BACTERIOLOGICAL ANALYSES OF URINE CONTAMINATED SOIL IN UNIVERSITY OF BENIN, UGBOWO CAMPUS

BY

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FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2022

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (HONS) IN

MICROBIOLOGY

NOVEMBER, 2022

CERTIFICATION

This is to certify that this project work was carried out by Osadolor Daniel in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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PROF. (MRS). F.I. AKINNIBOSUN **DATE**

(Head of Department)

APPROVAL

This project work is accepted in partial fulfillment of the requirement for the award of Bachelor of Science, B.Sc. (Hons). in the Department of Microbiology, University of Benin, Benin City.

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DEDICATION

This research work is dedicated to the most High God for his knowledge and inspiration towards me and my beloved parents, Mr. and Mrs. Osadolor for their assistance towards making my dreams come true.

ACKNOWLEDGEMENT

My hearty appreciation first of all goes to the Almighty God for his guidance and sustenance throughout the period of this research work till now.

My gratitude as well goes to my beloved parents, MR. and MRS. OSADOLOR for their financial support all these years.

A special note of thanks go to my supervisor, DR. (O. MRS). A. OLOGBOSERE for her close supervision to make this work a successful one.

I sincerely appreciate my Head of Department (HOD) PROF. (MRS). F.I. AKINNIBOSUN for her academic support. I also want to acknowledge all the staff (Academic and non-academy) of the department of Microbiology.

My appreciation also goes to my Lecturer Dr. (Mr.) Abraham, my friend Amara, Daniel, Yemi, for their installation during the time of this work.

ABSTRACT

Study was carried out to investigate bacteria in urine contaminated soils within University of Benin Ugbowo campus. Five soil samples were collected and analyzed using soil serial dilution and pour plating techniques. Nutrient agar was used for bacteria isolation and subculture. Urine contaminated soil samples were collected from Hall three, Life science complex and Library extension while control soil samples were collected from Hall two car park and Hall three. Bacteria isolated from these soil samples and their prevalence include *Bacillus subtilis* (80%), *Staphylococcus aureus* (40%), *Micrococcus luteus* (60%), *Escherichia coli* (20%), *Klebsiella pneumonia* (40%) and *Pseudomonas aeruginosa* (60%). Hall three control soil had the highest bacteria count while Life science complex had the lowest bacteria count with the range of 5.5×10^3 Cfu/ml and 7×10^3 Cfu/ml. Various biochemical test were carried out which include oxidase test, citrate test, indole test, TSIA test, to identify isolates based on their reaction to these test. Antibactrial susceptibility test was also carried out. Antibiotics used include Gentamycin, Colistin, Carbenicillin, Ciprofloxacin and Tetracyclin. Urine contaminated soils are threats to the public's health since they can be used as routes for the spread of infection.

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CHAPTER ONE

INTRODUCTION

1.1 Background study

Soil is a natural cultural media for the growth of many types of organisms. The inorganic and organic matter in the soil determines the fertility of the soil and enhance the growth of different microorganisms that play vital role in preserving the nutritional balance of the soil (Dada and Aruwa, 2014).

The highest concentration of organic matter and microorganisms is located in the topsoil and it is where most of the earth's biological soil activities occur. Hence, earth depend on soil to a great extent and as humans population grows, its depth , season of the year, state of cultivation, organic demand for food from crops increases, thereby making soil conservation very crucial. Some of the carelessness and activities carried out by humans are deforestation, pollution from man-made chemicals, over development and human wastes (Joanne *et al.,* 2008).

The primary recyclers of nutrients in the soil are bacteria and fungi. Microbial population found in soil decreases with depth and majority of microbial population is found in the upper 6-12 inches of the soil (Bridge and spooner, 2001). The kinds and number of organisms found in the soil depend on the temperature, aeration, nature of soil, matter and moisture. Different species of bacteria thrive in different microenvironment in the soil and on different food sources. No single cultural environment or isolation media can provide the necessary physical conditions and nutrients satisfactory for the growth of every viable cell (Elaine, 2009). Hence, each culture media or environment will only support the growth of cells that can adapt to its particular

nutrient and physical condition. A wide range of soil microorganisms are yet to be discovered. Much of the experience with bacteria involves disease (Hoglund *et al*., 2002).

