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The Detoxification of Petroleum Contaminated Coastal Plain Sandy Soil Using an Amended Vermicomposting Approach

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THE DETOXIFICATION OF PETROLEUM

CONTAMINATED COASTAL PLAIN SANDY SOIL USING AN

AMENDED VERMICOMPOSTING APPROACH

by

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B.S. December 1986, Indiana State University
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ABSTRACT

THE DETOXIFICATION OF PETROLEUM
CONTAMINATED COASTAL PLAIN SANDY SOIL USING AN
AMENDED VERMICOMPOSTING APPROACH

John Charles Kraemer
Old Dominion University, 1997
Co-Chairpersons: Dr. Rod Handy and Dr. Clare Houseman

This study explores the feasibility of utilizing an amended vermicomposting treatment approach for detoxifying petroleum hydrocarbon contaminated sandy soil. The bench-scale testing of gasoline and diesel contaminated soil using three test soil vessels and one control soil vessel was performed in a laboratory setting for a six week time period. The control soil received no treatment other than distilled water to maintain soil moisture. Treatment 1 consisted of direct application of liquid municipal biologic sludge on a weekly basis. Treatment 2 consisted of the addition of 30 *Eisenia foetida* earthworms and distilled water. Treatment 3 consisted of direct application of liquid municipal biologic sludge and 30 *Eisenia foetida* earthworms. The experimental findings showed the greatest reduction in Total Petroleum Hydrocarbon (TPH) concentration occurred in the soil receiving Treatment 3. In addition, the experimental data showed that the individual segments, biologic sludge alone and earthworms alone, provided significant reductions in TPH concentrations. However, the reductions of the individual segments did not exceed the performance of Treatment 3. This finding does, however, indicate that the interaction of the segments can lead to a higher rate of biodegradation within petroleum contaminated soils. The statistical operations performed on the test soils indicated a statistically significant reduction in TPH concentrations occurred in Treatment 3. In addition, the Lower 95% and Upper 95% prediction intervals and the Lower 99% and Upper 99% prediction intervals for Treatment 3 were the largest among the test and control soils. This suggests the amended vermicomposting treatment approach has merit and further studies should be conducted to show the technical feasibility of this treatment option.

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CHAPTER ONE

INTRODUCTION

Petroleum contaminated soils are a major domestic environmental issue in the United States. Petroleum contaminated soil is a direct outcome of large scale production, transportation, storage, and use of petroleum. Since there are over one million underground storage tanks in operating or abandoned service stations or industrial facilities, there is no doubt that urban areas will face soil contamination issues for many years to come. The current regulatory agencies estimate that up to 60% of these underground tanks leak. The Environmental Protection Agency (EPA) estimates that service station gasoline tanks could be losing 11 million gallons per year of product(Riser-Roberts, 1992). Remediation costs associated with leaking underground petroleum storage tanks can amount up to one million dollars per release incident. Thus, petroleum contaminated soils pose an enormous economic problem to industrialized areas.

Many urban areas across the United States have been identified as being contaminated due to the presence of anthropogenic chemicals and chemical products, such as petroleum products. In response to this condition, several

researchers have focused on biological treatment as one alternative for remedial action to detoxify contaminated sites. The biological approach is based on the inoculation of the soils at these sites with a homogeneous population of bacteria capable of catalyzing detoxification reactions. It is doubtful that pure cultures of an isolated microbial species can be deployed effectively in a bioengineering system under field conditions. Homogeneous populations of microorganisms cannot operate indefinitely on one or more organic compounds in the absence of other microorganisms. Additional microbes must be present to remove the waste products of the homogeneous community. In addition, a substrate may be incompletely catabolized by a single microbial community and a residual contaminant may persist in the soil environment. However, in the presence of multiple microbial species, the residual material may be detoxified even if the microbial community lacks the necessary enzymes required for the metabolism of the parent compound. Individually neither species can detoxify the contaminant, however, a heterogeneous microbial community of two or more species can act synergistically to completely detoxify or mineralize the substrate.

PURPOSE

The purpose of this discourse is to develop a basis for and to investigate a biological approach utilizing concepts associated with vermicomposting for the treatment of Virginia Coastal Plain sandy soil contaminated by petroleum compounds. This treatment approach is based upon the intrinsic properties related to microbial interactions within a heterogeneous community, soil mineralization, humic chemistry and earthworm activity in soil formation and stabilization. The paradigm associated with vermicomposting techniques will be modified to include a mixture of petroleum contaminated soil, secondary wastewater sludge and one biotechnologic class of earthworms.

PROBLEM

The problem which must be answered in this research is whether the amended treatment approach will detoxify petroleum contaminated soils and provide a stabilized soil end product. The research question which must be addressed is what effect will the treatment method have on petroleum contaminated soils. The general goal of this research was to determine the effects of vermial activity on the detoxification of the complex ring structures associated with petroleum pollutants within the soil matrix.

This determination was made by measuring the reduction of petroleum contamination within the test soil over time using single components of the treatment system and then by a total or holistic treatment system. A control experiment which utilized only water was used to determine contamination reduction due to natural attenuation, volatilization and degradation. The theoretical basis which was used in this research is founded on Darwin's theory of organic evolution and is characterized by the use of earthworms, biologic sludge, and soil/sludge humic chemistry to detoxify petroleum contaminants.

Biologic sludge obtained directly from a secondary treatment system at a municipal wastewater treatment facility in Hampton Roads, Virginia, was used as a source of a heterogeneous microbial population. Biologic sludge contains humic substances and enzymatic catalysts (Hartenstein, 1986) which may induce detoxification reactions by binding and inactivating the chemical constituents contained in petroleum hydrocarbons. Soil contains mineral catalysts, enzymatic catalysts and humic catalysts (Shindo and Hauge, 1984; Skujins, 1796; Hassett et al., 1987a) which may facilitate the breakdown of organic substrates.

The detoxification of petroleum products occurs more rapidly under aerobic conditions. Aerobic conditions within a vermicomposting treatment system can be maintained by the presence of a biotechnologic species of earthworms (Hartenstein, 1986). The vermial activity associated with the earthworms is thought to assist in accelerating the detoxifi- cation reactions by enhancing aeration of the soil through perturbation resulting from tunnelling and burrowing activities (Sorenson, 1974). These soil macroinvertebrates also provide the enzyme peroxidase (Hassett et al., 1988), which in the presence of hydrogen peroxide derived from microbes and the atmosphere, enhances the polymerization of phenolic compounds and binding of recalcitrant pollutants in soil (Bollagg and Loll, 1983).

In order to operationalize this innovative approach, the reduction in soil contamination was measured by interval data obtained through the use of an infrared spectrometer which measured the level of petroleum hydrocarbon contamination in parts per million. In addition, the independent variable was compared to a control group and a set of experimental groups to determine if the biological treatment measures were the proper indicators of detoxification reactions within the soil.

HYPOTHESIS

The hypothesis which was tested in this experiment is as follows:

H_1: Vermicomposting biotreatment will have a significant effect ($p<.05$) in reducing the level of petroleum hydrocarbon contamination in Coastal Plain sandy soil.

If the comparison of the holistic treatment data to the experimental components and control data indicate that there was no significant contamination reduction in the test soils, then the null hypothesis, H_o, will be accepted.

THEORETICAL FRAMEWORK

Charles Darwin's theory of organic evolution was based on his investigation, evaluation and description of biological agents, humus and earthworm internal and external functions in the formation of soil. In this theory, soil formation is considered to be a dynamic state in which the effects of the activity of organisms in changing their environment can be evaluated. This general theory leads to the consideration of each dynamic segment of the treatment system for its applicability to the holistic treatment methodology.

Charles Darwin was the first to recognize the dynamic state of humified soil or "vegetable mould" [sic] as the work of biologic agents within the soil. Darwin was not interested in the static state or soil horizon layers, but in the soil forming activity of earthworms within the soil. Darwin quantitatively estimated the rate of change to the surface layer of soil due to the actions of earthworms during their burrowing to the soil surface and their subsequent return to deeper soil regions. His observations led him to the belief that earthworms were of geological significance. His observations led to the current understanding of soil biology and contributed to the understanding of the genesis of soil humus and its role in soil stabilization (Satchell, 1983).

Darwin defines "vegetable mould" [sic] as "the uniformly fine soil particles of dark color which covers the whole surface of the land in every moderately humid country". This "vegetable mould" would reach to a depth of 40 centimeters and would have passed through the intestinal tract of earthworms several times. He believed that the primary work of earthworms to be "to sift the finer from the courser soil particles, to mingle the whole with the vegetable debris, and to saturate it with their intestinal

secretions (Darwin, 1881). This belief indicated that the
mixing actions of earthworms burrowing and their intestinal
secretions, or enzymes, played an important role in soil
formation. Darwin also indicated that earthworm casts are
brought to the surface soils during burrowing activities.

Darwin's attention of the tearing, shredding,
moistening, and consuming of vegetable matter and then
mixing the material with soil was important in describing
the color changes associated with organic matter
humification. His observations on the effects of "humus
acids" on soil oxides was a precursor to the current
concepts associated with the effects of humic substances in
the chelation of metals and their transportation throughout
the soil profile (Satchell, 1983). He believed acids
present in the earthworm digestive tract was similar to the
humus acids present in soil. However, current data
indicates that humic acids are polyelectrolytes and not
similar in structure to acids produced in the earthworm gut
(Satchell, 1983). Although Darwin's theory regarding the
composition of the acids within the earthworm gut was
incorrect, he was correct in identifying the importance of
the chemical interaction between the contents of the
ingested material and the biochemical constituents within

the gut. This is important in recognizing the biochemical interactions between the enzymes present within the gut of the earthworms and the substrate, soil and organic debris, in the ingested material.

Darwin refers to the mechanical grinding of soil particles within the alimentary canal of earthworms as a significant contribution to soil genesis (Satchell, 1983). Earthworms must ingest large quantities of water with the soil particles as a method of retaining them adjacent to each other. This action allows for the formation of soil aggregate stabilizing compounds to create bonds or bridges between the soil particles (Satchell, 1983). Therefore, Darwin's theory that earthworms and other macroorganisms act as promotional agents for microbial activities which form the natural and synthetic binding polymers necessary for soil humification or stabilization is an important aspect in the use of vermial actions for sludge-soil stabilization.

The natural polymers, humic substances, associated with Darwin's theory have been investigated by many researchers for a number of years. Humic substance composition can include low molecular weight materials, such as oligo-saccharides, to complex polydisperse humic polymers, fulvic acid, humic acid and humin (Satchell, 1983). Figure 1
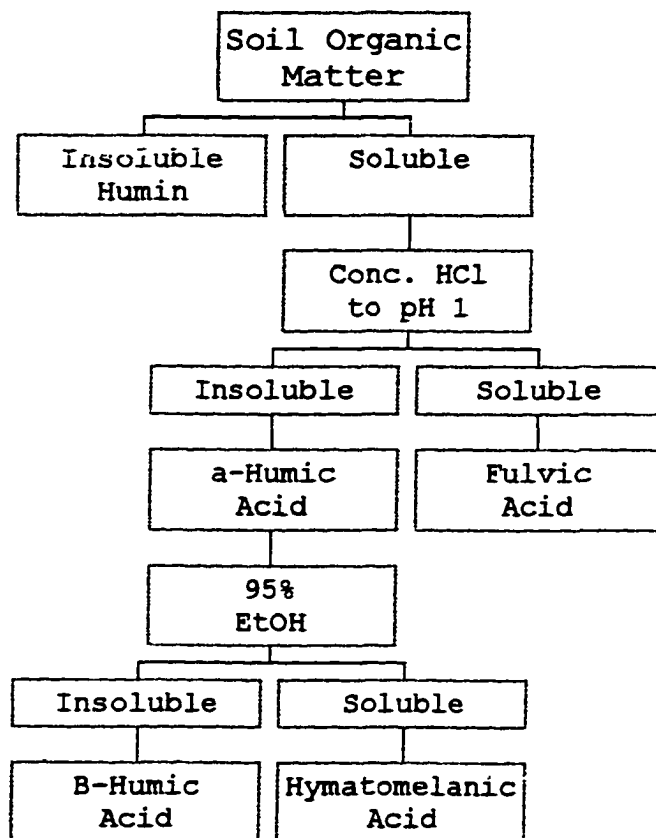
Figure 1.   Composition of Soil Organic Matter.

Source: Pierce and Felbeck, 1977.

provides an illustration of the composition of humic
substances. Compounds such as polysaccharides, polypeptides
and lignin-based compounds are synthesized by microbial
communities living within the organic substrates or formed
by biochemical actions on the parent substrates; therefore,
these compounds are considered as non-humic substances
within the soil matrix (Satchell, 1983). In order to
identify the structural composition of organic humic
polymers, it is necessary to classify repeating units and
the linkages of those units within a certain spatial
arrangement. Figures 2 through 4 provides an illustration
of the humic polymers present in humic substances.

Darwin's theory regarding the beneficial effects of
vermial activity in soils has led to extensive research
regarding the role of earthworms in soil stabilization.
Studies by Satchell (1967) and Edwards and Lofty (1972)
clearly show that soil structure is improved when an
adequate supply of earthworms is present. Polysaccharides,
muco-polysaccharides, and humic polymers resulting from
micro- and macroorganism metabolic activities were believed
to be responsible for binding soil particles together and
causing stabilized aggregates. Satchell's (1967) research
indicates that worm casts contain more water-stable
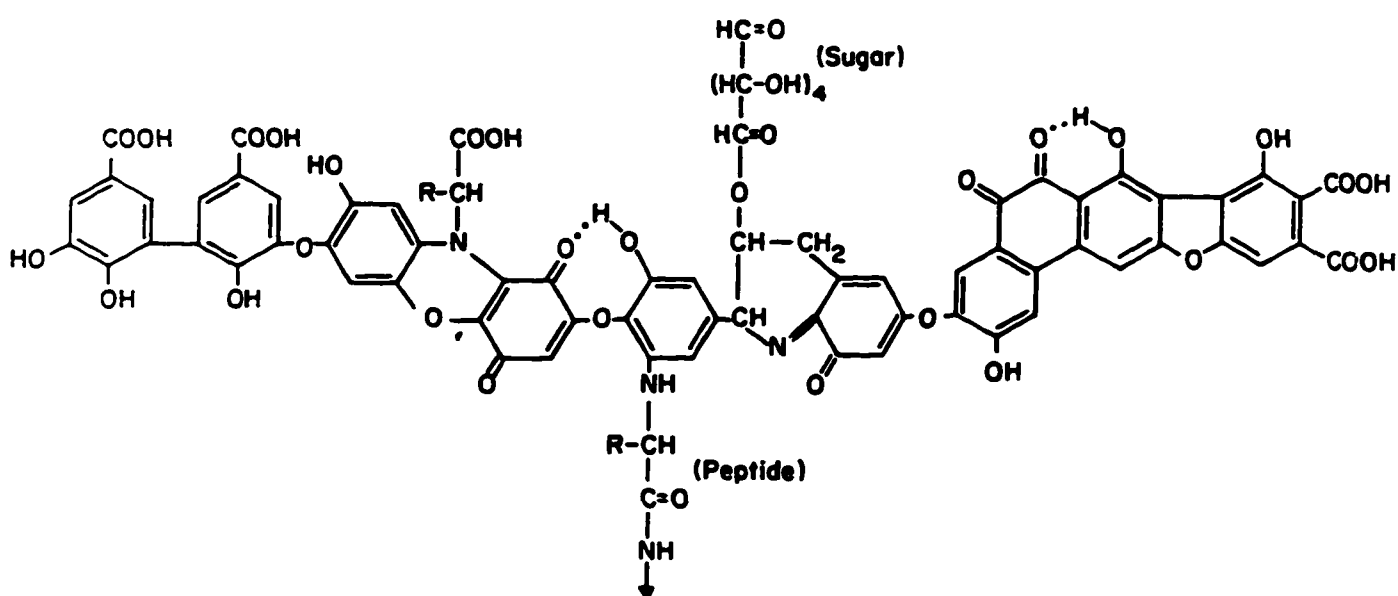
Figure 2. Postulated Structural Diagram of a Humic Acid.

Source: Stevenson, 1982.
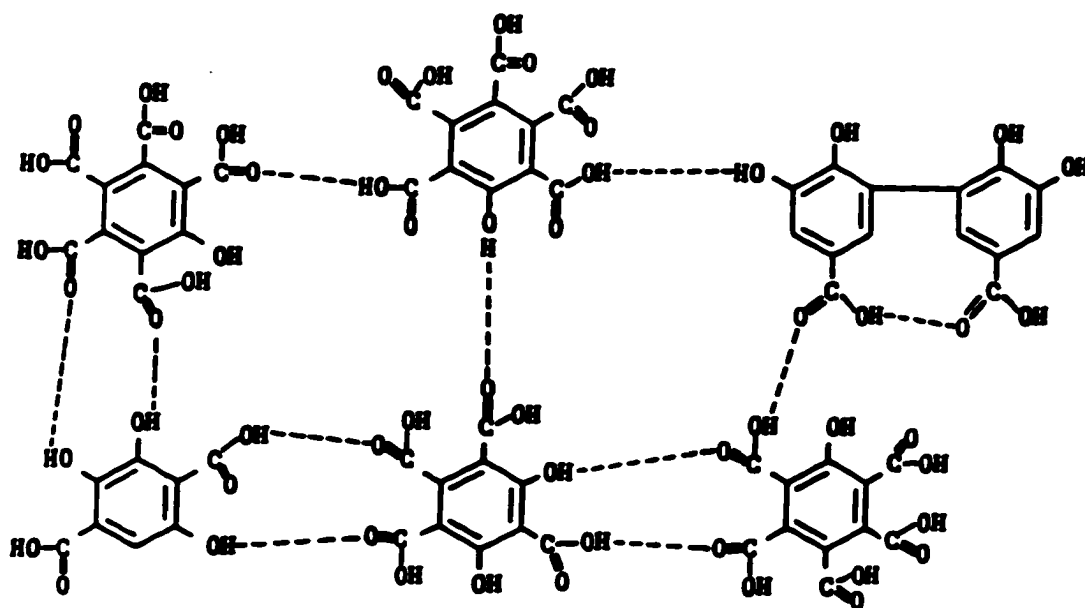
Figure 3. Postulated Structural Diagram of a Fulvic Acid.

Source: Schnitzer and Khan, 1972.
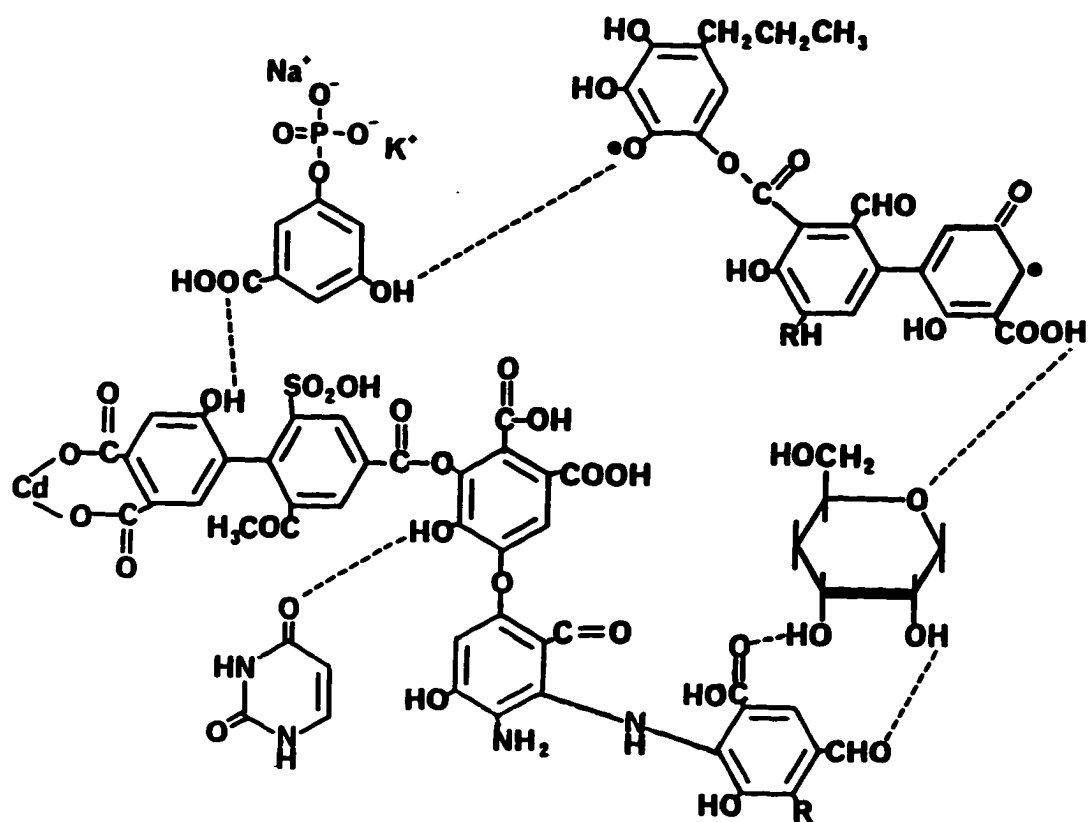
Figure 4. Model of a Humic Polymer.

Source: Bisesi, 1986.

aggregates and that vermially tilled soil was more stable
than non-vermially tilled soils.

Soil stability in vermially tilled soils resulted from
the mechanical binding of plant organic material or from the
growth of microorganisms within and on casts. It is thought
that the soil stabilizing components were produced by
microorganisms in the gut of the earthworm or within the
cast material after excretion (Satchell, 1983). The
microorganisms present in earthworm casts are similar to
those in soil (Satchell, 1967). Bhandari et al. (1967) show
that worm casts contained more organic carbon,
polysaccharides, and nitrogen than the parent soil used in
their research.

The nitrogenous, mucoprotein material deposited onto
the sidewalls and soil surfaces by burrowing and crawling
earthworms readily adheres to the various surface materials.
This body wall secretion stabilizes earthworm tunnels and
loose soil particles are formed into aggregates (Edwards and
Lofty, 1967). In addition, soil particles are mechanically
mixed in the earthworm gizzard during burrowing. Large
aggregates could not pass through the gizzard, therefore,
the formation of aggregate components must occur during the
ingestion process and result in aggregate formation after

passing through earthworms. The calciferous gland within the earthworm may provide specific amounts of calcium to facilitate divalent cation bridging with humic substances and negatively charged inorganic colloids (Satchell, 1983). Dry earthworm casts, in proximity to soil particles, would allow for the polymers secreted by the intestines of the earthworm and the humic substances and polysaccharides in the soil to form the bonds necessary for stabilization.

The organic litter materials contained in casts provide a growth substrate for microbial populations within the casts and in the soil. As humification proceeds within the soil, the binding capacity provided by the litter would decline. However, humic polymers would replace the bonds needed for soil aggregate stabilization (Satchell, 1983).

Darwin also observed that the transport of soil by earthworms plays an important role in creating a dynamic condition of environmental change. The constant burial of surface litter, mechanical mixing of organic matter with mineral soil, burrowing at depth, and casting at the surface all create an environment capable of supporting earthworm population shifts by stabilizing the soil aggregates (Kretzschmar, 1981, 1982). Burrowing activities introduce organic matter rich upper soils to lower soil strata.

The structural stability of the soil is increased by organic
matter decomposition and earthworm casts (Monnier, 1965).
The binding of organic matter and inorganic soil creates
greater porosity, aeration, and drainage amenable to
earthworm populations. This action not only provides soil
aggregate stabilization, but also a stable environment for
earthworm proliferation (Satchell, 1983).

The role of earthworms in the transfer of plant organic
matter and animal dung in grassland soils is well
documented. The mixing of organic matter with inorganic
soils facilitates the redistribution of nutrients within
soil profiles and enhances soil enzymatic activity
(Satchell, 1983). Earthworms increase nutrient levels in
soils by direct enzyme action on organic matter in the gut,
by reducing high carbon/nitrogen ratio organic matter into a
more usable low carbon/nitrogen ratio, and by releasing
metabolic products into the subsurface soils (Satchell,
1983).

The influence of earthworm activity on microbial
enzymatic activity is not only important in the
decomposition of organic matter in the environment, but also
to the vermiculture disposal techniques used in wastewater

sludge management. Hornor and Mitchell (1981) studied the effects of vermiculture treatment on sewage sludge and discovered that microbial populations within treatment vessels containing *Eisenia foetida* and sludge increased. In addition, they also found that *E. foetida* feeding activities enhanced aerobic decomposition within the treatment vessels. The effect of earthworms on domestic wastewater sludge microorganisms is extremely important in developing vermial based treatment options. Earthworm species have been shown by several researchers to reduce the number of pathogenic microorganisms in soil. These pathogens included *Serratia marcessens*, *Escherichia coli*, *Salmonella enteriditis*, and *Serratia typhyimurium* (Day, 1950; Brusewitz, 1959; Brown and Mitchell, 1981).

Activated wastewater sludge does not normally facilitate the growth of human enteric microorganisms due to its aerobic nature (Taber, 1976). The effect of earthworm burrowing in an aerobic sludge enhances the level of oxygen within the sludge and facilitates drying. Mitchell et al. (1980) found that aerobic bacteria levels were more than twice as numerous in 14 day old sludge after the introduction of the earthworm *E. foetida* as sludge without the earthworm. Since most human enteric microorganisms are

anaerobes, sludge conditioning and stabilization by earthworms appears to provide beneficial effects (Satchell, 1983).

Darwin's initial observations regarding the usefulness of earthworms in soil genesis and stabilization has led to the discovery of many facets of earthworm biotechnology or vermiculture. The theory of "humus substance" formation in soils due to the influences of earthworm activity provides a basis for the treatment of wastewater sludge. Therefore, the next logical step in this theory is to use the humification process and soil stabilization process to facilitate the remediation of organic chemical contaminated soils. The paradigm used to remediate the petroleum contaminated soils incorporates Darwin's theory of the formation of "vegetable mould" [sic] by the mixing of organic matter, microbial enzymes, earthworm casts, and soil.

BIOLOGICAL SLUDGE

The use of an activated sludge process by large municipal wastewater treatment plants has been an accepted treatment alternative for many years. This process mixes the effluent from a primary treatment clarifier with a source of healthy active aerobic microorganisms. As the

aerobic microorganisms are mixed with the wastewater, they convert the organic matter to stable solids and additional microbial growth occurs within the mixture. The effluent from the mixing tank consists of a suspension of organisms, undecomposed organic matter, humic colloids and water. In addition, the treated water contains very little Biological Oxygen Demand (BOD). The biologic sludge obtained from this type of treatment is a heterogeneous composition based upon the type of influent received into the treatment tank. Figure 5 illustrates the compositional heterogeneity of the constituents of biologic sludge. Table 1 provides a summary of the BOD and total suspended solid levels for the biologic sludge material obtained from the Hampton Roads Sanitation District's wastewater treatment plant used in this study.

The ability to metabolize the aromatic compounds associated with petroleum products and use them as a sole source of carbon and energy for growth is displayed by many of the organisms found in biologic sludge. Table 2 provides a summary of major genera of bacteria found in biologic sludge. To grow on such a compound, a microorganism must be able to break down at least a part of the chemical molecule to the single carbon components that are the intermediates in the central pathways of metabolism. These can be

Biologic Sludge

Water (70-99.5%)   Total Solids (0.5-30%)

Bound   Free   Inorganic (20-50%)   Organic (50-80%)
(encapsulated (present in
in microbes) extracellular
solution)

Soil   Anions   Cations   Humus   Microbes   Natural   Synthetic
sand NO_3^- Ca   humin   monera   Organics   Organics
silt SO_4^{-2} K   humic acid   protista   protein   pesticides
clay PO_4^{3-3} Mg   fulvic acid   fungi   carbohydrate
    Cl   Na   plantae   lipid
    CO_3^{-2} Pb   animalia   nucleic acid
    etc.   Cd   lignin
        etc.

Figure 5. Composition of Biologic Sludge.

Source: Bisesi, 1986.

