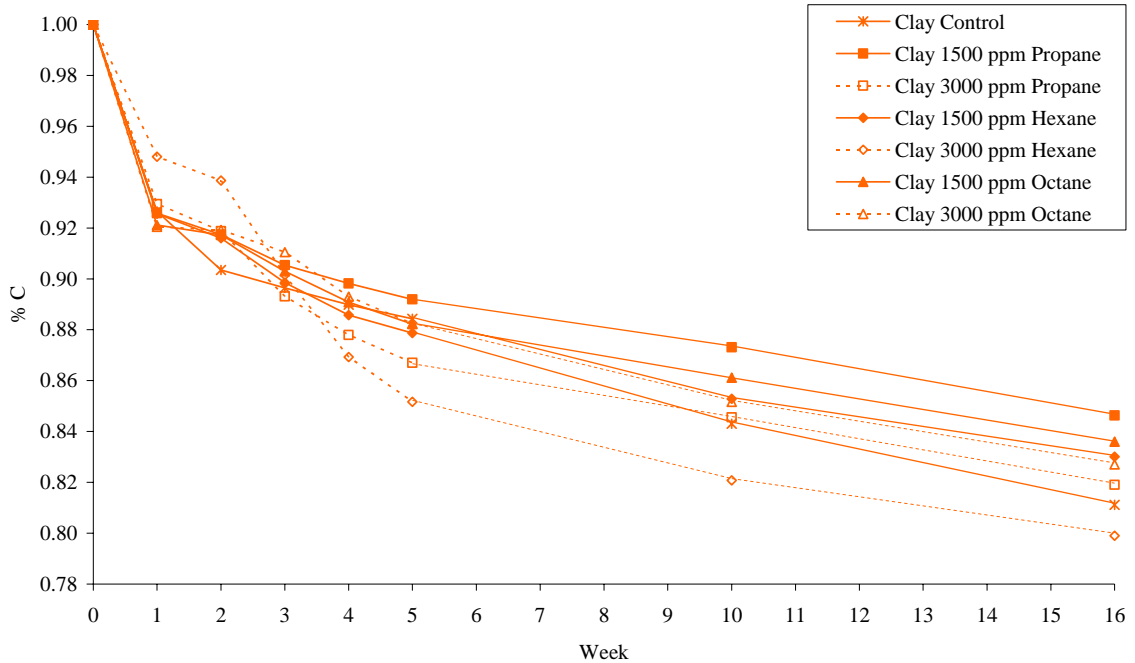
Figure 5. The percent carbon over 16 weeks of respiration for the clay soil and the clay soil contaminated with 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).

Sulfate

The loam control sample mineralized the highest amount of sulfate (SO_4^{-2}) compared to the clay and clay loam control samples. The clay control mineralized the least amount of SO_4^{-2} (Table 7). All the clay samples mineralized the least amount of SO_4^{-2}. However, the clay loam samples mineralized the most SO_4^{-2} compared to the loam samples except for their control samples. The Octane samples mineralized the least amount of SO_4^{-2} compared to the Propane and the Hexane samples. In the clay samples, Hexane mineralized the most SO_4^{-2} and in the clay loam and loam samples Propane mineralized the most SO_4^{-2}.

Table 7. The total amount of carbon respired, the initial amount of carbon from organic matter, the amount of carbon remaining in the soil after 16 weeks of respiration, the amount of sulfate mineralized, the amount of sulfur, and the carbon to sulfur ration for the clay, clay loam, and loam textured soils.