Urine is a pale yellow to amber fluid produced by the kidneys and it is composed of uric acid, urea, minerals, chloride, sulphur, nitrogen, ammonium, calcium, sodium, iron, carbolic acid, copper, manganese, potassium, copper, salts, hippuric acid, creatinine, vitamins A, B, C, and E, enzymes (Dada and Aruwa, 2014). Urea is abundant in the urine of humans and other mammals (Drangert, 2000). The urinary tract and bladder are usually sterile. However, the urethra may contain a few commensals. Some of these commensals are *Acinetobacter* species, Diphtheroids, Enterobacteria, and some skin microfloral such as Gram positive *Micrococci*, *Staphylococci* and Gram positive *Enterococci* (cheesebrough, 2006). Proper disposal of urine is important to prevent pollution and minimize the possibility of spreading disease. Some possible effect of uncontrolled urine disposal are that, it damages property value, it is disgusting, impacts the quality of life that live in the polluted area and it is a possible means of disease spread (Knuttson and Kiddljunggren, 2000; Hoglund *et al*., 2002).

In Nigeria, urine deposition in public places go on unchecked and has become a problem, a close examination of such soil macrocosm reveals clear discolouration, patchiness of soil, and pungent ammoniacal smell (Chukwu *et al*., 2018).

1.2. AIM AND OBJECTIVE

The aim of this research was to ascertain the bacteriological quality of urine contaminated soil in University of Benin, Ugbowo Campus. The specific objectives were to:

- Determine the total heterotrophic bacteria count of the soil samples.
- Isolate and identify bacterial isolates using standard microbiological techniques.
- Determine the susceptibility of bacterial isolates to antibiotics.

CHAPTER TWO

LITERATURE REVIEW

2.1. Soil properties

2.1.1. Soil structure

Although soil structure has been highly studied, it remains the least understood of those physical properties that have significant impacts on environmental quality, agricultural sustainability, crop production (Rattan, 1991). In simple terms, soil structure implies "size, shape, arrangement and packing of soil particles into identifiable units" (Rattan, 1991). Soil structure has high impact on aeration, water movement, resistance to erosion, and plant root growth.

2.1.2. Soil texture

Soil texture refers to the relative composition of sand, silt and clay (mineral particles < 2 mm) and is derived after the physical splitting and chemical scattering of the aggregates (Sandoval *et al*., 2012). Soil behavior is affected by soil compositions, including the retention capacity of nutrients and water (). Sand and silt are derived from physical weathering, while clay is gotten from chemical weathering. Texture is one of the most important properties of a soil, and it has a great effect on crop production, land use, and management. Nutrient retention and drainage capabilities are variables dependent on soil texture. The texture of a soil is considered a permanent soil attribute because it does not easily respond to change. There is less you can do to change soil texture because they are not easily alterable" (Gershuny, 1993). In agreement with Berry *et al*. (2007), "It is very expensive and thus unproductive advised to modify a soil's texture."

Clay content has retention capacity for nutrients and water. Clay soils resist wind and water erosion better than silt and sandy soils, because the particles are more tightly joined to each other. In medium textured soils, clay is often translocated downward through the soil profile and accumulates in the subsoil. Soil texture is the capacity of soils to store water and nutrients increases when their clay percentage increases since clay has a large surface area/unit volume and they can absorb large amounts of water and nutrients. A soil with sandy texture has difficulty in retaining water and thus nutrients are not made available to the plants growing in such soil. A clayey soil is poor in aeration thus debilitating the plant growth. Loamy soil is the best textured soil for crop production since it has all the beneficial aspects not found in the sandy and clayey soil. Soil texture is one of the most important properties of soil particularly when dealing with water movement through the soil. Soil mechanical and engineering are also influenced by water.

