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Project Title

Impact of Landfill Leachate on Iron Release from Northwest Florida Iron Rich Soils

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Final Report

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Abstract

Elevated iron concentrations have been observed in the groundwater downgradient of the landfills in Northwest Florida. It is suspected that microbial mediated iron reduction should be responsible for the iron release. The objective of this research is to investigate whether, and to what extent, microbial mediated iron reduction is responsible for iron release in the regions nearby landfills in Northwest Florida. It is also the goal of this research to characterize the microbial species that exist in the soils nearby landfills. In addition, the iron reduction rate is to be quantified to provide evidence that microbial mediated iron reduction is dominating and responsible for iron release in Northwest Florida.

This project started on August 1, 2006 and ended on July 31, 2007. In this research, we characterized soil samples collected from sixteen landfills (from fifteen Northwest Florida counties) through sieve analysis and quantifed and identified the bacterial species in the soil samples. In addition, we conducted laboratory iron reduction batch experiments using the sampled soils by reacting them with corresponding landfill leachate under chemical and biological conditions similar to the concerned site in the presence of identified bacteria. As a control, we also reacted soil samples with simulated storm runoffs. We monitored ferrous iron, ferric iron, dissolved oxygen, pH, and redox potential, etc. on a daily basis. Based on the plate counts, we found that there were around 0.03 million to 2.0 million colony-forming units (CFU) per gram of soils for the soil samples from Northwest Florida. Through morphology analysis, we identified that typical rod and cocci bacteria were the dominating species. In addition, we also identified potential iron reducing bacteria. According to the sieve analysis of the soil samples, we found a correlation of increased bacterial population in well-graded soils. The soils also showed an increase of water content with the increase of the percentage of fine particles. From laboratory batch experiments, we found that iron release was most pronounced for soil samples collected from Jackson County and Walton County reacting with the corresponding landfill leachate. The iron release for these sites can be as high as over 400 mg/L within 55 days. All the other samples produced less than 200 mg/L of iron release. Based on the laboratory experiments of iron release when landfill leachate reacted with iron rich soil under chemical and biological conditions of Northwest Florida landfills, ferrous iron was found to be released when landfill leachate contacted with iron rich Northwest Florida soil in the presence of cultured iron reducing bacteria. On the other hand, minimal ferrous iron release was observed in the absence of the cultured iron reducing bacteria. This indicated that iron release by landfill leachate reacting with iron rich soil was a microbial mediated process.

Key words: Landfill, Iron, Microbial, Leachate, and Florida.

1. Introduction

Landfill leachate is being blamed for elevated levels of iron and arsenic, especially iron observations in the groundwater from monitoring wells downgradient of landfills in Northwest Florida. It is suspected that the geochemical and geomicrobial iron reduction/oxidation processes are responsible for the iron release in the groundwater. In Florida, soils are mainly covered by poorly drained sandy soils of Myakka. Myakka soils are spodospl, acid soils characterized by a subsurface accumulation of humus and Al and Fe oxides. Although Myakka soil series is widely extensive in Florida, they can hardly be seen in any other states.

There is a possibility that iron-reducing bacteria reduce iron oxides to ferrous iron and release it to the groundwater when hydrocarbon rich landfill leachate contacts the soil. Researchers from Florida State University have demonstrated that a pure culture of *Shewanella oneidensis* strain MR-1 as well as enrichment cultures of iron-reducing bacteria are capable of conserving energy for growth with the structure Fe (III) bound in smectite clay as the sole electron acceptor[1-2]:

$$CH_2O + 2Fe_2O_3 + 3H_2O = CO_2 + 4Fe^{2+} + 8OH^- \quad (1)$$

This is a very important discovery since most of the iron on earth exists in the form of silicate minerals or iron oxides. When conditions permit, microbial mediated iron reduction and release may be the mechanism for elevated iron observations in groundwater.

In the regions near the landfills, there is also a possibility of iron release due to the oxidation of metallic iron, especially near the C&D landfills[3-4], which can occur under both aerobic and anaerobic conditions. Usually, the iron oxidation is a chemical oxidation process, which can be accelerated by anions in the leachate such as chloride, carbonate, and sulphate, etc. As municipal landfill leachate can cause depletion of dissolved oxygen of the contaminated groundwater owing to the high oxygen demand, chemical oxidation of metallic iron is most likely to occur under anaerobic conditions[5-7].

Many studies have also investigated the interactions of bacteria with metallic iron under both aerobic and anaerobic conditions. The oxidation or corrosion of zero-valent iron might be greatly stimulated by microorganisms[8-9]. Due to the limited solubility of oxygen in groundwater (especially near landfills where landfill leachate can deplete dissolved oxygen) and the rapid reduction of molecular oxygen by Fe^0 and Fe^{2+}, the oxidation usually occurs under anaerobic environments. Though under anaerobic conditions iron corrosion rates driven by chemical reactions may be reduced, biologically mediated anaerobic corrosion may occur at rates exceeding those seen under oxygenated conditions[10]. Anaerobic iron oxidation is frequently linked to the activity of sulphate-reducing bacteria[11]. There is evidence that sulphate-reducing bacteria are responsible for pitting the iron[10, 12]. Two iron oxidation mechanisms, indirect mechanism and direct mechanism, which may occur simultaneously at different extents, are involved in sulphate-reducing bacteria mediated iron oxidation[11]. The indirect mechanism refer to the oxidation of iron by hydrogen sulfide ($Fe + H_2S = FeS + H_2$).

Because sulphate-reducing bacteria use organic compounds (shown here as $[CH_2O]$) and H_2 for sulfate reduction, the net reaction is:

$$2[CH_2O] + \frac{4}{3}Fe + \frac{1}{3}SO_4^{2-} + \frac{2}{3}H^+ = 2HCO_3^- + \frac{4}{3}FeS + \frac{4}{3}H_2O \quad (2)$$

The direct mechanism is commonly attributed to sulphate-reducing bacteria such as *Desulfovibrio* species (can grow with organic substrate or H_2). These species can obtain electrons from metallic iron in a more direct manner than via free hydrogen:

$$4Fe + SO_4^{2-} + 4H_2O = FeS + 3Fe^{2+} + 8OH^- \quad (3)$$

This direct mechanism is also supported by a recent discovery of a newly isolated *Methanobacterium*-like archaeon that have a more direct access to electrons from iron than via hydrogen consumption[11]. For iron oxidation by both mechanisms, $Fe(OH)_2$ or $FeCO_3$ may precipitate in addition to FeS.

At Leon County Landfill, we found elevated levels of iron concentrations in the percolation from Lysimeters. With increased organic matter content, more obvious iron release was observed (Figure 1). We suspect that microbial mediated iron reaction processes compete with chemical processes, promoting and potentially dominating iron release near landfills where landfill leachate interacts with the soil since organic matter contributed to iron release (Figure 1). In Leon County landfills, we also found hydrogen sulfide during landfill gas emission sampling (Figure 2), which was believed to be produced during the sulphate reducing processes. Currently, we are not sure which microbial mediated iron release processes, i.e., iron reducing versus sulphate reducing (iron oxidation) should be responsible for elevated iron concentrations in the groundwater. It should also be noted that iron-reducing bacteria and sulphate-reducing bacteria coexist in the subsurface and compete for growth in most cases. In addition, we cannot eliminate the possible chemical iron oxidation without solid evidence.

Percolation from Lysimeter with Soil only

Percolation from Lysimeter with Soil and Organic Matter (Note: Darker Color or More Concentrated Iron Observation)

Figure 1. Microbial Mediated Iron Observation in Lysimeters

Figure 2. Landfill Gas Emission Sampling

This research explored the geochemical and geomicrobiological processes during which iron reduction occurs. We elucidated the dominating mechanism that was responsible for iron release in iron rich soils, especially in the iron rich soils that interact with landfill leachate. Our long-term goal is to derive the relationship of iron reduction processes with the hydrogeochemistry and geomicrobiology (e.g., pH, redox and microbial activity or degradation processes) of the iron rich soils and to develop modeling tools to predict and monitor the iron reduction and release processes. In regions near landfills, iron release from iron rich soils may be caused by one or more mechanisms when landfill leachate reacts with the soil, with certain mechanisms dominating upstream or downstream. Our goal for this research was to investigate possible iron release mechanisms at different geographically, hydrogeologically, geochemically and geomicrobiologically distinct sites using advanced analytical techniques. We focused on the regions near landfills in Northwest Florida but our discovery can be extended to cover any region with iron rich soils. Specific objectives of this research project included (1) collect soil samples from sixteen counties in Northwest Florida, (2) characterize the soil samples and identify the bacterial species existing in the soil samples, (3) culture microbial species that are responsible for iron reduction reactions, i.e., iron-reducing bacteria that mediate iron reduction reactions, and (4) elucidate iron release mechanisms by reacting iron rich soils collected from Northwest Florida with landfill leachate in the absence and presence of cultured iron-reducing bacteria to observe iron release. Eventually, results from this study can be applied beyond local and landfill perspective to any iron rich soils. In our future research, we will derive the relationship of iron reduction processes with the hydrogeochemistry and geomicrobiology of iron rich soils and develop modeling tools to predict and monitor the iron release processes.

2. Background

2.1 Soil Microbiology

Soils consist of many different components, mainly minerals, organic material, water, gases, and the living soil population. The major microbial groups in soils are viruses, bacteria, fungi, algae, and protozoa. Viruses are usually termed phages, in that they infect all other organisms including those in soils. The fungi are eukaryotic organisms that consist of molds, mildews, rusts, smuts, yeasts, mushrooms, and puffballs. The most common fungi are the eukaryotic algae which are made up of water moss, pond scum, seaweed, and red tide. The protozoa are unicellular organisms that are generally microscopic in size that in some instances reach macroscopic size. Of all living components in soil, the bacteria are the most numerous[13]. Termed prokaryotic due to a lack of nuclear membrane, bacteria are in fact the most abundant of all living organisms on earth.

2.1.1 Soil Bacterial Biota

The bacterial population in soils is a very diverse grouping that is classified based on several criteria. Bacteria can be classified based on the criteria of oxygen tolerance, morphological cell structure, energy source, and carbon source, etc.

The ability/inability to grow in the presence of oxygen is an important biochemical trait for bacteria. Bacteria are classified into three different categories based on this criterion. Aerobes are bacteria which must have access to oxygen; anaerobes grow only in the absence of oxygen; and facultative anaerobes can grow with or without an oxygen supply.

The morphological structure of the bacterial cell is characterized by three different groups: bacilli, cocci, and spirilli. Bacilli are rod shaped, and are the most abundant group in soil populations. Cocci are spherical-shaped and spirilli are spiral in shape. Spirilli are typically not common in soil bacterial populations. The bacilli have the ability to persist in unfavorable conditions, like those beneath a landfill with leaked hazardous chemicals. The bacilli produce endospores to survive the unfavorable conditions and can germinate when conditions improve for their growth. Also, they may have the ability to adapted to the hazardous chemicals and use them as an energy source.

Bacterial classification based on the type of energy source is most important in the field of remediation engineering in which the type and population of microbial species present in the soil can determine the system design. In general, microorganisms are classified as either heterotrophic or autotrophic based on how the microbes obtain the growth energy. Autotrophs are further classified as photoautotrophs or chemoautotrophs, i.e., photoautotrophs derive their energy through sunlight and chemoautotrophs through oxidation of inorganic materials. Heterotroph organisms dominate the soil substrata in terms of energy transformation, i.e., they require organic substrates to serve as an energy and carbon sources. The availability of these organic substances and other nutrients within the soil matrices are essential to the bacterial community. The bacteria use these substances to facilitate cell development.

2.1.2 Soil Bacterial Diversity

Since one of the objectives of this research was to identify the dominant bacterial species within the soil samples, it is important to understand the soil bacteria diversity. Due to the high variability of bacterial species in the soil, it is very difficult to determine the dominant species. However, the dominant bacterial species in the soil can be roughly estimated in terms of percentage of the total population based on plate count methods (Table 1)[12]. A more recent investigation into the dominant bacterial species in the soil was based on libraries of 16S rRNA and 16S rRNA genes[14]. Members of the phylum that make up the percentage of 16S rRNA libraries derived from soil communities were presented (Table 2)[14].

Table 1. Dominant Genus in Soil Bacteria[12]

Genus	% of Population*
Arthrobacters	40
Streptomyces	5 to 20
Pseudomonas	5 to 20
Bacillus	5 to 20

*Based on plate counts

Table 2. Dominant Phylum in Soil Bacteria[14]

Phylum	% Contribution to 16S rRNA Libraries
Protobacteria	39
Acidobacteria	20
Actinobacteria	13
Verrucomicrobia	7
Bacteroidetes	5
Chloroflexi	3
Planctomycetes	2
Gemmatimonadetes	2
Bacillus	2
Clostridium	2

2.1.3 Soil Properties

The nature, diversity, and activity of a soils bacterial community are determined by the adaptability of the organisms to adapt to or modify the environmental properties of soils. Temperature, pH, moisture, soil type, and redox potential are the most important soil/environmental parameters for bacterial growth. By modifying or adapting to these environmental parameters, a bacterial community can survive.

Temperature. Temperature within the soil matrix is important for the rate at which the chemical and biochemical reactions occur within bacterial cells and the surrounding environment. Typically, the optimal growth temperature for most bacteria is within the 25~30°C range[15]. A broader range where bacteria can grow is typically observed to be between 0 and 70 degrees Celsius. Microorganisms can be characterized based on these temperature tolerances or ranges. Psychrophiles can grow optimally below 20°C, mesophiles between 20°C and 40°C, and thermophiles above 45°C. Psychrophiles are not common in soil bacterial communities. Mesophiles, however, are the dominating type of bacterial species found in the soil while thermophiles are less common.

pH. The measurement of pH in the soil is essential to understand the types of microbial species within a specific soil. All microbial species have a pH range in which their cell functions are possible. Thus, each species has its optimum pH value for growth. Bacterial surface enzymes can also regulate the external pH so that internal cell functions are optimized. These surface enzymes are among the over 1000 surface enzymes[13] that are associated with internal functions as well as external functions such as membranes. The balance of the internal pH as affected by the external pH gradients within the soil is essential for bacterial growth. Also, the soil particles themselves affect the pH within a specific location. In particular, clay particles can greatly alter the soil pH owing to their negative charges that produce the double layer, thus increasing cations within the soil matrices. Like all soil properties, the soil pH is greatly dependent on other soil parameters such as water content and mineral content.

Moisture. The moisture availability in the soil is another major factor that affects microbial growth. Water serves as an essential component in cell processes, affects gas exchange in soil, transports nutrient supply to microbes, regulates soil temperature, and works as the growth medium for microbial colonies. The pores within soil matrices are filled with water and air. For microbial growth the water to air ratio in the pore is essential. Too much water content in the pores prohibits sufficient diffusion of oxygen, leading to an anoxic environment.

In soil matrices, water content is closely related to the water potential, which is determined by osmotic potentials. Consequently, water content in the soil matrices can be quantified by measuring the soil water potential. The general optimal water potential for microbial growth is approximately -0.05 MPa[13]. Water content can be expressed in terms of gravimetric and volumetric water content.

Soil Type and Structure. The soil habitat is comprised of many different components including minerals, plant roots, living soil microbes, decomposing organic matter, soil gases, and soil water, etc. All of these components come together to form suitable habitats for microbial development. The most dominant structural feature in the soil matrices is the clay-organic matter complexes. The clay particles are essential to the basic formation of these aggregates. This aggregation of the clays and organic matter complexes is one of the most important factors governing microbial activity in the soil. The forces within the soil

(freezing, thawing, and root growth, etc.) help mold these complexes into aggregates. These complexes form macroaggregates and their subunits microaggregates (Figure 3). Most microbial organisms do not exist in the pore spaces of the soil, rather they reside on the surfaces of these aggregates due to their bacterial fibrils on their surface and polysaccharides produced during metabolism. Furthermore, functional groups on microbial surfaces form bonds with receiving bonding sites on mineral surfaces. The formation of these aggregates further facilitates proper water infiltration and availability, oxygen tension, and nutrient movement as needed by the microbial community. Also, clay particles associated with the aggregates have been shown to assist in removing contaminants such as arsenic from groundwater[16].