Amendment		Total amount of CO_2-C respired	Initial C from Soil	Carbon Remaining in Soil	Mineralized Sulfate	Remaining Sulfur	C/S
		---------------------------mg--------------------------					
Clay	Control	328.66	1740	1411	8	1	1411 : 1
	1500 ppm Propane	267.38	1740	1473	272	18	82 : 1
	3000 ppm Propane	314.99	1740	1425	402	27	53 : 1
	1500 ppm Hexane	295.72	1740	1444	237	16	90 : 1
	3000 ppm Hexane	349.80	1740	1390	412	27	51 : 1
	1500 ppm Octane	285.29	1740	1455	195	13	112 : 1
	3000 ppm Octane	300.92	1740	1439	358	24	60 : 1
Clay Loam	Control	359.40	1030	671	11	1	671 : 1
	1500 ppm Propane	296.01	1030	734	433	29	25 : 1
	3000 ppm Propane	337.82	1030	692	657	44	16 : 1
	1500 ppm Hexane	308.91	1030	721	428	29	25 : 1
	3000 ppm Hexane	352.90	1030	677	518	35	19 : 1
	1500 ppm Octane	332.29	1030	698	423	28	25 : 1
	3000 ppm Octane	398.73	1030	631	503	34	19 : 1
Loam	Control	443.03	3030	2587	18	1	2587 : 1
	1500 ppm Propane	364.03	3030	2666	292	19	140 : 1
	3000 ppm Propane	399.58	3030	2630	585	39	67 : 1
	1500 ppm Hexane	360.31	3030	2670	280	19	141 : 1
	3000 ppm Hexane	427.67	3030	2602	452	30	87 : 1
	1500 ppm Octane	356.83	3030	2673	233	16	167 : 1
	3000 ppm Octane	381.15	3030	2649	428	29	91 : 1

Respiration of Carbon Dioxide

<u>Texture</u>

The respiration of CO_2 of the different soil textures indicated a correlation between respiration and clay content. The clay samples respired the least, the clay loam samples were in the middle, and the loam samples respired the most CO_2 (Figure 6). The sample respiring the least amount of CO_2 was the clay control and the most CO_2 was loam control. In general, the loam contaminated samples respired more CO_2 than the clay and clay loam samples. The clay sample respiring the least amount of CO_2 was the 1500 ppm of Propane. The clay loam samples consistently respired CO_2 between the loam and the clay samples.
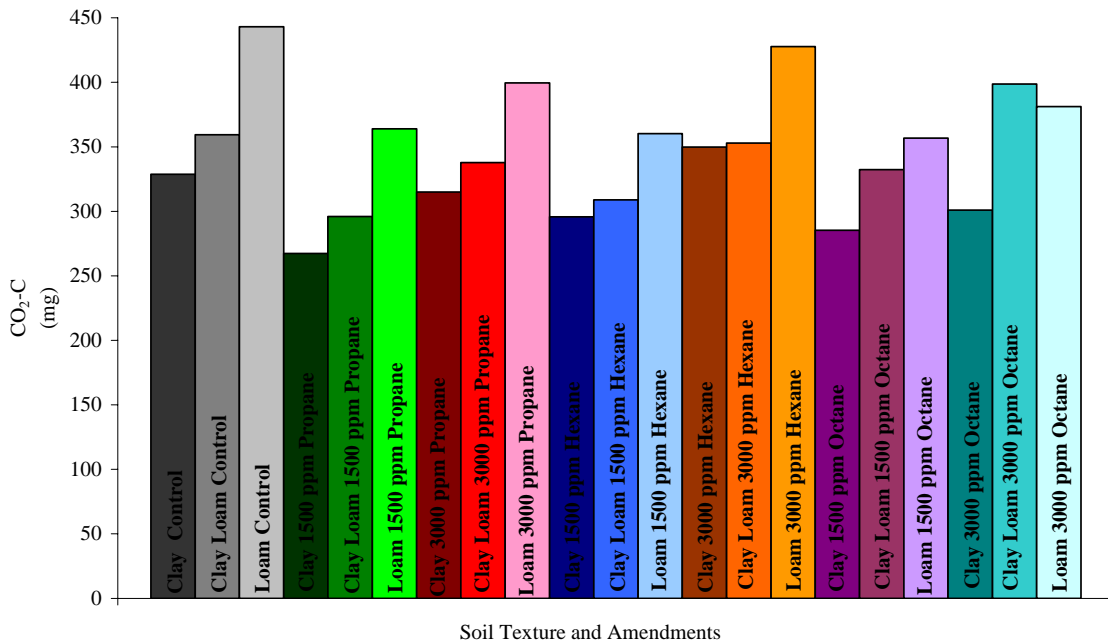

Figure 6. The total amount of carbon respired over 16 weeks of respiration for a clay, clay loam, and loam soils and these soils contaminated with 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).