2.1.3. Particle size distribution

Particle size distribution (PSD) of soil is assessed based on the amount and size of these mineral particles, usually by sieving and sedimentation analyses (Carter and Gregorich, 2006). PSD is a physical property that is usually used to categorize soils (Skaggs et al., 2001), and to estimate soil hydraulic properties with the use of pedotransfer functions (Rabot *et al*., 2018), being also an important variable for the productive and long lasting management of soils (Kettler *et al*., 2001; Di Stefano *et al*., 2010). PSD provides insight into the behavior of soil (Qi *et al*., 2018). PSD of a soil is alterable with soil formation processes, clay fraction mineralogy and soil organic carbon (SOC) (Kleber *et al*., 2015; Zúñiga *et al*., 2019). When it comes to the determination of PSD of a very reactive soil with a high SOC content the latter can become a problem. Micro-aggregates are resistant to dispersing agents, therefore additional procedures must be implemented to reach soil sample dispersion due to the binds of or-mic (mainly Al and

Fe) distributed in the soil (Wagai *et al*., 2020). Moreover, soil dispersion is incomplete, the drag resistance of particles increases due to their irregular shapes, reducing the sedimentation rate and reducing the soil clay fraction (Di Stefano *et al*., 2010). On the contrary, a prolonged dispersion to remove the cementing agents and separate the fractions can lead to false PSD results due to the disintegration of the primary minerals (Velescu *et al*., 2010). Therefore, soil textural class is sometimes adopted instead of PSD (Haller *et al*., 2015). The clay fraction (< 2 μm) consist of layered aluminosilicates, hydroxides, metal oxides, and non-crystalline aluminosilicates (e.g., allophane and imogolite). Soil aggregation, biological activity, and physico-chemical processes are influenced by this fraction (Yudina *et al*., 2018; Wiesmeier *et al*., 2019). A high clay content enhances the stability of aggregates (Six *et al*., 2002) because clay has a high specific surface area, which can stabilize organic compounds present in the soil (Wiesmeier *et al*., 2019; Clunes and Pinochet, 2021). A highly reactive mineral fraction generates strong or-mic (Kleber *et al*., 2015), which can alter the determination of clay and silt fractions and, consequently, of the degree of C stabilization (Plante *et al*., 2006).

2.2.4. Bulk density and porosity

Bulk density is simply the ratio of the weight of oven dry soil in an intact core to the volume of the soil core (White, 2009). It has been suggested as a measure of soil quality in different studies (Larson and Pierce, 1991; 1997; Glover *et al*., 2000; Sparling *et al*,. 2004; Pattison *et al*., 2008). Bulk density, however, is an insensitive measure of how root growth, air movement and water respond to soil structure (Kirchhof and Daniells, 2001). MacEwan, (2007) suggested that a comparison of field bulk density with the maximum bulk density determined by a Proctor compaction test for that soil may be a more useful measure than bulk density per se.

Soil compaction is a major challenge in agriculture because of its significant effect on productivity (Keesstra *et al*., 2016). The bulk density (ρb) of soil is regarded as a major parameter that is associated with soil compaction and many physical, chemical, and biological properties of soil. It is calculated as the ratio of the dried mass of soil to its total volume (Han *et al*., 2016; Walter *et al*., 2016). Soil bulk density can be derived from measurements of soil organic carbon using regressions methods, such as pedotransfer functions (PTFs) (Holmes *et al*., 2012; Rudiyanto *et al*., 2016; Yi *et al*., 2016). Accurate soil bulk density data is also indicates the level of soil porosity and moisture content as it depends on soil texture and structure. Such data also provide valuable information on soil compaction stress and can be used to calculate soil penetration resistance. Furthermore, some studies claim that the bulk density value of a soil is valuable indicator of its thermal properties (Russell *et al*., 2015). Bulk density value is influenced by the depth of soil; soil bulk density values can be used to estimate soil quality, productivity and carbon storage. Measurement of soil bulk density is important for deriving the physical properties of soil because bulk density has effect on many physical properties of soil.

Total porosity (TP) is the ratio of the volume of pores to the volume of soil. In practice, total porosity is derived from the bulk density by assuming a soil particle density of 2.65 g/cm3. Although the volume of soil root and water occupy is dependent on soil total porosity (Pagliai and Vignozzi, 2002), it does not give a measure of the number, size or shape of pores within the soil. Generally, soils with higher porosity have a higher ability to store gases, liquids, solutes and heat (Topp *et al*., 1997; Dexter, 2002). In a soil with a high content of expanding-lattice clays, such as montmorillonite, the total soil volume and porosity changes proportionately with changes in water content (White, 2003). The air-filled porosity at field capacity is the minimum volume

of air available at the wettest drained condition, and a critical value of 10–15% of the soil volume has been suggested (Dexter, 1988).