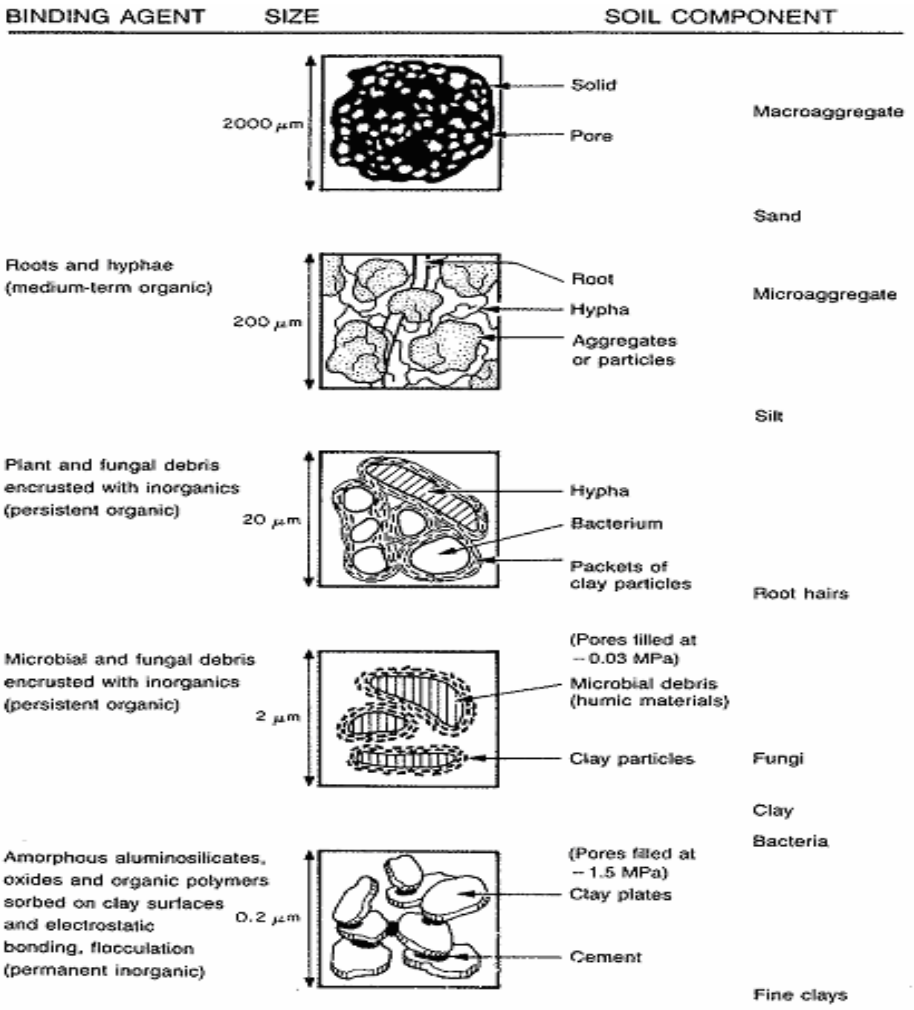

Figure 3. Soil Aggregates[13]

Redox Potential. The redox potential, an expression to describe the oxidizing or reducing capability of the surrounding environment, is also a major factor controlling soil biota. In the soil, oxygen is the major electron acceptor in these reactions. In scenarios when oxygen is limited, NO_3^-, Fe^{3+}, Mn^{2+}, and SO_4^{2-} can serve as electron acceptors. For this

research, the occurrence of Fe^{3+} serving as the electron acceptor, is of greater concern than the other reactions. When ferric iron is reduced, the thus produced soluble ferrous iron should be blamed for the elevated iron observations in the groundwater downgradient of the landfills.

2.2 Soil Microbial Structure Nearby Landfills

The structure of the soil microbial community nearby landfills has not been directly well studied. Many studies have characterized bacterial species that are present in groundwater downgradient of landfills as groundwater contamination is of concerns for human health. In general, landfills have been characterized in terms of zones of bacterial reactions (Figure 4). Beneath the aerobic region, the dominating anaerobic species are sulfate and iron reducing bacteria. Once sulfate and iron sources are depleted, methane production is the dominant reaction. Many studies have characterized the bacterial community that exists within contaminant plumes of landfills. A contaminated aquifer in the Netherlands was shown to have a high diversity of iron-reducing bacteria *Geobacteraceae* within the leachate plume when compared to an unpolluted aquifer[17]. In the same location, it was shown that *Protobacteria* were the dominant upstream of the contaminant plume. Gram-positive bacteria dominated the species beneath the landfill and downstream the *Protobacteria* reappeared, however they were different species from the upstream *Protobacteria*[18]. These differences in microbial diversity adjacent to the landfill were observed in groundwater samples but not in sediment samples. Also, a landfill in Denmark noted a shift in the bacterial community make-up with an increase in horizontal distance from the landfill[19-20]. It was noted that an increase of iron and sulfate reducing bacteria were more pronounced for locations close to the landfill.

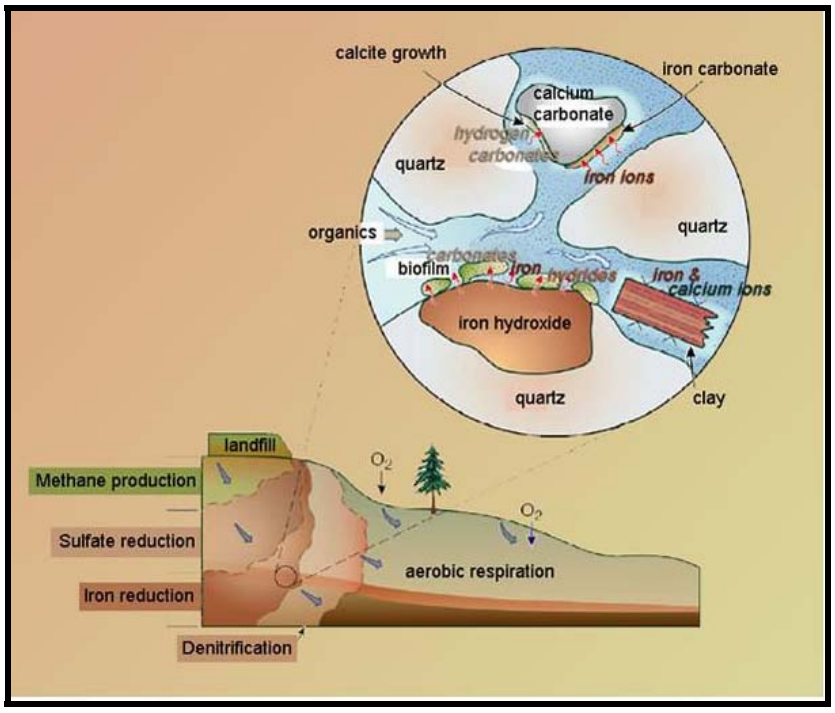

Figure 4. Oxidation and Reduction Zones Downstream of a Landfill[21]

Overall, most studies conclude that the iron and sulfate-reducing species existed beneath the landfill. It is also noted that the landfill leachate does alter the chemistry of the groundwater nearby the landfill, thus allowing favorable anoxic conditions for the iron and sulfate-reducing bacteria[22]. An aquifer contaminated by an anoxic landfill leachate showed distinct zones of sulfate reducing and iron reducing[23].

2.3 Soil Microbial Characterization

The characterization of the microbial community within a soil sample is a very useful tool in determining the overall health of the soil. Characterization is a very broad term that can cover many aspects of the soil microbes. For this research, the characterization that is of interest is the enumeration and identification of the bacterial species. The bacterial species of interest are the iron-reducing bacteria. Additionally, the most common soil bacteria were identified.

2.3.1 Microbial Enumeration

The most common and inexpensive methodology for the quantification or enumeration of the bacterial population within a soil sample is the plate count method. This method uses a general substrate or agar to propagate identifiable colony forming units (CFU) of growth. This method is considerably reliable for quantifying bacterial growth in a soil sample. Plate count methods favor faster growing bacteria thereby inhibiting slower growing bacteria. Also, the media used as the carbon source can favor certain species. It is possible to achieve up to approximately 50 to 60% of the bacteria present in soil samples through plate counts[24-25].

Other methods to determine the bacterial population of soil samples include direct microscopy, most-probable-number-procedures, and other microscopy methods. These methods are an estimation of the viable bacterial populations and are dependent of the species in question, media used for growth, and type of soil sample. Rather than estimating the population of the bacterial community, they are also common practices to measure the bacterial activity or biomass. By measuring activities such as respiration, it is possible to characterize the bacterial community through measuring gas production that is released by the bacteria.

2.3.2 Microbial Identification

There are several identification techniques for the determination of whether a bacterial isolate should be placed within a group of organisms known to fit within some classification scheme. Many different criteria are employed for bacterial identification. The techniques and tests that are suitable for the identification, however, depend on the type of bacteria or microorganisms that are being identified and how detailed of a classification is required. The common techniques used to characterize soil bacteria of interest include morphological identification, differential staining, differential media screening, serological methods, flow cytometry, phage typing, protein analysis, and comparisons of nucleotide sequences, etc.

Another technique that is commonly cited in current literature is PCR, or polymerase chain reaction, a method of amplifying specific regions of DNA found in an organisms genome by selectively catalyzing the replication of those regions. The replicated region is then compared to a database of organisms whose DNA has already characterized. This method was selected for this research due to the reliability of its results which has been verified in the current literature.

2.4 Microbial Mediated Iron Release

The microbial mediated iron reaction processes compete with chemical processes, promoting and potentially dominating iron release near landfills where landfill leachate interacts with the soil since organic matter can serve as energy and carbon sources for microbial activities. Iron-reducing bacteria and sulphate-reducing bacteria coexist in the subsurface and compete for growth in most cases. Some of the sulfate and iron reducing/oxidizing bacteria are listed in Table 3[13]. These bacteria exist under a specific range of soil parameters. Typically, the bacteria exist at approximately 30°C under acidic conditions. Most can exist in soil matrices as well as in water.

Table 3. Bacterial Species involved in Corrosion Processes[13]

Organism	Oxygen Requirement	Inorganic Components	Metabolic End-Products	Habitat	Optimal Range Temperature	pH
Sulfate Reducing *Desulfovibrio desulfuricans*	Anaerobic	Sulfate, thiosulfate	Hydrogen sulfide	Water, soil, mud oil reservoir	25-30	6-7.5
Sulfer Oxidizing *Thiobacillus thiooxidans*	Aerobic	Sulfur, thiosulfate	Sulfuric acid	Soil, water	28-30	2-4
Thiosulfate Oxidizing *Thiobacillus thioparus*	Aerobic	Thiosulfate, sulfur	Sulfur, sulfuric acid	Soil, water, mud sewage	28-30	7
Iron Bacteria *Crenothrix, Leptothrix, Gallionella*	Aerobic	Iron, manganese	Ferric or manganese oxides	Water	25	8
Iron Reducing *Shewanella oneidensis*	Facultative	Ferric Iron	Ferrous iron	Soil, mud, water	28-30	4-7
Nitrate Reducing *Thiobacillus dentrificans*	Facultative	Thiosulfate, sulfur, sulfide	Sulfate	Soil, water, mud peat	30	7-9
Hydrogen Utilizing *Hydrogenomonas*	Microaerophilic	Hydrogen	Water	Soil, water	28-30	7

The iron-reducing bacteria are also capable of causing the release of other contaminants that may be attached to soil minerals[26-27]. The Savannah River Site in South Carolina is well documented to contain many contaminants. It has been suggested that the increase in soluble iron may cause the release of many toxic metals and radionuclides which are frequently attached to Fe (III) oxides[28]. Aside from the increase of iron in groundwater, it is also important to characterize the microbial community to increase the knowledge of other contaminants release triggered by iron reducing bacteria.

3. Materials and Methods

3.1 Soil Sampling

The iron sources of the observed elevated iron observations are suspected to be from the Northwest Florida iron rich soils. Samples for this research were collected from sixteen different landfill sites in fifteen counties in Northwest Florida (Figure 5). The landfill location, name, type of landfill and current status are listed in Table 4.

Figure 5. Northwest Florida Counties

Table 4. Landfills in Northwest Florida Sampled in this Research

County	Landfill Name	Landfill Type*	Current Status
Bay	Steelfield	I & III	Active
Calhoun	Calhoun County	I	Closed
Escambia	Perdido	III	Active
Franklin	Franklin County	I & III	Active
Gadsden	Quincy-Byrd	I & III	Active -Class I closed
Gulf	Five Points	III	Active – NL
Holmes	Holmes County	I	Closed
Jackson	Springhill	I	Active
Leon	US 27 South	I & III	Active
Liberty	Liberty County	II & III	Active -Class II closed
Okaloosa	Baker	I	Closed
Santa Rosa	Santa Rosa Central	I	Active
Santa Rosa	Santa Rosa Holley	I	Closed
Wakulla	Lower Bridge	I & III	Active -Class I closed
Walton	Walton County Central	I & III	Active
Washington	Mudhill	I	Closed

* Class I & II waste = solid waste which is not a hazardous waste that can be disposed in a lined landfill; Class III wastes = yard trash, C&D debris, processed tires, asbestos, carpet, cardboard, paper, glass, plastic, furniture other than appliances, or other materials approved by DEP and not expected to produce Leachate. NL = No Leachate collection system.

In general, soil samples were collected 1 to 3 feet below the surface. The soil samples were immediately placed in either a Ziploc bag or a Styrofoam cooler and sealed. All samples were delivered to the laboratory on the day of collection and placed in refrigeration to maintain bacterial vitality. During transportation, the soil samples were stored in temperature-controlled containers. The soil classification based on soil surveys using the three common techniques, AASHTO, USCS, and USDA Soil Name is listed in Table 5.

Table 5. Soil Classification and Soil Identification*

County	Sample Depth	AASHTO	USCS	USDA Soil Name
Bay	1 ft	A-3	SP-SM	Lakeland sand **[11]**
Calhoun	2 ft	A-2; A-3	PT, SP-SM	Dorovan Pimlico Rutlege **[4]**
Escambia	1.5-2 ft	NA	NA	Arents Urbanland **[47]**
Franklin	2 ft	A-3	SP	Resota fine sand **[34]**
Gadsden	1.5-2 ft	A-7-5,-6	CL	Susquehann Sawyer Complex **[42]**
Gulf	2.5 ft	A-3	SP-SM	Mandarin fine sand **[38]**
Holmes	2 ft	A-6-2	SC	Stilson loamy sand **[40]**
Jackson	1.5-2 ft	A-6-3	SC	Dothan loamy sand **[10]**
Leon	2 ft	A-6; A-4	SC; CL	Orangeburg fine sandy loam **[35]**
Liberty	2 ft	NA	NA	NA
Okaloosa	2 ft	A-6	SM-SC	Dothan loamy sand **[30]**
Santa Rosa Central	1 ft	A-2-4	SP-SM	Lakeland sand **[46]**
Santa Rosa Holley	2 ft	A-2-4	SM	Troup loamy sand **[46]**
Wakulla	2 ft	NA	NA	Udorthents & Quartzipsamments **[3]**
Walton	2.5 ft	A-2-4; A-3	SP-SM	Lakeland sand **[29]**
Washington	2 ft	A-2; A-3	SP-SM	Lakeland coarse sand **[14]**

* AASHTO – American Association of State Highway and Transportation Officials
USCS – Unified Soil Classification System USDA – United States Department of Agriculture

3.1.1 Sieve Analysis

A sieve analysis was performed for all soil samples. The method employed for the sieve was a typical methodology used for engineering applications. 600 to 700 gram *in-situ* sample from each location was weighed for the sieve test. All samples were placed in an oven for approximately 10 days at 30°C. After samples were determined to be thoroughly dry, their dry weight was recorded. Soil samples were broken up with a pestle and mortar, after which the dry weight of the samples was determined. A stack of sieves were arranged from top to bottom in the respective order of decreasing sieve size openings, i.e. sieve number 4, 10, 20, 40, 60, 100, 140, 200, and Pan. The dry soil sample was placed on sieve number 4 and then closed and placed on a sieve shaker for 15 minutes. The total weight of soil retained on each sieve and the pan was then weighed. If a considerable amount of soil was retained on

the sieve number 200, a wash through was performed on that soil to determine if any additional soil could pass through to the pan. The following calculations were performed on the data obtained from the sieve test.

1. Percent mass soil retained on each sieve or n^{th} sieve (R_n)

$$\frac{\text{mass retained}}{\text{total mass}} \text{ X } 100 = R_n$$

2. Cumulative percent of soil retained on each sieve or n^{th} sieve (ΣR_n)

$$\sum_{i=1}^{i=n} R_n$$

3. Percent fines for each sieve

$$100 - \sum_{i=1}^{i=n} R_n$$

From these calculations, a grain size distribution plot was developed for each soil sample. Grain size was plotted on the log scale and percent fines was plotted on the natural scale. These plots were used for comparison with plate count data to investigate any correlation between grain size distribution and colony forming units.

3.1.2 Water Content

The water content for all sixteen soil samples was determined based on the sieve analysis described above in section 3.1.1. The soil samples were weighed pre-drying and post-drying. The difference in their weights divided by total dry weight yielded the water content, *w*. The water content of the samples is considered to play a very important role in quantifying the total plate counts or colony forming units that were observed from each soil sample.

3.1.3 Soil Iron Content

Following the extraction and oxidation of iron from the soil, soil iron content was then determined using spectrophotometric analysis techniques. In order for the substance to absorb certain wavelengths it is necessary for the given species to be colored. Some metals, such as iron, form highly colored complexes when react with the thiocyanate ion.

$$Fe^{3+}(aq) + 6SCN^{-}(aq) = [Fe(SCN)_6]^{3-}(aq) \qquad (4)$$

Because the thiocyanate complex is colored red, it will absorb at 447nm on the absorption spectrum.

3.2 Plate Counts

For enumeration of aerobic bacterial growth present in the soil samples, the plate count method was employed. A general nutrient agar was used as the growth medium. Sterile techniques were used throughout the entire process. For each soil sample collected, one gram of soil was diluted in sterilized tap water to obtain a concentration of bacteria that

was countable on the plates (Figure 6). Samples were vigorously mixed during dilution to assist in dislodging the bacteria from the soil particles. A total of 100 µl of diluted soil suspension was plated on three plates per soil sample. Sterilized water was spread on an agar plate to serve as the control.