Petroleum Hydrocarbons

There was no correlation between the Propane, Hexane, and Octane and respiration of CO_2 (Figure 7). In the loam samples the Octane respired the least amount of CO_2 compared to the Hexane and Propane. However, the 1500 ppm Propane respired more CO_2 than the 1500 ppm of Hexane and the 3000 ppm of Hexane respired more CO_2 than the 3000 ppm Propane (Figure 8). The clay loam sample indicated the 1500 ppm and 3000 ppm of Octane respired more CO_2 than the 1500 ppm and 3000 ppm of Hexane and the 1500 ppm and 3000 ppm of Hexane respired more CO_2 than the 1500 ppm and 3000 ppm of Propane. The 1500 ppm and the 3000 ppm of Hexane respired more CO_2 than the 1500 ppm and 3000 ppm of Propane and Octane in the clay samples (Figure 9). However, the 1500 ppm of Octane respired more CO_2 than the 1500 ppm of Propane and the 3000 ppm of Propane respired more CO_2 than the 3000 ppm of Octane (Figure 10).
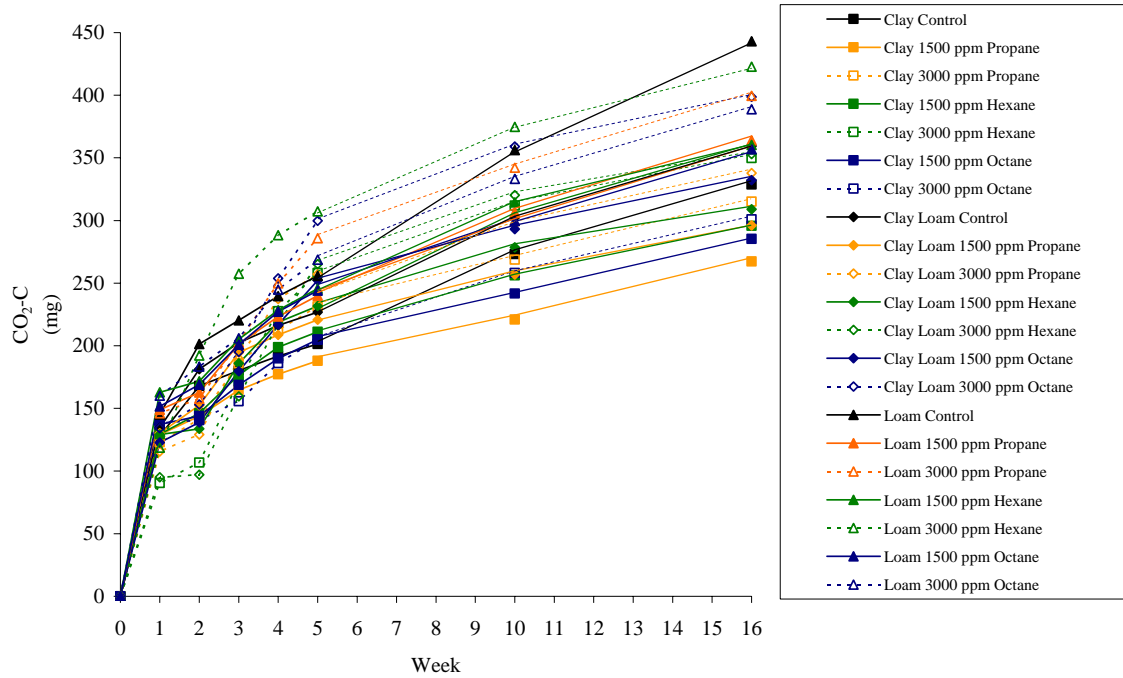
Figure 7. The cummulation of carbon respired over 16 weeks for the clay, clay loam, and loam soils and these soils contaminated with 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).
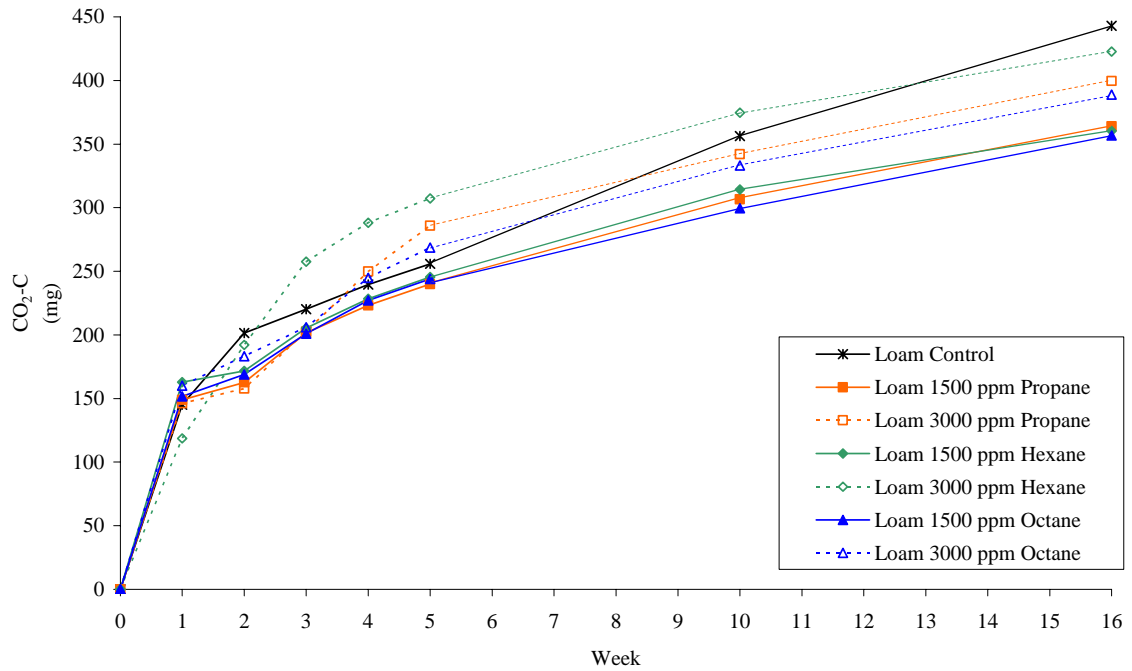