2.1.5. Soil consistency and soil strength

Soil consistency and soil strength is simply the capability of the soil to withstand the loss of structure through compaction, slaking caused by rainfall or irrigation, etc., and to resist penetration by plant roots and burrowing soil fauna. A soil with good physical quality should be strong enough to maintain its structure and hold plants upright but also weak enough to allow deep penetration by plant roots, soil flora and fauna (Topp *et al*., 1997). The moisture content at the moment of measurement affects the consistency or strength of the soil across the soil profile, hence it is important to test the soil at the same moisture content every time to allow for meaningful interpretation and comparison of results over time. A penetrometer can be used in the field to assess soil strength quantitatively or subjectively by measuring the force needed to crush air-dry aggregates. The latter measurement is done ideally in the winter after the soil has been fully moistened and allowed to drain without significant evaporation (Murray and Grant, 2007).

2.1.6. Infiltration and available water

Field infiltration is a liable indicator of the extent in which water penetrates through a soil profile. Infiltration, however, is affected by how rapidly water (through rainfall or irrigation) is supplied to the soil and the water content of the soil at the time that water is supplied. Matric suction is the main force supplying water into the soil when it is dry, but gravity becomes the main force when the soil is wet. Water infiltration in clay soils that crack when dry usually occurs preferentially down the cracks so that the soil wets up from the sides of aggregates as well

as from the surface (White, 2009). The distribution of water in soil has a strong influence on the distribution of root. The available water capacity (AWC) is the volume of water per unit depth of soil that is apparently available for plant growth. Its upper limit is set by the field capacity, and the lower limit is the permanent wilting point. While available water has been used by others as a soil quality indicator (Larson and Pierce, 1991; Gugino *et al*., 2009; 2012), one of the issues with using AWC is that the amount of available water will change with the rooting depth of the soil (White, 2009). Moderate degree of water stress is encouraged in some stages of plant growth. The intensity of water stress varies with the cultivar but water stress should not be regarded as the major factor for good quality fruit (Lanyon *et al*., 2004). A major challenge with the use of available water as a soil quality indicator is that the value obtained depends on soil type, rooting depth, topography and distance from the plant (White, 2009). This property is therefore not considered to be an important indicator of soil quality.

2.2.7. Soil resistivity

It is simply the measure of a soil's ability to retard the conduction of an electric current. The rate of galvanic, of metallic structures in contact with the soil can be affected by the electrical resistivity. Higher moisture content or increased electrolyte concentration can reduce the resistivity and increase the conductivity thereby increasing the rate of erosion. Soil resistivity value typically range from about 2 to 1000 ohm meter, but more extreme values are not unusual (Edwards, 1998).

Soil reactivity is generally evaluated through soil chemical indicators (Kleber et al., 2015), which are useful to determine the physicochemical associations between mineral and organic compounds (Dwivedi et al., 2019).

2.1.8. Soil color

Soil color has no effect on the behavior and use of soil; however it can indicate the composition of the soil and give clues to the conditions that the soil is subjected to (Brady *et al*., 2006). Soil has a wide range of color; gray, black, red, brown, yellow, and under the right condition green (Brady *et al*., 2006).Varying horizontal bands of color in soil often identify a specific soil horizon. The development and distribution of color in the soil results from chemical and biological weathering, especially ionic reactions. As the primary minerals in soil parent material weather, the elements combine into new and colorful compounds. Aerobic conditions produce uniform or gradual color changes, while reducing environments result in disrupted color flow with complex, mottled patterns and points of color concentration.

2.2. Physico-Chemical Properties in Soil Quality

2.2.1 pH

The pH level of the soil is the most important aspect because of how it affects all other soil parameters. As a result, pH is taken into account while analyzing any type of soil. A soil is described as acidic if the pH is less than 7, normal if the pH is between 6 and 8, and alkaline if the pH is greater than 8.5 (Mahajan and Billore, 2014)

2.2.2 Texture Soil

In order to categorize agricultural soils according to their physical texture, soil texture is a qualitative classification tool that is used in both the field and the lab. Different regions' soil has a different texture, which is largely determined by the size of the particles. The impact of soil texture on root penetration and aeration is evident. It has an impact on the soil's nutrient level as well. Electrical conductivity is an important indicator of soil texture (Osakwe, 2014)

2.2.3 Soil moisture

The soil's moisture content has a significant impact on how well nutrients are absorbed by the soil. The texture of the soil reflects the influence of soil moisture (Kekane *et al* 2015).