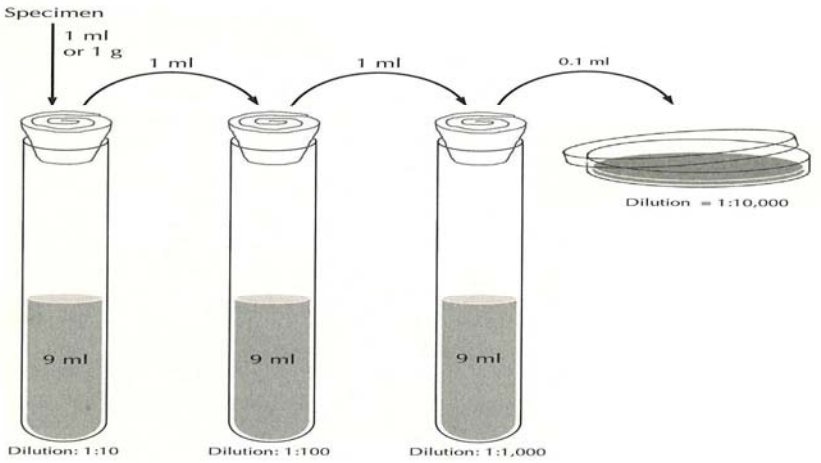

Figure 6. Plate Count Dilution

3.3 Aerobic Microbial Culture Cultivation and Identification

Aerobic microbial species were cultured using the following media and apparatus (Figure 7). The growth bottles received constant air supply for approximately two weeks. From these growth chambers, material was withdrew for identification both through morphology and PCR analysis. For the morphology identification, both mixed cultures and pure cultures were fixed on slides by heating and viewed under a bright field microscope. For the mixed culture, a small amount of soil/water material was withdrawn from the chamber and fixed onto the slide by heating.

Aerobic Species Culturing Media

Salt Solution

1. 10 grams $MgSO_4 \cdot 7H_2O$
2. 1.0 grams $MnCl_2 \cdot 4H_2O$
3. 0.4 grams $FeSO_4 \cdot 7H_2O$
4. Add three components above to 1.0 liter distilled water.

Growth Medium

1. Add 5.44 grams KH_2PO_4 and 6 ml salt solution/100 ml of growth medium.
2. Make medium to 1.0-liter total volume.

Aerobic Microbial Cultures

1. 10 grams of soil sample

2. 2.0 grams glucose (carbon source)
3. 100 ml of growth medium
4. Place all components in bottles and supply a constant air supply as seen in Figure 7.

Figure 7. Aerobic Bacterial Culturing

3.4 Anaerobic Species Cultivation and Identifiaction

The same culturing preparation used in the aerobic culturing was also used in the anaerobic culturing, with a few alterations. For anaerobic growth, the soil and media mixture were placed in a 750 ml flask and sealed. Approximately 5 ml of potassium hydroxide was placed in the arm tube to serve as the CO_2 entrapping device (Figure 7). This method helps to alleviate pressure in the system and closely mimic real world conditions. The system was allowed approximately one month of incubation before further analysis. For the morphological identification, a small amount of water, soil mixture was removed from the system. To avoid introduction of oxygen to the system, a needle was inserted through the rubber stopper. The same steps were then followed as were done with the aerobic species.

3.5 Polymerase Chain Reaction (PCR) Analysis

One of the more reliable and most cost-effective methodologies for identification of bacterial species is Polymerase Chain Reaction (PCR) analysis. The current literature regards PCR analysis as one of the most reliable means of bacterial species identification. Also, Dr. Reeves from Biology Department at Florida State University was consulted on this manner

and he also suggested employing the PCR analysis for identification of bacterial species. Brenda Bennison at Florida State University developed the methodology used in the PCR analysis[29]. The PCR analysis follows the following procedure:

Figure 8: Anaerobic Bacterial Culturing

1. An isolated pure colony is grown on media. The isolated colony is swiped with an applicator stick and inoculated in 50 µl of 10 mM EDTA in a 1.5 ml microfuge tube.
2. Boil for 5 minutes, vortex, and then centrifuge.
3. Prepared Master Mix that contained all ingredients for the reaction except the enzyme (Taq DNA Polymerase) and the sample DNA. Refer to Table 6: Master Mix Components for PCR Analysis.

Table 6: Master Mix Components for PCR Analysis

Component	**Quantity**
10X Taq Buffer	100 µl
50 mM $MgCl_2$	70 µl
5 mM dNTP mix	10 µl
1^{st} primer	50 µl
2^{nd} primer	50 µl
H_2O	705 µl

There were total of five samples tested, four soil samples and one known bacteria to serve as a control. A total of 500 µl of the Master Mix was used for the five samples with 99 µl per sample. A volume of 2.5 µl of the Taq polymerase was added to the 500 µl. From this, a total of 99 µl was added to separate labeled PCR tubes. A volume of 1 µl of the DNA from the samples prepared from step 2 mentioned above is added to each tube to bring the total

volume to 100 µl. Samples were then placed in a thermocycler and the program REEVES50 was initiated and run.

For analysis of the PCR reaction, a 1% agarose gel was poured. For the gel, 2 µl of the each DNA sample was mixed with 8 µl 2xTBE and 2 µl of gel loading buffer. Along with the samples, a molecular weight standard and a mass ladder were pipette into the wells. The gel was run at ~100 volts for one and a half hours, until the bromphenol blue band migrated at least two-thirds of the total gel length. The gel was then stained with ethidium bromide solution and agitated for 30 minutes. The gel was then rinsed and photographed in the transilluminator.

Upon verification of the PCR reaction by viewing the gel bands, the PCR samples were then purified for analysis by the sequencing laboratory. A QIAGEN QIAquick-spin PCR purification kit was used to purify the samples. After the purification, the samples were then labeled and sent to the FSU sequencing laboratory for amplification of the samples. Once the results were obtained, the sequences were entered into the National Center for Biotechnology Information (NCBI) website and identified based on the strands that have been previously identified.

3.6 Laboratory Iron Reduction Experiments

Laboratory iron reduction experiments were conducted using soil samples collected from landfill sites reacting with the corresponding leachate under chemistry and biology conditions similar to the concerned site in the presence of cultured iron-reducing bacteria (Figure 9). All the experiments were conducted in a sealed glass reaction vessel in the anaerobic chamber to mimic the situations in the subsurface where landfill leachate interacted with the soils. As a control, sampled soils also reacted with simulated storm runoffs. Throughout the course of the experiments, ferrous iron, ferric iron, and pH, etc. were monitored on a daily basis.

Figure 9. Student Conducts Batch Experiments

4. Results

4.1 Soil Characterization

The soil samples were characterized based on sieve analysis. The moisture content and the percent fines were determined from the sieve analysis. For each county, detailed sieve analysis is available in the Appendices. The sieve analysis is summarized in Table 7. All values reported are in grams of sand retained per sieve. All sieve runs were maintained at a low % lost after the test, with Washington County having the highest loss of 1.6%. The total weight that was used for each county varied due to variance in soil water present. A total of 500 grams of dry soil was required for an accurate sieve test. To assure this weight was retained after drying each soil, a total weight well above 500 grams was weighed out.

Table 7. Sieve Analysis of All Soil Samples

	Bay	Calhoun	Escambia	Franklin	Gadsden	Gulf	Holmes	Jackson
Sieve No.	Sand Retained	Sand Retained	Sand Retained	Sand Retained	Sand Retained	Sand Retained	Sand Retained	Sand Retained
4	2.0	1.5	3.2	0.2	15.4	0.4	6.5	10.0
10	10.6	6.8	25.1	1.4	6.8	1.4	21.1	24.5
20	24.6	71.6	194.7	2.8	85.6	1.7	91.1	86.2
40	165.5	251.1	300.5	26.4	358.0	7.2	184.9	145.5
60	326.9	207.8	155.3	367.9	115.3	418.6	149.1	140.0
100	76.4	88.4	50.8	244.4	40.8	217.1	67.2	97.5
140	5.2	23.4	9.7	10.4	13.3	5.4	24.3	40.2
200	1.3	14.5	6.0	1.6	3.7	0.4	11.4	25.2
Pan	1.4	23.7	9.0	8.7	3.4	1.5	15.6	27.1
Total	613.9	688.80	754.30	663.8	642.3	653.70	571.20	596.20
Weight Wet	647.9	827.20	840.70	799.2	668.7	678.60	617.20	638.80
Weight Dry	614.1	692.30	763.00	664.1	644.6	655.00	573.00	598.70
Water content	5.50	19.49	10.18	20.34	3.74	3.60	7.71	6.70
% lost	0.03	0.51	1.14	0.05	0.36	0.20	0.31	0.42
% Fines	0.23	3.44	1.19	1.31	0.53	0.23	2.73	4.55
	Leon	Liberty	Okaloosa	SR Central	SR Holley	Wakulla	Walton	Washington
Sieve No.	Sand Retained	Sand Retained	Sand Retained	Sand Retained	Sand Retained	Sand Retained	Sand Retained	Sand Retained
4	0.1	1.3	9.7	0.3	0.0	0.2	0.1	2.9
10	7.4	1.0	22.1	18.5	4.3	9.4	0.9	10.3
20	38.2	9.0	101.7	55.0	21.0	28.0	66.8	33.6
40	89.3	282.3	190.6	139.1	279.0	95.8	328.9	360.1
60	134.0	281.0	159.5	138.7	176.7	95.5	125.9	193.8
100	141.3	52.2	99.5	116.7	60.3	289.0	36.5	90.2
140	70.0	6.5	24.0	31.8	26.8	88.8	7.0	12.9
200	41.2	2.1	17.0	23.7	27.4	17.8	2.6	4.3
Pan	28.7	3.6	26.8	33.5	44.2	9.7	2.2	3.2
Total	550.2	639.0	650.9	557.3	639.70	634.20	570.90	711.3
Weight Wet	612.5	674.0	734.3	628.1	684.00	668.00	603.10	760.9
Weight Dry	550.3	643.1	651.0	559.6	644.40	635.70	572.40	723.2
Water content	11.30	4.80	12.80	12.24	6.15	5.08	5.36	5.21
% lost	0.02	0.64	0.02	0.41	0.73	0.24	0.26	1.65
% Fines	5.22	0.56	4.12	6.01	6.91	1.53	0.39	0.45

Franklin County and Calhoun County had the highest water content of 20%, and 19.5%, respectively. Gadsden and Gulf were determined to have the lowest water content, both at approximately 3.5%. The two Santa Rosa County samples had the highest percent fines, 6.9% for the Holley landfill, and 6% for the Central landfill. Gulf and Bay county were determined to have the lowest percent fines, both at 0.23%. Water content and percent fines were plotted for all the samples to derive any correlation (Figure 10). The plot shows a direct correlation between percent fines and water content. The water content increased with the increase of percentage of finer particles. This is due to the increase in surface area available for the water molecules to interact with. The finer particles refer to clay particles which interact with water molecules through surface charges and forces. It is well documented that clay particles have a very large capacity to hold water.

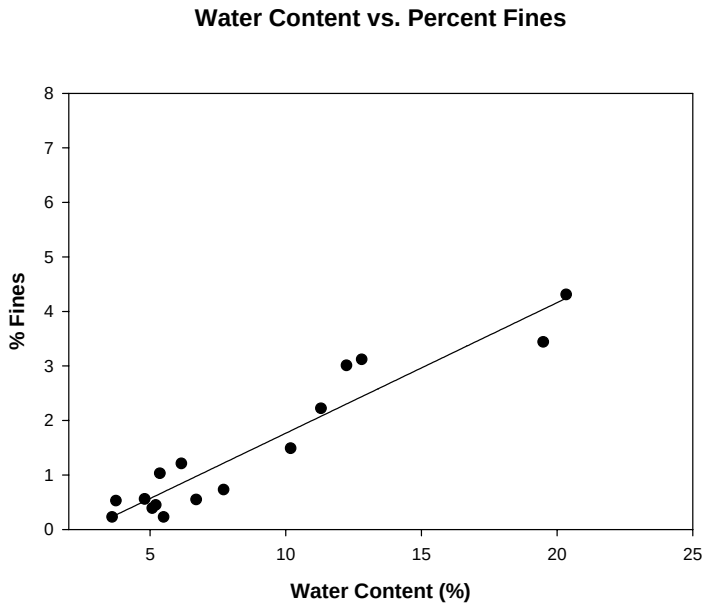

Figure 10. Water Content versus Percent Fines for all Soil Samples

The soil iron content for all the samples is listed in Table 8. Among the samples, Okaloosa has the most iron content, while Jackson County has the least.

4.2 Plate Counts

Plate counts were performed for all sixteen landfill locations from the fifteen counties in Northwest Florida (Table 9). Plates were allowed 48 hours of incubation before counting the colonies. The morphology of the colonies that formed was in general consistent across all samples. There were a few samples that developed a mold or fungus in addition to the bacterial colonies. The morphology of the colonies was circular in shape with variations in size, and color was typically a pale yellow to tan. Some plates would develop a light green pigmentation, which after consulting Dr. Reeves from Biology Department at Florida State University, was determined to be a reaction of *pseudomonas* bacterial species.

Table 8. Iron Content of the Soil Samples

County	Landfill Name	Iron Content (mg/g)
Bay	Steelfield	49.6
Calhoun	Calhoun County	84.1
Franklin	Franklin County	39.4
Gadsden	Quincy-Byrd	65.8
Gulf	Five Points	46.4
Holmes	Holmes County	91.2
Jackson	Springhill	34.0
Leon	US 27 South	43.8
Liberty	Liberty County	68.8
Okaloosa	Baker	119.9
Santa Rosa	Santa Rosa Central	83.2
Santa Rosa	Santa Rosa Holley	94.0
Wakulla	Lower Bridge	67.3
Walton	Walton County Central	90.0
Washington	Mudhill	84.3

Table 9. Plate Count CFU Count Ranked Highest to Lowest

County	CFU
Gadsden	1.97E+06
Liberty	9.50E+05
Leon	9.20E+05
Santa Rosa Holley	7.50E+05
Bay	7.00E+05
Calhoun	6.20E+05
Wakulla	4.60E+05
Holmes	4.20E+05
Washington	3.20E+05
Jackson	3.10E+05
Walton	1.40E+05
Okaloosa	1.20E+05
Escambia	1.00E+05
Gulf	9.00E+04
Franklin	4.00E+04
Santa Rosa Central	3.00E+04

The plate counts show that Gadsden County had the highest CFU of 1.97×10^6. Santa Rosa Central showed the lowest bacterial CFU of 3.00×10^4. The average CFU for all samples was 4.96×10^5. The CFU counts are an average of three replications for each soil sample. The average CFU for the soils falls within the range suggested for a soil with a healthy biological community[30-31].

4.3 Aerobic Species Characterization

The aerobic bacterial species were characterized based on morphology. Only a select representative soil samples were used in the identification using morphology. For the aerobic species, Okaloosa County and Liberty County were selected based on visual appearance of the soil. Okaloosa County (Figure 11) had a very dark red coloration indicating high iron content. Although the red coloration does not definitively indicate high iron content, it is usually indicative of a soil with a high iron and clay content. Liberty County was selected based on its dark brown and rich organic appearance.

Figure 11. Okaloosa County Soil Sample

A small amount of soil/water from each growth chamber was smeared on a slide and stained using Gram's Stain. These slides show a mixed culture of bacterial growth. Additionally, for Okaloosa, a pure culture was obtained from growth on an agar plate and also smeared and stained. Images were collected from slides using the FSU imaging laboratory (~500 × magnification). The species was further identified by PCR analysis and those results are discussed in section 4.5 PCR Analysis Results.

From Okaloosa County, two aerobic strains of *Bacillus* were positively identified. The gram positive bacteria *Bacillus cereus* (Figure 12) and *Bacillus subtilis* (Figure 13) were identified from pure cultures. These two *Bacillus* bacteria are well documented soil bacteria and are common in the depth zone where the samples were taken. Also, a culture from Franklin County was identified as *Bacillus thuringiensis*.