Figure 8. The cummulation of carbon respired over 16 weeks for the loam soil and the loam soil contaminated with 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).
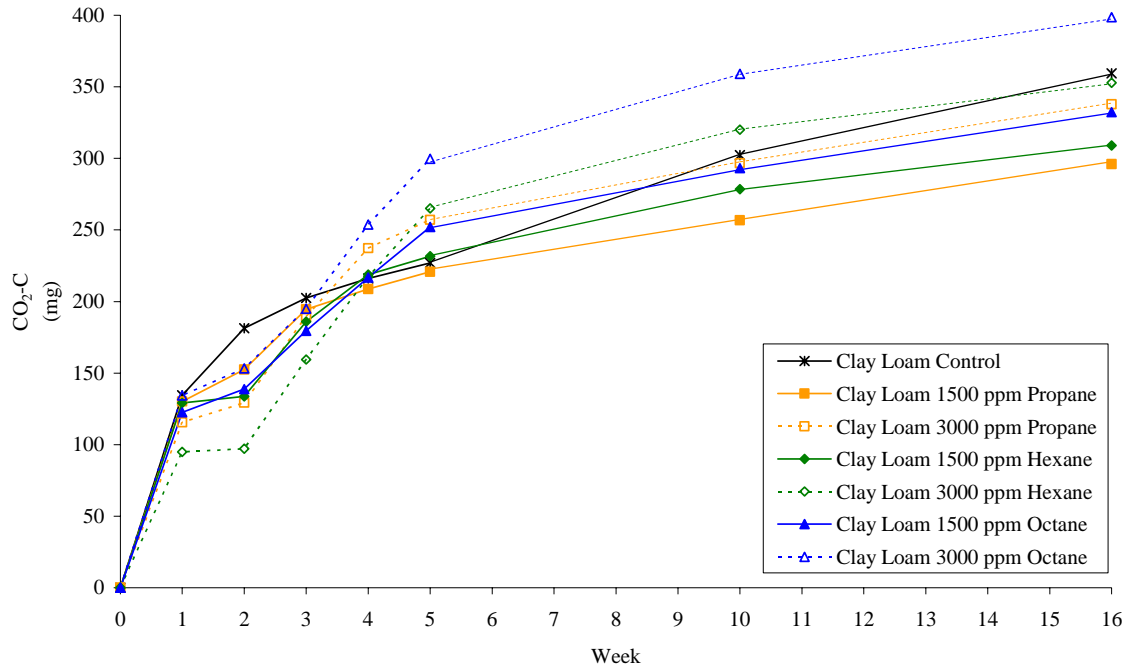
Figure 9. The cummulation of carbon respired over 16 weeks for the clay loam soil and the clay loam soil contaminated with 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).
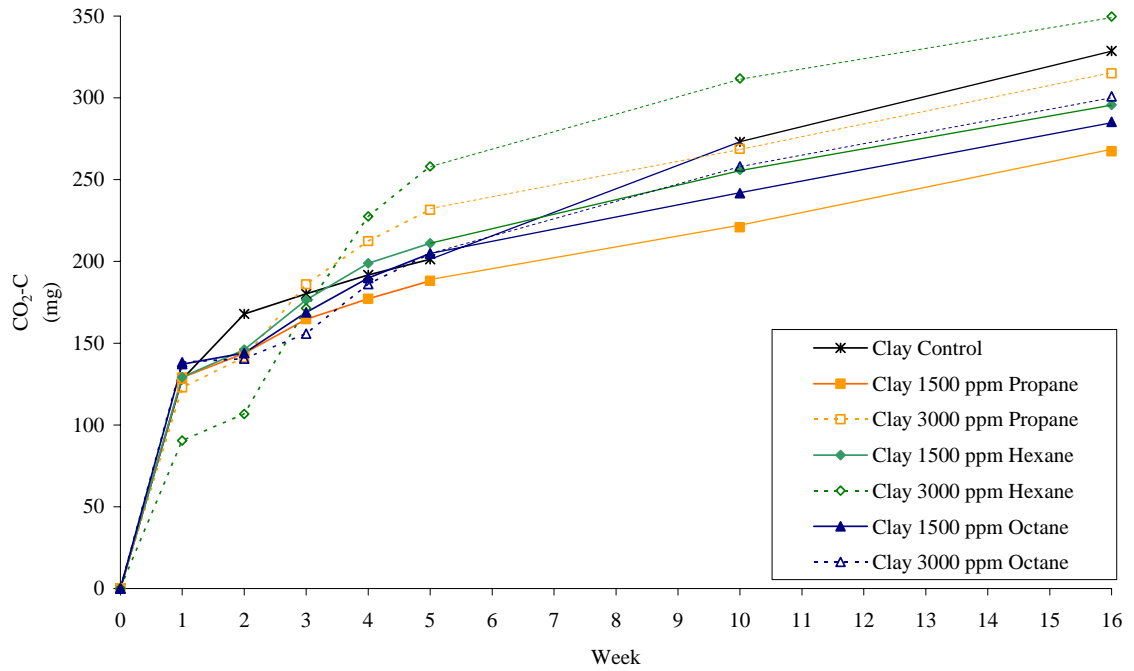