2.2.4. Temperature of the soil

The proportion of absorbed to lost energy determines the temperature of the soil. The temperature of soil varies from -20 to 60°C. The most significant characteristic of the soil is its temperature since it demonstrates how it affects the chemical, physical, and biological processes involved in plant growth. Season, time of day, and regional climate all affect soil temperature (Kekane *et al* 2015).

2.2.5. Electrical conductivity

Another crucial characteristic of soil is electrical conductivity, which is used to assess the soil's quality. It measures the number of ions in a solution (Tale and Ingole, 2015). A soil solution's electrical conductivity rises as the concentration of ions does. Electrical conductivity is a rapid, easy, and affordable way to assess the health of soils. It measures the number of ions in a solution. A soil solution's electrical conductivity rises as the concentration of ions does.

2.2.6. Nitrogen

The most important element that plants may acquire from the soil is nitrogen, which also serves as a growth barrier for plants (Gorde, 2013). Nitrogen makes up about 80% of the atmosphere. Nitrogen gas diffuses into the water, where blue-green algae can "fix" (convert) it to ammonia for use by the algae. Inorganic nitrogen and ammonia are other forms of nitrogen that

can infiltrate lakes and streams. Since nitrogen can enter aquatic systems in a variety of ways, there is a plentiful supply of nitrogen that is readily available in these systems.

2.2.7. Phosphorus

Every biological cell contains the essential ingredient phosphorus (Tale and Ingole, 2015). It is one of the most crucial micronutrients required for plant development. Most frequently, phosphorus limits the amount of nutrients that stay in plant nucleus and serves as an energy reserve.

2.2.8. Potassium

Potassium is a crucial element for the development of the plant and plays a significant function in a variety of physiological processes in plants (Solanki and Chavda, 2012). It is engaged in a wide range of plant metabolic processes, from the creation of plant sugars for a variety of metabolic requirements in plants to the regulation of photosynthesis and the formation of lignin and cellulose, which are used to construct cellular structural components.

2.2.9. Soil organic matter

It is a crucial characteristic of soil. The rate of soil erosion is accelerated by a lack of organic matter in the soil (Tale and Ingole, 2015). If there is soil organic matter available, then agricultural activities can exploit this soil. Animal manures, compost, and other organic materials may be put to the soil to add organic matter. Another potential cause of a lowered pH is the

existence of a larger amount of organic matter in the soil. Due to leveling, the organic matter content of the soil has dropped from the surface to the subsoil.

2.3. Metabolic diversity of soil bacteria

Bacteria are extremely diverse metabolically and can be split into four groups, based on their carbon and energy source: Photoautotrophs like cyanobacteria photosynthesise, thereby obtaining energy from sunlight and carbon by fixing carbon dioxide. An example of a cyanobacteria in soil is nostoc, which also fix nitrogen. Energy is derived by photoautotrophs from photosynthesis if an electron donor is provided (hydrogen or an organic compound) for reductive assimilation of carbon dioxide. Some photoautotrophs such as *Rhodopseudomonas*, will grow on organic substrates if oxygen is provided.

Chemoautotrophs fix carbon dioxide with reduced inorganic substrate and serves as a source of energy. The major energy sources for these organisms are ammonia, hydrogen, nitrite, hydrogen sulphide, and the ferrous ion (Fe2+). In soil, this group includes the bacteria involved in nitrification, such as and *Nitbactroer*, *Nitrosomonas* and *Thiobacillus*, which plays a role in acid mine drainage formation. Chemoheterotrophs require pre-formed organic molecules as their carbon and energy source. Some bacteria use simple carbon sources such as glucose whereas others degrade more complex substrates like carbohydrates and proteins. Although some bacteria, such as *Pseudomonas*, may utilize up to 100 different carbon sources for growth.

2.4. Soil bacteria phyla

Soil contains enormous microbial diversity. The total weight mass of organisms below temperate grassland can exceed 45 tons per hectare, equaling or exceeding above ground biomass (Ritz *et al*., 2003).