The remainder of the bacteria that were identified cannot be 100% accurately identified to genus and species name without DNA identification. Therefore, based on morphology, bacteria can be categorized in terms of class of species. The mixed culture bacteria were obtained from the growth chambers and placed on the slide and stained with Gram's stain. Two dominant bacteria observed in the Okaloosa sample are identified in Figures in 15 and 16. Figure 15 was a dominant species throughout the sample and had a head and tail morphology. Figure 16 showed a long rod-like bacteria that were also a

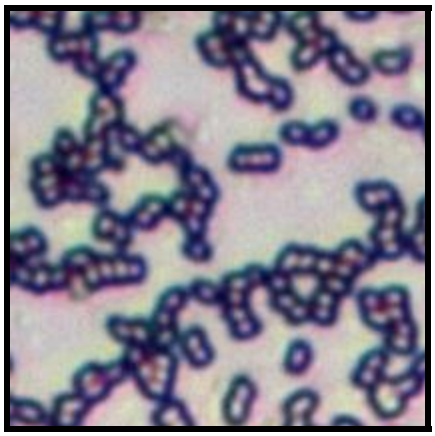

Figure 12. Okaloosa Co. Aerobic Sample
Bacillus cereus

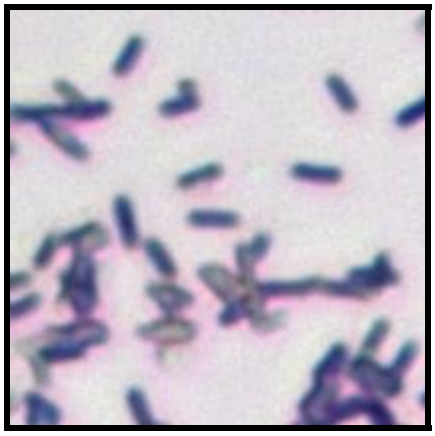

Figure 13. Okaloosa Co. Aerobic Sample
Bacillus subtilis

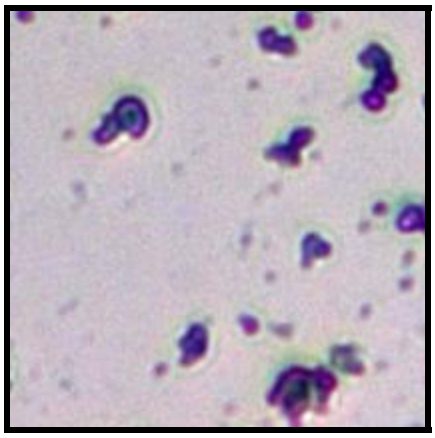

Figure 14. Liberty Co. Aerobic Sample
Unknown *Cocci* Bacteria

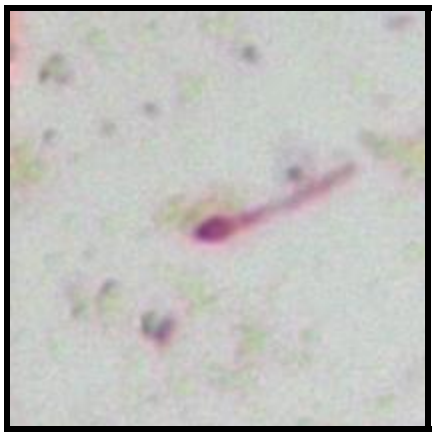

Figure 15. Okaloosa Co. Mixed Culture
Unknown Pseudomonal-like Bacteria

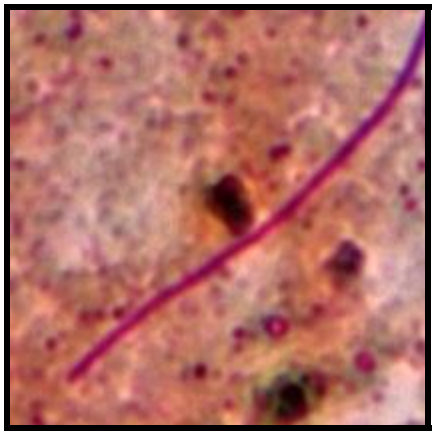

Figure 16. Okaloosa Co. Mixed Culture
Unknown Bacilli Bacteria

dominant species within the Okaloosa samples. The sample from Liberty County (Figure 14) contained a cocci-shaped bacteria that was the dominant species throughout the sample. Samples from other locations, such as Franklin County, Gadsden County, and Walton County were also viewed and the results showed a variation of the above mentioned bacteria. It was therefore assumed that these bacteria would be a clear representative group from all other samples. These bacteria listed in the figures are further analyzed in the Discussion section.

4.4 Anaerobic Species Characterization

The same methodology used for the aerobic identification was also used in the anaerobic identification. One pure culture was obtained for the anaerobic identification. From the Okaloosa sample, a strain of *Pseudomonas aeruginosa* (Figure 17) was identified through the PCR reaction (refer to section 4.5 for further discussion).

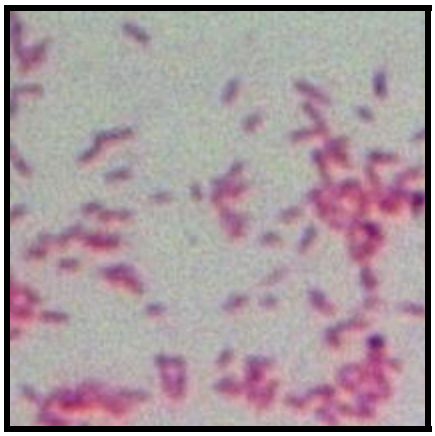

Figure 17. Okaloosa Co. Anaerobic
Pseudomonas aeruginosa

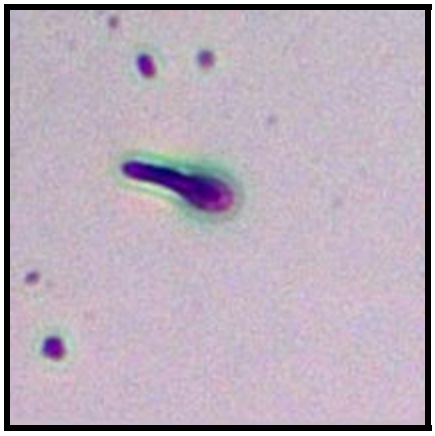

Figure 18. Liberty Co. Anaerobic Mixed Culture
Unknown *Pseudomonal*-like Bacteria

The remainder of the identification was obtained from other counties. In Figure 18 from Liberty County, a *Pseudomonal*-like bacteria similar to the one identified in the aerobic sample from Okaloosa County, was the dominant strain in that sample. From Walton County, a similar bacteria that had the head flagella morphology (Figure 19) was determined to be the dominant species in that sample. Gadsden County mixed culture samples (Figure 20) revealed a bacilli-like bacteria as the dominant species. For all samples, a total of three replications were made of the slides per sample and from those slides the dominant bacteria present was imaged. All images presented here followed this technique.

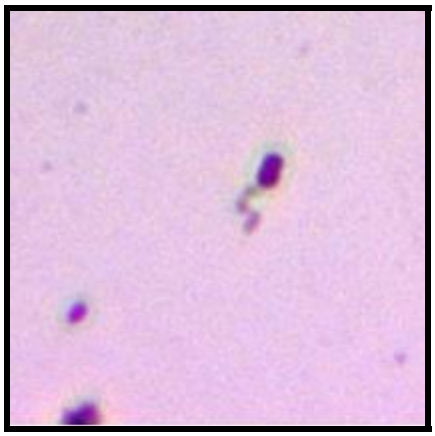

Figure 19. Walton Co. Anaerobic Mixed Culture
Unknown Pseudomonal-like Bacteria

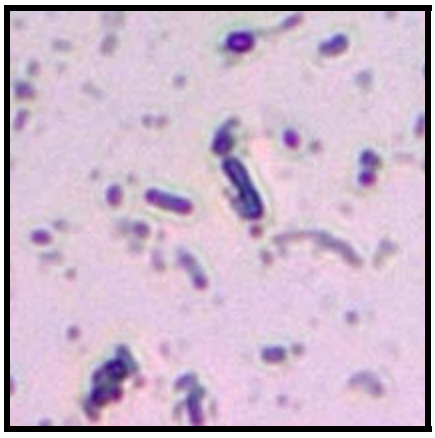

Figure 20. Gadsden Co. Anaerobic Mixed
Culture-Unknown Bacilli Bacteria

4.5 Polymerase Chain Reaction (PCR) Analysis

PCR analysis provided more accurate and reliable results. After going through the initial process as described in the Materials and Methods section, a 1% agarose gel was poured and the DNA samples were pipette into the wells within the gel. Voltage was then applied to the gel and allowed to run for approximately one hour and fifteen minutes. The resulting bands from the samples (Figure 21) were determined to be at the 1400 mark as indicated by the stepped band on the left side of the gel. This process was performed in Dr. Robert H. Reeves' laboratory in the Biology Department at Florida State University. Following the confirmation of the gel, the DNA samples were sent to the FSU DNA Sequencing Laboratory. The samples sent for identification were labeled and their subsequent identification are as follows:

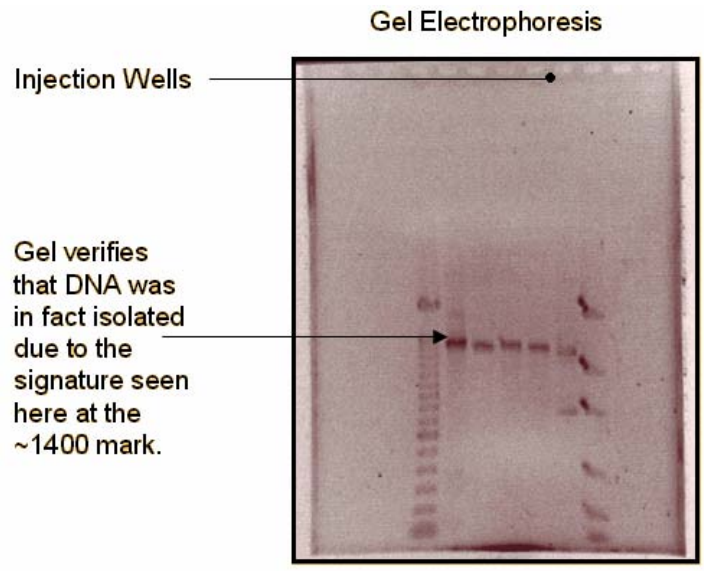

Figure 21. Gel Electrophoresis of DNA Samples

DNA Samples Analyzed by PCR	**Species Identified**
A. Okaloosa County Aerobic Sample	*Bacillus subtilis*
B. Okaloosa County Anaerobic Sample	*Pseudomonas aeruginosa*
C. Okaloosa County Aerobic Sample	*Bacillus cereus, Bacillus thuringiensis*
D. Franklin County Aerobic Sample	*Bacillus thuringiensis, Bacillus cereus*

Only four samples were chosen for the PCR analysis due to inability to culture a pure strain from other samples and also due to scheduling conflicts with the DNA Sequencing Laboratory. The DNA code obtained from the DNA laboratory were Blasted in the NCBI database (http://www.ncbi.nlm.nih.gov/) and the results present the top strain whose DNA code matches the samples code with the highest certainty. Samples C and D list the top strain match first, followed by the second closest match. The other samples results are a 100% match with the strain listed above.

4.6 Iron Reducing Batch Experiments

These experiments were performed in the presence of iron-reducing bacteria that was pre-cultured using the iron rich soils as base consortia (same setup as described in the Methods and Materials section). These results confirmed that iron reducing bacteria were present in the growth chambers that were used to isolate bacteria for identification in the PCR analysis and the morphology identification. Iron reduction results from the soil samples collected from different counties in Northwest Florida are shown in Figure 22.

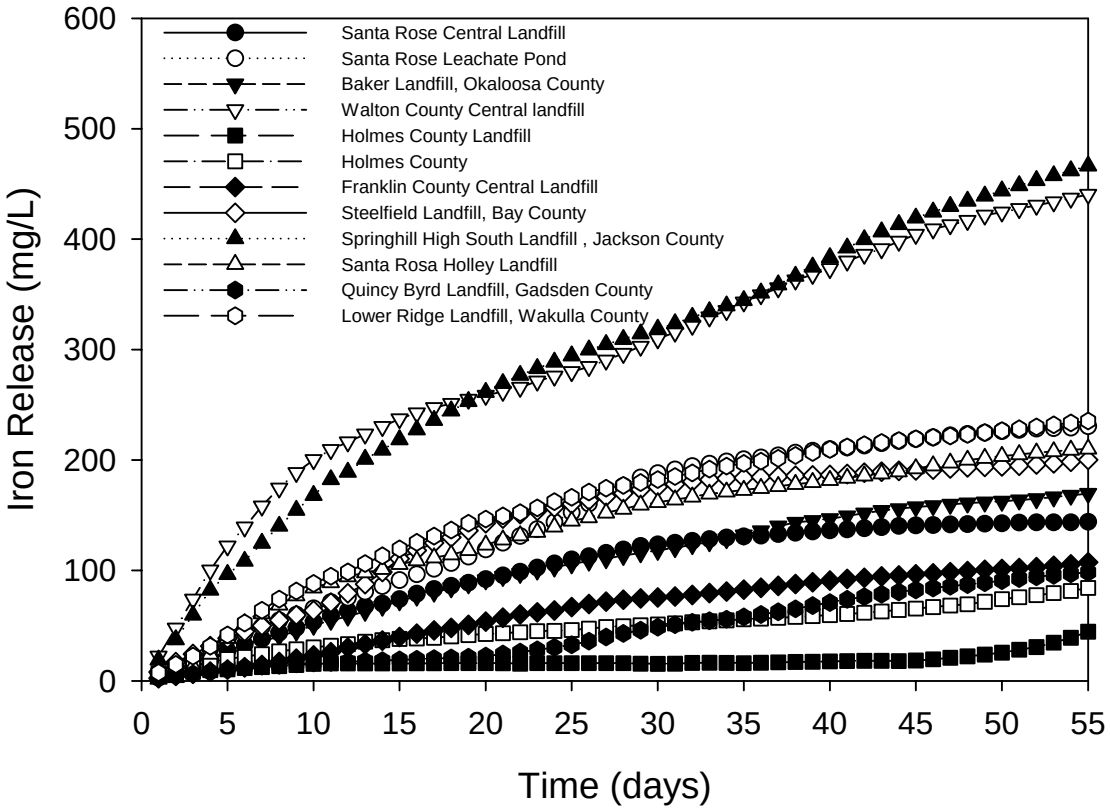

Figure 22. Laboratory Batch Experiments Using Soil Samples Collected from Landfill Sites Reacting with Corresponding Landfill Leachate

Iron release was most pronounced for soil samples collected from Jackson County (Spring Hill South Landfill) reacting with the corresponding landfill leachate. The iron release for this site can be as high as 450 mg/L within 55 days. Following Jackson County is the soil sample collected from Walton County, which produced iron release at a concentration of 420 mg/L within 55 days of reaction. All the other samples produced less than 200 mg/L of iron release. It should be noted that these reactions all occurred in the presence of iron-reducing bacteria pre-cultured using the iron-rich soil as base consortia.

5. Discussion

5.1 Soil Parameters versus CFU Plate Counts

Plate counts, PCR analysis, and morphological identification of the bacterial community provided a meaningful characterization of the soil adjacent to landfills in Northwest Florida. Common bacteria and possible iron-reducing bacteria were identified. The plate counts of the bacteria also provided meaningful data in evaluating the soil bacterial communities. The relationship that exists between the bacterial population and soil parameters of water content, percent fines, and the sieve analysis is further discussed in this section.

The results from the plate counts and the soil analysis were compared to determine any correlation between soil parameters of water content and percent fines and the bacterial counts. It is expected that the increase of water content and increase of percentage of fine particles would favor bacterial growth. Both soil parameters are essential for a healthy bacterial population within the soil matrices. Soil parameters, water content and percent fines are listed in Table 10. The correlation between the three parameters is illustrated in Figure 23.

Table 10. Water Content, Percent Fines, CFU, and Classification of all Soil Samples

Water Content			% Fines			CFU Counts		
1	Franklin	20.34	1	SR Holley	6.91	1	Gadsden	1.97E+06
2	Calhoun	19.49	2	SR Central	6.01	2	Liberty	9.50E+05
3	Okaloosa	12.80	3	Leon	5.22	3	Leon	9.20E+05
4	Santa Rosa	12.24	4	Jackson	4.55	4	SR Holley	7.50E+05
5	Leon	11.30	5	Okaloosa	4.12	5	Bay	7.00E+05
6	Escambia	10.18	6	Calhoun	3.44	6	Calhoun	6.20E+05
7	Holmes	7.71	7	Holmes	2.73	7	Wakulla	4.60E+05
8	Jackson	6.70	8	Wakulla	1.53	8	Holmes	4.20E+05
9	Santa Rosa	6.15	9	Franklin	1.31	9	Washington	3.20E+05
10	Bay	5.50	10	Escambia	1.19	10	Jackson	3.10E+05
11	Walton	5.36	11	Liberty	0.56	11	Walton	1.40E+05
12	Washington	5.21	12	Gadsden	0.53	12	Okaloosa	1.20E+05
13	Wakulla	5.08	13	Washington	0.45	13	Escambia	1.00E+05
14	Liberty	4.80	14	Walton	0.39	14	Gulf	9.00E+04
15	Gadsden	3.74	15	Bay	0.23	15	Franklin	4.00E+04
16	Gulf	3.60	16	Gulf	0.23	16	SR Central	3.00E+04