Figure 10. The cummulation of carbon respired over 16 weeks for the clay soil and the clay soil contaminated with 1-propane sulfonic acid (Propane), 1-hexane sulfonic acid (Hexane), and 1-octane sulfonic acid (Octane).

Carbon to Sulfur Ratio (C/S)

The clay loam samples had the lowest C/S ratios compared to the loam and clay samples. The loam samples had the highest C/S ratios compared to the clay and clay loam samples. It seems the type of texture of the soil did not have an effect on the C/S ratio. However, the initial amount of carbon and the type and rate of the petroleum hydrocarbons did effect the C/S ratio (Table 7). The loam samples contained the highest initial amount of carbon in the soil and had higher C/S ratios. The clay loam samples contained the least initial amount of carbon in the soil and had the smallest C/S ratios. The C/S values for the different petroleum hydrocarbons were hard to differentiate due to the influence of the initial carbon in the soil. The rate of the petroleum hydrocarbons did have a correlation with the C/S ratio. The 1500 ppm of petroleum hydrocarbon contaminant had higher C/S ratio values compared to the 3000 ppm of petroleum hydrocarbon contaminant.

Half Life

The half life of the clay loam control sample contained the shortest half life of 50.0 weeks compared to the clay control sample of 107.6 weeks and the loam control sample of 126.0 weeks. All of the loam samples had higher half life values compared to the clay loam and loam control samples and the clay loam samples had the lowest half life values.

The different concentration rates of petroleum hydrocarbons had no direct effect on the half life. The 1500 ppm and the 1300 ppm of Propane were similar for the loam,

clay loam, and loam samples. The 1500 ppm and the 1300 ppm of Hexane were similar

for the loam, clay loam, and loam samples. The 1500 ppm and the 1300 ppm of Octane

were similar for the loam, clay loam, and loam samples. However, the Hexane half life

values were higher than the Propane half life values in all three soil textures. The Octane

and the Propane samples were similar in the loam and clay loam soils. In the clay soil,

the Propane half life values were shorter than the Octane half life samples.

Statistics based on the Waller-Duncan Bayes LSD for Mean Separation using 1 % level of probability.

Clay

The 3000 ppm (high) of Hexane clay sample had the highest amount of CO_2

respired in all the clay samples. The high Hexane is significantly similar to control, high

Propane and high Octane and is not significantly to the 1500 ppm (low) of Hexane, low

Octane, and low Propane samples. The low Propane clay sample had the lowest amount

of CO_2 respired and was significantly similar to all the clay samples except the high

Hexane and control samples.

Clay Loam

The high Octane clay loam sample had the highest amount of CO_2 respired and

was not significantly similar to any of the other clay loam samples. The second highest

clay loam sample was the control and the control was significantly similar to the high

Hexane, high Propane, low Octane, and the low Hexane samples. The low Propane had

the least amount of CO_2 respired in the clay loam samples was only significantly similar to the low hexane sample.

Loam

The control loam sample had the highest amount of CO_2 respired and was significantly similar to the high Hexane and high Propane loam samples. All three of the high rate samples were significantly similar. The low Octane loam sample had the least amount of CO_2 respired and was significantly similar to the low Hexane, low Propane, high Octane, and high Propane samples.

Propane

The loam control sample had the highest amount of CO_2 respired in all the Propane samples and was significantly similar to the loam high Propane sample. The loam high Propane was significantly similar to the loam low Propane and was significantly different from all the other samples. The loam low Propane, clay loam control and clay loam high Propane were significantly similar. The clay loam high Propane, clay control, clay high Propane, and clay loam low Propane were significantly similar. The clay low Propane had the least amount of CO_2 respired and was only significantly similar to the clay loam low Propane sample.

Hexane

The loam control had the highest amount of CO_2 respired and was significantly similar to only the loam high Hexane sample. The loam low Hexane had the third highest rate CO_2 respired and was significantly similar to the clay loam control, clay

loam high Hexane, clay high Hexane, and clay control. The clay low Hexane had the least amount of CO_2 respired and was significantly similar to the clay loam low Hexane.