To examine the makeup of the in situ soil bacterial community, molecular methods have been applied. Less than 10 phyla of bacteria are prevalent in soil, despite the fact that bacteria have been classified into more than 100 different groups (Janssen, 2006). Phyla Proteobacteria, Acidobacteria, and Actinobacteria are widespread and frequently abundant, whereas Verrucomicrobia, Bacteroidetes, Firmicutes, Chloroflexi, Planctomycetes, and Gemmatimonadetes are typically less common. The estimated relative abundance of the major phyla varies between different soils (or samples). Although there are few phyla in soil, there are many species, according to comparisons with other environments (Nemergut *et al.,* 2011). Though it's possible that more than 10% of the sequences in a soil sample cannot be attributed to known phyla (Janssen 2006; Nacke *et al*. 2011).

Four of the Proteobacteria subphyla, including α, β, γ and ε -Proteobacteria, are frequently found in soil. The Proteobacteria are a metabolically varied group of organisms in multiple subphyla. Copiotrophs are members α, β, γ- Proteobacteria subphyla; they are more common in environments with high resource availability, such as rhizosphere soils (Fierer *et al*., 2007). While soils spiked with refractory carbon (cellulose, lignin, or tannin-protein) increased the relative abundance of α, β, and γ -Proteobacteria, adding low-molecular-weight carbon increased the relative abundance of β and γ -Proteobacteria (Eilers et al., 2010; Goldfarb *et al*., 2011).

Most significantly, members of Burkholderiales bacteria within the β-Proteobacteria rose in response to both chemically labile and recalcitrant chemicals (Goldfarb *et al*., 2011). Heterotrophic and autotrophic bacteria with a variety of metabolic processes are found in the α -Proteobacteria. *Sphingomonas* is one of the heterotrophs, and it can break down a variety of hazardous substances, including pentachlorophenol and polyaromatic hydrocarbons. They have also been connected to mineral weathering. The nitrogen fixers from the Rhizobiaceae, such as *Rhizobium*, *Mesorhizobium*, and *Bradyrhizobium*, which all have symbiotic interactions with legumes, are among the heterotrophs. The α -Proteobacteria also includes soil methane-oxidizers like *Methylophilus* and *Methylobacter*. Nitrite oxidizers from the genera *Nitrospira* and *Nitrobacter* as well as phototrophs from *Rhodospirillum* and *Rhodobacter* are included in the group of autotrophs.

Heterotrophs, autotrophs, and methanotrophs are all members of the β- Proteobacteria. The genus *Burkholderia*, *Alcaligenes*, and *Acidovorax* contain the most well-known heterotrophic organisms found in soil. Because they have a variety of metabolic processes and use simple sugars, amino acids, and refractory aromatic and phenolic chemicals as carbon substrates, *Burkholderia* species likely play a significant part in the turnover of carbon. Additionally, *Burkholderia* are said to fix nitrogen and encourage plant development. *Collimonas*, a heterotroph that can breakdown live hyphae and makes chitinase, is one of the heterotrophs (de Boer *et al*., 2004). The species of *Burkholderia* and *Collimonas* weather minerals (Uroz *et al*., 2007). The iron oxidizer *Thiobacillus*, the phototroph *Rhodocyclus*, and the ammonia oxidizer *Nitrosospira* are examples of autotrophs. *Methylomonas* is an illustration of a methanotroph that is a member of the β- Proteobacteria.

Heterotrophs, lithotrophs, and phototrophs are three types of γ- Proteobacteria found in soil. The heterotrophs *Pseudomonas* and *Xanthamonas* are among the most well-known. *Pseudomonas* species have a remarkable range of dietary needs. Most plants can grow on over 50 different substrates, and some can grow on over 100. Sugars, amino acids, fatty acids, alcohols, and hydrocarbons are some of these substrates. The photolithotrophs *Thiocapsa* and *Chromatium* are likewise members of the γ- Proteobacteria; they grow anaerobically in the presence of light using sulphide or elemental sulphur as an electron donor and carbon dioxide as a carbon source. Sulfate- and iron-reducing bacteria make up the majority of the Proteobacteria. *Desulfovibrio*, a sulfate reducer, develops anaerobically in soil using carbon sources like lactate or ethanol, which are found there where oxygen is depleted by organic matter breakdown. This group also includes the bacterial parasite *Bdellovibrio*.