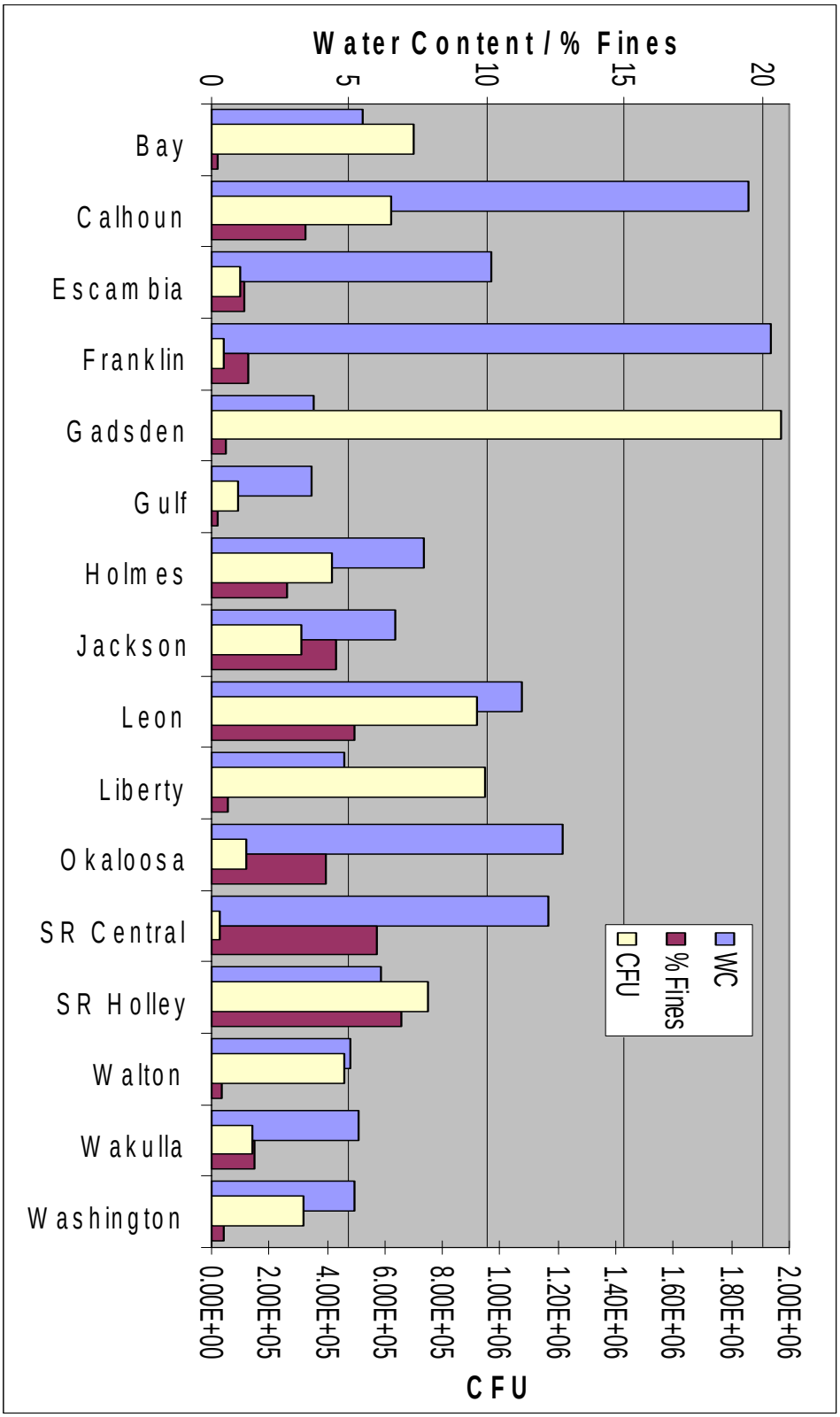

Figure 23. Water Content and Percent Fines versus CFU for all Soil Samples

The majority of the soils were identified as being loamy or fine sands. The sieve analysis of the soils revealed that the majority of the soils did exhibit a poor grading, meaning the soil particles were in general the same size (refer to the Appendices for individual soils sieve analysis). The comparison of the sieve analysis of the three parameters, water content, percent fines, and CFU counts is shown in Figure 24-27. These figures are similar, combining all sixteen soils sieve tests. The difference is the colors given to each soils sieve analysis is based on where that particular soil falls in a ranking of one of the three parameters, i.e., water content, percent fines, and CFU counts. Each figure represents only one of the three parameters. The color scheme indicates ranking of the parameter: yellow indicates a high ranking, blue indicates an average ranking, and red indicates a low ranking.

5.2 Aerobic Species Characterization

The characterization of the aerobic species was limited to a few soil samples, mainly Okaloosa County. This county was chosen due to the red appearance of the soil which suggests a high iron content. The bacteria that were identified, *Bacillus subtilis, Bacillus cereus, and Bacillus thuringiensis*, are all common bacteria that are found at the depth level where all of the soil samples were taken. For the aerobic species, the above mentioned species were identified from pure cultures through PCR analysis. For the morphology identification, the mixed cultures were used for identification. In Figure 28, the two mixed culture bacteria that were the dominant species present were an unknown rod bacterium and an unknown bacterium that resembles a *thiobacillus* as described in Figure 29. This classification is based solely on morphology and the fact that the bacteria were grown in the media conducive to iron reducing conditions. Both of the unknown rod bacteria are gram negative strains which are another strong characteristic for their classification for iron reducing bacteria. For proper identification of these two unknown bacteria, a DNA analysis would have to be performed from a pure culture. The difficulty of obtaining a pure culture of these bacteria lies in the nutrient agar used for culturing. The agar may only provide growth for the faster growing bacteria but not for the slower growing bacteria such as iron reducing bacteria. The selection of a different growth consortium would allow for the desired bacteria's growth.

5.3 Anaerobic Species Characterization

The anaerobic characterization provided additional problems of maintaining bacterial growth under anoxic conditions. While the growth chambers used in this research did provide anoxic conditions, the growth of the bacteria in the agar plated were more difficult to achieve. The one anaerobic species identified through PCR was the *Pseudomonas aeruginosa*, which is a facultative bacterium. A pure culture of the *pseudomonas* was achieved using the plate count method, however again it may have allowed the faster metabolic organisms to produce colonies first, thus out-competing the slower iron reducing bacteria. The PCR analysis bacteria was again obtained from