Table 1. Biologic Sludge Data From HRSD Atlantic Plant
          for 1996.

Month of Data Report	Average Activated Biosoild Influent Flow[1]	Average Activated Biosolid Influent BOD (mg/L)	Biosolid Total Suspended Solids (mg/L)
January	30.33 MGD	164 mg/L	82 mg/L
February	29.97 MGD	159 mg/L	75 mg/L
March	29.55 MGD	184 mg/L	83 mg/L
April	31.42 MGD	196 mg/L	92 mg/L
May	31.75 MGD	199 mg/L	150 mg/L
June	32.47 MGD	184 mg/L	73 mg/L
July	34.68 MGD	173 mg/L	63 mg/L
August	35.26 MGD	188 mg/L	137 mg/L
September	33.16 MGD	172 mg/L	68 mg/L

Source: HRSD personnel, personal communication.

Table 2. Principal Genera of Aerobic Bacteria in Activated
          Sludge.

Pseudomonas sp.	Bacillus sp.
Zoogloea sp.	Athrobacter sp.
Nocardia sp.	Microthrix sp.
Acintobacter sp.	Nitrosomonas sp.
Nitrobacter sp.	Achromobacter sp.

oxidized to provide energy or can serve as an initiating point for the biosynthesis of cell constituents. However, it may take several successive transformations or elementary reactions before the starting organic substrate is converted into central metabolites.

The control of the substrate decomposition is effected by enzymes or biocatalyst. Aerobic catabolism of aromatic substances uses a class of enzymes known as oxygenases. These are of particular importance in the ring fission reactions needed to detoxify petroleum products. The key step in the degradation of aromatic compounds is the opening of the stable ring structure to provide aliphatic intermediates. The ring is activated for cleavage by the insertion of hydroxyl groups and most substrates have at least two such substitutes situated ortho or para to each other (Mudrack and Kunst, 1986). For example, benzene is converted into catechol and toluene to 3-methylcatechol by hydroxylation.

Oxygenase, both of the monooxygenase type that insert one atom of an oxygen molecule into the substrate and of the dioxygenase type that insert two atoms, have roles in the metabolism of organic substrates. Several aromatic hydrocarbons are converted into o-dihydroxy compounds by

simultaneous introduction of two hydroxyl groups catalyzed by a dioxygenase and intermediate in this process is the corresponding cis-dihydrodiol to produce catechol. The dioxygenases involved are complex enzymes with more than one interacting protein (Gibson and Subramanian, 1984).

The known pathways for aerobic oxidation of aromatic compounds involve ring fission by dioxygenase enzymes and the activities of these and the pathways for conversion of the products to central metabolites have all been documented (Bayly and Barbour, 1984; Dagley, 1989). These enzymes are found in biologic sludge and would therefore aid in the ring fission processes needed to detoxify petroleum contaminated soils.

Enzymes needed to metabolize cellulose material are present within biologic sludge, however, cellulose is typically a poor biodegradable substance. This difficulty in degradation creates problems for sewage treatment processes. The use of a vermicomposting process for the treatment of wastewater residuals aids in eliminating the cellulose material from the sludge material. Earthworms digest cellulose material as they burrow through the sludge. Therefore, the use of a soil-amended version of the vermicomposting treatment system would provide an enhanced

medium for cellulose removal.

SOIL PROPERTIES AFFECTING DETOXIFICATION

Soil is divided into liquid, solid, and gaseous phases. The solid phase is divided into inorganic and organic fractions. Figure 6 illustrates the main components of soil constituents. The significant fraction of soil which impacts soil formation is the transformation of organic matter to humus. There are four stages of development which occur during the transformation of soil organic matter to humus:

(1) decomposition of soil biomass, including lignin, into simple organic compounds;

(2) microbial metabolism of the simple organic compounds;

(3) nutrient cycling between soil organic matter and microbial biomass, and;

(4) microbial-mediated polymerization of the cycled organic compounds.

This transformation process leads to the formation of humic substances. Humic substances can be classified as humic acids (soluble in base), fulvic acids (soluble in base and acid) and humin (insoluble in acid and alkali). These
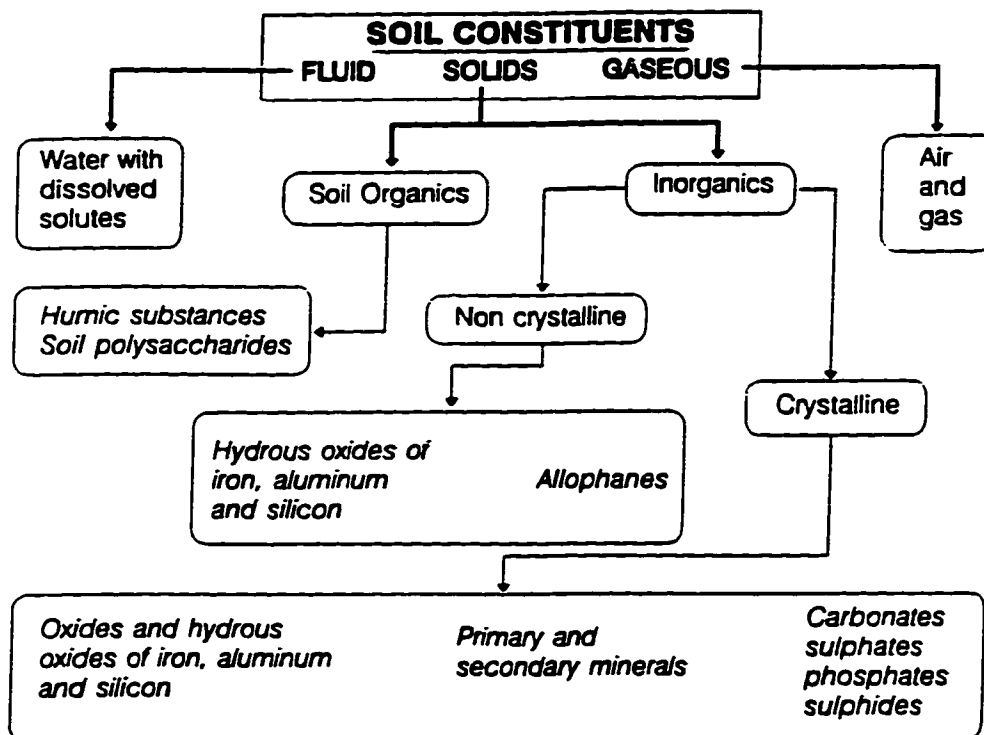
Figure 6.   Constituents of Soil.

Source: Brady, 1990.

three fractions are also present in biologic sludge.
Therefore, the introduction of biologic sludge in the soil
treatment system should significantly increase the amount of
humic materials and result in facilitating detoxification
reactions within the treatment matrix. The four
characteristics which form the importance of humic
substances to soil detoxification include the following:

(1) polyfunctionality: The existence of a
heterogeneous mixture of functional groups and
reactivity;

(2) macromolecular charge: The development of anionic
character on a macromolecular framework;

(3) hydrophilicity: The tendency to form strong
hydrogen bonds with water molecules solvating
polar functional groups (e.g., COOH, -OH), and;

(4) structural lability: The capacity to associate
intermolecularly and to alter molecular
conformations in response to changes in pH,
reduction-oxidation potentials, and functional
group binding (Sposito,1989).

Studies have shown that humic substances possess high
recalcitrancy in non-disturbed soil environments (Stevenson,
1982). The addition of sludge to the soil environment has

been effective in raising the sorption and degradation properties of the treated soil (Fairbanks and O'Connor, 1984). The retention of the potential contaminants in soil due to the presence of higher concentrations of humic substances minimizes their movement through the soil strata and prolongs their exposure to soil catalysts for transformation.

The detoxification of organic compounds within the soil matrix is catalyzed by enzymatic reactions, soil mineralization processes, and by free radical interactions. Free radical interactions are due to the presence of unpaired electrons within the organic chemical's structural form. The concentration of free radicals within soil and sludge are based on the presence of humic substances and lignin. Free radicals are highly reactive and tend to form bonds with other atoms present within the matrix (Senesi and Schnitzer, 1977). In addition, free radical oxidations require less activation energy than is needed to either cause a cleavage of a covalent bond or a polar oxidation (Dragun and Helling, 1985).

Free radical oxidations occur as a series of two or more steps. The first step involves the removal of a single electron from a molecule. This step, according to Dragun

and Helling (1985), may occur by one of the following mechanisms: (1) thermal dissociation of molecules with weak covalent bonds; (2) disruption of molecules subjected to radiant energy; (3) disruption of molecules by high-energy particles or electrons, or; (4) single electron transfer to ions of transition elements consisting of incomplete inner electron shells.

The second step in this process consists of the addition of the free radical to another molecule. The free radical may join with another free radical or, more likely, join with another molecule. The resulting product may be the formation of another free radical or the reaction may proceed until oligomers and finally polymers are formed (Dragun and Helling, 1985).

The final step in the free radical oxidation reaction pathway involves the end of free radical production or in the termination of the free radicals formed in the previous step. This can be accomplished by coupling, disproportion-ation, or abstraction. Examples of these three steps are provided in Figures 7 to 9 (Dragun and Helling, 1985). The aromatic structures associated with petroleum products are capable of undergoing free radical oxidation based upon their structural characteristics and water solubilities.
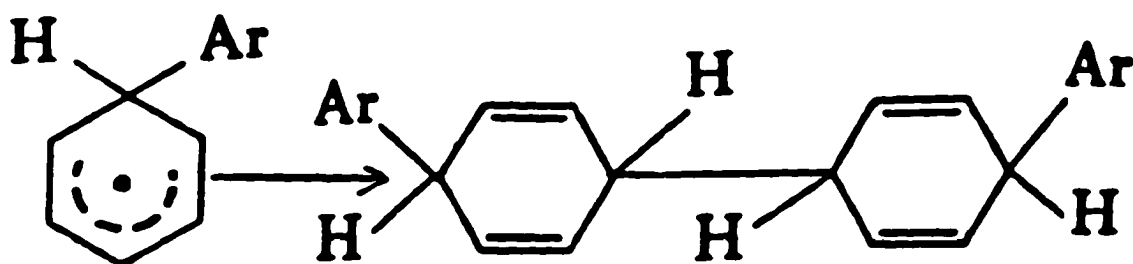
Figure 7. Free Radical Termination By Simple Coupling.

Source: Dragun and Helling, 1985.
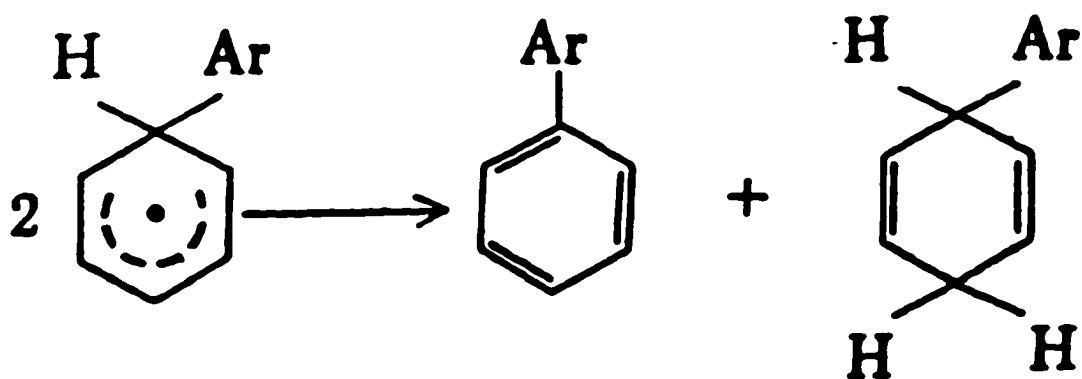
Figure 8. Free Radical Termination By Disproportionation.

Source: Dragun and Helling, 1985.
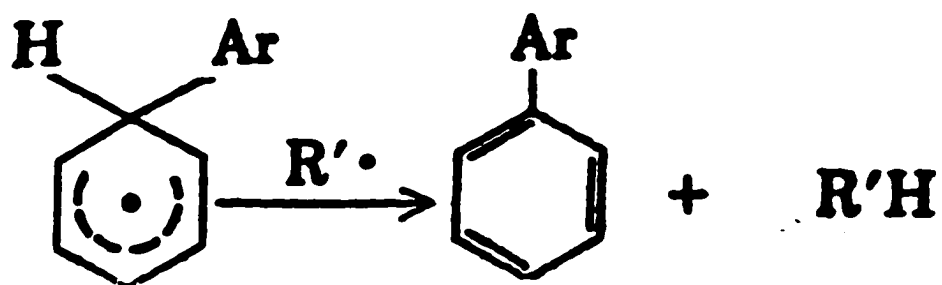
Figure 9. Free Radical Termination By Abstraction.

Source: Dragun and Helling, 1985.

The concentration of free radicals within soil and sludge is based on the degree of humification within the material. Concentrations of 10^{17} to 10^{19} of unpaired electrons spin freely in each dry gram of humic substance (Hassett et al., 1987 a,b). Studies have shown that humic substances have intermolecular interactions with organic contaminants, therefore, it can be logically questioned that as the amount of humic substances increases, the transformation or degradation abilities of the material should increase as well (Stevenson, 1972; Khan, 1972). A correlation between the nature of organic matter applied to soil and enzymatic activity was shown by Ross (1966). Increased microbial and enzymatic activity has been correlated with increased quantities of land applied sludge. Approximately 50 soil enzymes have been identified and these occupy 6 classes of enzymes. These enzymes include those capable of biocatalyzing ring fission in anthropogenic organic compounds (Alexander, 1980). These soil enzymes, due to their ubiquity, can also be found in sludge. A summary of the six classes of enzymes is provide in Table 3. A summary of the major groups of soil enzymes are presented in Table 4.

Organic matter residues contained in soils are composed

Table 3.   Six Classes of Enzymes in Sludges/Soils.

CLASSES OF ENZYMES	ACTIONS OF ENZYMES
1. Oxo-reductases	Catalyze redox processes in cells
2. Transferases	Catalyze the transfer of organic groups from a donor to an acceptor
3. Hydrolases	Catalyze hydrolytic reactions
4. Lyases	Cleave chemical bonds by methods other than hydrolysis
5. Ligases	Accelerate the combination of two molecules
6. Isomerases	Catalyze internal rearrangement of molecules

Source: Alexander, 1981.

Table 4. Soil Enzymes and Their Reactions.

Soil Enzyme	Reaction
1. OXIDOREDUCTASES	
Dehydrogenase	$XH_2 + A \longrightarrow X + AH_2$
Catalase	$2H_2O_2 \longrightarrow 2H_2O + O_2$
Peroxidase and Polyphenol oxidase	$A + H_2O_2 \longrightarrow$ oxidized $A + H_2O$
Catechol oxidase (phenolase, tyrosinase)	o-Diphenol $+ 1/2O_2 \longrightarrow$ o-quinone $+ H_2O$
Diphenol oxidase	p-Diphenol $+ 1/2O_2 \longrightarrow$ p-quinone $+ H_2O$
Urate oxidase (uricase)	Uric acid $+ O_2 \longrightarrow$ allantoin $+ CO_2$
2. TRANSFERASES	
Transaminase	$R_1R_2-CH-NH_3^+ + R_3R_4CO \longrightarrow R_3R_4-CH-NH_3^+ + R_1R_2CO$
Transglycosylase and levansucrase	$nC_{12}H_{22}O_{11} + HOR \longrightarrow H(C_6H_{10}O_5)_nOR + nC_6H_{12}O_6$
3. HYDROLASES	
Phosphatase	Phosphate ester $+ H_2O \longrightarrow R-OH + PO_4^{-3}$

Table 4. Soil Enzymes and Their Reactions (continued).

Nucleotidases	Dephosphorylation of nucleotides
Lipases	Triglyceride + $3H_2O$ --> glycerol + fatty acid
B-Fructofuranosidase (saccharase, sucrase)	B-Fructofuranosidase + H_2O --> R-OH + fructose
Cellulase	Hydrolysis of B-1,4-glucan links in cellulose
Alpha- and Beta- Glucosidase	R-OH + glucose ROH + glucose
Proteinase	Hydrolysis of proteins to peptides and amino acids
Cathespin and pepsin and trypsin	Hydrolysis of proteins
Deaminase	Monocarboxylic acid amide + H_2O --> monocarboxylic acid + NH_3
4. LYASES	
Aspartic acid decarboxylase	Aspartic acid --> analine

Source: Skujins, 1967.

of polysaccharides, lignins, proteins, lipids, aliphatic acids, phenols, and many other substances (Martin and Focht, 1977). The large polymeric units which compose biological residues in soil must be broken down into smaller units that microbial cells may use for energy and cell synthesis. Polysaccharides, such as cellulose, are broken into simpler sugars. Proteins are broken down to form peptides and amino acids. Lipids are oxidized by Beta-oxidation and produce acetic acid units. Lignin is decomposed and it releases simple phenolic units to the soil. These phenols are then degraded into aliphatic compounds, carbon dioxide, and water (Dagley, 1967). Figure 10 provides an illustration of the degradation of lignin.

The humic acid type molecules are complex polymers of phenolic units linked with amino acids, peptides, amino sugars, and other organic constituents (Flaig, 1966; Kononova, 1961; Stevenson and Butler, 1969). Lignin molecules and phenolic polymers that are released during biochemical reactions can be transformed by Beta-oxidation of side chains, by the addition of hydroxyl groups, by oxidation of methyl groups, and by decarboxylation into numerous phenolic compounds (Martin and Focht, 1977). Figure 11 provides an illustration of this degradation
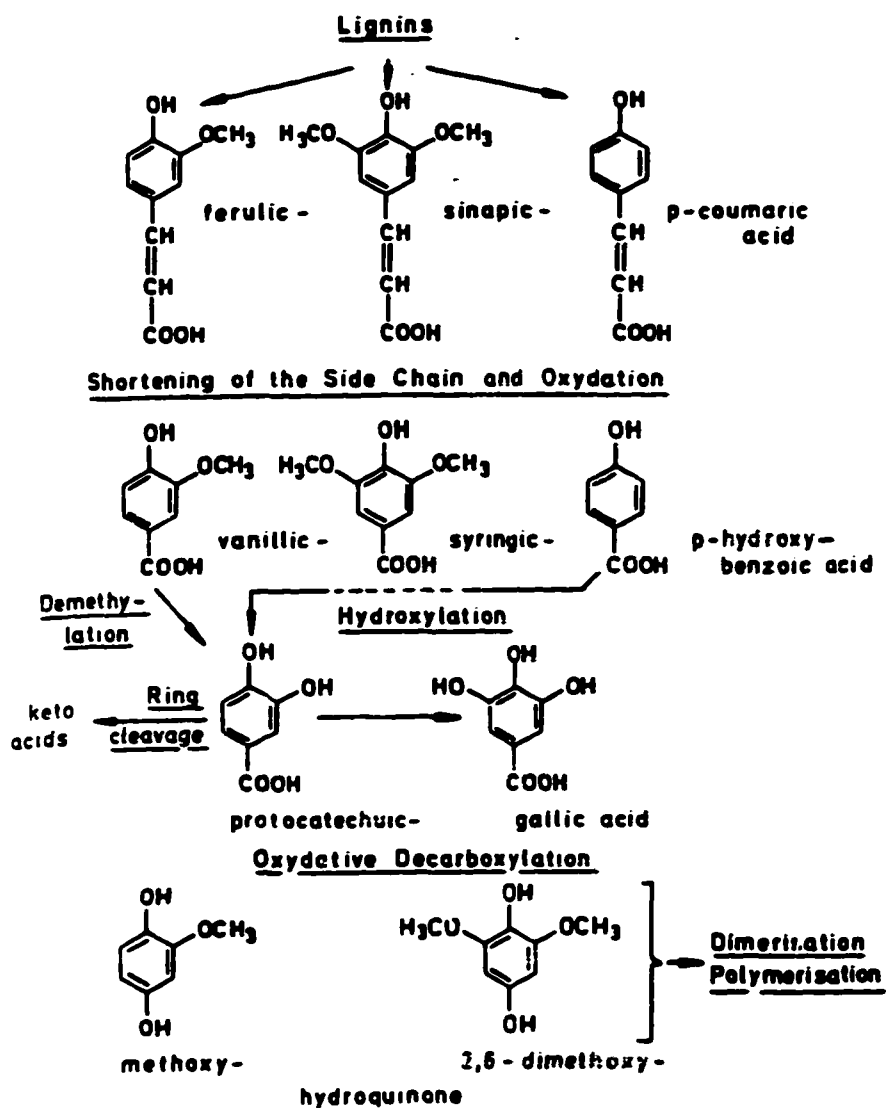
Figure 10. Degradation of Lignin in Soil.

Source: Martin and Focht, 1977.
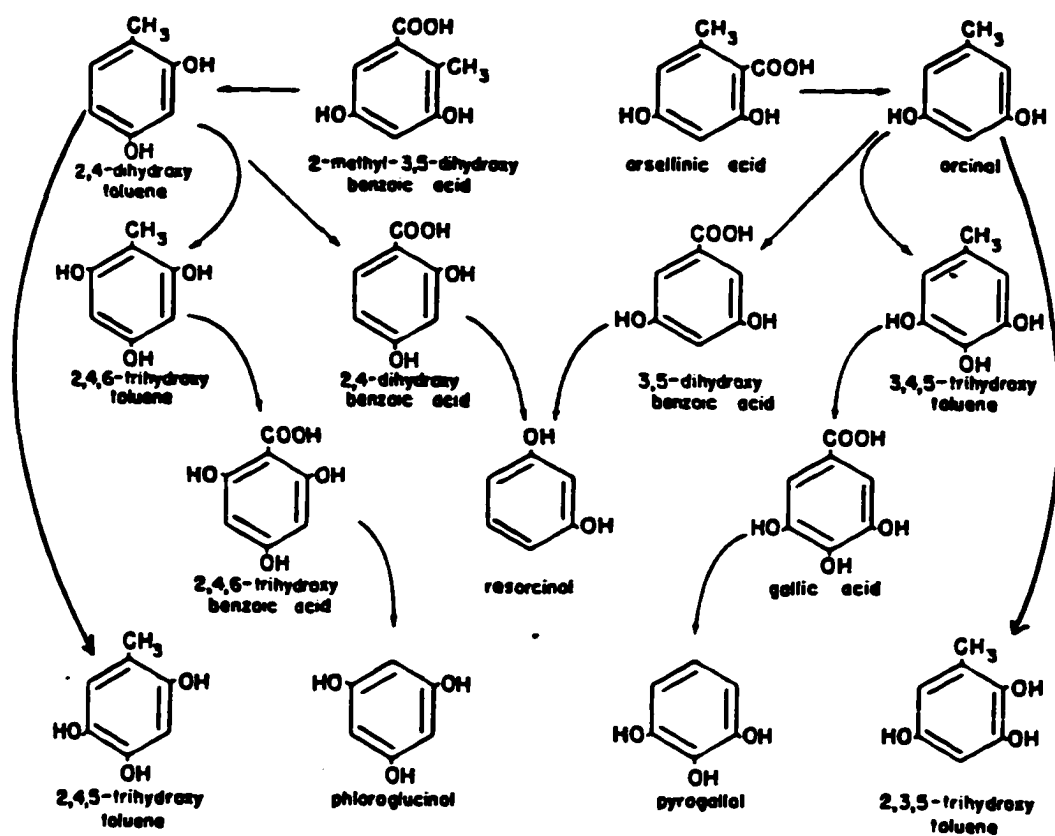
Figure 11. Microbial Transformation of Orsellic and
cresorsellinic acids.

Source: Martin and Focht, 1977.

pathway. Other phenolic compounds may undergo
polymerization reactions with peroxidase or phenolase
enzymes, as illustrated in Figures 12 and 13. Free radical
formation may also occur during these polymerization
reactions. These radicals are available for linkage to
other organic compounds present in the soil. The formation
of humic acids occurs during these reactions and can be
simply illustrated as shown in Figure 14.

The importance of the soil environment in the retention
of humic substances illustrates the potential for
detoxification reactions. A modified vermicomposting
methodology can potentially be an excellent treatment
alternative for remediating organic pollutants such as
petroleum hydrocarbons based upon the humic nature and
microbial activity of soils and sludge.

EARTHWORM ACTIVITY

As earthworms are the principal tillers of the soil,
they are responsible for the mixing of soil nutrients,
enzymes and humic substances within the soil matrix. A
significant reduction in or the elimination of earthworm
communities due to soil contamination can lead to soil
structure deterioration. This action can preclude soil
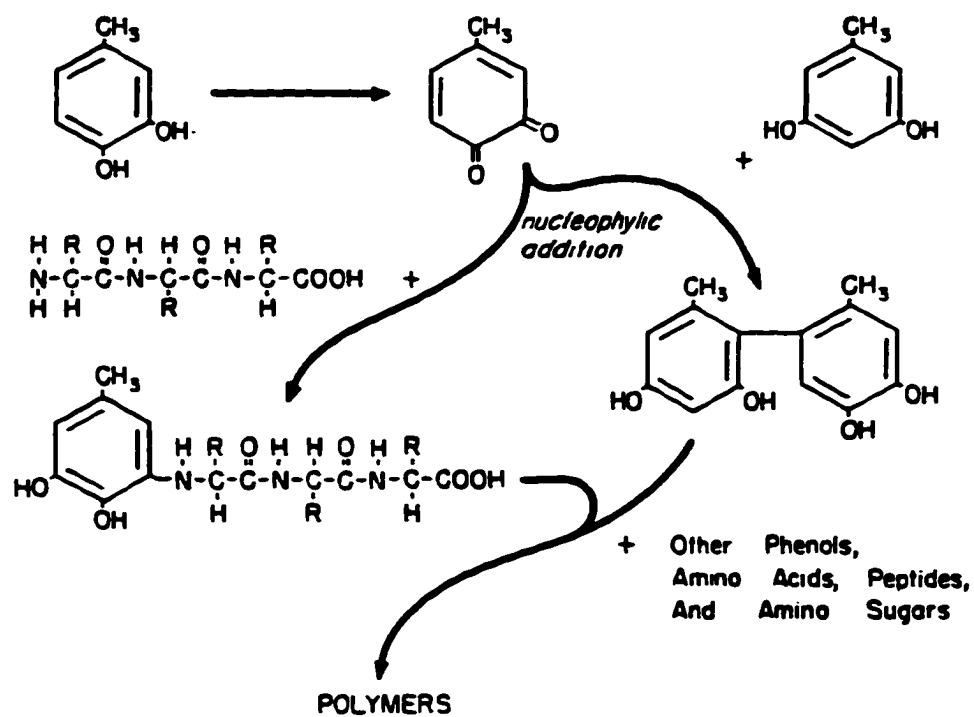
Figure 12:   Potential Oxidative Polymerization Reactions

Involved in the Formation of Humic Polymers.

Source: Martin and Focht, 1977.
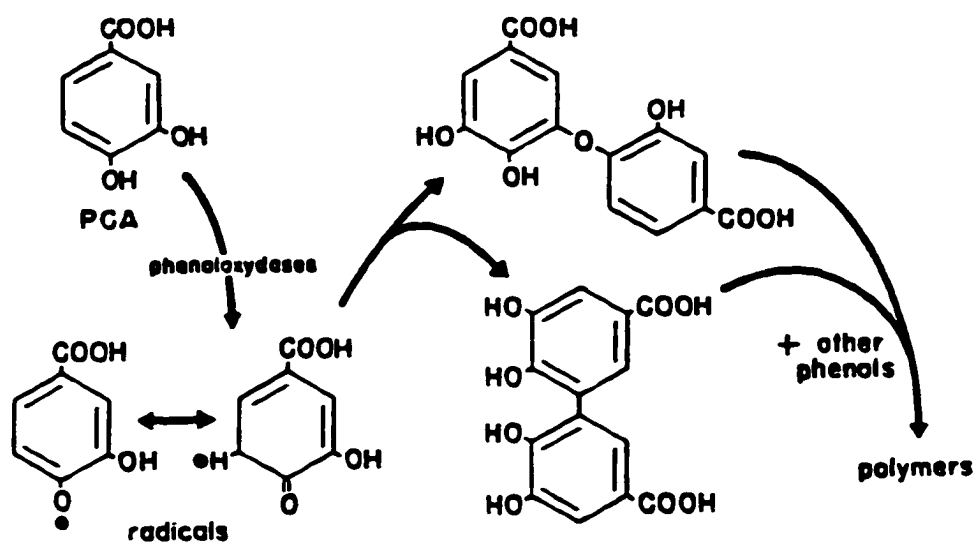
Figure 13. Potential Enzymatic Oxidative Polymerization Reactions of Phenols Involving Free Radical Formation.