Octane

The loam control had the highest amount of CO_2 respired pertaining to the Octane samples and was significantly similar to the clay loam high Octane sample. The low high Octane had the third highest amount of CO_2 respired and this sample was significantly similar to the clay loam high Octane, clay loam control, and the loam low Octane. The clay control had the firth highest amount of CO_2 respired and was significantly similar to the clay loam control, loam low Octane, and the clay loam low Octane. The clay low Octane had the least amount of CO_2 respired and was significantly similar to the clay high Octane, clay loam low Octane, and the clay control.

Statistical Summary

The loam control had the highest amount of CO_2 respired compared to all the samples and was only significantly similar to the loam high Hexane. The clay loam high Octane had third highest amount of CO_2 respired and was significantly similar to the loam high Hexane and the loam high Propane. The loam high Octane had the fifth highest respiration rate and was significantly similar to the clay loam high Octane and loam high Propane. The loam low Propane had the sixth highest respiration rate and was significantly similar the loam high Octane and the loam low Hexane, loam low Octane, clay loam control, clay loam high Hexane, clay high Hexane, and the clay loam high Propane. The clay control had the thirtieth highest respiration rate and was significantly similar to the loam low Hexane, loam low Octane, clay loam control, clay loam high

Hexane, clay high Hexane, and the clay loam high Propane and the clay loam low Octane. The clay high Propane had the 15th highest rate and was significantly similar to the clay loam high Propane, clay control and the clay loam low Octane and the clay high Octane, clay loam low Propane, clay low Hexane, and tithe clay low Octane. The clay low Propane had the least amount of carbon dioxide respired of all the samples and was significantly similar to the clay low Octane and the clay low Hexane.

CONCLUSION

Soil texture was very influential on the rate of soil respiration. The loam samples containing the least percentage of clay had the highest soil respiration rate. The clay samples contained the highest percentage of clay and had the lowest soil respiration rates. The clay loam soil respiration rates were between the loam and clay samples.

There was no correlation between soil respiration and the three petroleum hydrocarbons. However, the different rates of contamination inhibited the rate of respiration. The control samples for the loam, clay loam, and clay soils respired more CO_2 than the 1500 ppm and 3000 ppm petroleum hydrocarbons. This clearly indicated both the lower and higher rates of contaminate had an inhibitory effect on respiration. The different rates of contamination also effected the rate of respiration. The higher petroleum hydrocarbon concentration in the soils the lower the amount of CO2 was respired. The 1500 ppm of the petroleum hydrocarbons in the individual soils allowed more respiration than the 3000 ppm of petroleum hydrocarbons.

There was no direct relationship between the concentration of sulfate released by mineralization and the respiration rates of the different textures due to their different amounts of organic matter. The clay loam samples mineralized the highest concentration of sulfate and the loam mineralized the least concentration of sulfate. However, the type of petroleum hydrocarbon and the different rates of contamination did have a relationship on the concentration of sulfate released by mineralization. The octane the least

concentration of sulfate released and the propane had the highest concentration of sulfate released. The lower rate of petroleum hydrocarbons released lower amounts of sulfate compared to the higher rate.

The C/S ratio was highest in the loam soil and lowest in the clay loam. The relationship between the C/S ratio and texture was inconclusive probably due to the different amount of organic matter in each soil. The Propane contaminated soil had the lowest C/S ratio compared to the Hexane and Octane. The Octane contaminated soil had the highest C/S ratio. In addition, the different rates of contamination influenced the C/S ratio. The 1500 ppm rate had higher C/S ratios compared to the 3000 ppm rate.

The half life values were not dependant on the clay, clay loam, and loam soil textures. The different rates of 1500 ppm and 3000 ppm of petroleum hydrocarbons had no direct effect on the half life. The 1500 ppm and the 1300 ppm of Propane were similar for the loam, clay loam, and loam samples and it was the same for the Hexane and Octane half life values. The different types of petroleum hydrocarbons were individual influenced by the soil texture they were contaminated in. The Hexane half life values were higher than the Propane half life values in all three soil textures. The Octane and the Propane samples were similar in the loam and clay loam soils. In the clay soil, the Propane half life values were shorter than the Octane half life samples.

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