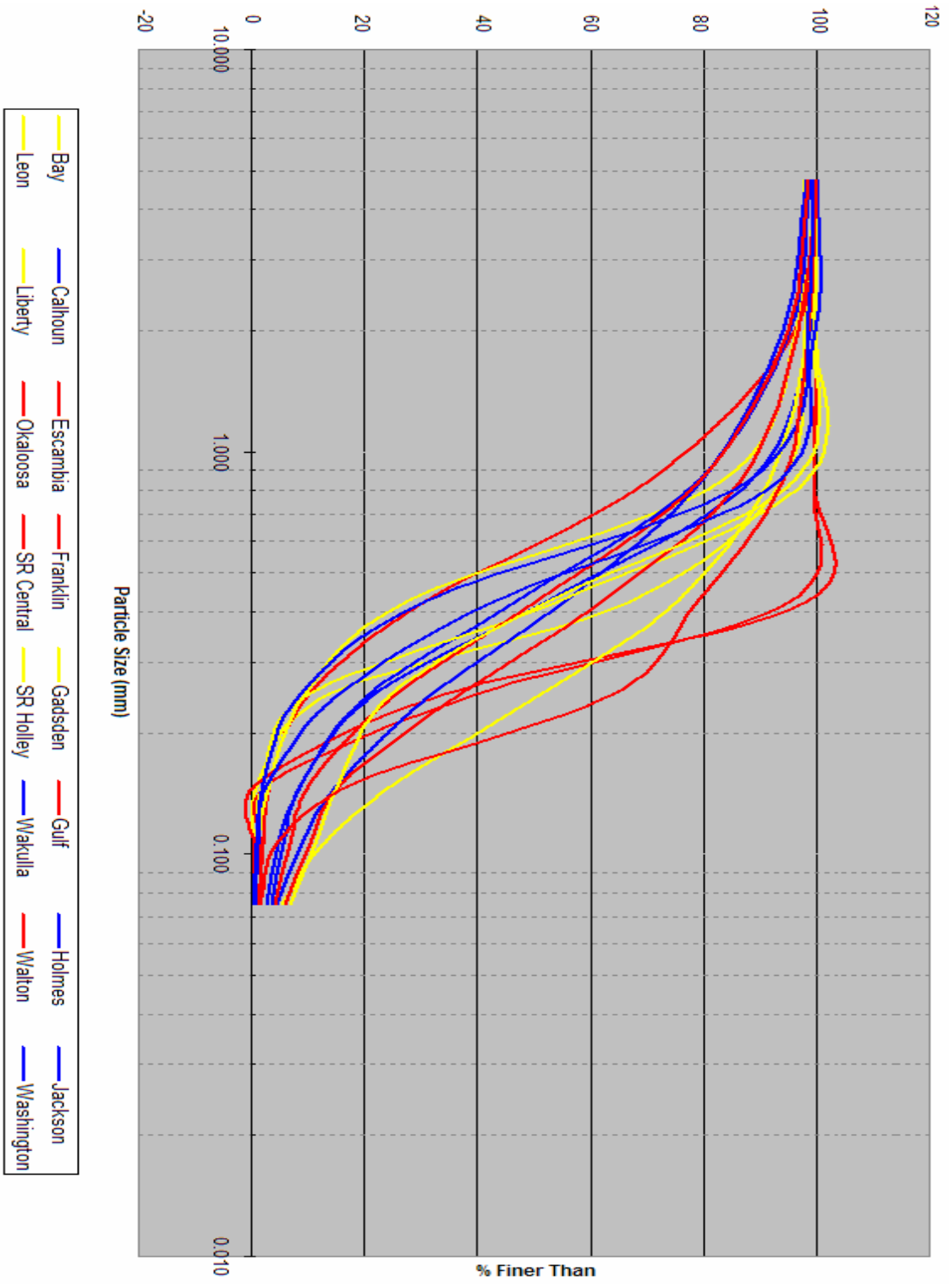

Figure 24. Comparison of Grain Size Distribution and CFU Plate Counts

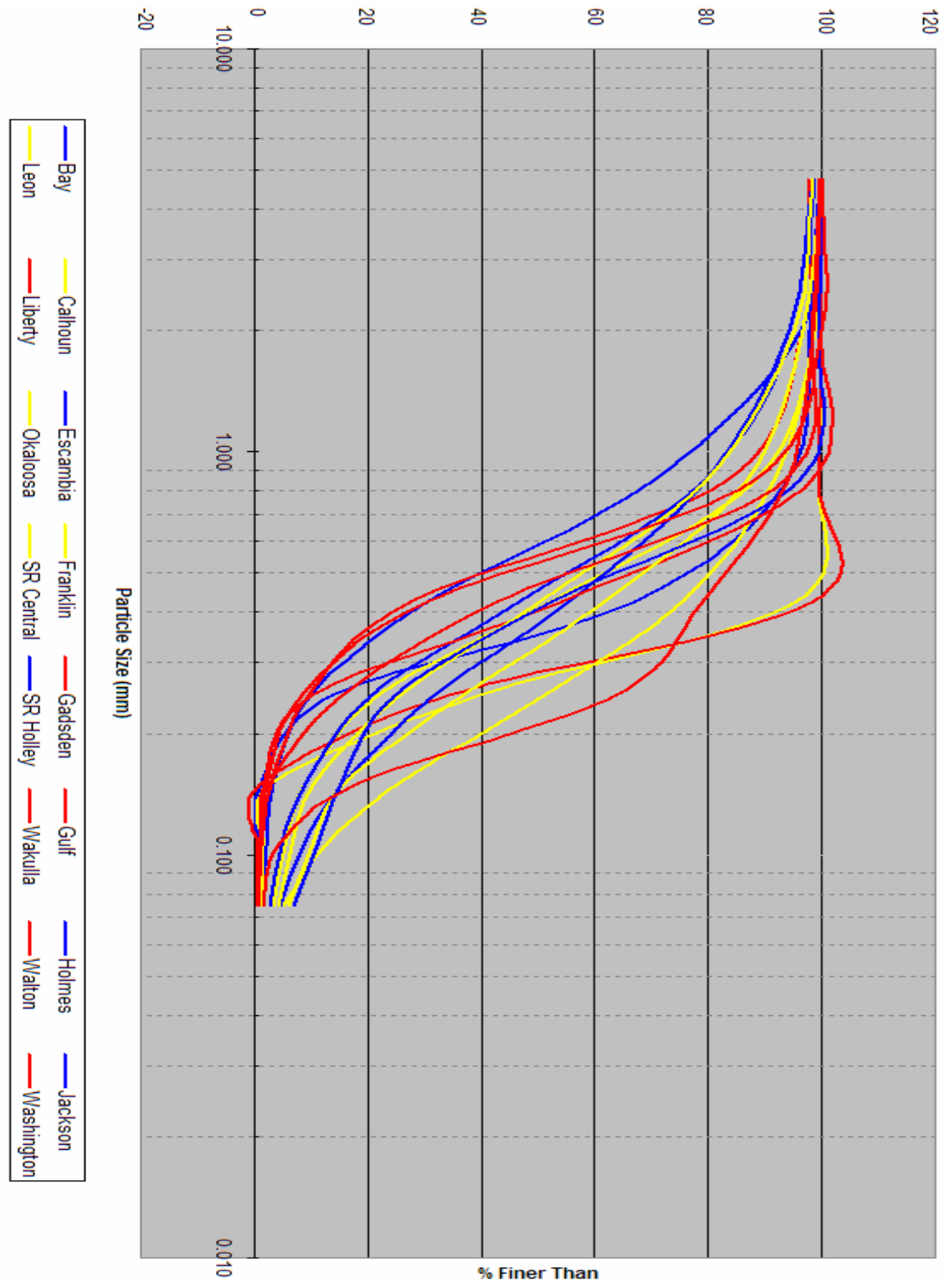

Figure 25.Comparison of Grain Size Distribution and Water Content

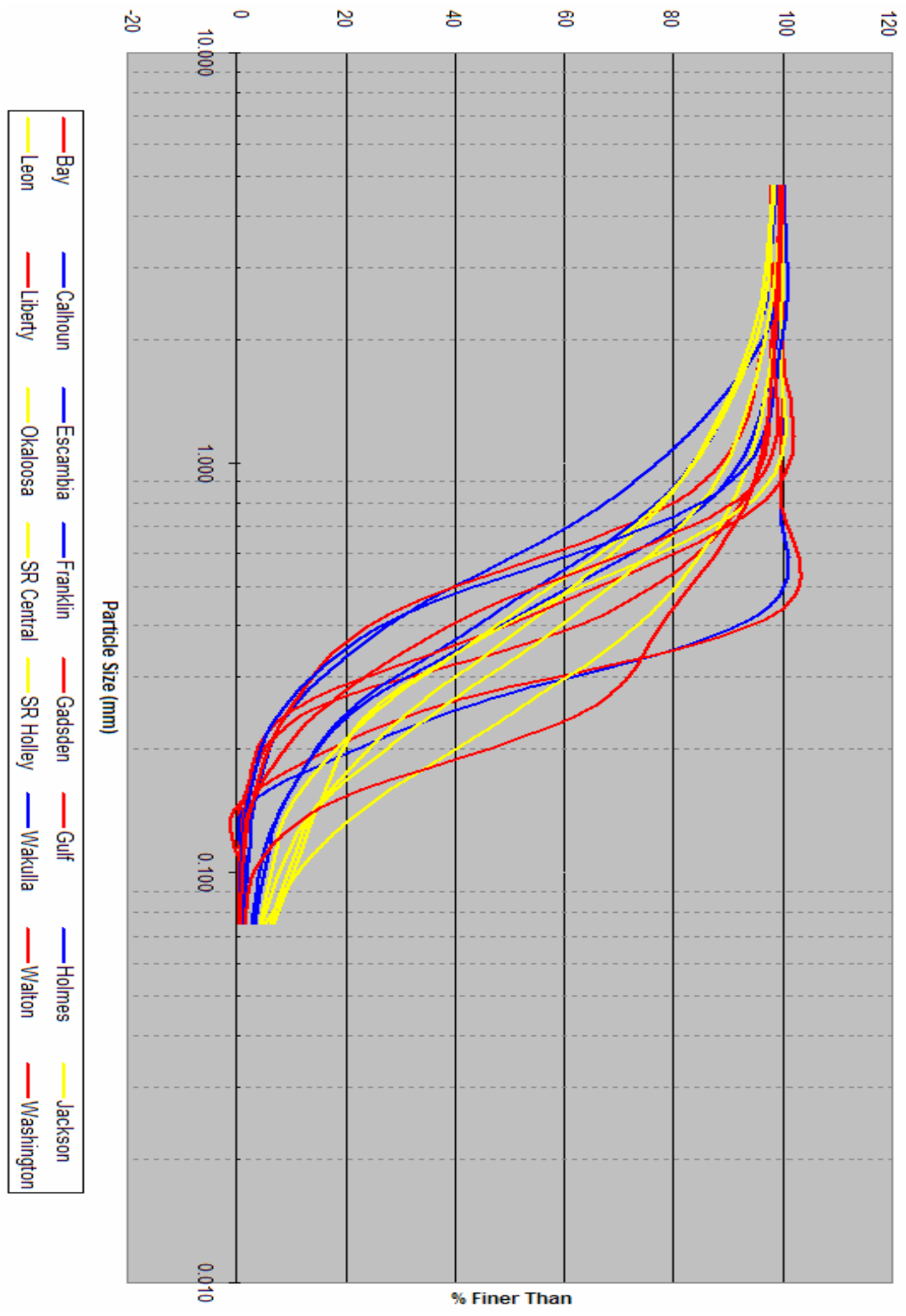

Figure 26. Comparison of Grain Size Distribution and Percent Fines

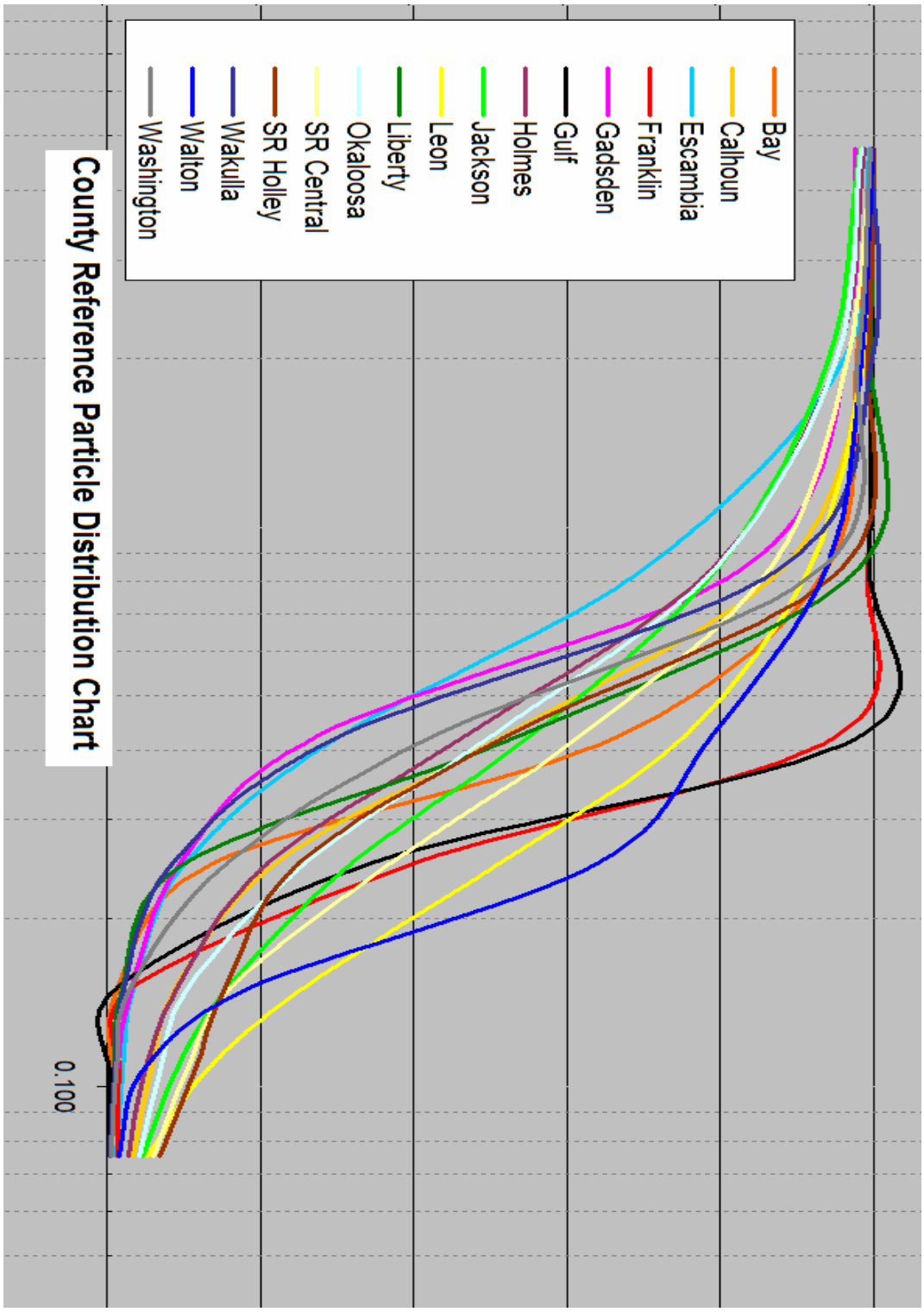

Figure 27. County Reference For Grain/Particle Size Distribution Charts

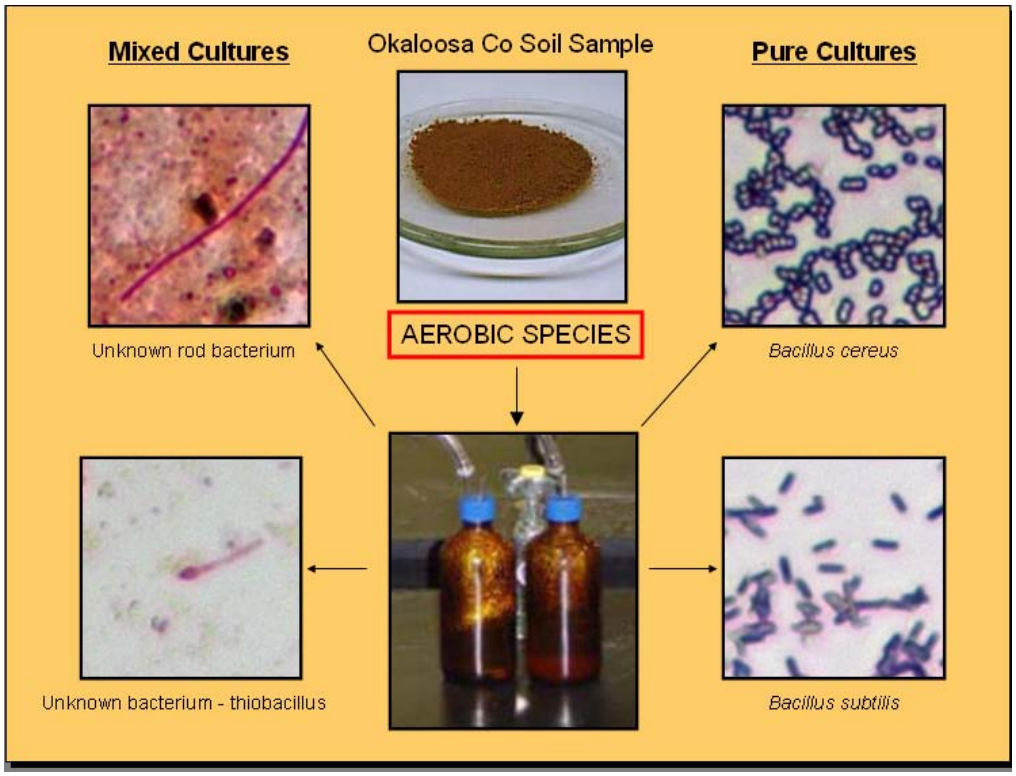

Figure 28. Aerobic Bacterial Species Identification

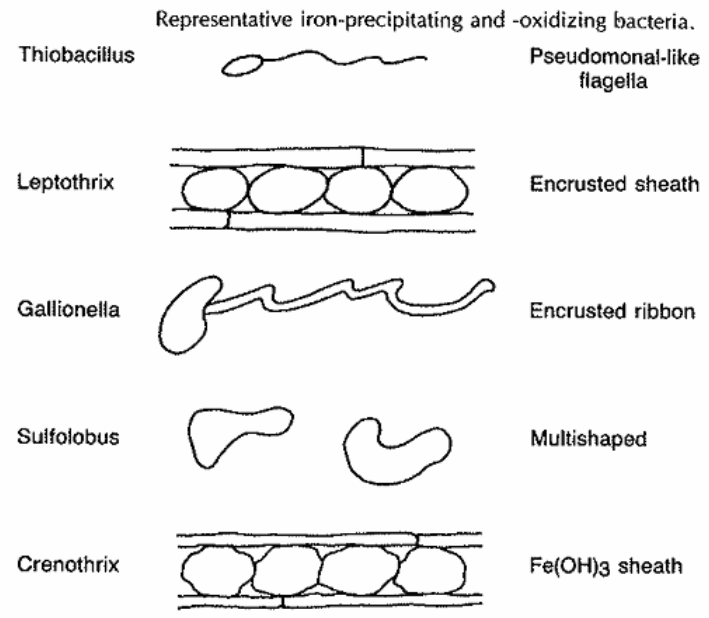

Figure 29. Morphology of Some Iron-Precipitation/Oxidizing Bacteria[13]

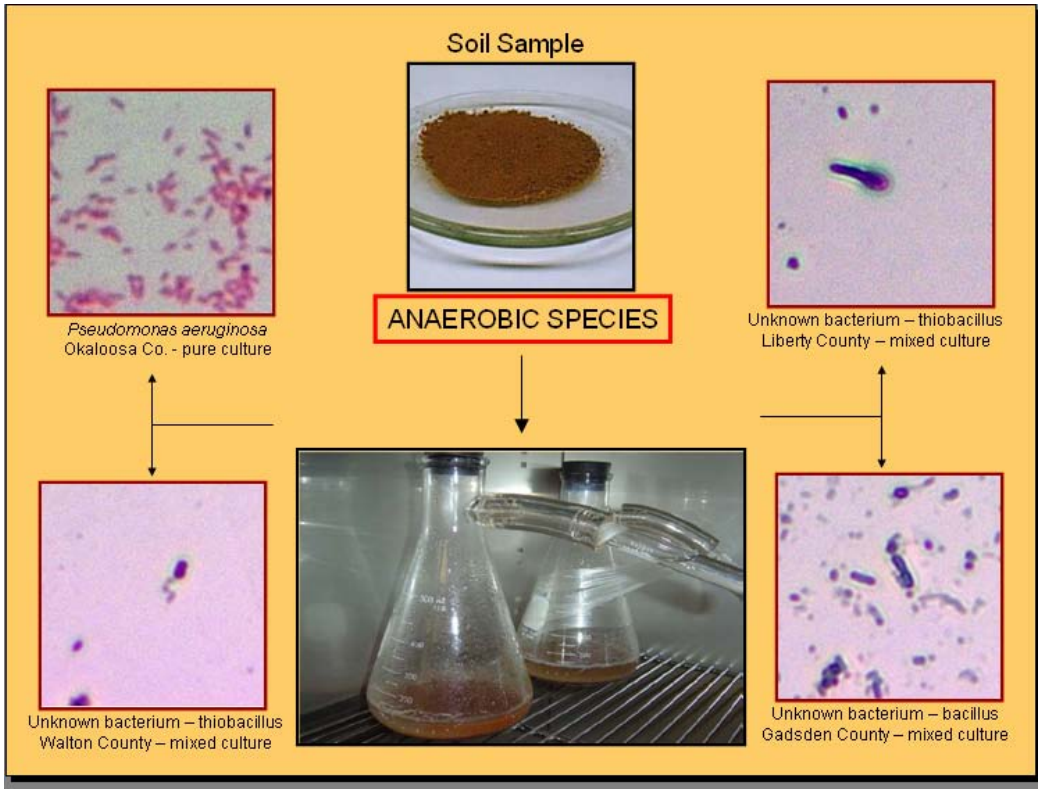

Figure 30. Anaerobic Bacterial Species Identification

Okaloosa County as was the aerobic species. The mixed culture species were obtained from Gadsden, Liberty, and Walton Counties (Figure 30). Liberty and Walton Counties both have similar bacteria that resemble a *thiobacillus*, an iron reducing strain. Gadsden county samples contained a bacillus-like bacterium that was also gram positive. The Walton County bacteria were a gram-negative strain and the Liberty County bacteria were gram-positive strains. The bacteria morphology closely resembles the *thiobacillus* mentioned earlier (Figure 29) but again this is based solely on morphology and the conducive conditions within the anaerobic growth chambers for iron reducing bacteria proliferation.

5.4 Iron Reducing Processes

It is suspected that microbial mediated iron reduction should follow the following reaction:

$$CH_2O + 2Fe_2O_3 + 3H_2O = CO_2 + 4Fe^{2+} + 8OH^- \qquad (5)$$

Consequently, pH of the solutions in the reactors should increase with the proceeding of the reactions. Monitored results were consistent with above prediction except for reactions using the soil samples collected from Holmes County. Instead of increase, pH decreased with the proceeding of the reactions. By monitoring the pH variation, we found that the pH of the leachate was very low for Jackson County and Walton County. The low pH favored the iron reduction process as described above. Consequently, higher iron release was observed for these samples.

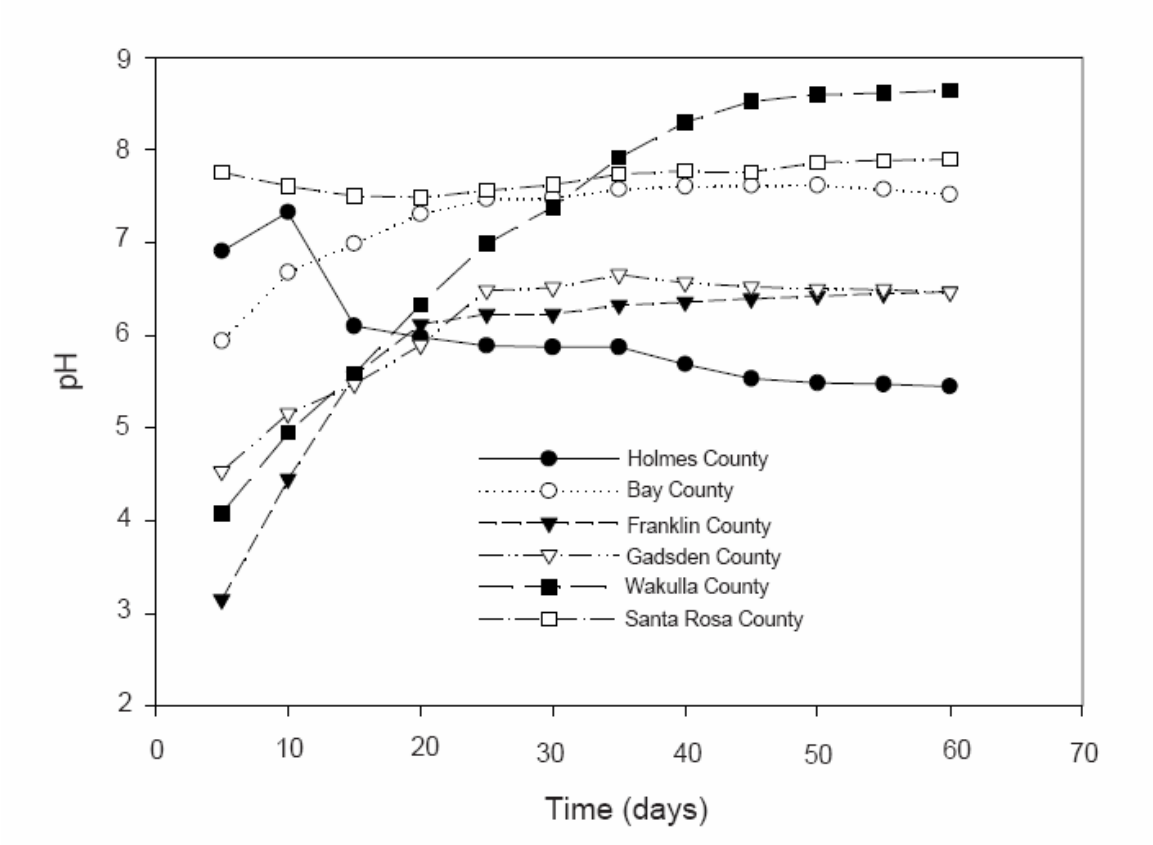

Figure 31. pH Profile of the Batch Reaction

6. Conclusions

The characterization of the aerobic and anaerobic bacterial species present in sixteen different landfills throughout fifteen counties in Northwest Florida identified common soil bacteria present in the soil. A total of four bacteria were identified based on DNA analysis using PCR. Potentially iron reducing bacteria were identified based on morphology.

The sieve analysis of the soil samples showed a tendency for well graded soils to favor bacterial growth. However the higher water content and higher percent fines present in the soil showed a decreased bacterial population. The average bacterial population from the plate count method was 5.0×10^5 CFU, which falls within the normal range of bacterial populations in soil samples.

Based on the laboratory experiments of iron release when landfill leachate reacted with iron rich soil under chemistry and biology conditions of Northwest Florida landfills, ferrous iron was found to be released when landfill leachate contacted with iron rich Norwest Florida soil in the presence of cultured iron reducing bacteria. On the other hand, minimal ferrous iron release was observed in the absence of the cultured iron reducing bacteria. This indicated that iron release by landfill leachate reacting with iron rich soil was a microbial mediated process.

The ultimate goal of this research is to help understand the possible mechanisms for elevated iron in the groundwater near landfills of concern. This research is broadly applicable since landfills are present throughout the world and the monitoring of their effects on groundwater supply is essential for our increased water demands. Characterization of the microbial community as well as the geochemistry, and the soil is an important step in evaluating possible groundwater contamination related to landfills and possible other waste disposal sites.

7. Future Work

We suspect that the iron release also is associated with the microbial sulphate (gypsum) reduction processes within the landfills. The current research is focused on the iron release when landfill leachate contacts with iron rich Northwest Florida soils. On the other hand, the existing zero-valent metallic iron in the landfills can be oxidized by dissolved oxygen or other oxidative substances to ferrous ion and ferric iron. The oxidation or corrosion of zero-valent iron might also be greatly stimulated by microorganisms. The aerobic iron oxidation may not lead to iron release to the groundwater owing to the possible precipitation of ferric iron in the subsurface.

Once oxygen is depleted, sulphate-reducing bacteria become active, which are also responsible for H_2S production:

$$SO_4^{2-} + 2[CH_2O] + H^+ \rightarrow H_2S + H_2O + HCO_3^- + CO_2$$

The sulphate reduction process is frequently linked to the anaerobic iron oxidation process, which should be directly responsible for groundwater iron contamination. Two iron oxidation mechanisms, indirect mechanism and direct mechanism, which may occur simultaneously at different extents, are involved in the sulphate reduction process. The indirect mechanism refers to the oxidation of iron by hydrogen sulfide ($Fe + H_2S = FeS + H_2$), while the direct mechanism is commonly attributed to sulphate-reducing bacteria of *Desulfovibrio* species that can grow on organic substrate or H_2. *Desulfovibrio* species can obtain electrons from metallic iron in a more direct manner than via free hydrogen. This direct mechanism is also supported by a recent discovery of a newly isolated *Methanobacterium*-like archaeon that have a more direct access to electrons from iron than via hydrogen consumption. Once sulphate concentration is low, anaerobic methanogenic bacteria begin to dominate and CH_4 emission becomes obvious.