Source: Martin and Focht, 1977.
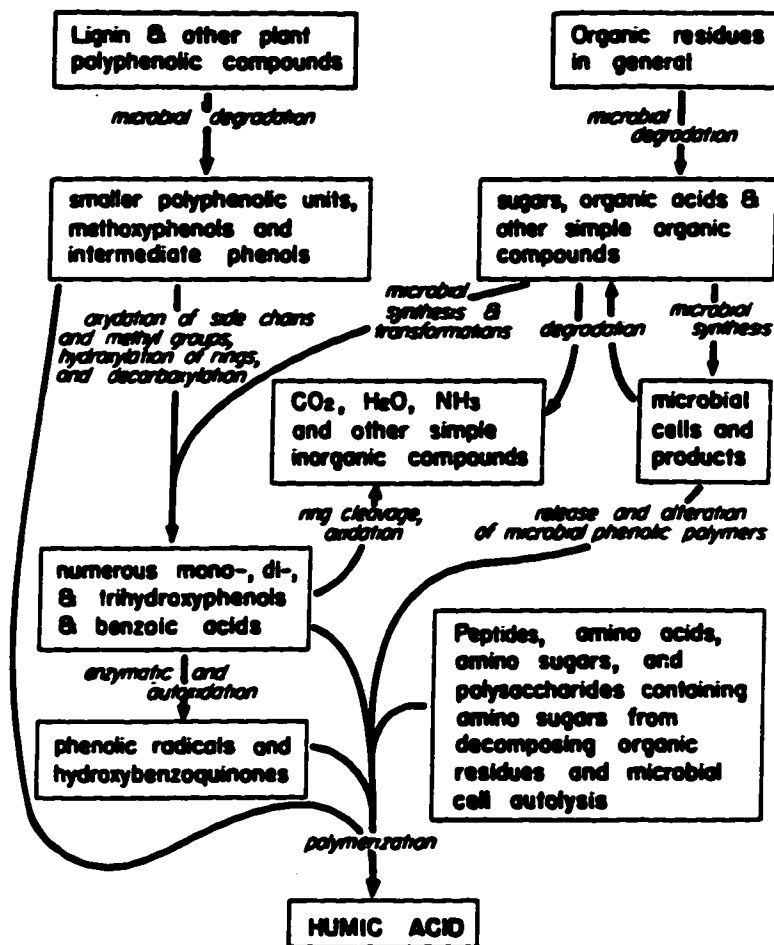
Figure 14. Formation of Humic Acid in the Soil.

Source: Martin and Focht, 1977.

microbial diversity, enzymatic activity, and inhibit biodegradation of organic pollutants. Alternatively, the treatment of soil with biologic sludge can increase earthworm activity through the introduction of nutrients, organic matter and a heterogeneous community of microorganisms. The increased activity of the earthworms facilitates increased microbial and enzymatic activity in the soil environment and a subsequent beneficial effect on biodegradation processes.

The intestinal microflora of earthworms plays a symbiotic role and assists earthworm nutrition by transforming complex humic compounds into simpler, more readily assimilated substances by catalyzing numerous hydrolytic and oxidative decomposition reactions. Several enzymes have been identified in earthworms (Laverack, 1963; Tillinghast, 1967; Nelson et al., 1976; Hartenstein, 1982). Table 5 provides a summary of these enzymes. These enzymes are important in biologic sludge decomposition and stabilization and contribute to the detoxification of organic pollutants in the soil environment.

The importance of earthworms in the incorporation of substrates into the soil ecosystem is well established. Earthworms mechanically break up organic matter and mix it

Table 5. Earthworm Enzymes Supporting Organic Matter

Decomposition and Detoxification.

Amylase	Lipase
Cellulase	Chitinase
Deaminase	Epoxidase
Peroxidase	Catalase
Protease	

within the mineral soil horizons. This action results in
the following occurrences:

(1) redistribution of nutrients to plant systems and
to soil microorganisms;

(2) blockage of phosphate-sorbing sites on soil
components, thereby increasing phosphate
availability to plant systems;

(3) expansion of the degree of mixing within the soil
microbial community and thereby facilitating
further biocatalyst reactions (Syers and
Springett, 1983).

The third discussion point mentioned above has the
greatest impact on biodegradation processes. The increased
enzymatic activity will provide a mechanism for degrading
chemical constituents found in the soil material ingested by
the earthworms. An accompanying tenet to an increased
enzyme production is that organic matter decomposition will
increase. This is important because as the organic matter
decomposes, free radical production will also increase. The
resulting free radicals will then in turn cause hydrolytic
and oxidation-reduction reactions with the anthropogenic
organic chemicals in the soil environment. These reactions
should assist in the detoxification of the organic chemicals

in the soil.

Earthworms require an assimilable source of organic carbon and nitrogen for survival. These nutrients are obtained from the organic matter ingested by earthworms. Earthworm activity affects the chemical composition of soils and the distribution of nutrients throughout the soil horizons. The major activities of earthworms which may have an impact on biodegradation or detoxification processes include the following:

(1) fecal material or casts consisting of thoroughly mixed organic and inorganic materials are deposited on the soil surface and in earthworm burrows. This provides the formation of organic matter enriched soils;

(2) soil microorganisms are ingested along with organic litter and inorganic soil. This affects the concentrations of microorganisms in cast material and in the resulting decomposition processes of cast material;

(3) the thin layer of soil adjacent to earthworm burrows is affected by the secretion of nitrogenous wastes and a secretion of mucus from the earthworms. In addition, the increased air

flow through the soil affects oxygen content (Lee, 1985).

These activities are all important in maintaining the processes which affect microbial growth and diversity and nutrient availability. These are the basic principles of the amended vermicomposting process. Earthworm casts contain some level of nutrients within their structure (Parle, 1963; Graff, 1970; Vimmerstat and Finney, 1973). Although earthworms do not increase the total amount of nutrients within the soil environment, they are capable of increasing nutrient availability and thereby increasing nutrient recycling within the soil (Sharpley and Syers, 1977).

Earthworms chemically influence nutrient activity in the soil by direct enzyme actions on the organic matter contained within the intestine and by metabolizing organic materials. The nutrient metabolites are released into the soil environment. Earthworm casts have been shown to contain increased microbial populations (Atlavinyte, 1975) and enhanced nitrification (Parle, 1963). The relationships between earthworm activities, soil properties, and nutrient availability are highly interactive and complex.

The physical effects of earthworms on soil systems

result from burrowing and cast production. Geophagous and detritivorous species of earthworms ingest and excrete large quantities of inorganic soil particles during burrow formation. The mixing of organic and inorganic fractions of soil during burrowing activities aid in the building of a stabilized soil structure. The water stability of the soil system aggregates is enhanced by the burial of organic matter during burrowing and the burrow walls within the soil are enriched with diverse microbial populations as mucus and waste products are deposited. The pore space of the soil system is increased through burrowing activities, thereby increasing the aeration, infiltrability, and holding capacity of the soil.

Lavelle (1983) divided earthworms into five ecological categories; epigeic, anecic, oligohumic, mesohumic, and polyhumic. Figure 15 provides a representation of these categories based on ecological habitat. The ecological variations in earthworm communities can be based upon their interactions with soil organic matter, microflora and fauna. Bouche (1977) divided earthworms into three classes; epigees (species which live above the mineral soil layer), aneciques (species which live in burrows in mineral soil layers), and endogees (species which live and feed in the mineral soil
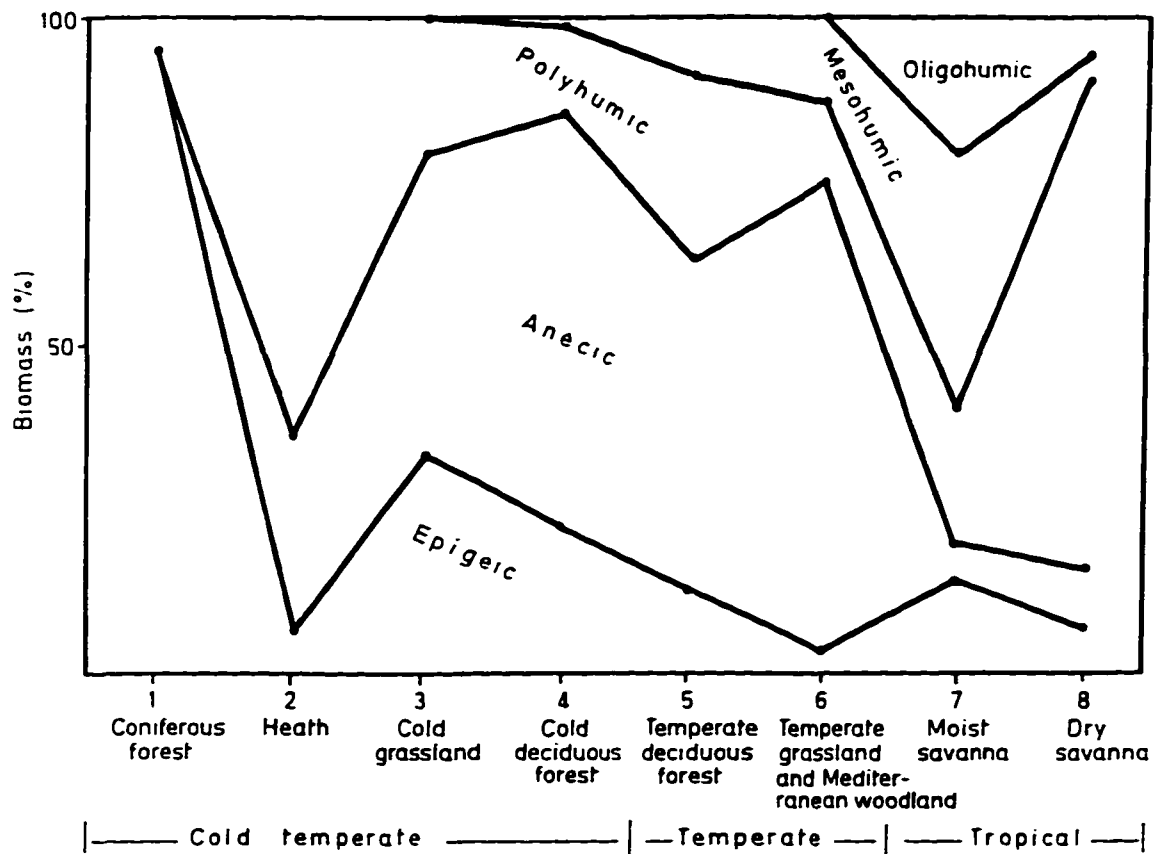
Figure 15.   Earthworm Categories Based on Ecological

Habitat.

Source: Lavalle, 1983.

layers). Hartenstein (1986) provides new biotechnologic nomenclature for Bouche's classifications. These new names are annelepigeo, annelendogeo, and anneldiageo. Annelepigeo class earthworms live above the mineral layer of soil and feed at the surface. Annelendogeo class earthworms live in the mineral layer and tunnel to the surface to feed. Anneldiageo class earthworms live in the mineral layer of soil and burrow within the layer to feed.

Eisenia foetida are annelepigeo class earthworms, therefore, they dwell in surface soils and tunnel to the surface to feed and deposit their casts. Their function is to ingest organic matter at the surface and to incorporate that material into the upper soil surfaces. They also provide aeration to the upper soil layer by their tunneling activities. In addition, they provide channels through any sludge material applied to the soil surface. This natural action provided by this earthworm makes it an ideal candidate for use in the proposed research study.

Many species of earthworms, especially those which construct horizontal burrows, utilize organic matter from within the soil matrix as a food source and then deposit casts in the pore space of the soil. Casts deposited in the subsurface contribute to soil formation or pedogenesis.

Those casts deposited on the surface are potentially more important in terms of soil stabilization (Bouche, 1981). The comparison of the particle sizes in casts and surrounding soils has led to the belief that the physical grinding of the ingesta mechanically alters the size of the particles (Edwards and Lofty, 1977). This mechanical alteration is important in the decomposition of organic matter in the soil profile. The physical effects on soils attributed to burrowing activities include the following:

(1) they are constructed channels rather than interstitial spaces between aggregates;

(2) they are continuous in vertical and horizontal planes, often penetrating from the surface to depths of tens or hundreds of centimeters;

(3) they are of the largest soil pores based on their length and width;

(4) their construction involves the deposition of illuviated materials on the walls which may effect physical changes in soil structure;

(5) they are lined with a protein rich mucus which provides temporary stabilization, and;

(6) they provide a pathway for the movement of surface water, air and large organic particles to deeper

soil horizons (Lee, 1985).

The role of earthworm activities on both the chemical and physical characteristics of soils is well documented. It is important that these effects be considered in organic chemical detoxification. Earthworms provide the perturbation needed for aeration to nonsurficial contaminated soils. Earthworms also provide a concentrated microbe and nutrient laden material to soils in their casts which are essential for detoxification reactions. Therefore, the role of earthworms in this amended vermicomposting treatment method is easily discovered and should be utilized.

SUMMARY

The principles of this amended vermicomposting treatment alternative encompasses the theory of organic matter evolution. The synergistic actions of microbial enzymes, soil physical and chemical properties, and earthworm activities act to degrade contaminated soils and provide a stable soil/sludge environment. The previous discussions have provided a scientific basis for considering the usefulness of an amended vermicomposting approach which can utilize the dynamic conditions that pervade the processes involved in organic matter evolution and organic

chemical degradation.

SIGNIFICANCE OF THE RESEARCH

The benefits associated with this proposed treatment methodology include remediation cost effectiveness and technical efficiency. A wastestream, liquid biologic sludge, could be utilized to accelerate natural detoxification reactions in contaminated soils. This method would not only provide a simple, effective way of disposing of the wastestream, but it would provide an efficient method of soil detoxification. The underlying scientific theory and technologic principles are available. Demonstration through the proposed research may lead to the development of an economically and technologically feasible method for detoxifying contaminated land areas in the Commonwealth of Virginia and elsewhere. In addition, the treatment method may create a new outlet for the marketing and sale of biologic sludge from municipal wastewater treatment plants. The revenue generated from the sale of the biologic sludge may assist municipalities in maintaining their fiscal responsibilities for the treatment of urban wastewaters.

ASSUMPTIONS

The biodegradation of petroleum compounds has received much attention over the past decade and one of the most researched areas has been _in situ_ bioremediation. The use of microbial populations to detoxify petroleum is not a new idea, but the use of a heterogeneous population of microbes delivered via biologic sludge application is an innovative approach. It is assumed in this research that a dynamic soil environment will provide the foundation for biologic sludge decomposition and stabilization and for earthworm burrowing and biocatalyst activities. The use of a sandy soil should provide an environment amenable to humic substance reactions, free radical formation, and aeration activities.

The temperature range used in the experiment (20°-25°C) has been shown to be an optimum temperature for earthworm activity. It is assumed that this temperature range will not cause degradation alone and will not confound the data.

The analytical method used for measuring petroleum contaminant concentrations was performed within the established quality assurance/quality control standards designed for the method. The method has been accepted by federal and state regulatory agencies for determining petroleum hydrocarbon concentrations at contaminated sites.

A sandy soil representative of the Coastal Plain soil formations of Virginia was used in this experiment. It is assumed that the soil will react in the laboratory to the treatment in the same manner as it would react under field conditions. However, not all climatic variables can be controlled in the field as in the laboratory.

SCOPE OF THE RESEARCH

This research project was designed to examine the applicability of the innovative treatment technology to detoxify a Coastal Plain sandy soil. The use of a homogeneous microbial community to biodegrade petroleum contamination has been a long studied issue, however, no conclusive findings have been reported for in situ treatments. There has been a concern that a homogeneous microbial community cannot survive and proliferate in the soil environment. Competition between microbial communities has usually ended in the destruction of the homogeneous population.

This research focuses on the use of a heterogeneous microbial community, which in turn stimulates soil enzymatic and humic chemistry, to degrade toxic soil pollutants. The role of enzymes has received much attention in previous

studies. It has been shown that the enzymes present in liquid biologic sludge and soil are important mechanisms in detoxification reactions. The importance of humic chemistry to detoxification reactions is less understood, but it is a reasonable assumption that free radical interaction with chemical pollutants will result in the ring separation of aromatic pollutants. Since gasoline and diesel are composed of aromatic compounds, it was concluded that ring separation should occur in the soil environment as a result of the free radicals introduced in the biologic sludge.

The use of earthworms to till and perturbate the contaminated soil allows for the soil/sludge matrix to become better aerated. In addition, the earthworm casts should provide a highly enriched microbial and enzymatic source to the soil environment. These casts not only provide basic chemical mechanisms to induce detoxification reactions, but also provide soil stabilizing properties to the parent soil material.

LIMITATIONS

The study is limited to the detoxification reactions which occur in Coastal Plain sandy soil only. The same general theory applies to all classifications of soil, but

the experiment is designed to encompass only sandy soils which are located in the Coastal Plain Region of the Commonwealth of Virginia.

A second limitation of this study is the temperature range in which the study occurred. Since the temperature range is between 20^0 to 25^0 C, the results can only be discussed in this range. Field temperatures do not remain in this range during seasonal variations. This may be an important limitation in the use of this treatment method.

A third limitation of this study is sample collection. The samples were obtained from the soil matrix in such a manner (See Procedure Section) to prevent cross contamination. The samples were randomly obtained from the soil matrix based on a table of random numbers. It was expected that this manner of sample collection provided unbiased samples for analysis and represent the true detoxification reactions which occurred in the soil/sludge matrix.

CHAPTER TWO

LITERATURE REVIEW

Vermicomposting utilizes biotechnologic species of earthworms to accelerate the stabilization of organic materials. In this process, earthworms are provided an optimum environment to consume and metabolize municipal wastewater sludge. The production of fecal material or casts by the earthworms during feeding on sludge material provides soil conditioning properties to the material. Therefore, vermicompost provides a sludge product that contains a high water retention capacity and excellent growth enhancement properties. The earthworms identified as active participants in the vermicomposting process are the branding worms Eisenia foetida and red worm Lumbricus rubella (Atwitter, 1994).

Earthworm conversion of wastewater sludge is a relatively simple process which requires only earthworms, earthworm beds, and sludge material. The process usually requires that deterred and digested sludge is placed in the worm beds and a bulking agent (e.g., wood chips) is added to the sludge. The bulking agent facilitates keeping the bed

in an aerobic state, especially if the water content of the sludge is high. The earthworms within the bed consumes the sludge material and produces the soil enhancing casts throughout different elevations within the sludge. The earthworms are then separated from their castings by the use of a horizontally rotating drum screen. The isolation of the earthworms from their castings performs two important functions; (1) casts are toxic to earthworms, therefore, separation is needed to maintain the population within the sludge, and; (2) the casts are the primary end product of the vermicomposting process and are marketed as soil conditioning amendments to both private and public entities (Cheremisinoff, 1994). The Fallbrook Sanitary District of Fallbrook, California was the first installation to utilize the vermicomposting technique in the early 1980's and discovered that there was a large demand for the compost product and initiated a large scale production project in 1986 (Harris et al., 1990). The process at this facility uses anaerobically digested sludge as the base material. The sludge is placed into drying beds for 30 to 60 days until the sludge is 15% to 18% solids. After the solids content reaches the desired level, the sludge is mixed with straw and then placed into vermicomposting beds for

processing (Atwitter, 1994).

The vermicomposting beds are described by Atwitter (1994) as being approximately eight feet wide and of varying lengths. The earthworms are placed into the sludge material at a rate of one pound per cubic foot of sludge. New sludge material is added to the bed at a rate of four to six inches per week and new straw is added approximately once per month to provide adequate aeration. The worms move the top layer of newly applied sludge and the bottom portions of the bed are stabilized sludge material. The stabilized material is removed to a screening facility and the top layer is returned to the vermicomposting bed to act as seed for the next freshly applied layer of new sludge (Atwitter, 1994). Figure 16 provides a description of the vermicomposting process.

The daily monitoring of the vermicomposting bed is a time consuming effort, but it is of great importance in facilitating the stabilization of the sludge material. Moisture, pH, temperature and feed level management is crucial in providing an optimal environment for the earthworms in the vermicomposting bed.

The stabilized sludge product is made available to public and private entities as a growth-enhancing soil
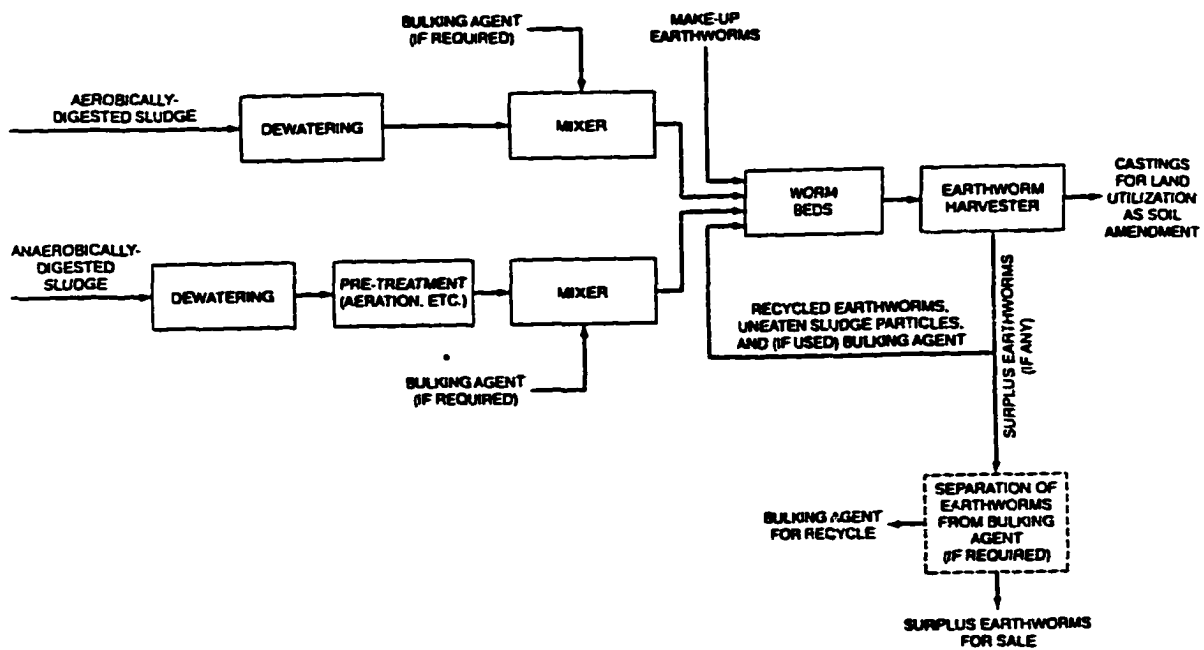
Figure 16. Vermicomposting Process for Sludge Treatment.

Source: Atwitter, 1994.

amendment product. This approach to wastewater sludge treatment has been readily accepted by the general public and the demand for the product far exceeds the production capabilities of the treatment facility (Atwitter, 1994).

Land application of stabilized sludge material has long been an acceptable practice in agricultural areas. The Environmental Protection Agency has shown that the land application of sludge poses less carcinogenic risk than sludge incineration, 6×10^{-4} and 7×10^{-3}, respectively (Atwitter, 1994). However, the beneficial use of the stabilized sludge product is not limited to agricultural settings. The material is also useful in silviculture and mining-site soil enrichment.

The Hampton Roads Sanitation Department (HSRD) of Norfolk, Virginia, does not use the vermicomposting technique for composting wastewater sludge. However, HSRD does utilize windrow composting and produces approximately 11,000 cubic yards of material for direct sale under the product name of "Nutri-Green". The typical users of this composted sludge material can be broken down as follows: 40% to the landscape industry, 30% to municipalities and, 20% to the general public (Logsdon, 1990; Donovan, 1990). Currently, HRSD does not utilize the biologic sludge

produced at any of its treatment plants for soil amendment functions.

Approximately 33% of the wastewater sludge generated in the United States is effectively reused by land application or sold to the general public (EPA, 1988). Table 6 provides a summary of the amount of sludge produced in the United States and a description of the disposal option used by these facilities. Table 7 provides a summary of the disposal methods and the number of facilities which use each method (EPA, 1988). The beneficial reuse of wastewater sludge has quickly become a viable disposal alternative for most treatment plants since the promulgation of USEPA 40CFR Part 503 to address the Clean Water Act's requirement that the EPA develop a regulation for the use or disposal of sewage sludge. The final Part 503 rule which was revised in 1993 encompasses three broad categories of use or disposal: (1) land application to agricultural land, silviculture opportunities and mine reclamation sites;(2) disposal applications to specifically designated land or sewage sludge only landfills, and; (3) incineration at sludge-only facilities. The Part 503 regulations classify sewage sludge into two classes, Class A and Class B. All sewage sludge that is used for land application must meet, at a minimum,

Table 6.   Estimated Sludge Production/Disposal In U.S..

Disposal Practice	Total Mass in Dry Metric Tons x 10^3
Incineration	864.7
Land application: Agriculture	1170.9
Land application: Silviculture	32.3
Land application: Compost	150.2
Land application: Public contract	166.1
Land application: Reclamation	65.8
Land application: Sale	71.1
Co-disposal: Landfill	1818.7
Surface disposal: Dedicated site	258.8

Source: EPA, 1988

Table 7.   Number of POTWs[1] and Sludge Disposal Practices.

Disposal Practice	No. of POTWs Using Practice	Quantity of Sludge Disposed[2]	
Incineration	381	864.7	(16.1%)
Land application	4,657	1,785.3	(33.3%)
Codisposal: Landfill	2,991	1,818.7	(33.9%)
Surface disposal	1,351	553.7	(10.3%)
Ocean disposal	133	335.5	(6.3%)

Source: EPA, 1988

1: Publically Owned Treatment Works
2: Dry metric tons x 10^3

Class B requirements.

The objectives of wastewater composting techniques have traditionally focused on the bioconversion of putrescible organics into a stabilized form and a material free of human pathogenic organisms. In addition, malodor problems associated with wastewater sludge are often abated due to the presence of highly stable organic structures within the sludge end product. Composting can cause considerable drying within municipal sludge. Therefore, the decomposition and drying of wastewater sludge can reduce the cost of handling and increase the potential reuse of compost material (Haug, 1993).

Organic composts provide a number of beneficial effects on the land to which it is applied. It provides a rich source of organic matter for building soil humus which is a necessary constituent for soil structure and moisture holding capacity. In addition, the organic matter in sludge reduces the bulk density of soils and increases their aggregate stability and hydraulic conductivity. Secondly, stable compost can reduce the amount of plant pathogens in the soil matrix, thereby, improving plant resistance to disease. Thirdly, compost provides a rich source of essential micronutrients to soil (Haug, 1993).

High moisture organic wastewater sludge provides a unique management problem. Normal composting procedures (e.g., windrow and static pile) are effective in converting wet sludge material to a more usable form. Most municipal biosolids contain approximately 70%-80% water, therefore the composting procedures may not function as efficiently as expected due to low operating temperatures. In addition, high moisture content within the organic sludge material requires the need to maintain a large pore space within the compost material to provide adequate aeration for stabilization to occur (Haug, 1993).

Sludge reuse as a soil amendment improves the overall productivity of the soil by enhancing the organic matter content, by providing aggregate stabilization, by improving the water retention potential and cation exchange potential, and by raising the nutrient levels.