We plan to conduct further experiments to provide the possible iron release mechanisms that are responsible for the water quality deterioration. Specifically, based on the available iron and sulphate data, we can predict whether the iron release is associated with the microbial mediated sulphate (gypsum) reduction within the landfills. By further analyzing the water chemistry data such as pH, we can advance our knowledge of iron release by screening out the direct or indirect iron release mechanisms.

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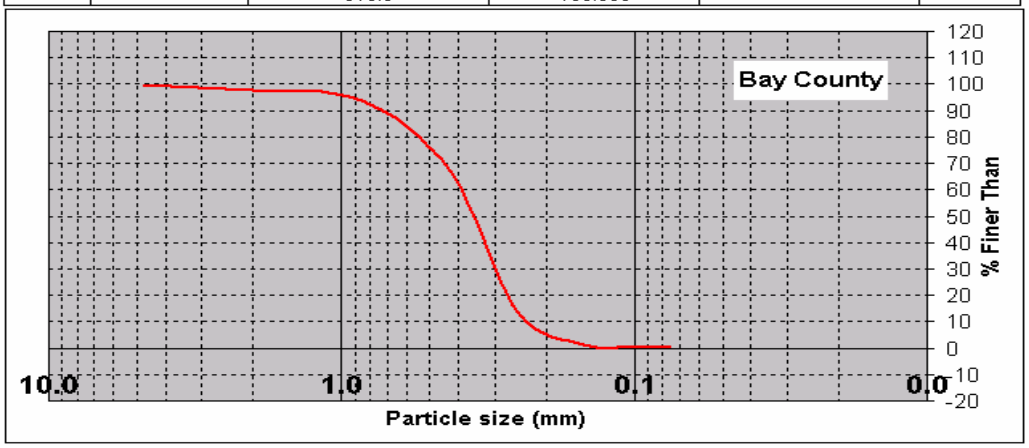
1. Chen, G., Abichou, T. and Chanton, J. Impact of Landfill Leachate on Iron Release from Northwest Florida Iron Rich Soil, Technical Exchange on Iron at Landfills, Destin, FL, Oct. 2006.
2.. Subramaniam, P., Penagonda Srinivasa Ranga, V., and Chen, G. Microbial mediated iron transformation, 83rd ACS Annual Florida Meeting and Exposition, Orlando, FL, May, 2007.

Related publications where the sponsorship from Hinkley Center for Solid and Hazardous Waste Management for this project has been acknowledged:
1. Chen, G., Abichou, T., Tawfiq, K. and Subramaniam, P. K. (2007) Impact of Surface Charge Density on Colloid Deposition in Unsaturated Porous Media, Colloid Surface A., 302, 342-348.

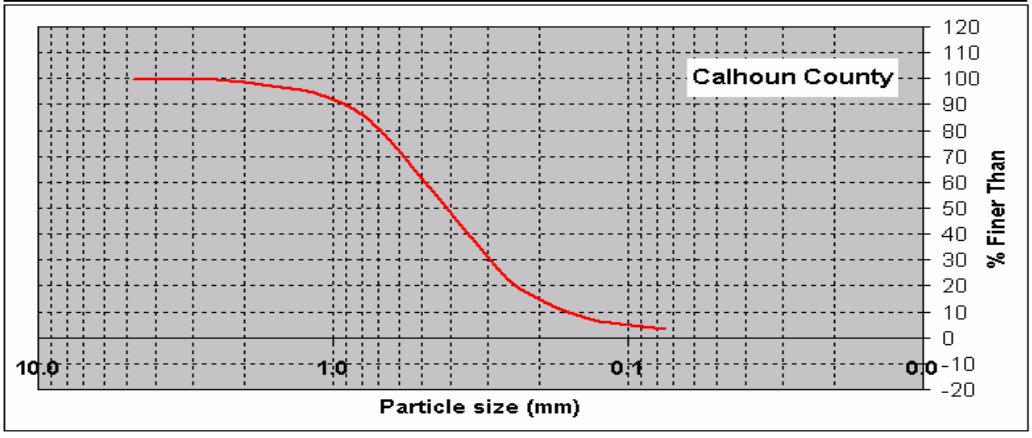
Appendix 1

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	2.0	0.326	0.326	99.67
10	2.000	10.6	1.727	2.052	97.948
20	0.850	24.6	4.007	6.060	93.940
40	0.425	165.5	26.959	33.018	66.982
60	0.250	326.9	53.250	86.268	13.732
100	0.150	76.4	12.445	98.713	1.287
140	0.106	5.2	0.847	99.560	0.440
200	0.075	1.3	0.212	99.772	0.228
Pan	NA	1.4	0.228	100.000	0.000
		613.9	100.000		



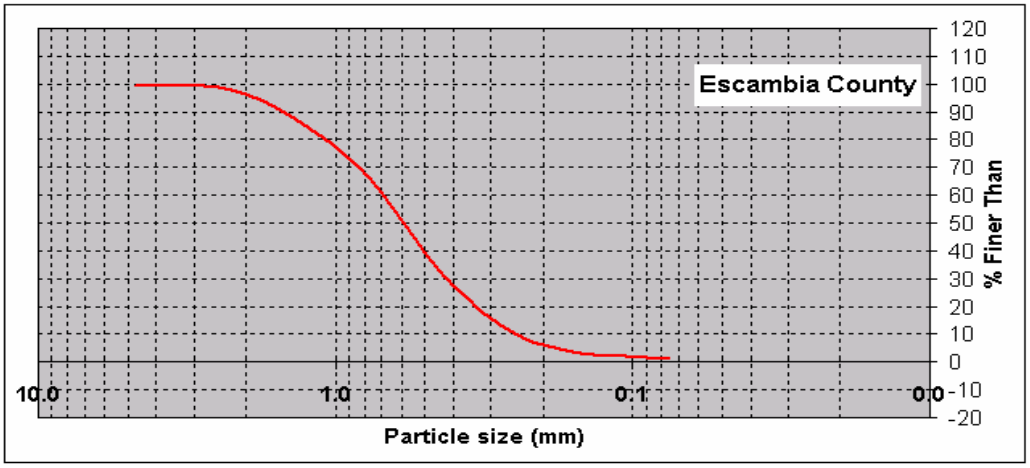
Bay County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	1.5	0.218	0.218	100
10	2.000	6.8	0.987	1.205	98.795
20	0.850	71.6	10.395	11.600	88.400
40	0.425	251.1	36.455	48.055	51.945
60	0.250	207.8	30.168	78.223	21.777
100	0.150	88.4	12.834	91.057	8.943
140	0.106	23.4	3.397	94.454	5.546
200	0.075	14.5	2.105	96.559	3.441
Pan	NA	23.7	3.441	100.000	0.000
		688.8	100.000		



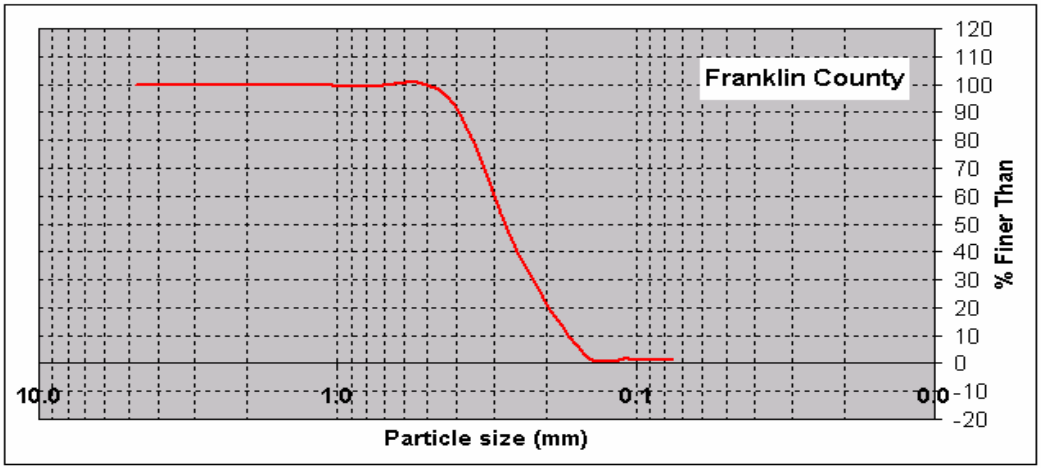
Calhoun County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	3.2	0.424	0.424	100
10	2.000	25.1	3.328	3.752	96.248
20	0.850	194.7	25.812	29.564	70.436
40	0.425	300.5	39.838	69.402	30.598
60	0.250	155.3	20.589	89.991	10.009
100	0.150	50.8	6.735	96.725	3.275
140	0.106	9.7	1.286	98.011	1.989
200	0.075	6.0	0.795	98.807	1.193
Pan	NA	9.0	1.193	100.000	0.000
		754.3	100.000		



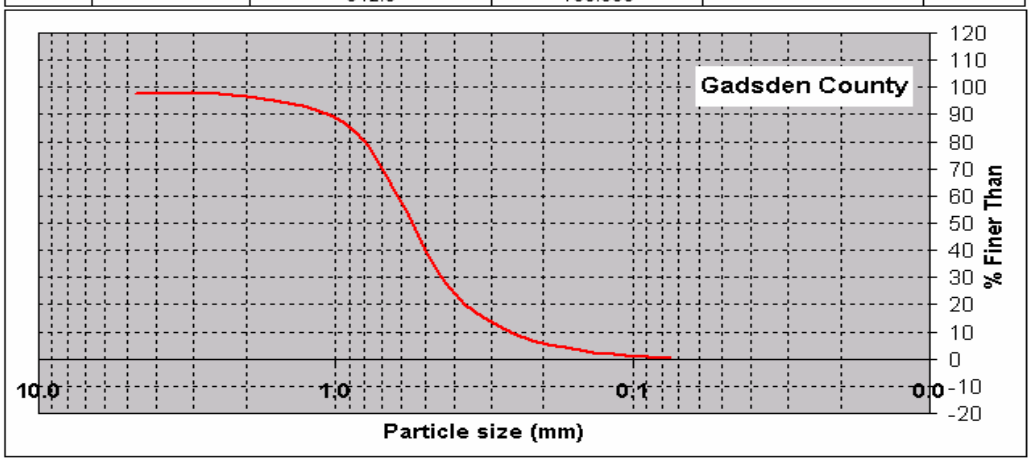
Escambia County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	0.2	0.030	0.030	100
10	2.000	1.4	0.211	0.241	99.759
20	0.850	2.8	0.422	0.663	99.337
40	0.425	26.4	3.977	4.640	95.360
60	0.250	367.9	55.423	60.063	39.937
100	0.150	244.4	36.818	96.882	3.118
140	0.106	10.4	1.567	98.448	1.552
200	0.075	1.6	0.241	98.689	1.311
Pan	NA	8.7	1.311	100.000	0.000
		663.8	100.000		



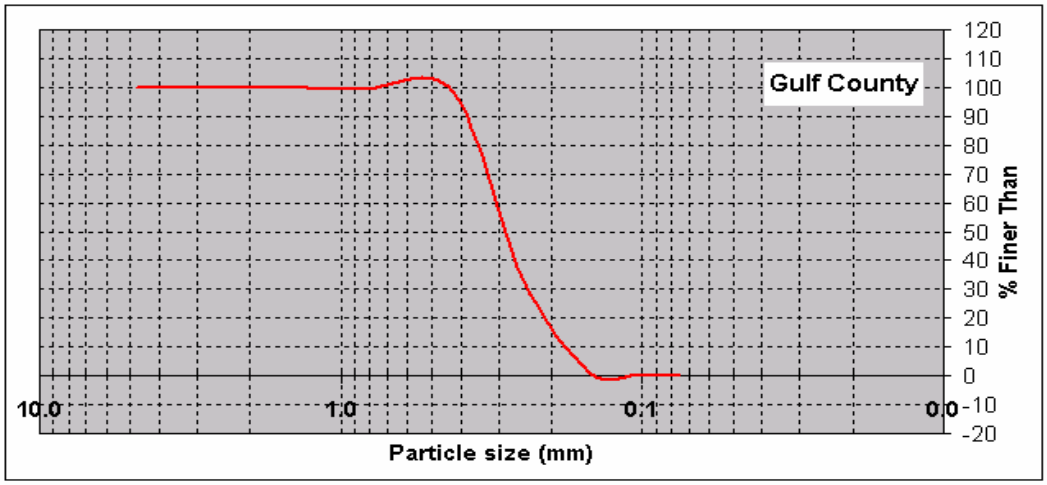
Franklin County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	15.4	2.398	2.398	98
10	2.000	6.8	1.059	3.456	96.544
20	0.850	85.6	13.327	16.783	83.217
40	0.425	358.0	55.737	72.521	27.479
60	0.250	115.3	17.951	90.472	9.528
100	0.150	40.8	6.352	96.824	3.176
140	0.106	13.3	2.071	98.895	1.105
200	0.075	3.7	0.576	99.471	0.529
Pan	NA	3.4	0.529	100.000	0.000
		642.3	100.000		



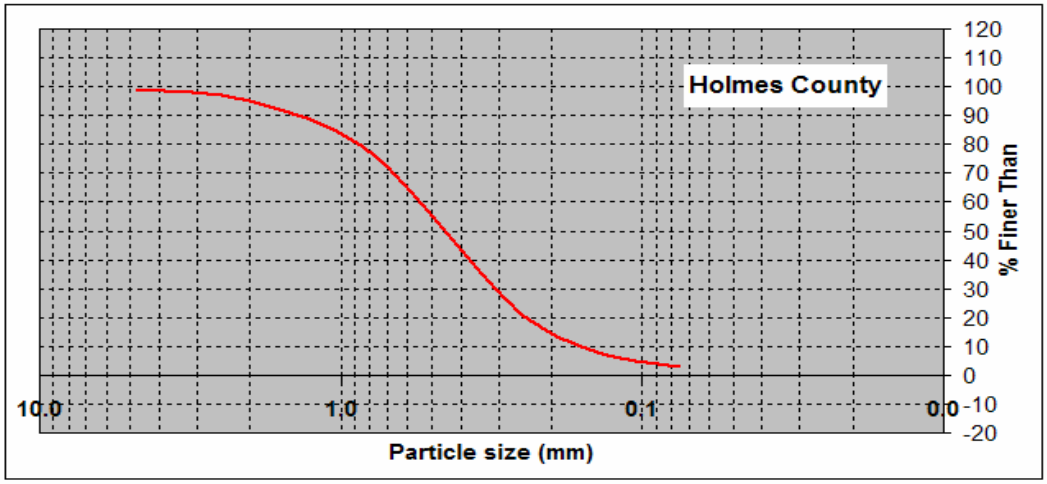
Gadsden County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	0.4	0.061	0.061	100
10	2.000	1.4	0.214	0.275	99.725
20	0.850	1.7	0.260	0.535	99.465
40	0.425	7.2	1.101	1.637	98.363
60	0.250	418.6	64.035	65.672	34.328
100	0.150	217.1	33.211	98.883	1.117
140	0.106	5.4	0.826	99.709	0.291
200	0.075	0.4	0.061	99.771	0.229
Pan	NA	1.5	0.229	100.000	0.000
		653.7	100.000		



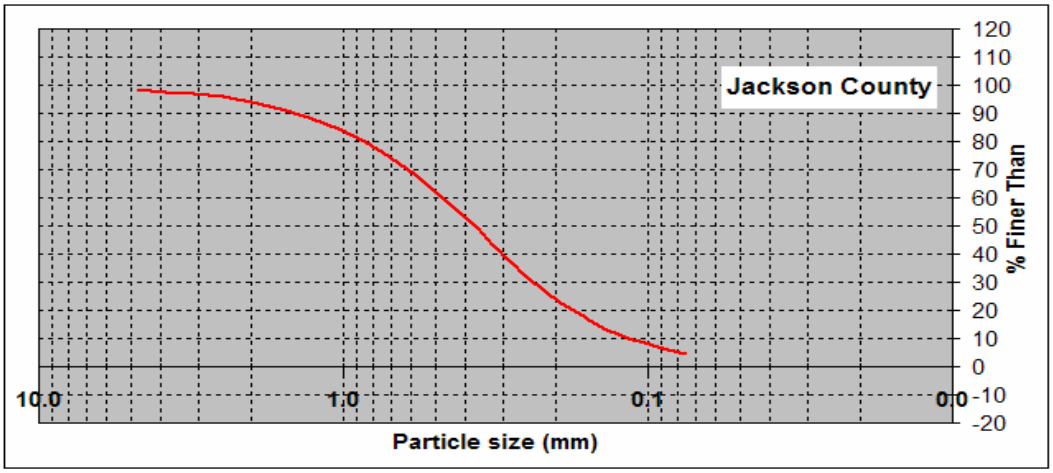
Gulf County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	6.5	1.138	1.138	99
10	2.000	21.1	3.694	4.832	95.168
20	0.850	91.1	15.949	20.781	79.219
40	0.425	184.9	32.370	53.151	46.849
60	0.250	149.1	26.103	79.254	20.746
100	0.150	67.2	11.765	91.019	8.981
140	0.106	24.3	4.254	95.273	4.727
200	0.075	11.4	1.996	97.269	2.731
Pan	NA	15.6	2.731	100.000	0.000
		571.2	100.000		