One of the main concerns associated with the land application of sewage sludge has been the belief that human pathogenic microorganisms will accumulate within the treated areas and cause adverse health effects in the human population adjacent to the site. A recent EPA study found that the pathogen levels in processed sludge are low (Cheney, 1990). The pathogenic organisms in sludge products

have a short residence time in soils. Studies showed that after one year the density of pathogenic organisms in sludge amended soils was approximately equivalent to non-amended soils (Atwitter, 1994).

A study reported by Surampalli et al.(1995) discusses the effects of long term land application of secondary treatment biosolids on groundwater and surface soils. The stabilized biosolids were placed at a rate of 6.03 dry tons per acre per year. The lifetime of the land application site was reported at 8-15 years. The results of the study indicate that groundwater was not adversely affected by the biosolid application. In addition, the bacteriological soil data indicate that the measured levels of fecal coliform and fecal streptococci were close to background levels.

A common link between all biological treatment methods of wastewater sludge is that the stabilization capacity of the method is dependent upon the metabolic reactions of the microorganisms within the treatment matrix. During biological treatment, the degradation of high molecular weight, energy rich materials are broken down to low molecular weight, low energy compounds. Secondary sludges have a low solids content (0.5%-2.0%) and are difficult to thicken and dewater (EPA, 1979). Organic compounds found in

domestic wastewater sludge consist of biodegradable

substances in a colloidal and suspended form (Cherisinoff,

1994). Organic substrates found in domestic wastewater

sludge are typically protein (40%-60%), carbohydrates (25%-

50%), and fats (10%) (Cheremisinoff, 1994).

The contents of activated biologic sludge, referred to

as mixed liquor suspended solids (MLSS), are composed of

active microbial mass, non-active microbial mass, non-

biodegradable organics, and inorganic mass. In a con-

ventional activated wastewater sludge system, the active

microbial mass composes approximately 30% or less of MLSS

(Cheremisinoff, 1994). The control of substrate

decomposition is effected by the enzymatic reactions

occurring within the treatment vessel. Enzymes have the

following intrinsic properties:

(1) they are effective in small amounts;

(2) they survive biochemical reactions unchanged, and;

(3) they accelerate the speed in which equilibrium is

achieved (Mudrack and Kunst, 1986).

Biologic degradability is limited by the molecular structure

of the material to be degraded and by the composition of the

biomass in proximity of the substance. The biomass must be capable of providing the necessary enzymes to degrade the substrate. Therefore, a diverse biocoenosis that inhabits and proliferates in a material balanced for all microorganisms, such as biologic sludge, provides an opportunity for contributing many diverse enzymes to degrade an organic structure (Mudrack and Kunst, 1986).

Sludge stabilization, as described by Hartenstein (1981), is a process in which "readily decomposable material is converted into colloidal, humified lignoproteinaceous material and human and animal pathogens are rendered ineffective or disappear". During stabilization or humification, sludges undergo decarboxylation, dehydration and demethanation. An equilibrium is established between the production and oxidation of humic substances and the aerobic stabilization process ultimately produces carbon dioxide and water as fresh applications of sludge are added to the stabilized or humified older sludge. Hartenstein (1981) notes several studies which support this theory of organic sludge humification. In addition, several studies indicated that the organic material contained in sludge do not significantly accumulate in aerobic soils (Waksman, 1936; Kononova, 1975) regardless of loading rate frequency

(Waksman, 1936; Thomas and Bendixen, 1969).

As illustrated in Figure 1, humic substances are composed of humic acids, fulvic acids and humin. Humic acids and fulvic acids are not single chemical entities described by a simple chemically-defined structural formula. Both are defined as a model structure composed of the same basic structural units and the same type of functional groups (Stevenson, 1982). The basic macromolecular units are composed of aromatic, phenolic, quinonic and heterocyclic components which are randomly condensed and or linked by aliphatic oxygen, nitrogen or sulfur bridges. In addition, the surface of the macromolecule contains chains of aliphatic, glucidic, amino acidic, and lipidic composition and also chemically-active functional groups comprised of carboxylic, phenolic and alcoholic OH, aldehydric and ketonic units (Senesi and Chen, 1989). A representation of the macromolecular structure of humic and fulvic acids is provided in Figure 17. These macromolecules are capable of forming water soluble and water insoluble complexes with inorganic ions and hydrous oxides and may interact with clay minerals and hydrophobic organic compounds (Hartenstein, 1981). Approximately 10^{18} stable free radicals are present per gram of humic or fulvic
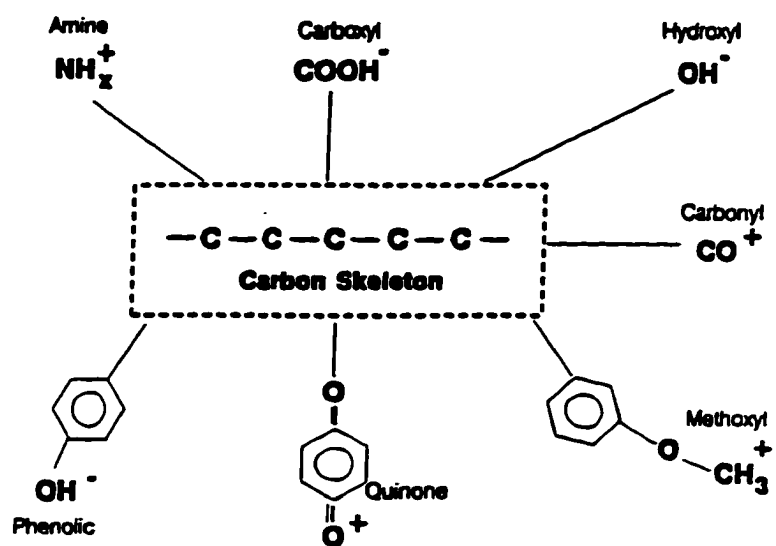
Figure 17. Functional Groups Attached to Humic Substances.

Source: Brady, 1990.

acids (Hartenstein, 1981).

Humic acid constituents are composed as 50%-60% carbon, 30%-40% oxygen, 3%-6% hydrogen, 0.5%-6% nitrogen and 0%-2% sulfur (Schnitzer et al., 1973; Griffith and Schnitzer, 1975). The average functional group composition of humic acids consists of 2%-7% OH, 3%-10% carboxyls, 2%-5% phenolic OH, 0%-2% alcoholic OH, 0.5%-4% carbonyls, 1%-2% quinones, and 1%-4% ketones (Schnitzer et al., 1973; Hatcher et al., 1981).

Fulvic acids contain more oxygen and sulfur than humic acids. They also contain less carbon, hydrogen and nitrogen than humic acids. The constituents of fulvic acid consist of 40%-50% carbon, 40%-50% oxygen, 4%-7% hydrogen, 1%-3% nitrogen and 1%-4% sulfur. The functional groups of fulvic acids include 1.5%-6% carboxyls, 2%-6% phenolic OH, 2.5%-4% alcoholic OH, 2%-2.5% quinones, 1.5%-2% ketones and 0.3%-5% carbonyls (Schnitzer and Khan, 1972; Griffith and Schnitzer, 1975; Chen et al., 1977).

Humic substances are complex polymers with high molecular weights ranging from hundreds to tens of thousands. The high molecular weight of these substances should enable the formation of van der Waals bridges between soil components, including organic contaminants. In

addition, the high number of functional groups attached to
humic substances increases their interactive and
complexation reactions with soil constituents, both
naturally occurring and anthropogenic.

Wastewater solids consist of a high proportion of
labile organic constituents which may be removed during
treatment processes. When wastewater sludge is applied to
soil, the decomposition rate is dependent upon the degree of
stabilization and humification. Mitchell et al., (1978)
show that aerobically digested sludge lost 48% of its
organic matter in contrast to anaerobically digested sludge
which lost from 28% to 36% of its organic matter after being
applied to grassland soils. This occurrence may lead to the
supposition that the greater loss of organic matter was due
to the reactivity of the aerobic sludge within the soil
matrix. Therefore, it may be reasonable to also predict
that an aerobic liquid secondary sludge material contains
the same inherent reactivity as deterred digested sludge.

One of the major objectives of utilizing the activated
sludge process and aerobic digestion is to maximize oxygen
concentrations within the wastewater sludge. This action
increases the amount of terminal electron acceptors (oxygen)
within the sludge material (Eckenfelder and O'Connor, 1961).

When sludge is added to soil, it becomes a source of electron donors and may deplete oxygen and alternate electron acceptors causing a depression of Eh and an increase in anaerobic metabolites. Therefore, the use of earthworms to provide aeration to the sludge amended soils should create an environment with more terminal electron acceptors and facilitate soil/sludge stabilization.

The principal differences between wastewater sludge and mineral soils are: (1) the higher concentrations of organic matter in sludge; (2) the greater stability of organic matter in soils due to humification, and; (3) the presence in soils of mineral matter as a stable layer of crystals rather than as polydispersed simple salts in wastewater sludge (Mitchell et al., 1977). Mitchell et al. (1977) conducted a series of studies which examined the role of the earthworm, Eisenia foetida, in the stabilization of sludge. Earthworms play an important role in soil by grinding detrital material, by affecting the density and composition of soil microbial communities and by altering the physical and chemical properties of soil (Edwards and Lofty, 1972; Satchell, 1967). These actions facilitate the stabilization of the organic matter in wastewater sludge into humic substances. Their studies show that the

decomposition rate of sludge organic matter was significantly increased after the ingestion of the sludge by the earthworm, E. foetida. An accelerated decomposition rate of about 2- to 5-fold was observed during the studies. These rates were dependent upon sludge characteristics and ambient temperatures. In addition, the dynamic microbiological content of sludge also contributes to the fluctuating stabilization rates during humification. The production of casts by the feeding earthworms also provides greater surface areas and microsites for microbial degradation of organic matter. Other studies have also shown that earthworm activity in soil increases the infiltration rates of water (Slater and Hopp, 1947; Guild, 1955; Satchell, 1958; Ehlers, 1975). This increase is of importance when considering the use of a liquid biologic sludge as an inoculating agent for the degradation of synthetic organic substances in soil. The ability of water to percolate to lower regions within the proposed treatment method allows for the soil moisture to remain at a level necessary for microbiological proliferation and stimulate detoxification reactions within the soil matrix.

Hartenstein and Hartenstein (1981) show that the earthworm E. foetida is capable of digesting a volume of

activated sludge that was approximately 20 times its own volume in 10 days. These authors also found that the earthworms did not ingest all the activated sludge which was available to them. This occurrence may have resulted from the contact between casts and activated sludge. Earthworm castings are toxic to E. foetida (Kaplan et al., 1980) and may render the activated sludge material which has incidental contact with the castings as toxic. Hartenstein and Hartenstein (1981) proffered a value of 18% as the amount of sludge ingested by earthworms present in their study. Therefore, the remaining sludge was stabilized by activities associated with the heterotrophic microbial community present in sludge. Earthworm casts exhibited a lower pH, higher Eh, greater cation exchange capacity, lower concentrations of phenols and nitrogen, and higher concentrations of nucleic acids and phosphorus than the sludge materials used in their study. The higher Eh value indicated that aerobic metabolism was favored above anaerobic metabolism. This observation was also demonstrated by the absence of a malodorous castings. The higher cation exchange capacity of the castings may be an indication of the appearance of soil minerals resulting from sludge stabilization or mineralization. The higher cation

exchange capacity might be indicative of the formation of humic polymers within the stabilized sludge since humic polymers are known to have high cation exchange capacities (Holtzclaw and Sposito, 1979). The decrease of phenolic concentrations may have occurred due to the presence of earthworm gut microflora capable of decomposing phenolic compounds or by the incorporation of the phenols into the complex humic polymers resulting from stabilization of the sludge and earthworm casts (Neuhauser et al., 1978).

Hartenstein (1982) studied the effects of aromatic compounds, humic compounds, and lignins on the growth of $E.$ foetida. The study investigated the toxicity of low molecular weight compounds with simple functional groups identified as secondary metabolism products and high molecular weight compounds such as lignins and humic acids. The outcome of the study showed that benzene was not toxic at normal concentrations, but did cause significant growth reduction at an 8% (80,000ppm) concentration. Toluene was found to be toxic at a concentration of 4% (40,000ppm) and caused a growth rate reduction at 1% (10,000ppm) concentration. However, both humic acids and lignins were not shown to be toxic or cause a growth rate reduction at any concentration. This information indicates that

petroleum product constituents (i.e., benzene and toluene) are not completely toxic to the earthworm E. foetida and that it can be used as a facilitator for the degradation of petroleum compounds within certain concentrations.

Earthworms require microorganisms, cellulose, and soil as essential ingesta for growth and survival. The microorganisms present in soil and sludge serve as primary consumers and decomposers of labile organic components. In addition, earthworms accelerate the oxidation of organic matter by their ingestion of microbial species (Hartenstein and Neuhauser, 1985) and increased aeration of soil (Abbot and Parker, 1981). As earthworms feed on microorganisms, they may also decrease the number of pathogenic microbes within the sludge material (Amaravadi et al., 1990). The earthworms E. foetida and L. terrestris were found to have bactericidal activities associated with the enzyme peroxidase (Hassett et. al., 1988). In addition, Amaravadi et al., (1990) show that E. foetida exhibited virucidal activity during experimentation.

Laverack (1963) identifies the presence of lichenase, protease, cellulase, chitinase, amylase, and lipase in tissue extracts from earthworm alimentary tracts. These enzymes allow earthworms to feed on soluble organic matter

(i.e., glucosides, amino acids, fatty acids) and transform them to readily assimilated substances. In addition, these enzymes provide a mechanism for the metabolism or cometabolism of organic pollutants during biochemical reactions within the soil microbial communities.

An earthworm requires a large amount of water to enter and contact its body. This requirement is due to an earthworm's cutaneous respiratory system. Earthworms can obtain water from their food or by direct ingestion and contact with water from soil surface films or from water-filled pore spaces within the soil matrix. They must obtain oxygen by absorption of solution through the cuticle on their body. The cuticle of earthworms consists of layers of unbanded collagen fibers lying in a fibrillar material containing mucopolysaccharide. Gland cells in the epidermis secrete a mucous type lubricant for the body wall during tunnelling and for maintaining the worm surface moist during respiration. The mucus producing cells were called reticulate monochromatic cells by Richards (1978) and they produce a carboxylated acid mucus which has a low viscosity. This acid material provides a respiratory surface film for the earthworm (Lee, 1985). The carboxylic acid material and mucopolysaccharide material remain on the soil particles

encountered during tunnelling and provide soil aggregate stability.

Hartenstein (1986) discusses the use of domestic sewage sludge for remediating toxic soil sites. He states that "the cost and time for soil cleanup could be substantially reduced by (i) capitalizing on urbanization; (ii) using sewage sludge as a source of metabolic energy and a soil as a physicochemical reagent; (iii) deploying established trickling filter technology as a tool; (iv) considering the principle of recurring perturbation by which the rate of decomposing toxic organic matter can be maximized, and; (v) using earthworms". These observations are important in the recognition of the opportunities available to the utilization of an existing heterogeneous population of microorganisms present in sewage sludge to remediate contaminated soils. In addition, the perturbation of soil and sludge materials by earthworms illustrates the importance of maintaining an aerobic system in which biodegradation may occur.

ROLE OF HUMIC SUBSTANCES

Humus is a complex mixture of resynthesized products of decay. Its principal components are predominantly protein-

aceous materials derived from microbial synthesis and lignin (Hausenbuiller, 1985). Humic substances usually possess a large surface area (500-800 m^2/g) and a cation exchange capacity of 200-400meq/100g. Soil humus provides several beneficial properties which include: (1) increases soil water holding capacities; (2) increases soil buffering capacities; (3) promotes a heterogeneous microbial population in soil; (4) increases nutrient availability to plants; (5) improves soil physical properties, and; (6) reduces the toxicity of toxic substances of both natural and anthropogenic origin (Martin and Focht, 1977).

Humic particles are negatively charged and are able to hold cations in adsorbed form. The predominant sites for negative charge adsorption is located at the phenolic (-OH) and carboxylic (-COOH) groups. These groups are binding sites for the retention of cations such as magnesium, calcium, potassium (Hausenbuiller, 1985). This action will bind the heavy metals present in sludge and allow them to be used as nutrients for plant growth and earthworm proliferation.

A second major source of organic matter in soil is derived from decayed tissue, mostly from microbes, that has not been chemically altered to a semistable form. This type

of material can comprise up to 33% of soil organic matter.
Figure 18 illustrates the organic matter cycle within the
soil environment. This diagram shows that the simpler
compounds in organic residues are rapidly degraded and
become mineralized end products or new microbial tissue.
Resistant compounds persist for longer periods and remain as
humic substances. Eventually, these resistant components
are transformed into simple end products (Hausenbuiller,
1985). This same organic matter cycle applies to the
biologic sludge material placed onto soil.

In order to assess the role of humic substances in the
fate and transport of pollutants in soil, it is important to
consider the potential interaction between the pollutants
and humic substances. Humic substances interact with and
adsorb to synthetic organic chemicals due to their
polyelectrolytic nature. The presence of a large variety of
chemically reactive functional groups attached to humic
substances provides a mechanism for binding to organic
chemicals such as pesticides, petroleum hydrocarbons, and
surfactants (Senesi and Chen, 1989). Because of the
functional groups and hydrophilic and hydrophobic binding
sites associated with humic substances, physical and
chemical adsorption and hydrolysis are the major mechanisms
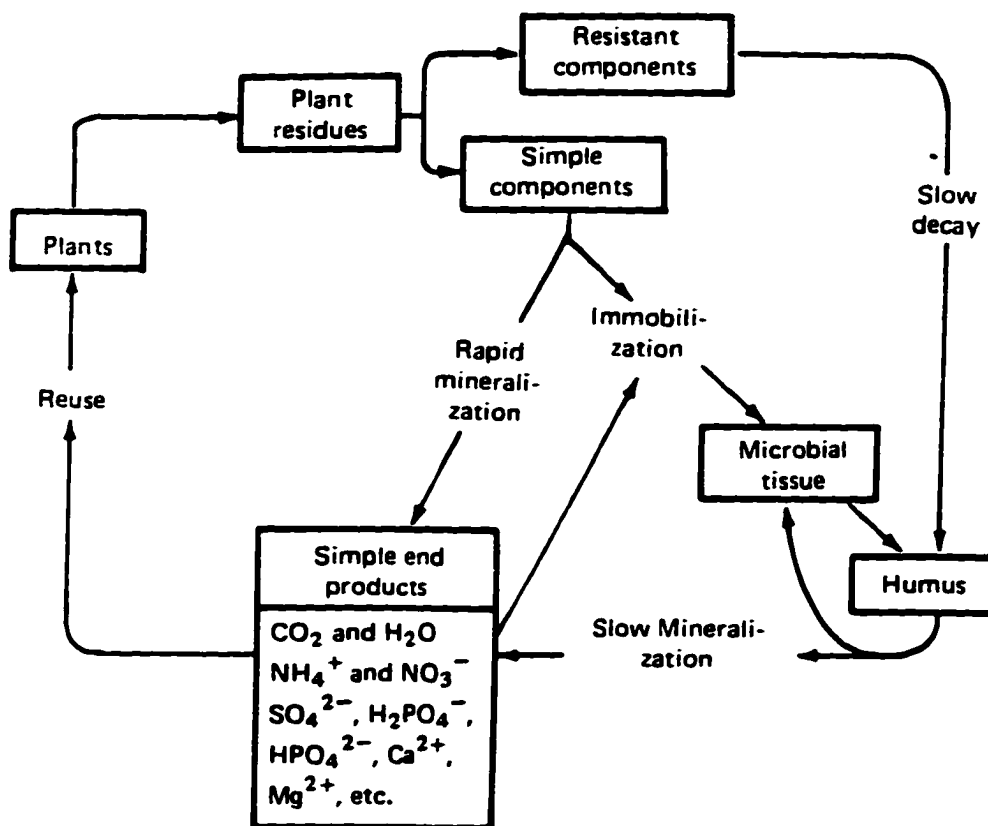
Figure 18: Soil Organic Matter Cycle.

Source: Hausenbuiller, 1985.

of organic substance degradation in the soil environment.
Also, chemical bonds of various strengths and stabilities
are formed between humic substances and organic chemicals.
These bonds range from weak, partially reversible, physical
associations to strong, irreversible covalent bonds
(Stevenson, 1972; Hayes and Swift, 1978). All of the
aforementioned physical and chemical processes can affect
chemical degradation, detoxification, residue persistence,
mobilization and transport processes, and bioavailability of
organic chemicals in soil.

Sorption represents the most important interaction
mechanism exerted by humic substances on the fate and
transport of toxic organic chemicals in the soil and sludge
environments. Adsorption controls the quantity of free
organic chemicals in soil solution. Once an organic
chemical is adsorbed by humic acids, it can be easily
desorbed, desorbed with difficulty, or remain sorbed
permanently. The most important properties of an organic
chemical which determines the type of interaction it will
have with a humic substance molecule includes the chemical
character of the molecule, the shape and configuration of
the molecule, the pK_a value (acidity) of the molecule, the
pK_b value (basicity) of the molecule, the water solubility

of the chemical, and the molecule's polarity and/or charge
distribution (Bailey and White, 1970).

Several types of bonding mechanisms occur simul-
taneously in the adsorptive interactions between humic
substances and organic chemical pollutants. These
mechanisms include ionic bonding, hydrogen bonding, van der
Waals attractions, ligand exchange, charge-transfer
(electron donor-acceptor) processes, covalent bonding
(chemical and/or enzyme mediated), and hydrophobic bonding
(Senesi and Chen, 1989).

Ionic bonding applies to a small number of organic
chemicals which become cations in solution or can accept a
proton to become cationic. Adsorption by ionic bonding
occurs through ionized carboxylic and phenolic hydroxyl
groups on the surface of humic substance molecules
(Schnitzer and Kahn, 1972).

The presence of oxygen- and nitrogen-containing
functional groups, as well as, hydroxylated and amino groups
on humic substances suggests that hydrogen bonding is an
important adsorption mechanism for organic chemicals that
contain similar complimentary groups. The organic
chemicals, however, will be in competition with water
molecules for these binding sites (Senesi and Chen, 1989).

Van der Waals forces are characteristically weak bonds that operate in all adsorbent-adsorbate interactions. They result from short range dipole-dipole or dipole-induced dipole or induced dipole -induced dipole attractions. These interactions assume particular importance in the adsorption of nonionic and non-polar molecules or portions of molecules on similar sites of the adsorbent humic molecule (Burchill et al., 1981). These forces are additive and their contribution increases with the size of the molecule and with its capacity to adapt to the adsorbent surface.

The presence of groups that possess an electron-deficient acceptor (quinone) and an electron rich donor (activated aromatic ring) in humic substances and in an organic pollutant which possess similar traits allows the possibility of an interaction based on the formation of electron donor-acceptor or charge-transfer binding between structural moieties (Senesi and Chen, 1989).

The formation of covalent bonds leads to highly stable and potentially irreversible bonding of organic pollutants or their degradation reaction intermediates or products to humic substances. This bond formation is generally mediated by chemical, photochemical, or enzymatic catalysts. Enzyme-mediated, oxidative cross-coupling mechanisms lead to the

incorporation of stable anilines and phenols by humic
substances (Senesi and Chen, 1989).

Enzymaticaly-mediated oxidative coupling reactions are
not only important in the synthesis of humic substances, but
also for the incorporation of organic pollutants into humic
substances. Oxidative-coupling enzymes are metal containing
and belong to the groups of monophenol monooygenases and
peroxidases. These enzymes catalyze the oxidation and
covalent linking of phenolic compounds and aromatic amines
to each other or to humic substances. The most important
monooxygenase, laccase, contain copper and require oxygen
for their activation. Peroxidases contain a heme group and
require hydrogen peroxide for activation.

Phenolic compounds are believed to be coupled by
laccases and peroxidases by involving free radicals (Sjoblad
and Bollag, 1981). Free radicals are atoms that contain
unpaired electrons and have a high affinity to react and
form bonds with other atoms. The concentration of free
radicals appears to increase as humification proceeds.
Concentrations of 10^{17} to 10^{19} of these unpaired electrons
spin freely in each dry gram of humic substance. Any
organic molecule is potentially vulnerable to free radical
interaction.

Since most aromatic organic chemical pollutants that release phenols or anilines during degradation could potentially bind to humic substances through enzyme catalyzed polymerization reactions, this process can be used to minimize or remove organic pollutants from soil environments. This event has significance in the use of the amended vermicomposting treatment method.

Hydrophobic adsorption has been proposed as a mechanism for retention of nonpolar organic compounds or organic compounds having nonpolar regions. These nonpolar regions bind to the hydrophobic regions of humic substances. This type of adsorption is caused by a weak solute-solvent interaction within the soil. Water molecules are not competitors with nonpolar chemicals for binding sites at the surface of humic substances. Hydrophobic binding sites on humic substances include aliphatic side chains or lipid segments and lignin-derived moieties with high carbon content and limited polar groups. Hydrophobic retention acts as the partitioning of a solvent to a non-specific surface (Hance, 1965).

MICROBIAL ACTIONS IN ORGANIC COMPOUND DEGRADATION

Since the 1930's, the mechanism of aerobic

mineralization has been studied. It became obvious that several specific enzymes acting in sequence were needed to provide the nutritional needs of a single microorganism. Stephenson (1949) illustrates the importance of enzymatic reactions when his research showed that Pseudomonas putida could utilize 77 compounds as its sole source of carbon within a synthetic medium. His study indicates that hundreds of specific enzymes must function at a cellular level as needed.

Karstrom (1930) states that bacterial enzymes fall within two distinct categories; those present at all times (constitutive) and those present in full amounts only when their substrate of a metabolic precursor of that substrate was present (adaptive). Changes in cellular enzyme complement by physiological mechanisms and genetic variations operate as a method of enhancing the response of microbial populations within soil to new circumstances created by the addition of organic matter and organic chemicals to that arena. Therefore, it is not surprising to note that the catabolic pathways associated with Pseudomonas putida and similar microorganisms active in organic chemical mineralization are adaptive to their environmental circumstances. According to the principle of simultaneous

adaption, organic compounds which are intermediates in a catabolic pathway are oxidized immediately by cells grown with a precursor of that compound. Compounds that are oxidized using other unrelated pathways or occur as intermediates earlier in the pathway are oxidized after a lag time period needed to synthesize the specific enzyme (Jacob and Monod, 1961). In addition, studies performed by Hegeman (1966) and Ornston (1966, 1971) indicate that groups of enzymes were induced in blocks containing enzymes for earlier catabolic steps with later intermediates functioning as enzyme inducing agents. Bull and Slater (1981) indicate that many processes within the soil are carried out by a consortia of microorganisms.

Microbial consortia are microbial communities that interact in a beneficial way to promote cell growth and proliferation. Many microbial processes, including organic chemical mineralization and humification of wastewater sludge, are dependent upon the actions of a consortia rather than a pure microbial culture. Consortia are more often suited to situations in which the environment and competing populations cannot be effectively controlled, such as in a soil/sludge mixture (Tiedje et al., 1994). The advantages of using a consortia include: (1) providing a range of

microorganisms providing a stable process; (2) providing
metabolic interactions essential for reaching the desired
catabolic pathways, and; (3) providing genetic diversity to
facilitate the distribution of enzymes within the treatment
matrix. Diversity of microorganisms allows for the
stabilization of the catabolic process. This diversity can
be characterized into four types: (1) kinetic diversity;
(2) tolerance to stress; (3) substrate diversity, and; (4)
organism positioning (Tiedje et al., 1994).

Kinetic diversity controls the process rates of
chemical degradation and stabilization. Microbial treatment
systems must be capable of processing high and low
concentrations of pollutants. A microbial community must
contain species with a broad range of affinities for
substrates (K_s) and maximum growth rate values in order to
process varying concentrations of pollutants.

A consortia chosen for remediation alternatives may
contain species that have different kinetic optima if native
microorganisms which have the same degradation capacity are
found in large numbers that have already assumed kinetic
diversity. Soil environments used for the treatment of
petroleum hydrocarbon contamination may already contain 10^5
out of 10^7 bacteria per gram of soil capable of degrading

petroleum substrates.

Tolerance to stress is important to microbially-mediated remediation techniques because many contaminated sites contain several organic pollutants or environmental conditions which are potentially toxic. Conditions such as high/low pH values, chlorinated solvent contamination, low soil moisture content, extreme temperature fluctuation, and periods of low or no oxygen content can all adversely affect soil microorganism actions.

Substrate diversity or the ability of a microbial community to utilize more than one substrate as an energy source, is also important in biodegradation. Most microorganisms have substrate specific enzymes, therefore, a diverse group or consortia of microorganisms that contains a wide range of enzymes proffers the most effective method of broadening the degradation pathways of organic pollutants (Tiedje et al., 1994).

The strategies used to isolate microorganisms in organic chemical detoxification studies has failed to recognize that most soil environments are capable of sustaining the growth of a diverse microbial community having a wide range of metabolic capabilities. Many heterogeneous communities have shown a beneficial

relationship between the various population species rather than acting as individual or isolated populations (Slater and Lovatt, 1984).

Biodegradation or detoxification occurs more rapidly in the presence of mixed cultures of microbes than with pure isolates. This appears to be especially true for cultures isolated from a complex environment such as activated sludge. Herbert et al., (1976) attempts to demonstrate the nutritional needs between 50 different microbial species isolated from activated sludge, however, the study only succeeded in approximately 15% of the trials. The number of successes are not as important as the realization that the heterogeneous microbial population relies, as a whole, on its members to provide an environment suitable for growth.

An additional study provided by Slater and Lovatt (1984) discusses the ability of a four member microbial community that was isolated from activated sludge to utilize a specific substrate, linear alkyl benzene sulfonate (LAS), as a carbon source. Two of the four members were not capable of sustaining growth on the substrate; however, two were capable of growth. Each of the microorganisms complemented each other and synergistically caused a LAS detoxification rate greater than the two individual

microorganisms alone. The results indicated that the four individual species were not able to cause ring cleavage alone, but as a four member consortia significant cleavage occurred. This study provides strong evidence that metabolic cooperation or cometabolism is present within activated sludge material.

DETOXIFICATION OF PETROLEUM HYDROCARBONS

Over the last few decades, large scale releases of chemicals and petroleum products have occurred. Figure 19 provides a summary of the potential fates of those chemicals released into the terrestrial environment. Microorganisms are capable of catabolizing any organic compound that structurally resembles a naturally occurring compound. An organic compound may undergo nonenzymatic or enzymatic reactions when encountered by a subsurface soil microorganism, however, most detoxification reactions are facilitated by enzymatically mediated microbiological reactions (Riser-Roberts, 1992). The enzymatic reactions act synergistically with abiotic chemical reactions, such as with soil humic colloids.

A number of soil factors can affect detoxification rates in the terrestrial environment. The primary factors
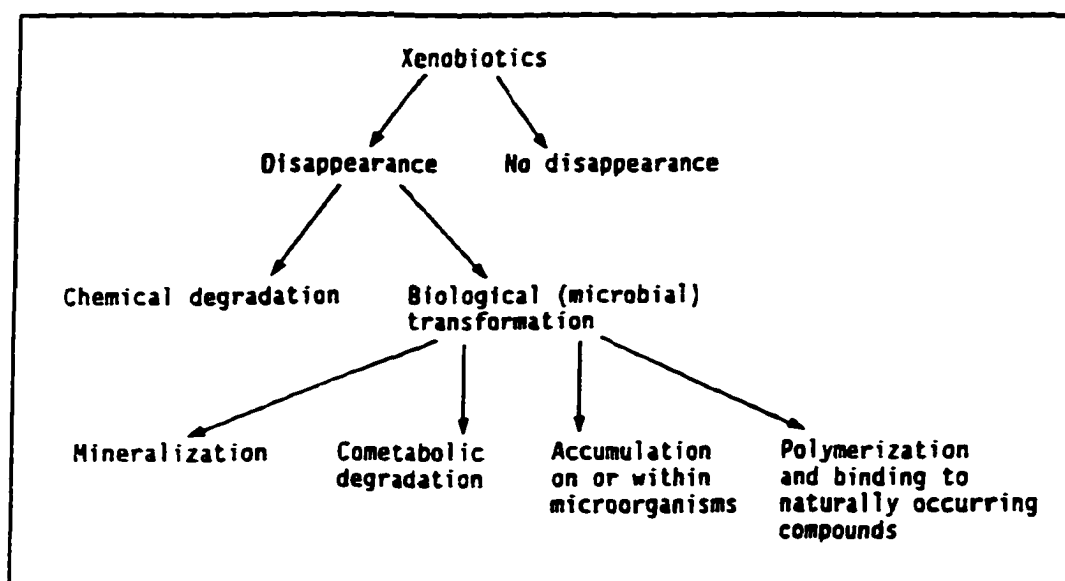
Figure 19.   Fate of Anthropogenic Chemicals in the Soil

Environment.

Source : Leisinger, 1983.

that affect the metabolic actions of microorganisms in the soil include soil moisture content, oxygen content, temperature, organic matter content, acidity, and inorganic nutrient supply (Alexander, 1977). Moisture is important to microbial actions because it composes a major component of cell protoplasm. However, extreme amounts of water cause a reduction in gas exchange dynamics in the soil environment and this may limit the amount of oxygen that is available for metabolic reactions for aerobic bacteria. The optimum soil moisture level for supporting aerobic biologic activity is 50%-70% of the soil's moisture holding capacity (Alexander, 1977).

Soil pH can adversely affect a microorganism's ability to sustain metabolic activity at a level necessary for detoxification. The pH range which is best suited for soil microorganisms is between the pH units of 6-8. For most petroleum hydrocarbon degraders the optimum pH for soil is slightly above 7 (Clark, 1979).

Soil temperature can affect the detoxification rate of organic chemicals. The general rule that biochemical reactions increase as soil temperatures increase. The optimum soil temperature for promoting petroleum detoxification by aerobic microorganisms is a range between

15°C to 30°C (Englert et al., 1993).

Aerobic microorganisms require that at least eleven essential macronutrient and micronutrient inorganic elements be present in soil. These nutrients include nitrogen, phosphorus, sodium, potassium, sulfur, calcium, magnesium, iron, manganese, copper, and zinc. The soil availability and the soil capacity of these nutrients should be optimized to achieve maximum detoxification rates (Englert et al., 1993).

The nutrients present in the soil environment provide three important functions: (1) providing materials required for protoplasmic synthesis; (2) supplying the energy needed for cell growth and biochemical reactions, and; (3) serving as electron acceptors released in the reaction (Alexander, 1977). In aerobic microorganisms, oxygen serves as the electron acceptor. Anaerobes or facultative anaerobes utilize either an organic product of metabolism or an inorganic substance as electron acceptors. Table 8 provides a summary of the nutrients needed by soil microbes for survival.

Energy sources for soil heterotrophs, both aerobic and anaerobic microorganisms, include cellulose, hemicelluloses, lignin, carbohydrates, hydrocarbons, sugars, proteins, amino

Table 8. Nutrients Required By Soil Microbes For Survival.

Nutrient Need	Sources
1. Energy	Organic compounds, Inorganic compounds, Sunlight
2. Electron Acceptors	Oxygen Organic compounds NO_3^-, NO_2^-, SO_4^{2-}, CO_2
3. Carbon	Organic compounds CO_2, HCO_3^-

4. Growth Factors
  a. Amino acids
 	
  b. Vitamins
  c. Others
	Analine, aspartic acid,
	glutamic acid, etc.
	Thiamine, folic acid, etc.
	Purine bases, choline,
	peptides, inositol

5. Minerals	N, P, K, Mg, S, Fe, Ca, Mn, Cu, Co, Mo

Source: Alexander, 1977.

acids, and organic acids. The conversion of these organic substances to oxidized products provides energy which can be used for the synthesis of cell protoplasmic constituents (Alexander, 1977).

Table 9 provides a list of heterogeneous soil bacteria which are capable of detoxifying petroleum hydrocarbons in the soil environment. In decreasing order, Pseudomonas, Arthrobacter, Alcaligenes, Corynebacterium, Flavobacterium, Achromobacter, Micrococcus, Nocardia, and Mycobacterium appear to be the most consistently isolated hydrocarbon detoxifying bacteria in soil (Englert et al., 1993).

Once chemicals, such as petroleum hydrocarbons, enter the microorganism, it is catabolized by one of three metabolic processes; aerobic respiration, anaerobic respiration, and fermentation. Aerobic respiration uses oxygen as the terminal electron acceptor in the oxidation of organic chemicals. In addition, oxygen may be incorporated into metabolic products through the actions provided by the oxidase enzymes (Englert et al., 1993). Anaerobic respiration uses inorganic substrates as its terminal electron acceptor.

Hydrocarbon substrates can be degraded by fermentation by utilizing phosphorylation as the terminal electron

Table 9. Hydrocarbon-Degrading Species In Soil.

Achromobacter	Acinetobacter
Arthrobacter	Bacillus
Corynebacterium	Flavobacterium
Alcaligenes	Brevibacterium
Cytophaga	Erwinia
Micrococcus	Mycobacterium
Nocardia	Proteus
Pseudomonas	Sarcina
Serratia	Spirillium
Streptomyces	Vibrio

Source: Bossert and Botha, 1984.

acceptor. Fermentation occurs independent of molecular oxygen and depends upon organic compounds to become terminal electron acceptors. It results in the production of a wide variety of inorganic and organic products (Speece, 1983).

Petroleum hydrocarbons can be divided into four major groups; the saturates, the aromatics, the asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and the resins (pyridines, quinolines, carbozoles, sulfoxides, and amides) (Colwell and Walker, 1977). Studies have shown that the degradation rates of petroleum hydrocarbons are related to chemical structure (Bossert and Bartha, 1984; Brink, 1981; Gibson, 1980; Ribbons and Eaton, 1982). The n-alkanes, n-alkylaromatics and aromatics in the C_{10} to C_{22} range are readily degradable. Those n-alkanes, alkyl-aromatics and aromatics in the C_5 to C_6 range are biodegradable and also volatilize readily into the soil environment (Englert et al., 1993).

Petroleum products also contain a wide variety of additives, such as detergents, octane improvers, antioxidants and anticorrosives, and combustion aids. These additives have a wide range of chemical structures, but these are also capable of being detoxified in the soil environment (Englert et al., 1993). Table 10 provides a

Table 10. Selected Molecular Fragments Available to
Microbial Transformation.

Alcohols	Aldehydes
Aliphatics	Amides
Amines	Aromatics
Carboxylic acids	Esters
Ethers	Glycosides
Hydroxyl amines	Ketones

Source: Englert et al., 1993.

summary of organic molecular fragments associated with
petroleum hydrocarbon products that can be ameliorated by
soil microorganisms. In addition, Alexander (1981) provides
detailed data pertaining to the reactions for chemical
transformation, cleavage, and conjugation reactions due to
microbial detoxification reactions. This data is provided
in Tables 11 and 12.

The aromatic hydrocarbons are important components of
refined petroleum products. Aromatic hydrocarbons are
broadly defined as benzene and other compounds that exhibit
similar behavior (Morrison and Boyd, 1973). The important
aromatic compounds associated with petroleum hydrocarbons
include benzene, ethylbenzene, toluene and xylenes. The
aromatic constituents of petroleum hydrocarbons are all
derivatives of benzene; therefore, there is one common
detoxification reaction that leads to the mineralization of
benzene, ring cleavage.

Marr and Stone (1961) indicates that catechol is the
ring cleavage substrate in benzene detoxification.
Molecular oxygen serves as a reactant in two steps of the
catabolism of benzene. In each reaction step, both atoms of
the oxygen molecule are incorporated into the benzene
substrate. The enzymes which act as biocatalysts in these

Table 11. Categories of Microbial Transformation
Reactions.

Dehalogenation	Deamination
Decarboxylation	Methyl oxidation
Hydroxylation	Beta oxidation
Epoxide formation	Nitrogen oxidation
Sulfur oxidation	Sulfoxide reduction
Reduction of triple bond	Reduction of Double bond
Oxime metabolism	Nitro metabolism
Nitrile-amide metabolism	Hydration of double bond

Source: Alexander, 1980.

Table 12. Soil Microbial Cleavage Reactions.

Molecular Fragment	Reaction[1]
Ester	RC(O)OR' --> RC(O)OH
Ether	ArOR --> ArOH ROCH$_2$R' -->ROH
C-N Bond	R(R')NR" --> R(R')NH or RNH$_2$ RN(Alk)$_2$ --> RNHAlk or RNH$_2$ RNHCH(R')R" --> RNH$_2$ RNH$_2$CH$_2$R' --> RNH
=NOC(O)R	RCH=NOC(O)R --> RCH=NOH
Peptide	RNHC(O)R' --> RNH$_2$ or HOOCR"
C--S Bond	RSR' --> ROH and/or ESR'
C--Hg Bond	RHgR' --> RH and/or Hg

Source: Alexander, 1890.

1: R= organic fragment, Ar= aromatic, Alk= alkyl

reactions are called dioxygenases (Sheldon and Kochi, 1981).
Rittmann et al. (1994) provide the following stoichiometry
of dioxygenase-catalyzed reactions:

$$S + O_2 \rightarrow SO_2$$

where S = benzene substrate and O_2 = molecular oxygen.

The reactions causing ring cleavage and microbial
detoxification requires that the aromatic ring of benzene be
made reactive or destabilized. This ring activation occurs
when the dioxygenase is inserted into the benzoid ring and
results in the formation of benzene dihydrodiol (Ribbons and
Eaton, 1982; Gottschalk, 1986). This process may require
reducing equivalents in the form of nicotinamide-adenine
dinucleotide (NADH). The resulting dihydroxylated
nonaromatic intermediates are then dehydrogenated to form
catechols. Aromaticity is restored to the ring cleavage
substrate during the formation of catechol. The reactions
leading to the detoxification are illustrated in Figure 20
(Rittmann et al., 1994).

The use of a hydroxylating dioxygenases to activate the
benzene ring for cleavage are multicomponent systems that
resembles the enzymes monooxygenases. In this type of
reaction, a flavoprotein acts as an electron acceptor from
NADH and passes the electrons through a ferredoxin to the

O_2

NADH + H·   NAD·   NAD·   NADH
                                                                          + H·

benzene   .   benzene dihydrodiol   catechol
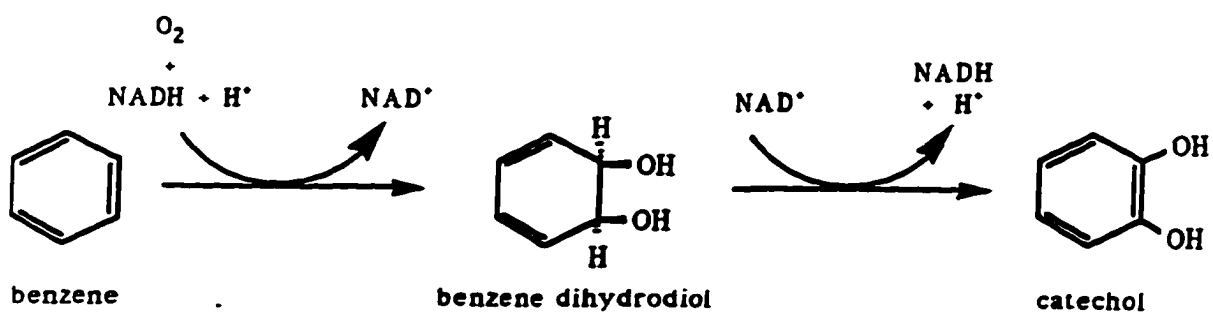
Figure 20:   Oxidation of Benzene to Catechol.

Source: Rittmann et al., 1994.

dioxygenase (Rittmann et al., 1994). The reduced dioxygenase then reacts with molecular oxygen and the benzene substrate (Gibson and Subramanian, 1984). Figure 21 provides an illustration of the different functions of the components of this pathway with the flavoprotein acting as the reductase of the non-heme-iron protein (Hopper, 1978).

Catechol can be catabolized by ring cleavage and this cleavage can occur by one of two pathways: (1) the ortho-cleavage pathway which causes the benzene ring to be broken between the two carbon atoms with hydroxyl groups or, (2) the meta-cleavage pathway which causes the aromatic ring to be broken between the hydroxylated carbon atom and an adjacent unsubstituted carbon atom (Gottschalk, 1986). Dioxygenase-catalyzed reactions cause the ring fission in both of these pathways. Although the metabolic pathways for each reaction pathway differ, they both lead to the forma-tion of tricarboxylic acid (TCA) cycle intermediates or to substrates that can lead to the formation of TCA intermediates. The ortho-cleavage pathway (Figure 22) produces the intermediates acetate and succinate and the meta-cleavage pathway (Figure 23) forms pyruvate and acetaldehyde (Rittmann et al., 1994).

Alkyl-substituted benzenes such as ethylbenzene,
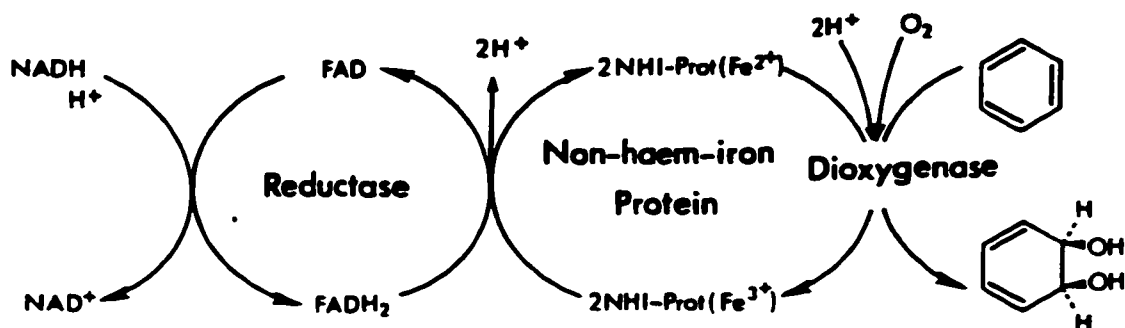
Figure 21:   Benzene Detoxification Using Protein Components

and Dioxygenase.

Source: Hopper, 1978.
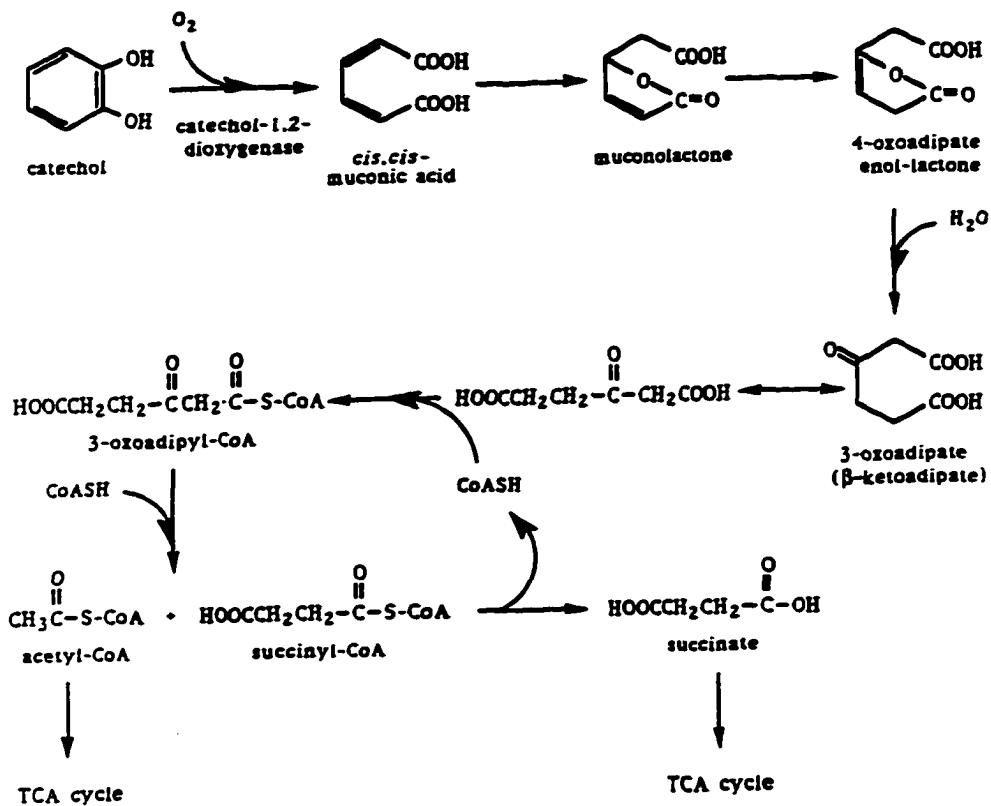
Figure 22. Ortho-cleavage for Catechol Catabolism.

Source: Rittmann et al., 1994.
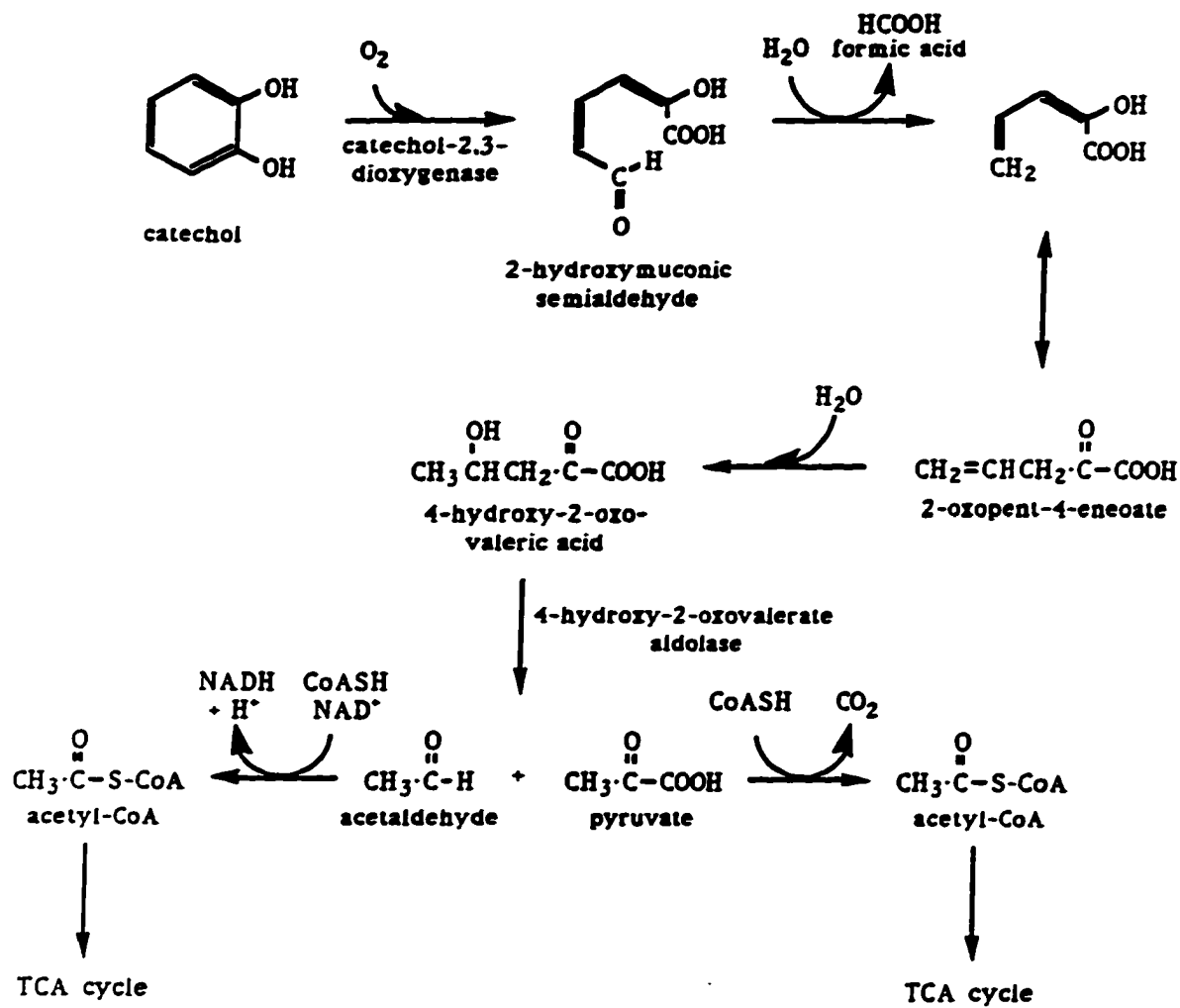
Figure 23. Meta-cleavage for Catechol Catabolism.

Source: Rittmann et al., 1994.

toluene, and xylenes are petroleum hydrocarbon compounds that are associated with environmental contamination due to gasoline and diesel fuel releases. These compounds can serve as sources of carbon and energy for several species of microorganisms including Pseudomonas, Nocardia, and Achromobacter (Gibson and Subramanian, 1984). Metabolic catabolism can be initiated by the oxidation of the alkyl side chain or the benzene ring.

The catabolism of toluene by Pseudomonas aeruginosa is a side chain initiated reaction relying on the enzyme monooxygenase (Ribbons and Eaton, 1982; Gibson and Subramanian, 1984). This reaction pathway converts toluene to benzyl alcohol and then converts benzyl alcohol to benzoic acid by dehydrogenation. Benzoic acid acts as a substrate capable of inserting oxygen into the benzene ring. This insertion then leads the reaction to the production of catechol (Rittmann et al., 1994). Figure 24 illustrates the oxidation of toluene by Pseudomonas aeruginosa.

The oxidation of toluene and ethylbenzene by Pseudomonas putida provides an example of detoxification initiated by dioxygenase-catalyzed ring hydroxylation. This reaction pathway leads to the formation of 3- or 4-methyl catechols or ethylcatechols (Ribbons and Eaton, 1982; Gibson
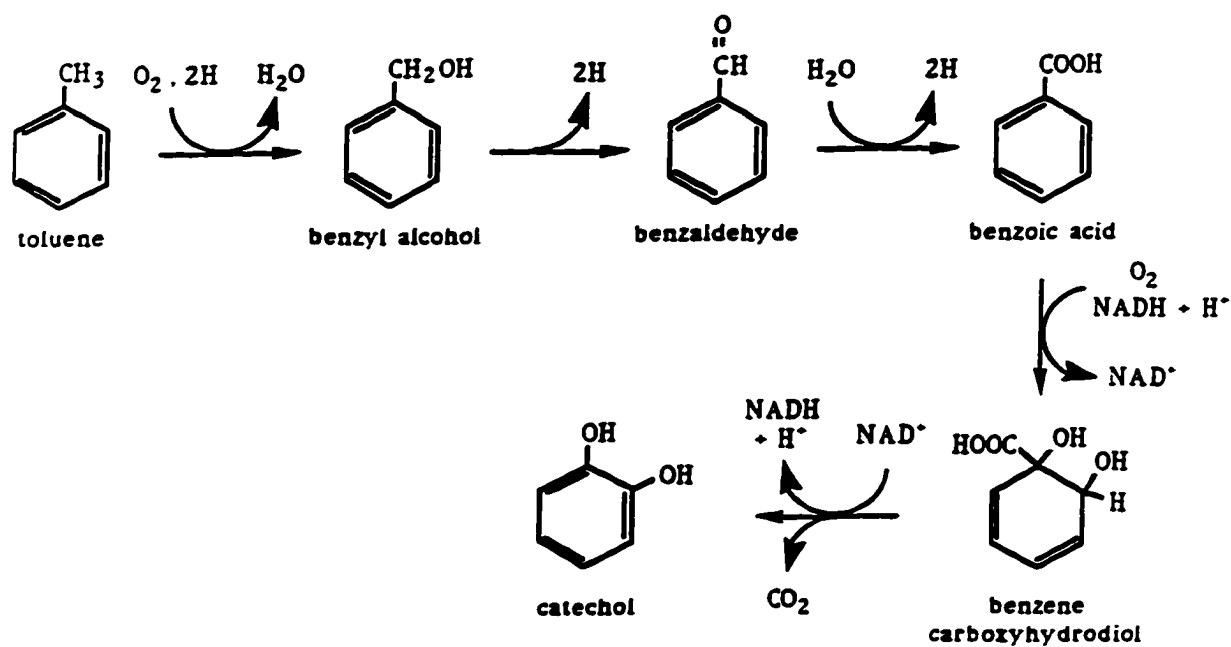
Figure 24: Oxidation of Toluene by Pseudomonas aeruginosa.