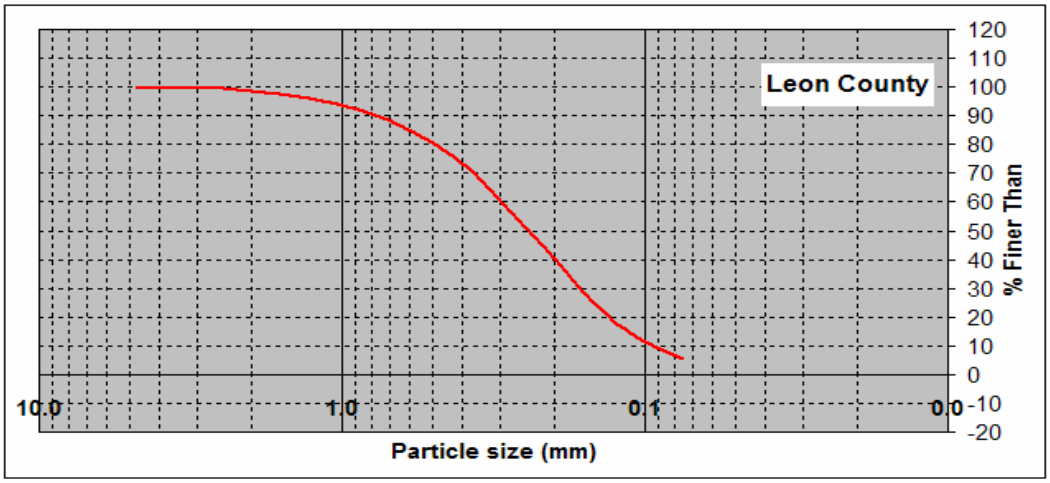
Holmes County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	10.0	1.677	1.677	98
10	2.000	24.5	4.109	5.787	94.213
20	0.850	86.2	14.458	20.245	79.755
40	0.425	145.5	24.405	44.649	55.351
60	0.250	140.0	23.482	68.131	31.869
100	0.150	97.5	16.354	84.485	15.515
140	0.106	40.2	6.743	91.228	8.772
200	0.075	25.2	4.227	95.455	4.545
Pan	NA	27.1	4.545	100.000	0.000
		596.2	100.000		



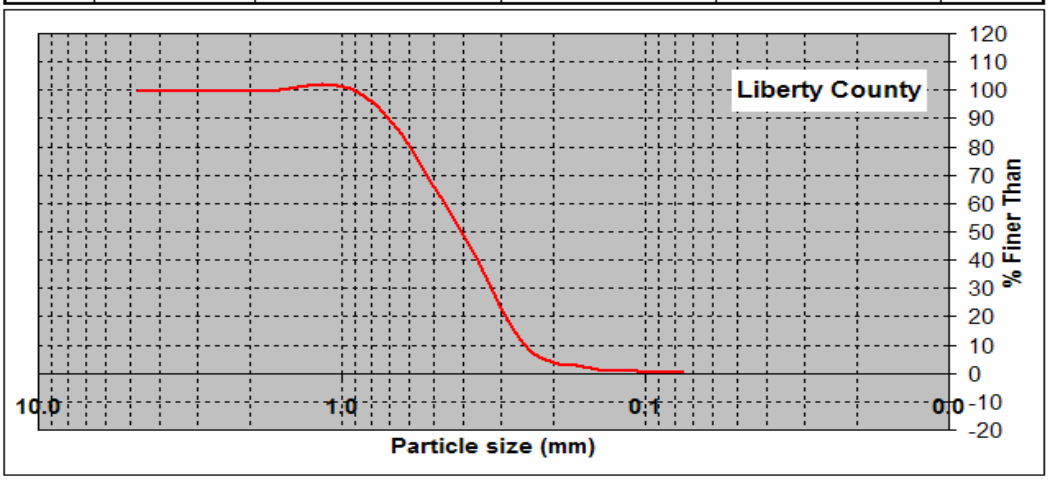
Jackson County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	0.1	0.018	0.018	100
10	2.000	7.4	1.345	1.363	98.637
20	0.850	38.2	6.943	8.306	91.694
40	0.425	89.3	16.230	24.537	75.463
60	0.250	134.0	24.355	48.891	51.109
100	0.150	141.3	25.682	74.573	25.427
140	0.106	70.0	12.723	87.296	12.704
200	0.075	41.2	7.488	94.784	5.216
Pan	NA	28.7	5.216	100.000	0.000
		550.2	100.000		



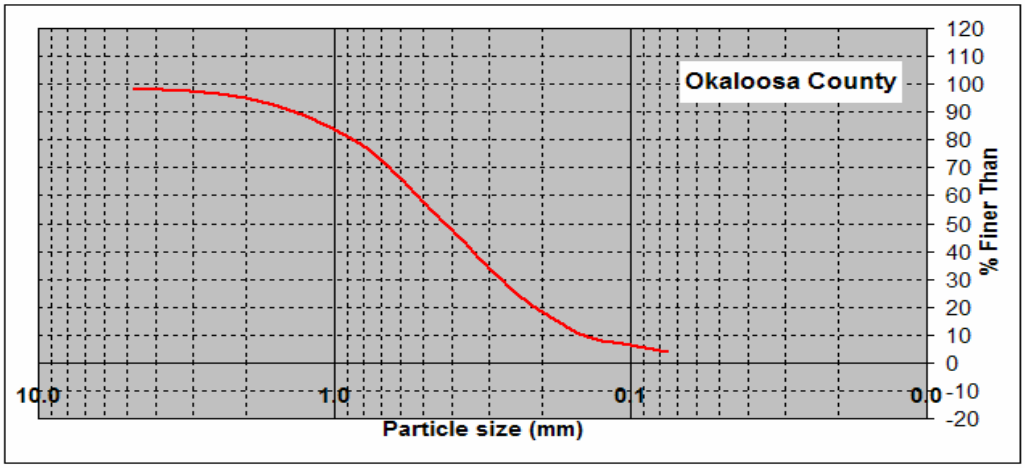
Leon County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	1.3	0.203	0.203	100
10	2.000	1.0	0.156	0.360	99.640
20	0.850	9.0	1.408	1.768	98.232
40	0.425	282.3	44.178	45.947	54.053
60	0.250	281.0	43.975	89.922	10.078
100	0.150	52.2	8.169	98.091	1.909
140	0.106	6.5	1.017	99.108	0.892
200	0.075	2.1	0.329	99.437	0.563
Pan	NA	3.6	0.563	100.000	0.000
		639	100.000		



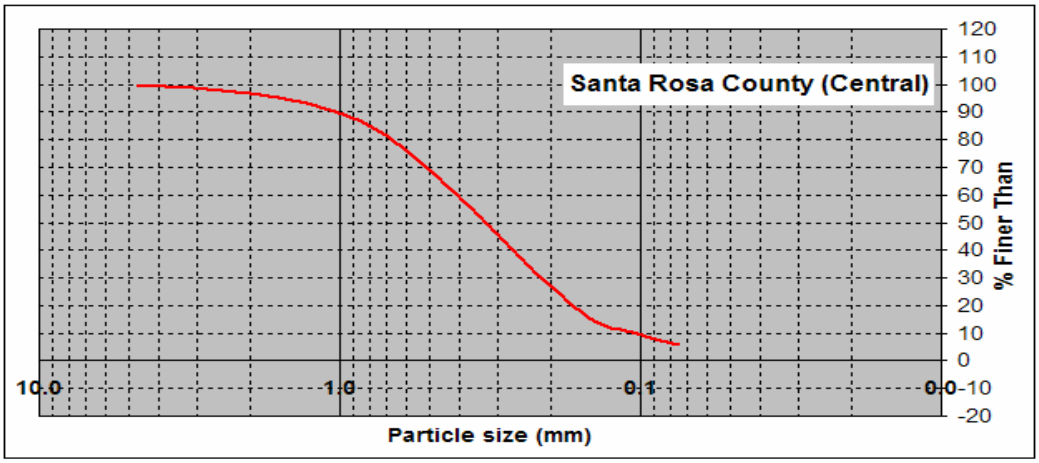
Liberty County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	9.7	1.490	1.490	99
10	2.000	22.1	3.395	4.886	95.114
20	0.850	101.7	15.625	20.510	79.490
40	0.425	190.6	29.283	49.793	50.207
60	0.250	159.5	24.505	74.297	25.703
100	0.150	99.5	15.287	89.584	10.416
140	0.106	24.0	3.687	93.271	6.729
200	0.075	17.0	2.612	95.883	4.117
Pan	NA	26.8	4.117	100.000	0.000
		650.9	100.000		



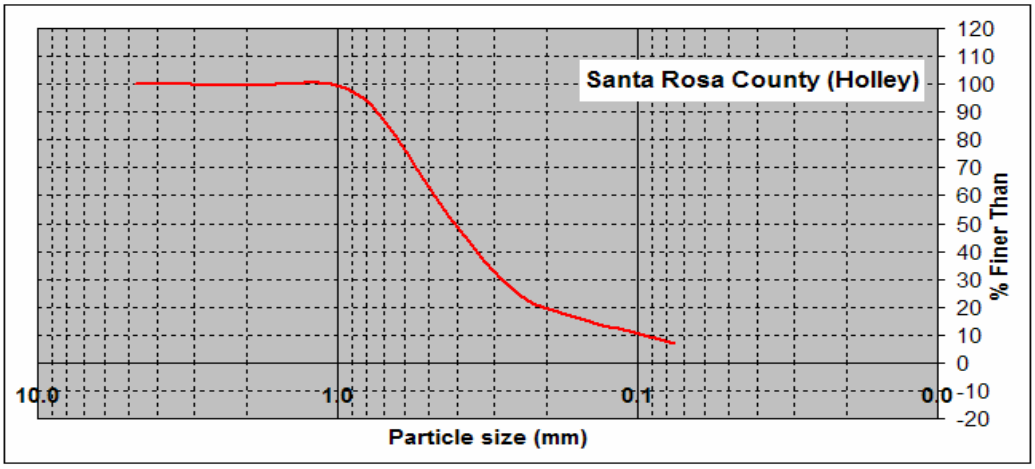
Okaloosa County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	0.3	0.054	0.054	100
10	2.000	18.5	3.320	3.373	96.627
20	0.850	55.0	9.869	13.242	86.758
40	0.425	139.1	24.960	38.202	61.798
60	0.250	138.7	24.888	63.090	36.910
100	0.150	116.7	20.940	84.030	15.970
140	0.106	31.8	5.706	89.736	10.264
200	0.075	23.7	4.253	93.989	6.011
Pan	NA	33.5	6.011	100.000	0.000
		557.3	100.000		



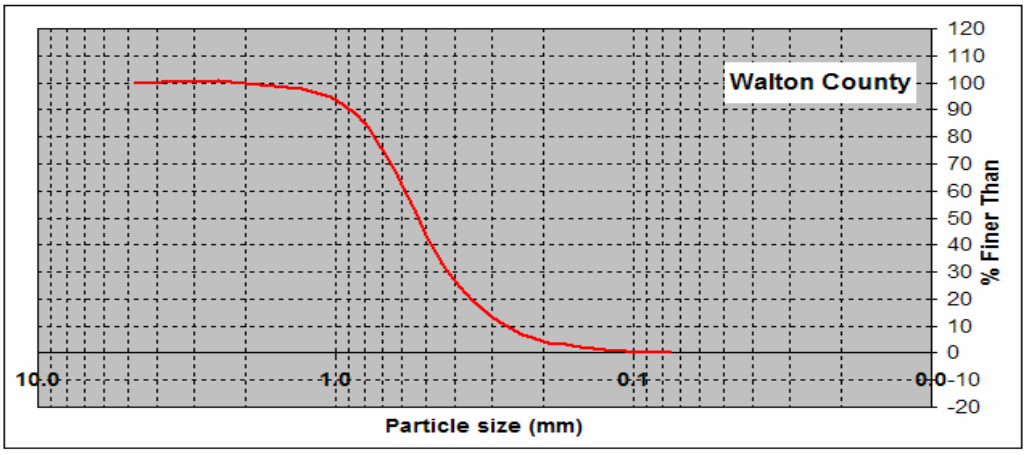
Santa Rosa County (Central) Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	0.0	0.000	0.000	100
10	2.000	4.3	0.672	0.672	99.328
20	0.850	21.0	3.283	3.955	96.045
40	0.425	279.0	43.614	47.569	52.431
60	0.250	176.7	27.622	75.191	24.809
100	0.150	60.3	9.426	84.618	15.382
140	0.106	26.8	4.189	88.807	11.193
200	0.075	27.4	4.283	93.091	6.909
Pan	NA	44.2	6.909	100.000	0.000
		639.7	100.000		



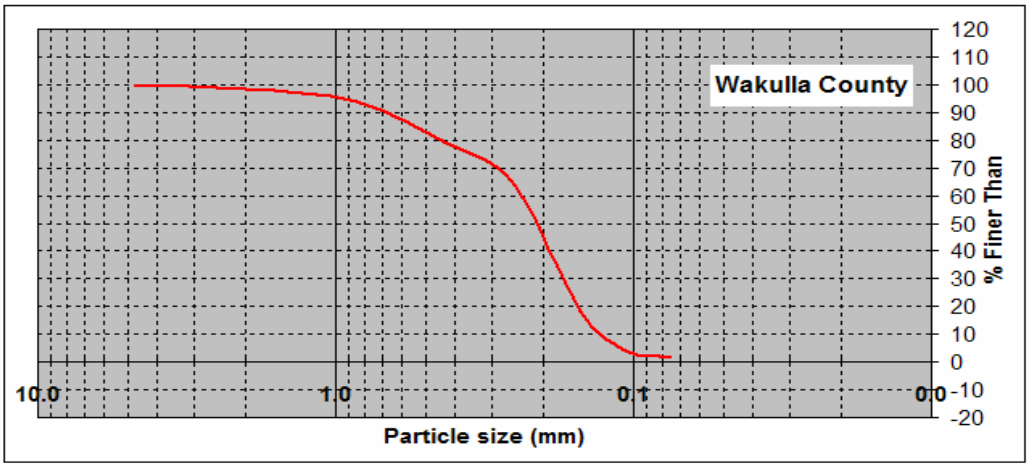
Santa Rosa County (Holley) Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	0.1	0.018	0.018	100
10	2.000	0.9	0.158	0.175	99.825
20	0.850	66.8	11.701	11.876	88.124
40	0.425	328.9	57.611	69.487	30.513
60	0.250	125.9	22.053	91.540	8.460
100	0.150	36.5	6.393	97.933	2.067
140	0.106	7.0	1.226	99.159	0.841
200	0.075	2.6	0.455	99.615	0.385
Pan	NA	2.2	0.385	100.000	0.000
		570.9	100		



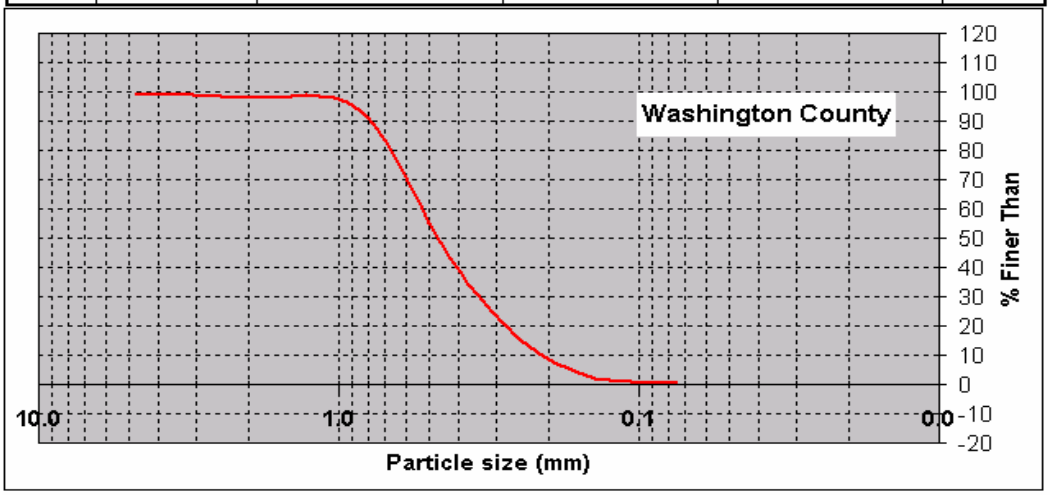
Walton County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	0.2	0.032	0.032	100
10	2.000	9.4	1.482	1.514	98.486
20	0.850	28.0	4.415	5.929	94.071
40	0.425	95.8	15.106	21.034	78.966
60	0.250	95.5	15.058	36.093	63.907
100	0.150	289.0	45.569	81.662	18.338
140	0.106	88.8	14.002	95.664	4.336
200	0.075	17.8	2.807	98.471	1.529
Pan	NA	9.7	1.529	100.000	0.000
		634.2	100.000		



Wakulla County Sieve Analysis

Sieve #	Grain Size (mm)	Mass of Soil Retained (g)	% of mass retained	Cumulative % Retained	% Finer
4	4.750	2.9	0.408	0.408	100
10	2.000	10.3	1.448	1.856	98.144
20	0.850	33.6	4.724	6.580	93.420
40	0.425	360.1	50.626	57.205	42.795
60	0.250	193.8	27.246	84.451	15.549
100	0.150	90.2	12.681	97.132	2.868
140	0.106	12.9	1.814	98.946	1.054
200	0.075	4.3	0.605	99.550	0.450
Pan	NA	3.2	0.450	100.000	0.000
Σ		711.3	100.000		



Washington County Sieve Analysis