Source: Rittmann et al., 1994.

and Subramanian, 1984). These alkylcatechols are further oxidized by meta-cleavage. Several microbial species, including **Nocardia** sp., **Achromobacter** sp., and **Pseudomonas** sp., are capable of oxidizing toluene and ethylbenzene utilizing this pathway. Figure 25 provides an illustration of this pathway for toluene and Figure 26 provides an illustration of this pathway for ethylbenzene.

Nocardia sp. can utilize o-xylene as a food source by ring hydroxylation. This action gives 3, 4-dimethylcatechol as a product. The next step in the detoxification reaction leads to the meta-cleavage of the 3, 4-dimethylcatechol and the subsequent formation of acetic acid, propionaldehyde, and pyruvate (Gibson and Subramanian, 1984). This species is one of few that can use o-xylene as a sole source of carbon. However, many other species are capable of utilizing m- and p-xylenes as sole carbon sources (Rittmann et al., 1994).

The several bacterial species that can utilize m- and p-xylene must first oxidize a methyl group. This oxidation produces toluic acid and then oxidizes the aromatic ring. The oxygen insertion into the aromatic ring occurs at the carbon atom containing the carboxyl group. Decarboxylation occurs on the ring structure and then dehydrogenation of the
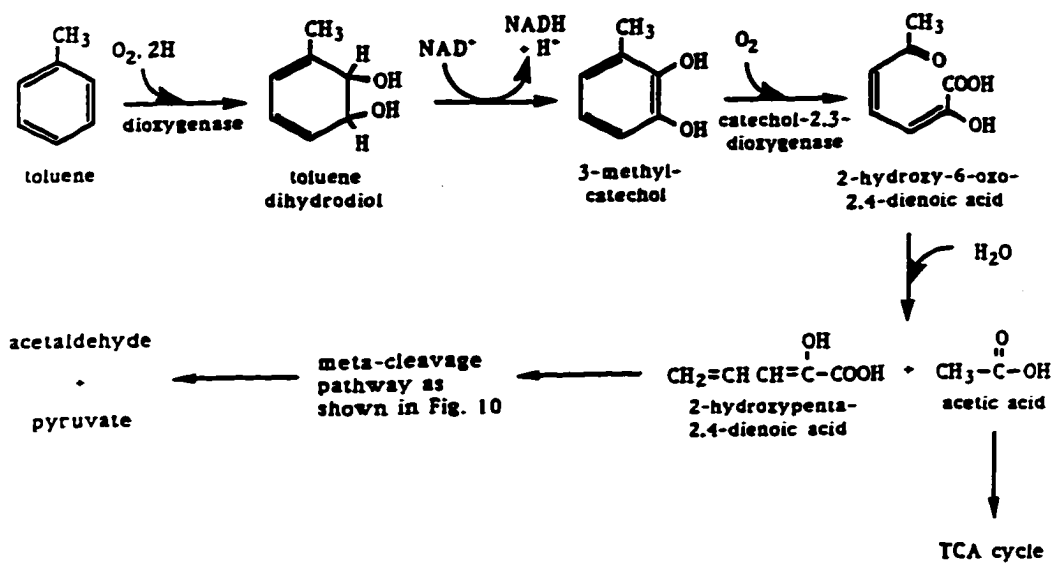
Figure 25: Toluene Hydroxylation by Pseudomonas putida.

Source: Rittmann et al., 1994.
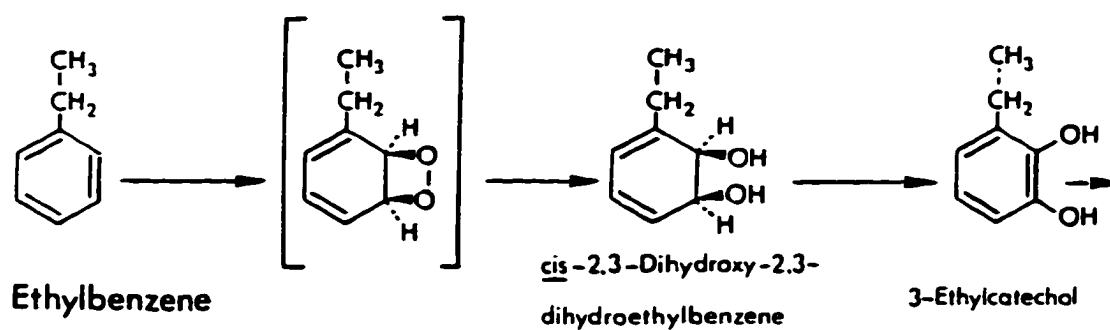
Figure 26. Dihydroxylation of Ethylbenzene.

Source: Hopper, 1978.

diol produces methylcatechol (Ribbons and Eaton, 1982). The detoxification reaction of m-xylene is illustrated in Figure 27.

The detoxification pathways described for aromatic hydrocarbons have shown the importance of the oxygenase enzymes, catechols, and substituted catechols as ring fission substrates. The conversion of catechol to metabolites useful in the central metabolic pathways of microorganisms may occur by one of two distinct pathways (Hopper, 1978). The ortho-fission pathway causes a cleavage of the aromatic ring between the two carbon atoms bearing hydroxyl groups. This pathway is illustrated in Figure 28. The meta-fission pathway causes a cleavage of the aromatic ring between a carbon atom bearing a hydroxyl group and the adjacent carbon without such a group. This pathway is illustrated in Figure 29.

Some microorganisms can not produce the enzymes needed for the complete detoxification of petroleum hydrocarbons. These microbial species rely on other microorganisms to produce the inducer enzymes necessary for initiating detoxification reactions. This process is called "co-metabolism". Co-metabolism refers to the oxidation of substrates without using the energy derived from such a
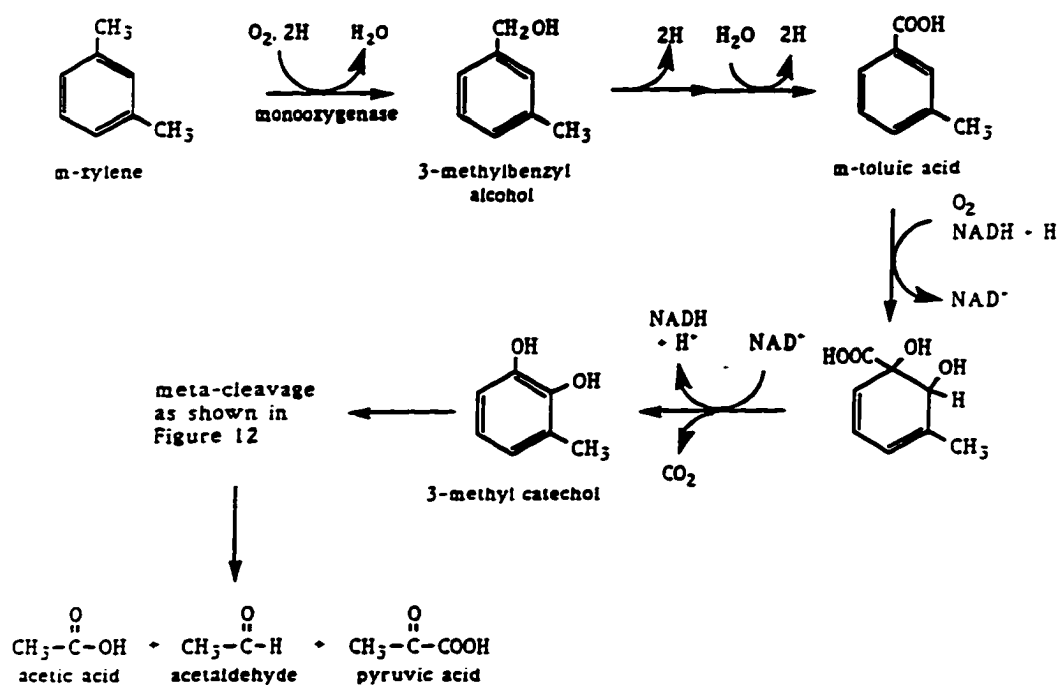
Figure 27. Hydroxylation of m-Xylene by Pseudomonas putida.

Source: Rittmann et al., 1994.
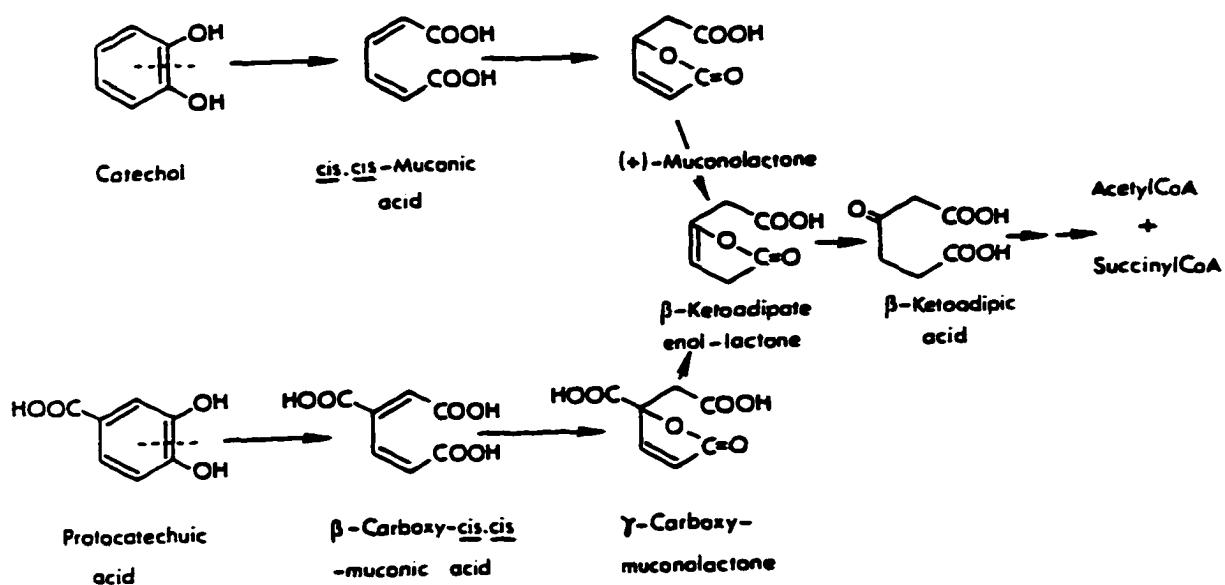
Figure 28. Ortho-fission of the Aromatic Ring.
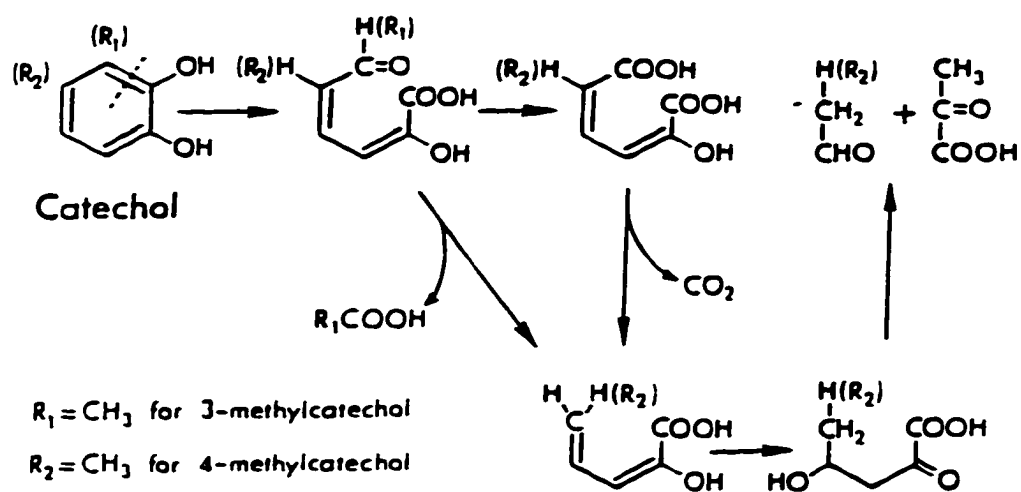
Source: Hopper, 1978.

Figure 29. Meta-fission of the Aromatic Ring.

Source: Hopper, 1978.

reaction to support microbial growth. Broadbent and Norman (1946) indicate that soil organic matter becomes a higher nutrient source for soil microorganisms when additional, more readily decomposable organic matter is provided to the original material. Horvath (1972) shows that microorganisms capable of co-metabolizing organic pollutants become enriched when degradable analogues of the pollutants are added to the microbial system. Both of these examples indicate that co-metabolic reactions can be elevated by the addition of fresh organic matter and degradable analogues of pollutants are added to the microbial environment. This induction of co-metabolic reactions is one of the highlights of utilizing an amended vermicomposting approach for contaminated soil detoxification.

Table 12 provides a partial list of microbial species known to possess co-metabolic properties. These micro-organisms are present in soil and biologic sludge, therefore it is believed that co-metabolism of pollutants will be enhanced in the soil environment when biologic sludge is applied to petroleum contaminated soil. Examples of organic substances subject to co-metabolic processes include o- and p-xylene, ethylbenzene, and 3-methylcatechol (Horvath, 1972).

Table 13. Cometabolic Microbial Species In Soil.

Achromobacter sp.	Arthrobacter sp.
Aspergillus niger	Azobacter sp.
Bacillus sp.	Brevibacterium sp.
Flavobacterium sp.	Microbacterium sp.
Micrococcus sp.	Hydrogenomonas sp.
Nocardia sp.	Pseudomonas sp.
Streptomyces sp.	Trichoderma sp.
Vibrio sp.	Xanthomonas sp.

Source: Horvath, 1972.

The oxidation of petroleum hydrocarbons produces a wide variety of products that are generally more water soluble than the parent compounds. The remaining parent compounds may become more mobile or water soluble themselves. In addition, oil-water emulsions may form as oxidation proceeds (Blakebrough, 1977). It has been shown that the availability of oxygen, temperature, soil acidity, redox potential, nutrient content, and humic substances all have profound effects on the detoxification of hydrocarbon contaminants. High levels of organic matter encourage microbial activity, but may also slow the oxidation of hydrocarbons by providing alternate nutrient sources. However, co-oxidation may be stimulated by the presence of additional organic material and by the introduction of fresh microbial communities (Blakebrough, 1977).

CHAPTER THREE

METHODOLOGY

OBJECTIVE

The purpose of this study was to evaluate the effect of an amended vermicomposting treatment system on the degradation of petroleum hydrocarbon contaminated sandy soil. The effectiveness of the treatment method and all other experimental trials and controls was measured using a standardized analytical method for petroleum hydrocarbon content (Method 8015). The measured results for each treatment or control were then compared to determine significant reductions in petroleum hydrocarbon content within the contaminated soil.

RESEARCH QUESTION

The research question in this experiment was designed to determine if the amended vermicomposting treatment method had a significant effect on reducing the levels of petroleum hydrocarbon content in a contaminated sandy soil. In order to test the research question, a contaminated soil sample was treated with various segments of the total amended process and with the entire process. A control

sample received no treatment other than a weekly application of distilled water to maintain water moisture within the sample. Weekly measurements of the sample were obtained to determine changes within the treatment matrices. The sampling period was established for six weeks. A pilot study was performed to determine the best application rate for the biologic sludge and indicate if the treatment reductions in contamination became static over the period of testing.

DESIGN

The type of quasi-experimental design utilized in this discourse is the Equivalent Materials Design. According to Campbell and Stanley (1963), the basis of the argument for using this design is the use of equivalent samples of materials to which the experimental variables being compared are applied. Equivalent time samples were obtained for each treatment system or control group. The experiment was conducted using a four group repeated measures design. Equivalent materials are required since the nature of the experimental variable is such that the effects are enduring and different treatments and repeats of treatments were applied to non-identical content. The soil samples obtained

from each treatment and control will be from different locations within the sample matrix. Since the samples were not identical and the treatments were continued for six weeks, this study design provided the most effective method for detecting actual changes in each sample due to the type of treatment received by each trial.

VARIABLES

The independent variables in this research are those involved with the innovative soil treatment approach. These include biologic sludge, earthworms, and a combination of the two. These variables are manipulated during the life of the experiment. The dependent variable in this experiment is the measured concentrations of petroleum hydrocarbons obtained from each week of the experiment. It was measured in parts per million (ppm) as determined by the analytical protocol used in the laboratory.

HYPOTHESIS

The hypothesis which was tested in this research is whether the use of the amended vermicomposting treatment approach affected the petroleum contaminated soil in such a way as to significantly lower the concentration of petroleum

hydrocarbons. The test hypothesis was as follows:

H_1: The amended vermicomposting treatment method will have a significant effect in reducing the level of petroleum contamination in Coastal Plain sandy soils ($p < .05$).

The null hypothesis can be stated as follows:

H_0: The amended vermicomposting treatment method will not have a significant effect in reducing the level of petroleum hydrocarbon contamination in Coastal Plain sandy soils ($p > .05$).

TREATMENT GROUPS

Three treatment groups were used for the pilot study and a separate set of four treatment groups were used for the verification study. The pilot study was composed of a control group [gasoline contaminated soil with distilled water to keep the soil moist, (i.e., 0.5 gallons per week)], Experimental Group 1 [gasoline contaminated soil with liquid biologic sludge (i.e., 0.5 gallons per week)], and Experimental Group 2 [gasoline contaminated soil with liquid biologic sludge (i.e., 0.5 gallons per week) and 30 Eisenia foetida earthworms]. The pilot study was conducted

for four weeks to determine if the application rate of sludge and the volatilization rate of the gasoline has an adverse effect on the measured outcomes of the experimental verification study. It also showed if the degradation reactions within the treatments leveled out during the pilot study time period. The four week time period for the pilot study was chosen based on the review of treatability study efforts performed by testing laboratories on soils receiving biological treatments for petroleum contamination.

The verification study consisted of four groups. These groups consisted of the following: (1) Group 1: contaminated soil and 0.5 gallons of distilled water added per week; (2) Treatment 1: contaminated soil + 0.5 gallons of biologic sludge added per week; (3) Treatment 2: contaminated soil + 30 Eisenia foetida earthworms + 0.5 gallons of distilled water added per week, and; (4) Treatment 3: contaminated soil + 0.5 gallons of biologic sludge added per week + 30 E. foetida earthworms. This experiment ran for a six week time period.

VALIDITY FACTORS

The research design selected for this experiment provides the mechanism to control all threats to internal

validity (Campbell and Stanley, 1963). History is controlled for by the presentation of treatments in numerous occasions. The occurrence of extraneous events during the experiment should not explain the results of the various treatments under this design. Maturation is controlled for in this design because many observations were collected during the experiment. Testing was controlled for in this design because many tests were performed on each group in the experiment. Instrumentation was controlled for in this design since the analytical instrument used in the experiment was calibrated prior to beginning each analytical session and calibration checks were used during the analysis work according to method specifications. Regression was controlled for in this design because its effects are usually a negatively accelerated function of elapsed time. Selection was controlled for in this design because random sampling points in the same soil matrix were used for each sampling period. Mortality was controlled for in this design because no sample was dropped from the experiment for any reason.

External validity problems associated with this design include the interaction of testing and X; the interaction of selection and X; reactive arrangements and multiple-X

interference. The interaction of testing and X is important because the experimental effect might be specific to the contaminant subject to repeated testing. The interaction of selection and X considers the limitation of the effects of the experimental variable to a specific sample and to the possibility that this reaction would not be typical to some more general universe of interest. Reactive arrangements are less involved in this experiment because of the heterogeneity of the materials and the greater possibility that the test materials has no inherent awareness that it is receiving different treatments. Multiple-X interferences result from the cumulative effects of the treatment being used during the experiment. Since the treatments were given over a several week time period, this threat should be considered; however, it was hoped that the long term application of the amended treatment would result in cumulative effects and thereby degrading the contaminant under study.

This research design was chosen because of the number of treatments provided to the same type of materials over a long period of time. This design provides a method of measuring the changes which occurs within each treatment group and control group during the time frame of the

experiment. The effects of the continually-applied treatments to each group can be measured using the selected research design. Therefore, this design provides a method of considering the research as a time series experiment using the same basic material as the test medium.

SAMPLE POPULATION

The samples used in this study were obtained from one cubic foot of sandy soil that had been contaminated with diesel fuel and gasoline. The sample size was approximately 230 grams in weight and this sample was divided into three equivalent subaliquots. Each subaliquot was analyzed for Total Petroleum Hydrocarbon content for each sampling episode.

Random sampling was accomplished by insuring that, at any stage of sampling, the selection of a particular sample unit was not influenced by other units that have already been selected. The method used to insure randomness in sample selection was the assignment of numbered areas within the sample matrix and the selection of sample areas using a table of random numbers. This selection methodology was used for each sampling event during the experiment.

The whole soil sample obtained from the soil corer was

homogenized and placed into clean sample jars for analysis.
This type of sampling, termed composite depth sampling,
provided reliable information regarding average petroleum
contaminant values in each sample. However, any information
pertaining to the variability of contamination with depth is
lost. In addition, the mixing of the sample precludes its
use for the measurement of individual volatile compounds
within the sample.

METHOD OF DATA COLLECTION

The study relied upon an experimental approach for data
collection. Experimental and control groups were used to
determine the overall effects of the treatment methods on
detoxifying or degrading the petroleum contaminated soil.
Each sampling episode for each group provided three data
points which analytically describe the concentration of
petroleum contaminant in each sample. This type of sampling
was done for each week of the laboratory experiment.

The main advantage of this type of experiment was
the isolation of the experimental variable over time. A
type of experimental media was chosen and a determination of
its characteristics was made at the onset of the
experiment. Following the administration of an experimental

stimulus, measurements were obtained from each treatment and control group. Differences among the treatments were recorded for each group and little concern was given to extraneous factors.

MATERIALS

The materials used in the experiment include seven cubic feet of sandy soil, gasoline and diesel fuel to contaminate the soil, 3.5 feet of river rock, seven-five gallon polyethylene round containers with spigots, distilled water, one stainless steel soil probe, 90 *Eisenia foetida* earthworms, non-petroleum based detergent for decontamination of soil sampling equipment, 100 230 gram glass jars with teflon-lined lids, one cooler for temporary sample storage, ice for cooling the samples during transport to the analytical laboratory, and one gas chromatograph with all appropriate apparatus (See APPENDIX A).

METHOD OF SAMPLE ANALYSIS

The analytical method used for measuring the changes of petroleum hydrocarbon levels within each experimental group was EPA Method 8015 as described in SW-846. This analytical method provides a mechanism for determining the volatile

(gasoline) and semi-volatile (diesel) petroleum hydrocarbon levels in soil and water. The method is sensitive to levels of 0.5 parts per million (ppm) for gasoline and 5.0 ppm for diesel in soil. EPA Method 8015 is an analytical technique used for performing gas chromatography analysis on soil and water samples.

In order to determine the volatile hydrocarbon level in each soil sample, a 5 gram sample of the soil was extracted by helium sparging on a purge and trap and analyzed by gas chromatography using a flame ionization detector (FID) and a packed column. The determination of semi-volatile organic concentrations in each sample, a 5 gram sample was extracted with 50:50 acetone/methylene chloride using sonication, and then concentrated to a 1 milliliter volume using a Kuderna-Danish appartus. The sample was then analyzed by gas chromatography using a FID and a capillary column.

A stock solution of 10,000 mg/l was prepared by weighing 250 mg of diesel fuel into a 25 ml volumetric flask and bringing to volume with methanol. From this stock solution a series of standards were prepared as follows:

STANDARD #	AMT. ADDED	VOLUME	FINAL CONC.
1	0.100ml	10ml	100 mg/l
2	0.250ml	10ml	250 mg/l
3	0.500ml	10ml	500 mg/l

All standards were clearly labeled with the appropriate standard number, concentration, and preparation date. The standards were prepared at the beginning of the experiment and on the beginning of the fourth week of the experiment.

In order to process the soil samples for volatile fraction analysis, the following sample preparation procedure was used:

1. A 5 gram soil sample was weighed and placed into a purging chamber and the chamber was then connected to the purge-and-trap system on the gas chromatograph.

2. The plunger was then removed from a 5ml Luerlock-type syringe and filled with reagent water. The reagent water was added to the purge chamber containing the soil sample.

3. The purge cycle was then started and the system was leak checked with water. The purge cycle was completed in 11 minutes. At the end of the purge

cycle the sample is ready for desorption. The
internal temperature within the gas chromatograph
(GC) rose to 150°C and the GC and integrator
automatically started to analyze the sample.

4. When the desorption of the sample was completed,
   the purging chamber was emptied. The chamber was
   washed with a minimum of two 5ml flushes of reagent
   water to avoid cross contamination in subsequent
   analyses.

The sample preparation technique used for semi-volatile
fraction analysis included the following:

1. A 30 gram soil sample was weighed into a 250 ml
   clean beaker and 60 grams of anhydrous sodium
   sulfate was added to the sample. The mixture was
   stirred to insure that the sample was free-flowing.

2. 50 ml of methylene chloride and 50 ml of acetone
   were measured into a 100 ml graduated cylinder and
   then added to the sample mixture.

3. The entire mixture was sonicated at 100% power for
   three minutes with a 0.5 second pulse.

4. At the end of the three minute period, the mixture
   was filtered and the extract was collected in a 500
   ml Erlenmeyer flask.

5. Steps 3 and 4 were repeated two additional times.

6. The Kuderna-Danish apparatus was then assembled and the extract was added to the evaporative flask and the apparatus was placed into a hot water bath (80-90°C). Boiling chips were added to the concentrator tube.

7. When the volume of the liquid reached 1 ml, the apparatus was removed, drained, and allowed to cool for 15 minutes.

8. The Snyder column was removed and the flask and its lower joints were rinsed with 1-2 ml of methylene chloride into the concentrator tube. A two-ball micro-Snyder column was attached to the concentrator tube and a clean boiling chip was added to the tube. The column was placed into the hot water bath until the liquid reached a volume of 0.5 ml. The column was then removed from the water bath and drained and cooled for 15 minutes. The micro-Snyder column was removed and the lower joint was rinsed with 0.2 ml of solvent into the collector tube. The sample volume was then adjusted to 1.0 ml and then placed into the GC for analysis.

PILOT STUDY

A pilot study was used to determine if the selected biologic sludge application rate of 0.5 gallons per week would result in causing hydraulic overload in the sandy soil. This was of concern because if the soil pore space had become overfilled with liquid, the application rate must be reduced to prevent pore space congealment which would have severely impeded the treatment.

The pilot study was also used as a method of measuring the effect of the biologic sludge on soil pH. It was expected that the sludge material would aid in keeping the soil at or near a neutral pH which would, in turn, enhance microbial activity.

A third use of the pilot study was to determine if the earthworms would be adversely affected by the contaminant in such a manner as to deter them from burrowing into the soil matrix within the testing vessel. If the worms had been deterred from performing their burrowing function, then the use of the innovative treatment technology for petroleum degradation must be reconsidered.

A final rationale for using the pilot study was to determine if the sample collection method refrained from causing cross contamination within the test groups. If

problems with outlying sample results were identified, then the soil sampling approach would have been amended to address this concern.

The pilot study consisted of three groups which included a control group, a biologic sludge treatment group, and a biologic sludge and earthworm treatment group. The control group consisted of gasoline contaminated soil and received 0.5 gallons of distilled water per week. This frequent wetting should have reduced volatilization rates from the control soil. Treatment Group 1 consisted of gasoline contaminated soil and liquid biologic sludge which was applied at a rate of 0.5 gallons per week. Treatment Group 2 consisted of gasoline contaminated soil, 0.5 gallons of biologic sludge applied at a rate of 0.5 gallons per week, and 30 **Eisenia foetida** earthworms. The top surface of the soil matrix was divided into nine approximately equal plots. Each treatment and control group was sampled once a week for four weeks and the sampling locations were from plot numbers determined by a table of random numbers. Plots were sampled until a sample weight of 230 grams was attained. The samples were placed in clean glass jars with teflon lined lids and immediately placed into an ice filled cooler for transport to the laboratory. The samples were

run within allowable holding times to insure protocol
integrity. The pilot study samples were replicated three
times to insure that accurate results were obtained.

PILOT STUDY RESULTS

The effects of soil pH on the survival of earthworms
has been well documented. Earthworms rarely inhabit soils
with a pH less than 4.0 to 4.5. *Eisenia foetida* have been
shown to be sensitive to soils with a pH of less than 4.0
(Satchell, 1983).

In order to assess the ability of *E. foetida* to
facilitate the alteration of pH in Coastal Plain sandy soil,
the following test procedure was used: (1) three standard
Petri dishes (20mm x 100mm) were filled with 50 grams of
sandy soil with a pH of 4.2 and two *E. foetida* earthworms
were then placed on the soil; (2) three standard Petri
dishes were filled with 50 grams of sandy soil with a pH of
4.2 and 10 grams of filtered biologic sludge was added to
the soil surface, and; (3) three standard Petri dishes were
filled with 50 grams of sandy soil with a pH of 4.2, 10
grams of filtered biologic sludge was added to the soil
surface, and 2 *E. foetida* earthworms were placed on the soil
surface. Each of the Petri dishes received 20ml of

deionized water each week for four weeks to maintain a moist environment for the earthworms. The pH of each soil sample was measured at the end of the four week testing period. In addition the survival rate of the earthworms was noted. The results of the pH test and survival data are provided in Table 14.

Table 14 shows that the earthworms placed in the unamended dishes did not survive for one week. Also, the pH of the unamended soil remained unchanged at the end of the four week testing period. The trials which utilized the sludge amendment both showed an increase in soil pH. This occurrence illustrates the buffering capacity of biologic sludge on low pH soils. The trials which included both filtered biologic sludge and earthworms demonstrated the highest pH gain.

This testing sequence leads to the possibility that the presence of biologic sludge and the burrowing activities of E. foetida can have a beneficial effect on improving the acidic quality of Coastal Plain sandy soil. This buffering capacity can improve biodegradation processess by creating an improved soil environment. To test this hypothesis, a pilot testing program was designed to determine if a combination of soil microbes, earthworms, and biologic

Table 14. Response of **E. foetida** to pH Changes[1].

Test #	Initial pH	Earthworm Survival (days)	Final pH
1	4.2	6	4.2
2	4.2	5	4.0
3	4.2	6	4.2
4	4.2	---	6.1
5	4.2	---	6.3
6	4.2	---	6.1
7	4.2	20	7.1
8	4.2	24	6.9
9	4.2	21	7.1

[1] At 20°C.

sewage sludge can catalyze the biodegradation processes in petroleum hydrocarbon contaminated soils.

The second series of experimental tests was performed using the three groups discussed in an earlier segment of the pilot study section of this chapter. The purpose of this series of tests was to determine the ability of biologic sludge and earthworm activity to facilitate biodegradation processes within petroleum contaminated sandy soils. The results of this phase of the pilot test are provided in Table 15.

The results shown in Table 15 indicate that the greatest reduction in soil petroleum contaminant levels occurred in the treatment containing earthworms and biologic sludge. This result indicates that earthworms provide a mechanism for mixing biologic sludge through the soil profile during burrowing activities. This mixing incorporates humic materials and microbial populations within the sludge into the soil matrix. The detoxification of petroleum contaminated soils using humic chemistry, enzyme catabolism, earthworm casting and sludge stabilization processes offers a methodology for the breakdown of ring structures associated with organic chemical contaminants. Therefore, based on the initial

Table 15. Response of Petroleum Contaminated Sandy Soil to
Treatment Alternatives[1].

Trial #	Initial TPH []	Week 1 TPH []	Week 2 TPH []	Week 3 TPH []	Week 4 TPH []
A1	2500	2300	2100	2100	2000
A2	2500	2400	2400	2200	2100
A3	2600	2400	2400	2200	2000
B1	2500	2000	1700	1400	1200
B2	2500	1900	1700	1300	1000
B3	2600	2000	1800	1500	1200
C1	2500	2000	1100	540	400
C2	2500	1900	1000	630	520
C3	2600	1900	1100	610	450

[1] TPH results in parts per million.

findings of the pilot study, a verification study using the amended vermicomposting approach is warranted.

PROCEDURE USED IN MAJOR RESEARCH STUDY

The major study, which consisted of three treatment groups and one control group was performed as described in the steps provided below.

STEP 1

Approximately six inches of clean river rock was placed into the bottom of each of the five gallon polyethylene containers to insure that the spigot of each container was not filled with the contaminated sandy soil. One cubic foot of diesel fuel contaminated soil was placed on top of the river rock. The surface of the soil matrix was divided into nine approximately equal plots. One of these plots per treatment vessel was chosen at random using a table of random numbers for sampling at one week intervals for six weeks. Four experimental groups were used for this experiment:

Control Group: contaminated soil + 0.5 gallons of distilled water per week;

Treatment 1: contaminated soil + 0.5 gallons of biologic sludge per week;

Treatment 2:   contaminated soil + 30 E. foetida

earthworms + 0.5 gallons distilled water

per week;

Treatment 3:   contaminated soil + 0.5 gallons biologic

sludge per week + 30 E. foetida

earthworms.

A sample of 230 grams was collected from each group

using a stainless steel soil corer which had a depth of one

foot and an outside diameter of 20 centimeters. The soil

corer was decontaminated before each use by washing with a

non- petroleum based detergent, rinsing with tap water, air

drying, and then rinsing with distilled water and allowed to

air dry again.

STEP 2

Each soil sample of 230 grams was divided into three

equal subaliquots in the analytical laboratory. These

subaliquots were prepared and analyzed for Total Hydrocarbon

Content using SW 846 Method 8015. All analytical work was

performed within the allowed holding time of 14 days. The

appropriate quality assurance/quality control mechanisms for

the chosen analytical method was followed (See APPENDIX A).

STEP 3

The water contained in each treatment or control group

vessel was drained from the treatment container through the spigot. The water was visually examined for clarity to determine if the biologic sludge was being utilized within the soil or simply passing through the soil matrix.

TREATMENT OF DATA

Since the object of this research was to determine the effectiveness of the amended vermicomposting treatment approach, the data was used to determine the differences between the holistic treatment and the remaining treatment and control groups. As interval data was gathered for each group, it was possible to determine the mean, variance, and standard deviation for each group. The measurement data which was gathered for this experiment is demonstrated by the generic illustration given in TABLE 16.

The interval data was used to perform t-tests on the means of the control and treatment soils used in the research. The t-test was performed on the initial mean TPH concentrations and the final (Week 6) mean TPH concentrations for the control and treatment soils. The t-score was then compared to the reported 0.05 level of significance to determine if the differences between the means were significant. In addition, regression analysis and stepwise

Table 16. Data Table Example for Verification Study.

	Control	Treatment 1	Treatment 2	Treatment 3
Week1	A1	B1	C1	D1
	A2	B2	C2	D2
	A3	B3	C3	D3
Week2	A4	B4	C4	D4
	A5	B5	C5	D5
	A6	B6	C6	D6
Week3	A7	B7	C7	D7
	A8	B8	C8	D8
	A9	B9	C9	D9
Week4	A10	B10	C10	D10
	A11	B11	C11	D11
	A12	B12	C12	D12
Week5	A13	B13	C13	D13
	A14	B14	C14	D14
	A15	B15	C15	D15
Week6	A16	B16	C16	D16
	A17	B17	C17	D17
	A18	B18	C18	D18

regression was performed on the data to determine the
significance of the treatment variables on the overall
reduction of TPH concentrations. Stepwise regression
analysis allows the computer program to use different
combinations of independent variables to identify the best
interaction possible. The "differences" between the initial
TPH concentration and the subsequent six week trial
concentrations were used to determine treatment
effectiveness. Quadratric forms were used in the model to
account for time differentials between measurements and the
nesting of soil samples within each sampling episode. The
analysis of variance for the regression model provided the
information necessary to determine the significance of each
treatment. In addition, the regression model provided Lower
95% and Upper 95% prediction intervals for each treatment,
thereby, making it possible to illustrate which treatment
would provide the largest reduction in TPH concentration
within the test soils. The Lower 99% and Upper 99%
prediction intervals were then calculated for the treatment
and control soils to illustrate which treatment would have
the largest reduction in TPH concentration within the test
soils.

CHAPTER FOUR

DATA ANALYSIS

OVERVIEW

In order to assess the success or failure of the experimental treatment process utilized in this study, a total of 84 soil samples were obtained for Total Petroleum Hydrocarbon (TPH) analysis. Table 17 provides a summary of the TPH concentrations obtained from these samples. It can be observed from this table that the initial TPH concentrations for all the test groups were similar in range, 7,150ppm to 8,490ppm. The mean TPH concentrations for each group was calculated on a weekly basis and Table 18 provides a summary of these values. It is readily noted that the greatest reduction in test soil means occurred in Treatment 3. The overall mean changes in each group were: (a)Control Group: 7,533ppm to 4,690ppm; (b) Treatment 1: 7,983ppm to 1,077ppm; (c) Treatment 2: 7,542ppm to 3,303ppm, and; (d) Treatment 3: 8,133ppm to 330ppm.

Graphs 1 through 4 illustrates the overall changes in the mean TPH concentrations of each test group during the six week study. Graph 5 provides a comparison of the mean TPH values for all test soils used in the experiment. This

Table 17. Summary of TPH Measurements From Verification
Study.

Table 17. TPH Measurements From Verification Study.				
	Control	Treatment 1	Treatment 2	Treatment 3
Initial	7425	7890	7355	8490
	7150	8120	7760	8060
	8025	7940	7510	7850
Week 1	7270	8250	7120	10980
	7100	8920	7940	10680
	8100	8860	7450	9980
Week 2	7200	8000	6650	8400
	7000	7920	7140	8260
	7900	8210	7000	7020
Week 3	6920	5450	5600	6820
	6710	4650	5920	6110
	6980	5100	5150	5930
Week 4	5210	4780	4750	3050
	4600	3750	4810	2910
	4780	3980	3840	2260
Week 5	5180	2120	4920	1790
	4810	2050	4450	1810
	4920	1980	3960	1100
Week 6	4950	1080	3310	420
	4500	1100	3240	360
	4620	1050	3360	210

Table 18. Summary of Mean TPH Measurements From
          Verification Study.

Table 18. Mean TPH Measurements From Verificaton Study.			
	CONTROL	TR1	TR2	TR3
WEEK 0	7533	7983	7542	8133
WEEK 1	7490	8677	7503	10547
WEEK 2	7367	8043	6930	7893
WEEK 3	6870	5067	5557	6287
WEEK 4	4863	4170	4467	2740
WEEK 5	4970	2050	4443	1567
WEEK 6	4690	1077	3303	330

Graph 1. Mean TPH Concentrations In Control Soil Samples.
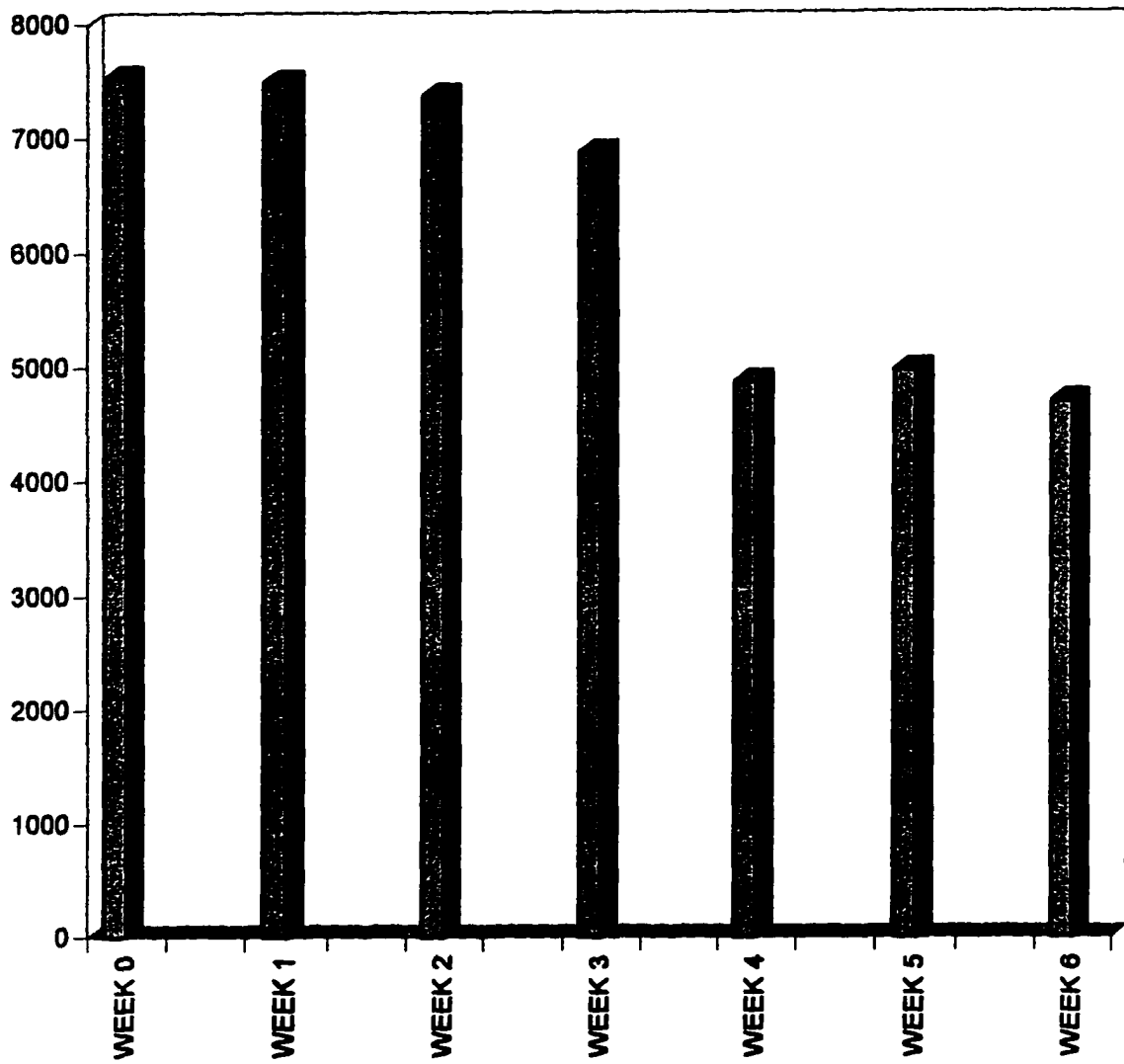
Graph 2.   Mean TPH Concentrations In Treatment 1 Soil
           Samples.
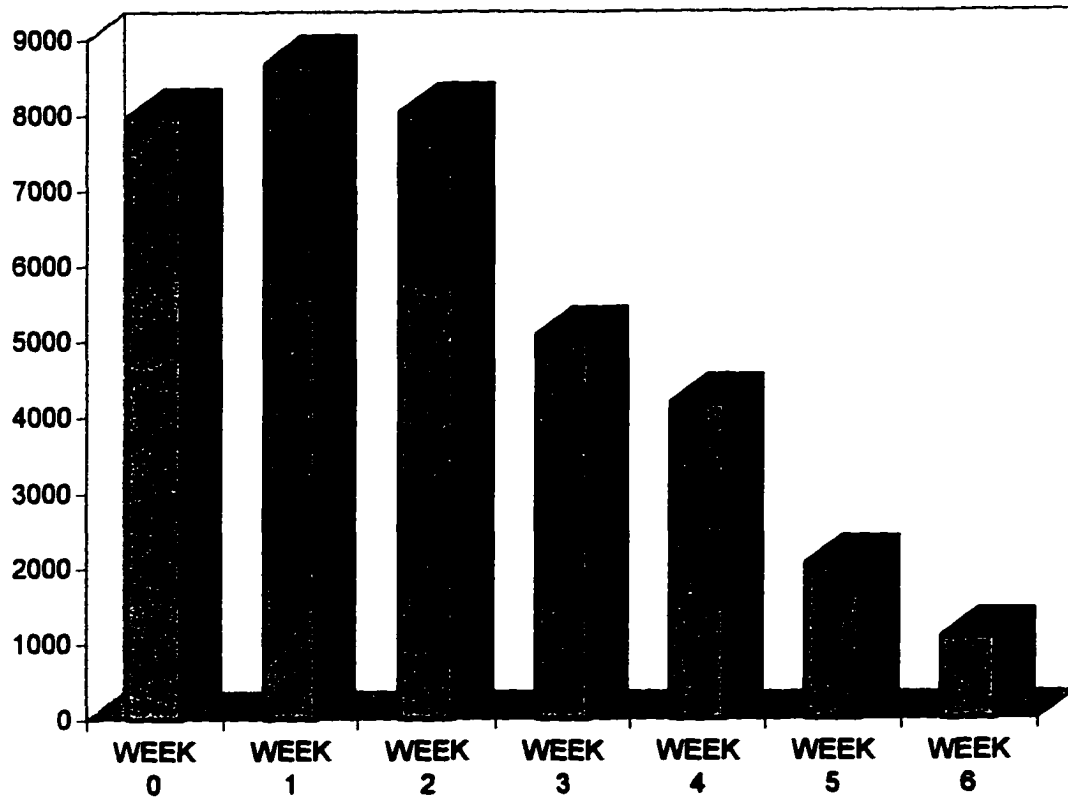
Graph 3.   Mean TPH Concentrations In Treatment 2 Soil
   Samples.
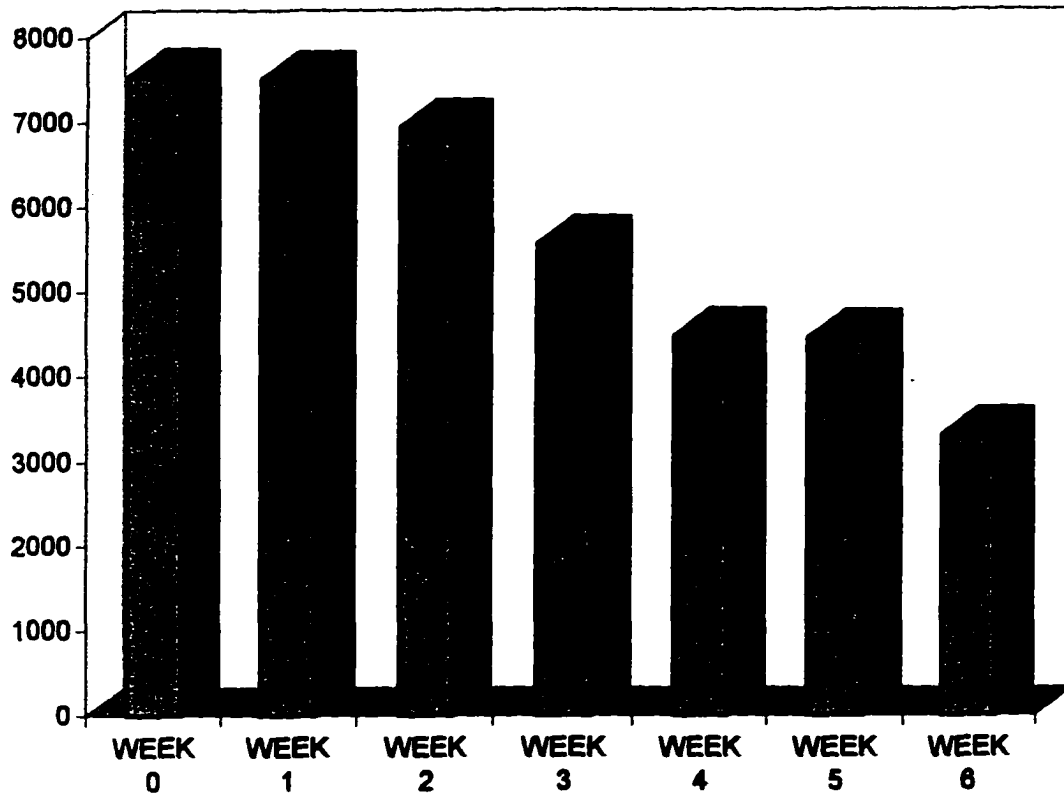
Graph 4. Mean TPH Concentrations In Treatment 3 Soil
   Samples.
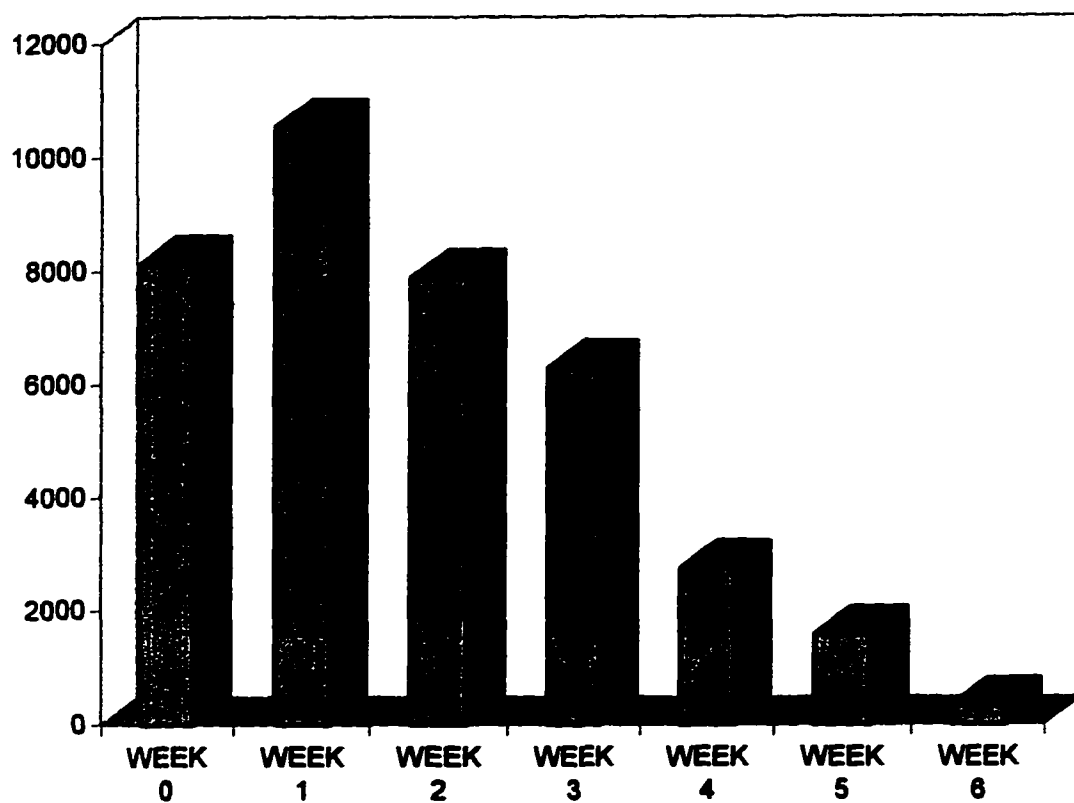
Graph 5. Mean TPH Concentrations In All Test Soils.
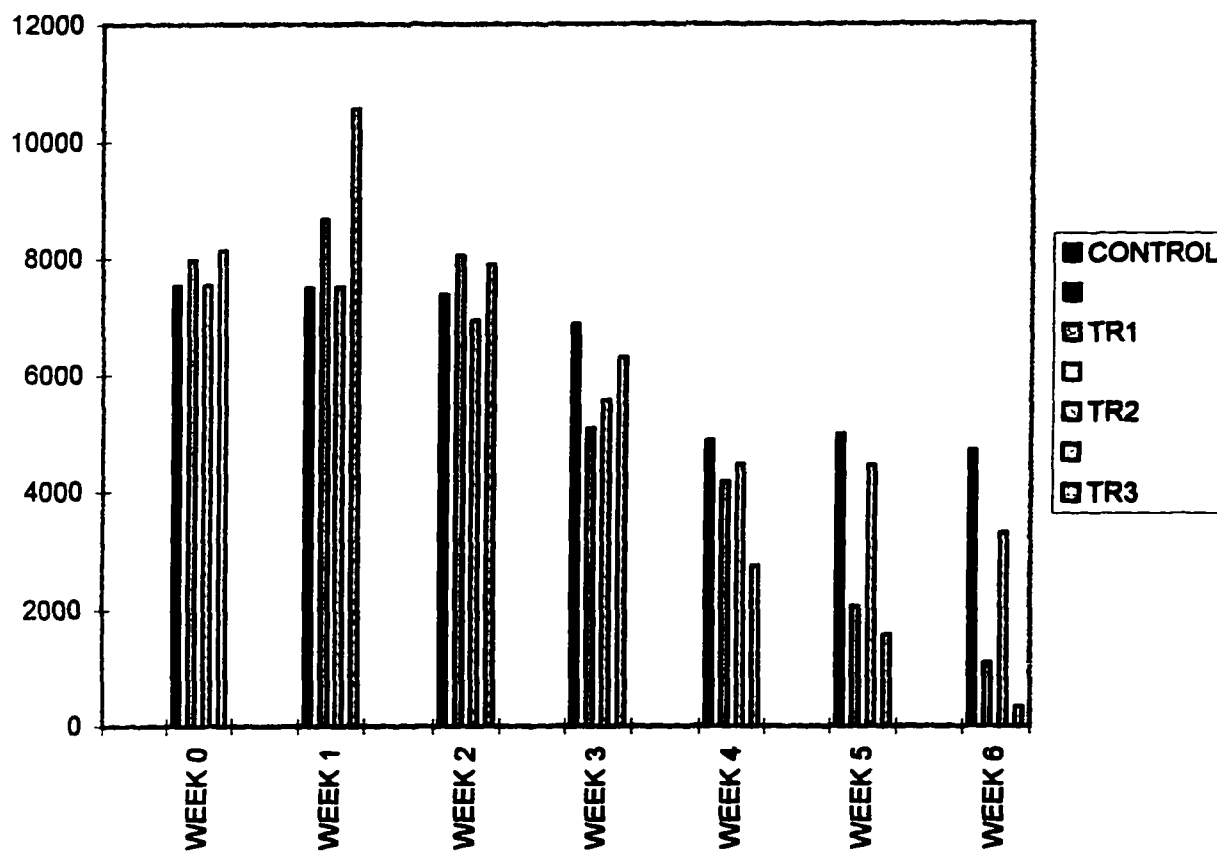
graph shows that the greatest reductions in mean TPH values occurred in Treatments 1 and 3. The initial control soil TPH mean was 7,533ppm and the final TPH mean was 4,690ppm. The overall mean difference TPH concentration for the control soil was 2,843ppm.

STATISTICAL ANALYSIS

The initial Treatment 1 soil TPH mean was 7,983ppm and the final TPH mean was 1,077ppm. Therefore, the overall mean difference TPH concentration was 6,906ppm.

The initial Treatment 2 soil TPH mean was 7,542ppm and the final TPH mean was 3,303ppm. Therefore, the overall mean difference TPH concentration was 4,239ppm.

The initial Treatment 3 soil TPH mean was 8,133ppm and the final TPH mean was 330ppm. Therefore, the overall mean difference TPH concentration was 7,803ppm.

The comparison of the difference between the initial mean TPH concentration and the final or Week 6 mean TPH concentration for each test does not provide a description of the effectiveness of amended treatment process on TPH reduction. The test statistic or t-score for all the treatments and the control indicated that all groups showed a statistically significant difference at the 0.05 level. The calculated t-scores for each group are as follows:

Control Group: t-score = 11.95; Treatment 1 Group: t-score

= 118.56; Treatment 2: t-score = 42.21, and; Treatment 3: t-

score = 65.88. All of these t-values exceed the t-score of

3.182 at 0.05 for 3 degrees of freedom.

The mean values for each group were used to obtain a

"difference" measurement which described the difference

between the weekly mean TPH concentrations and the initial

TPH concentration for each test soil. These "difference"

measurements were used in a regression model to assess a

functional relationship between the dependent variable (TPH)

and the independent variables (Control and Treatment 1

through 3).

Dummy variables were assigned to each of the treatments

used in the study due to the different treatment periods

from which the soil samples were obtained for analysis. In

addition, the dummy variables were used in the establishment

of quadratic equation forms used in the regression model.

The following distinctions were made for each dummy variable

in the SAS program:

If Treat=B Then Dum1=1, else Dum1=0;

If Treat=C Then Dum2=1, else Dum2=0;

If Treat=D Then Dum3=1, else Dum3=0.

The treatments were modeled with the calculated

differences by using the following equations:

L1= Dum1*Diff;

L2= Dum2*Diff;

L3= Dum3*Diff.

Quadratic forms were then used to model the interaction of the dummy variables with the square of the differences:

Q1= Dum1*Diff*Diff;

Q2= Dum2*Diff*Diff;

Q3= Dum3*Diff*Diff.

Quadratic forms assume that all variables and constants are real. In addition, the use of quadratic forms allows for the making of a nonsingular linear transformation of the variables into a new and simpler form.

The regression program considering all of the variables was then run to obtain prediction intervals for the means of each level of the variables and for confidence intervals for the means of each level of the variables. These intervals are shown in Table 19. In addition, the analysis of variance values and parameter estimates are provided in Table 20. The reported F-value for this regression is 44.291 and the p-value is 0.0001. The F-value exceeds the reported $F_{(.95,11,12)}$ value of 2.69, therefore the null hypothesis can be rejected. Also, significance is shown in

Table 19. Mean Prediction Intervals and Confidence Intervals From Regression Analysis.

Jbs	Dep Var TPH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Lower95% Predict	Upper95% Predict	Residual
1	-43.3300	262.3	514.966	-859.7	1384.3	-1408.5	1933.0	-305.6
2	-166.7	-516.9	314.894	-1203.0	169.2	-1932.3	898.5	350.3
3	-663.3	-1237.9	346.283	-1992.4	-483.4	-2687.7	211.9	574.6
4	-2670.0	-1900.7	346.283	-2655.2	-1146.2	-3350.4	-450.9	-769.3
5	-2563.3	-2505.2	314.894	-3191.3	-1819.1	-3920.6	-1089.8	-58.1184
6	-2843.3	-3051.5	514.966	-4173.6	-1929.5	-4722.3	-1380.8	208.2
7	693.3	1029.3	514.966	-92.7240	2151.3	-641.5	2700.1	-336.0
8	60.0000	-719.1	314.894	-1405.2	-32.9968	-2134.5	696.3	779.1
9	-2916.7	-2405.8	346.283	-3160.3	-1651.3	-3855.6	-956.0	-510.9
10	-3813.3	-4030.9	346.283	-4785.3	-3276.4	-5480.6	-2581.1	217.5
11	-5933.3	-5594.2	314.894	-6280.3	-4908.1	-7009.6	-4178.8	-339.1
12	-6906.7	-7095.9	514.966	-8218.0	-5973.9	-8766.7	-5425.2	189.3
13	-38.3400	89.0411	514.966	-1033.0	1211.1	-1581.7	1759.8	-127.4
14	-611.7	-938.6	314.894	-1624.7	-252.5	-2354.0	476.8	326.9
15	-1985.0	-1874.5	346.283	-2629.0	-1120.0	-3324.3	-424.8	-110.5
16	-3075.0	-2718.8	346.283	-3473.3	-1964.3	-4168.6	-1269.0	-356.2
17	-3098.3	-3471.4	314.894	-4157.5	-2785.3	-4886.8	-2056.0	373.1
18	-4238.3	-4132.4	514.966	-5254.4	-3010.4	-5803.2	-2461.6	-106.0
19	2412.7	2542.7	514.966	1420.7	3664.7	871.9	4213.5	-130.0
20	-240.0	-189.9	314.894	-876.0	496.2	-1605.3	1225.5	-50.0986
21	-1846.7	-2607.7	346.283	-3362.2	-1853.2	-4057.5	-1157.9	761.0
22	-5393.3	-4710.8	346.283	-5465.2	-3956.3	-6160.5	-3261.0	-682.6
23	-6566.7	-6499.0	314.894	-7185.1	-5812.9	-7914.4	-5083.7	-67.6171
24	-7803.3	-7972.6	514.966	-9094.6	-6850.6	-9643.3	-6301.8	169.2

Table 20. Analysis of Variance and Parameter Estimates.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	11	157287432.01	14298857.455	44.291	0.0001
Error	12	3874083.3244	322840.27703		
C Total	23	161161515.33			

Root MSE	568.19035	R-square	0.9760
Dep Mean	-2510.44250	Adj R-sq	0.9539
C.V.	-22.63308		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for HO: Parameter=0	Prob > |T|
INTERCEP	1	1099.673000	1016.4098024	1.082	0.3006
DUM1	1	1739.668000	1437.4205275	1.210	0.2495
DUM2	1	108.648000	1437.4205275	0.076	0.9410
DUM3	1	4490.327000	1437.4205275	3.124	0.0088
DIFF	1	-866.513536	664.96306622	-1.303	0.2170
DIFFSQ	1	29.107321	92.99197503	0.313	0.7596
L1	1	-974.370143	940.39978672	-1.036	0.3206
L2	1	-298.598714	940.39978672	-0.318	0.7563
L3	1	-2338.198607	940.39978672	-2.486	0.0286
Q1	1	1.726429	131.51051228	0.013	0.9897
Q2	1	16.725000	131.51051228	0.127	0.9009
Q3	1	128.273393	131.51051228	0.875	0.3486

the p-value of 0.0001. The R-square value of 0.9760
illustrates a 97.6% chance that the TPH reductions are due
to the interactions of the treatments on the test soils.
However, the Prob> /T/ values for each variable are not
significant. These values are also provided in Table 20.

In an effort to obtain significant Prob> /T/ values,
another regression approach, stepwise regression, was
performed on the data. Stepwise regression inserts
variables in turn until the regression equation is
satisfactory. The order of variable insertion is determined
by utilizing the partial coefficient as a measure of the
importance of each variable not yet used in the equation.
The comparison of the partial F scores with a preselected F
percentage point within the equation allows for the
retention or deletion within the equation. The preselected
percentage point used in this study was 0.1. If a tested
variable provides a non-significant contribution, it is
removed from the model and an appropriate fitted regression
equation is then computed for all the remaining variables in
the model.

The results of the stepwise regression model are
provided in Table 21. Table 22 provides the prediction
intervals for the means of each of the variables and the

Table 21. Stepwise Regression Model Results.

Stepwise Procedure for Dependent Variable TPH

Step 1   Variable DIFF Entered   R-square = 0.74400146   C(p) =107.79419128

	DF	Sum of Squares	Mean Square	F	Prob>F
Regression	1	119904403.21429	119904403.21429	63.94	0.0001
Error	22	41257112.115764	1875323.2779893		
Total	23	161161515.33005			

Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
INTERCEP	2070.30750000	637.43238352	19782337.590260	10.55	0.0037
DIFF	-1308.78571429	163.67752608	119904403.21429	63.94	0.0001

Bounds on condition number: 1,   1

Step 2   Variable Q3 Entered   R-square = 0.83402954   C(p) = 64.85227439

	DF	Sum of Squares	Mean Square	F	Prob>F
Regression	2	134413464.11316	67206732.056578	52.76	0.0001
Error	21	26748051.216894	1273716.7246140		
Total	23	161161515.33005			

Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
INTERCEP	1855.72686416	529.16361106	15664661.625006	12.30	0.0021
DIFF	-1147.85023741	143.07226840	81984618.916858	64.37	0.0001
Q3	-91.96312964	27.24774187	14509060.898870	11.39	0.0029

Bounds on condition number: 1.124957,   4.49983

Step 3   Variable Q1 Entered   R-square = 0.92026546   C(p) = 23.80339462

	DF	Sum of Squares	Mean Square	F	Prob>F
Regression	3	148311376.38486	49437125.461619	76.94	0.0001
Error	20	12850138.945192	642506.94725962		
Total	23	161161515.33005			

Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
INTERCEP	1561.13134460	381.13125142	10779722.809289	16.78	0.0006
DIFF	-926.90359774	112.17159315	43871369.075254	68.28	0.0001
Q1	-95.20938715	20.47122825	13897912.271702	21.63	0.0002
Q3	-123.00896517	20.47122825	23198695.073904	36.11	0.0001

Bounds on condition number: 1.370838,   11.66533

Table 21. Stepwise Regression Model Results (Continued).

Step 4   Variable DUM2 Entered   R-square = 0.93523035   C(p) = 18.33293677

	DF	Sum of Squares	Mean Square	F	Prob>F
Regression	4	150723140.42098	37680785.105244	68.59	0.0001
Error	19	10438374.909073	549388.15310909		
Total	23	161161515.33005			

Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
INTERCEP	1690.51136259	357.80077755	12264021.930881	22.32	0.0001
DUM2	-817.96234656	390.39617136	2411764.0361197	4.39	0.0498
DIFF	-870.57067125	107.15301744	36264236.944631	66.01	0.0001
Q1	-111.30450900	20.42904876	16308293.405829	29.68	0.0001
Q3	-139.10408702	20.42904876	25471984.418002	46.36	0.0001

Bounds on condition number: 1.466105.   22.5741

Step 5   Variable DIFFSQ Entered   R-square = 0.95081888   C(p) = 12.55116146

	DF	Sum of Squares	Mean Square	F	Prob>F
Regression	5	153235411.56173	30647082.312346	69.60	0.0001
Error	18	7926103.7683214	440339.09824008		
Total	23	161161515.33005			

Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
INTERCEP	2902.17601323	599.94778876	10304047.008441	23.40	0.0001
DUM2	-871.36905292	350.22440011	2725829.6547582	6.19	0.0229
DIFF	-1769.30540179	388.29991650	9142358.7936444	20.76	0.0002
DIFFSQ	131.03166677	54.85757872	2512271.1407513	5.71	0.0281
Q1	-116.58649095	18.42271217	17635045.198525	40.05	0.0001
Q3	-144.38606897	18.42271217	27047722.382984	61.42	0.0001

Bounds on condition number: 24.46179,   263.2955

Step 6   Variable DUM3 Entered   R-square = 0.95939105   C(p) = 10.27194385

	DF	Sum of Squares	Mean Square	F	Prob>F
Regression	6	154616915.36140	25769485.893567	66.94	0.0001
Error	17	6544599.9686480	384976.46874400		
Total	23	161161515.33005			

Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
INTERCEP	2637.66411437	578.08357887	8014785.4345176	20.82	0.0003
DUM2	-722.02740668	336.82462166	1769024.6678821	4.60	0.0468
DUM3	908.70594919	479.69414954	1381503.7996734	3.59	0.0753
DIFF	-1769.30540179	363.07033430	9142358.7936444	23.75	0.0001
DIFFSQ	138.62530979	51.44963433	2794825.2551242	7.26	0.0154
Q1	-113.59965802	17.29771572	16603923.427706	43.13	0.0001

Table 21. Stepwise Regression Model Results (Continued).

	Q3	-177.74747401	24.63481734	20042060.416055	52.06	0.0001

Bounds on condition number: 24.61119. 342.829

Step 7 Variable L3 Entered R-square = 0.97183677 C(p) = 6.05905404

	DF	Sum of Squares	Mean Square	F	Prob>F
Regression	7	156622686.42945	22374669.489922	78.87	0.0001
Error	16	4538828.9005984	283676.80628740		
Total	23	161161515.33005			

Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
INTERCEP	1956.45413556	558.45974821	3481604.3733662	12.27	0.0029
DUM2	-722.02740668	289.13365936	1769024.6678822	6.24	0.0238
DUM3	3633.54586444	1104.37451550	3070805.9195924	10.83	0.0046
DIFF	-1290.83648810	359.87771602	3649693.4152761	12.87	0.0025
DIFFSQ	73.12435029	50.57001374	593146.16444961	2.09	0.1675
L3	-1913.87565476	719.75543204	2005771.0680495	7.07	0.0171
Q1	-113.59965802	14.84853399	16603923.427706	58.53	0.0001
Q3	84.25636399	100.77604960	198296.29319483	0.70	0.4154

Bounds on condition number: 132.6271. 2016.841

Step 8 Variable Q3 Removed R-square = 0.97060635 C(p) = 4.67327818

	DF	Sum of Squares	Mean Square	F	Prob>F
Regression	6	156424390.13626	26070731.689376	93.56	0.0001
Error	17	4737125.1937933	278654.42316431		
Total	23	161161515.33005			

Variable	Parameter Estimate	Standard Error	Type II Sum of Squares	F	Prob>F
INTERCEP	2154.11933684	501.44854504	5142244.3372390	18.45	0.0005
DUM2	-726.65115408	286.51030805	1792410.0618541	6.43	0.0213
DUM3	2847.50880672	574.29485934	6850562.5388737	24.58	0.0001
DIFF	-1437.92945220	311.15061297	5951134.6641376	21.36	0.0002
DIFFSQ	94.34087252	43.35311703	1319547.6024738	4.74	0.0439
L3	-1325.50379832	149.69001981	21849535.190910	78.41	0.0001
Q1	-114.20938296	14.69874319	16823219.324216	60.37	0.0001

Bounds on condition number: 24.32055. 374.7052

All variables left in the model are significant at the 0.1000 level.
No other variable met the 0.1000 significance level for entry into the model.

Table 21.   Stepwise Regression Model Results (Continued).

Summary of Stepwise Procedure for Dependent Variable TPH

Step	Variable Entered	Removed	Number In	Partial R**2	Model R**2	C(p)	F	Prob>F
1	DIFF		1	0.7440	0.7440	107.7942	63.9380	0.0001
2	Q3		2	0.0900	0.8340	64.8523	11.3911	0.0029
3	Q1		3	0.0862	0.9203	23.8034	21.6308	0.0002
4	DUM2		4	0.0150	0.9352	18.3329	4.3899	0.0498
5	DIFFSQ		5	0.0156	0.9508	12.5512	5.7053	0.0281
6	DUM3		6	0.0086	0.9594	10.2719	3.5885	0.0753
7	L3		7	0.0124	0.9718	6.0591	7.0706	0.0171
8		Q3	6	0.0012	0.9706	4.6733	0.6990	0.4154

confidence intervals for the means of each level of the variables. These intervals are shown in Table 22. In addition, the analysis of variance values and parameter estimate values are provided in Table 23. The reported F-value for this stepwise regression is 69.599 and the Prob>F value is 0.0001. The F-value exceeds the reported $F_{(.95,5,18)}$ value of 2.77, therfore, the null hypothesis can be rejected. Also, significance is shown in the Prob>Fvalue of 0.0001. The R-square vale of 0.9508 indicates a 95.08% chance that the TPH reductions in the test soils are due to the interactions of treatments on the test soils. In essence, the R-square value or the coefficient of multiple determination measures the percentage of the variation in the dependent variable which is explained by variations in the independent variables considered together. These values are provided in Table 23. Finally, the Prob> /T/ values show significance in the stepwise regression model.

One factor which must be considered when using the SAS program output is the mean square value. The lowest mean square value should be used when making prediction statements from regression data. The mean square value for the stepwise regression model was 440339.09824. However, the mean square value for the normal regression model was

Table 22. Mean Prediction Intervals and Confidence Intervals From Stepwise Regression Analysis.

Obs	Dep Var TPH	Predict Value	Std Err Predict	Lower95% Mean	Upper95% Mean	Lower95% Predict	Upper95% Predict	Residual
1	-43.3300	1263.9	314.272	603.6	1924.2	-278.7	2806.5	-1307.2
2	-166.7	-112.3	211.900	-557.5	332.9	-1575.8	1351.2	-54.3519
3	-663.3	-1226.5	242.873	-1736.7	-716.2	-2711.0	258.1	563.1
4	-2670.0	-2078.5	271.836	-2649.6	-1507.4	-3585.1	-572.0	-591.5
5	-2563.3	-2668.6	307.765	-3315.1	-2022.0	-4205.3	-1131.8	105.2
6	-2843.3	-2996.5	445.847	-3933.2	-2059.8	-4676.1	-1316.9	153.2
7	693.3	1147.3	312.469	490.8	1803.8	-393.6	2688.3	-454.0
8	60.0000	-578.7	203.822	-1006.9	-150.4	-2037.1	879.8	638.7
9	-2916.7	-2275.7	236.193	-2772.0	-1779.5	-3755.5	-795.9	-640.9
10	-3813.3	-3943.9	281.149	-4534.6	-3353.3	-5458.0	-2429.8	130.6
11	-5933.3	-5583.2	352.904	-6324.6	-4841.8	-7162.2	-4004.2	-350.1
12	-6906.7	-7193.6	529.195	-8305.4	-6081.8	-8976.8	-5410.5	287.0
13	-38.3400	392.5	397.042	-441.6	1226.7	-1232.1	2017.2	-430.9
14	-611.7	-983.7	310.512	-1636.0	-331.3	-2522.9	555.5	372.0
15	-1985.0	-2097.8	313.429	-2756.3	-1439.3	-3639.6	-556.0	112.8
16	-3075.0	-2949.9	309.795	-3600.8	-2299.1	-4488.5	-1411.3	-125.1
17	-3098.3	-3539.9	307.765	-4186.5	-2893.3	-5076.7	-2003.2	441.6
18	-4238.3	-3867.9	414.482	-4738.7	-2997.1	-5511.6	-2224.1	-370.5
19	2412.7	1119.5	312.469	463.0	1776.0	-421.4	2660.5	1293.2
20	-240.0	-689.9	203.822	-1118.1	-261.6	-2148.3	768.6	449.9
21	-1846.7	-2525.9	236.193	-3022.2	-2029.7	-4005.7	-1046.1	679.3
22	-5393.3	-4388.7	281.149	-4979.4	-3798.0	-5902.8	-2874.6	-1004.6
23	-6566.7	-6278.2	352.904	-7019.6	-5536.8	-7857.2	-4699.2	-288.4
24	-7803.3	-8194.4	529.195	-9306.2	-7082.6	-9977.6	-6411.2	391.1

Sum of Residuals   0
Sum of Squared Residuals   7926103.7683
Predicted Resid SS (Press) 13966026.611

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Table 23. Analysis of Variance and Parameter Estimates
From Stepwise Regression Analysis.

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Prob>F
Model	5	153235411.56	30647082.312	69.599	0.0001
Error	18	7926103.7683	440339.09824		
C Total	23	161161515.33			

Root MSE	663.58051	R-square	0.9508	
Dep Mean	-2510.44250	Adj R-sq	0.9372	
C.V.	-26.43281			

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	T for HO: Parameter=0	Prob > |T|
INTERCEP	1	2902.176013	599.94778876	4.837	0.0001
DIFF	1	-1769.305402	388.29991650	-4.557	0.0002
DIFFSQ	1	131.031667	54.85757872	2.389	0.0281
Q1	1	-116.586491	18.42271217	-6.328	0.0001
Q3	1	-144.386069	18.42271217	-7.837	0.0001
DUM2	1	-871.369053	350.22440011	-2.488	0.0229

322840.27703. Therefore, the normal regression model should be used to predict the intervals of contaminant reduction in each treatment.

The Lower 95% and Upper 95% prediction values for the final week of the study were as follows: (a) Control Group: -4722.3 and -1380.8; (b) Treatment 1 Group: -8766.7 and -5425.2; (c) Treatment 2 Group: -5803.2 and -2461.6, and; (d) Treatment 3 Group: -9643.3 and -6301.8. This data indicates that Treatment 3 provided the highest predicted drop in TPH concentrations within the treatment and control soils. However, it must be noted that the Lower 95% prediction value of Treatment 1 falls within the Treatment 3 prediction range.

In order to calculate the Lower 99% prediction value and the Upper 99% prediction value for the test soils, the t-value of 12 degrees of freedom (3.055) at 0.01 level of significance was utilized. This value is based upon the 12 degrees of freedom in the error term of the regression model. The 3.055 value was multiplied by the standard error prediction value provided in Table 22. The resulting numerical result was then added and subtracted from the prediction value to arrive at the 99% prediction values. The Lower and Upper 99% prediction values for the treatment

and control soils were as follows: (a) Control Group: -4624.72 and -1477.78; (b) Treatment 1 Group: -8669.12 and -5522.68; (c) Treatment 2 Group: -5705.62 and -2258.78, and; (d) Treatment 3 Group: -9545.82 and -6399.38. Treatment 3 had the highest predicted reduction in TPH concentrations within the treatment and control soils. However, it must be noted that the Lower 99% prediction value for Treatment 1 falls within the Treatment 3 prediction range.

An important point to consider is that when prediction intervals or confidence intervals are calculated based on prior calculated intervals within a given treatment, that the final level has an uncertainty factor associated with it. In the case of the calculated Lower 99% and Upper 99% prediction intervals, the true interval is approximately 97.5%.

CHAPTER FIVE

FINDINGS AND DISCUSSION

OVERVIEW

The purpose of this study was to determine the ability of an amended vermicomposting treatment technique to detoxify petroleum contamination present in Virginia Coastal Plain sandy soil. This amended treatment alternative utilized biological sludge and the earthworm *Eisenia foetida* to cause ring fission of the aromatic hydrocarbons present in petroleum products within a soil matrix. The reductions in TPH concentrations were measured using EPA Method 8015 for petroleum contaminated soil and analytical measurements were obtained on a weekly basis from each test soil.

The analytical data indicated that the largest reduction in TPH concentration during the six week trial period occurred in the test soil treated with the amended vermicomposting technique (Treatment 3). The initial mean TPH concentration within the Treatment 3 soil was 8,133 ppm and the end mean concentration was 330 ppm. Therefore, the total reduction in TPH concentration within the Treatment 3 test soil was 7,803 ppm.

The second largest TPH reduction occurred within the

Treatment 1 (biologic sludge only) test soils. The total reduction of mean TPH concentration was 6,906 ppm. The reduction of TPH using this method is not surprising since the addition of the heterogeneous community of micro-organisms present in biologic sludge provided a new source of naturally-occurring petroleum degrader microorganisms to the test soil.

The reduction of the mean TPH concentrations within the Treatment 2 (E.foetida only) soils was greater that the control mean TPH reduction of the control soil, 4,239 ppm and 2,573, respectively. This result indicates that the earthworm activity could have caused the reduction of the TPH concentrations within the Treatment 2 soil. Therefore, the combination of the earthworm activity and increased microbiological community within Treatment 3 soils had a shared impact on reducing the TPH concentration. This occurrence provides limited, but positive, proof that the amended vermicomposting approach was successful in TPH detoxification in Virginia Coastal Plain sandy soil.

DISCUSSION OF STATISTICAL TESTING

The performance of a t-test on the Treatment 3 data indicated that the null hypothesis was rejected at the 0.05

level of significance. This result also provides data supporting the effectiveness of the amended treatment technique on the petroleum contaminated soil. However, since the t-test data for the Control, Treatment 1 and Treatment 2 soils were also significant at the 0.05 level, a regression analysis was performed to determine if significance existed for the treatments.

The regression analysis showed significance, $F>.0001$, for Treatment 3. However, the most useful data output generated by the regression analysis was the ability to describe the Lower 95% and Upper 95% predictive values for the expected TPH concentration reduction in the coastal plain soil. In addition, the regression model also provided a mechanism for calculating the Lower 99% and Upper 99% predictive values for TPH reduction. Treatment 3 showed the highest predictive reduction potential of all test soils at both the 99% and 95% Lower predictive value. This data also supports the hypothesis that the amended vermicomposting treatment technique can effectively reduce the concentration of TPH in Virginia Coastal Plain sandy soil.

FURTHER RESEARCH POTENTIAL

The findings of this research study opens two potential

paths of future research, urban wastewater treatment plant operations and direct scientific study of the amended vermicomposting treatment alternative.

The use of biologic sludge as a catalyst for the biodegradation or detoxification of contaminated soil may present urban wastewater treatment plants with a new market for the secondary biologic sludge produced at their facilities. Many urban wastewater plants are currently composting the sludge and selling the dried sludge material as a soil enhancement product to the general public. This method requires that the municipal wastewater plants keep the sludge for a long period of time and use a large portion of storage space to accomodate the drying process. The use of liquid biologic sludge as a part of the amended vermicomposting treatment technique would reduce the amount of liquid residual sludge that would require treatment and drying at the wastewater facility. In addition, the sale of the biologic sludge would create a new market for the municipal wastewater material and increase the finances available to the wastewater treatment plant or the municipality in charge of the operation of the plant.

The potential scientific research which may be conducted using the amended vermicomposting approach

includes, but is not limited to, the following topics:

1. Large-scale testing of the amended vermicomposting process in order to determine if the predictive values identified in the bench scale project remain constant.

2. Further study on the potential of earthworms to have both viricidal and bactericidal actions on the pathogenic microorganisms present in wastewater sludge material.

3. Further study on the multicomponent mixtures of petroleum products and their respective biodegradation rates in order to provide some predictive values for degradation rates.

4. Determine the ability of the amended vermicomposting treatment approach to degrade other organic contaminants such as pesticide residues and other recalcitrant organic compounds found in contaminated soils.

5. Determine the impact of free radical interactions on the degradation of organic pollutants.

6. Determine if a combination of surface dwelling earthworms and deeper burrowing earthworm classes increases the degradation rate of organic pollutants.

The limited scope of the bench-scale study performed for this research provided an indication that the amended

treatment provided the largest decrease in petroleum
hydrocarbon concentration in the sandy soil. Three
additional tests could be performed to strengthen the
results of the study. The measurement of microbial
respiration rates within each soil microcosm would indicate
any increased aerobic activity occurring in the soils
receiving liquid biologic sludge. A second way to strengthen
the results of this study would have been to measure the
benzene, toluene, etheylbenzene, and toluene (BTEX) levels
within the water collected from each soil microcosm. If the
measured BTEX levels within the collected water did not show
the migration of the petroleum products from the soil to the
water, then degradation of the petroleum products is
occurring within the soil pore space prior to becoming
suspended in pore space water. A third method which could
be used to strengthen the study results would be to perform
the experiment on different sandy soil types (i.e., sandy
clay or silty sands) and measure the degradation, if any,
from these soils. Finally, the entire experiment could be
performed in an outdoor setting to determine how the
external environmental events and seasonal variations would
affect the degradation potential of the amended treatment
system.

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APPENDIX A

GAS CHROMATOGRAPHY NEEDS

Method 8015 is used to determine the concentration of petroleum hydrocarbons by gas chromatography (GC). The samples may be introduced into the GC following solvent extraction, by direct injection, by purge-and-trap, or by vacuum distillation. Diesel range organics may be prepared for analysis using a solvent extraction method and gasoline range organics may be introduced into the GC by purge-and-trap, automated headspace, vacuum distillation, or other appropriate techniques. Detection of petroleum hydrocarbons is performed using a flame ionizing detector (FID).

The recommended GC columns for this method include:

(A) Column 1: 8 ft. x 0.1 in. id stainless steel or glass column packed with 1% SP-1000 on Carbopack-B 60/80 mesh or equivalent.

(B) Column 2: 6 ft. x 0.1 in. id stainless steel or glass column packed with n-octane on Porasil-C 100/120 mesh (Durapak) or equivalent.

(C) Column 3: 30 m x 0.53 mm id fused silica capillary column bonded with DB-Wax or equivalent, 1 um film thickness.

(D) Column 4: 30 m x 0.53 mm id fused silica capillary column chemically bonded with 5% methyl silicone, 1.5 um film thickness. Capillary columns are needed for petroleum hydrocarbon analysis.