There are only a few known genera of γ- Proteobacteria. The twisted to spirilloid *Helicobacter* and *Campylobacter* have both been found in dirt. Both species live in animal digestive tracts and may penetrate soil when feces are deposited. *Burkholderia*, *Collimonas*, and members of the Rhizobiaceae family are among the protozoa that are frequently found in the rhizosphere.

In soils, Acidobacteria are common and their relative abundance rises as soil pH drops (Lauber *et al*., 2009). The 16S rRNA gene analysis reveals a great diversity in this phylum. In soils, there are more than 20 different subgroups, although it has been claimed that members of subgroups 1, 2, 3, and 4 are the most prevalent (Jones *et al*., 2009). Due to their lack of representation in soil culture collection very little is known about their metabolic capabilities

The Verrucomicrobia, like the Acidobacteria, seem to be common in soil and may be oligotrophs, which may account for their lack of representation in culture collections (Janssen, 2006). Verrucomicrobia's ecology is still not well understood. The class Spartobacteria, of

subdivision 2, which is believed to dominate Verrucomicrobia in grasslands and subsurface soil strata at 10–50 cm depth, is the main group of Verrucomicrobia discovered in soil (Bergmann *et al*., 2011). Free-living taxa and endosymbionts connected to nematodes of the genus *Xiphinema* are found in this class. *Chthoniobacter flavus*, a free-living aerobic soil heterotroph, has been discovered to be most closely related to the majority of phylotypes in soil (Bergmann *et al*., 2011).

When labile carbon sources are added to soil, the relative abundance of Actinobacteridae rises (Goldfarb *et al*., 2011). *Arthrobacter*, *Rhodococcus*, *Streptomyces*, and *Mycobacterium* are among the Actinobacteria that make up the subclass Actinobacteridae and have been isolated from soil. They are various aerobic heterotrophs in terms of metabolism. The potential of *Streptomyces* to create antibacterial substances is well known. *Rubrobacter* and *Solirubrobacter* are members of the Rubrobacteridae family. In soil culture collections, both genera are uncommon. *Rubrobacter* are very common in desert soils and may be radiation-resistant (Holmes *et al*., 2000). The acid-tolerant ferrous iron oxidizer *Acidimicrobium feroxidans* is one of the few cultivated representatives of the Acidimicrobidae that have been discovered in soil.

Lactic acid bacteria and endospore-forming bacteria are both Firmicutes members. The anaerobic genus *Clostridium* and the aerobic to facultative anaerobic genus *Bacillus* are two of the best-known genera of endospore formers in soil. Plant polysaccharides are just one of the many carbon sources that Bacillus breaks down. While others fix nitrogen or denitrify, some are fermentative. Due to its broad metabolic capabilities, *Clostridium* may ferment cellulose, starch, pectin, and sugars. After adding recalcitrant C compounds, there is an increase in the relative abundance of Clostridiales in the soil (Goldfarb *et al*., 2011). Long-term survival in soil during dry periods has been connected to the production of endospores. Aerotolerant anaerobic lactic

acid bacteria, such as *Lactobacillus*, are frequently isolated from decomposing plant matter. Sphingobacteria are usually found among Bacteroidetes bacteria that are frequently isolated from soil. They participate in the aerobic breakdown of complex organic molecules like cellulose, starch, proteins, and chitin. They might be crucial for destroying plant matter in soil. There are reports of numerous close cousins of the Sphingobacteria genus *Chitinophaga* in soil. Members of this genus are gliding, filamentous, and chitinolytic. They might utilise insects and fungus hyphae in the soil as carbon sources. Bacteroidetes have been hypothesized to be copiotrophs because their relative abundance in soil may increase after carbon addition (Fierer *et al*., 2007; Eilers *et al*., 2010). With rising soil pH, Bacteroidetes and Actinobacteria tend to grow in relative abundance (Lauber et al. 2009). Because so few representatives of the phyla Gemmatimonadetes, Chloroflexi, and Planctomycetes have been cultured, very little is known about the physiology, genetics, and ecology of soil bacteria. A few soil-based Gemmatimonadetes isolates have been found; they are aerobic heterotrophs and members of subphylum 1. According to DeBruyn *et al*. (2011), they are suited to low soil moisture levels. There are reportedly many *Gemmatimonas* species in the soil. On oligotrophic conditions, Chloroflexi-related aerobic heterotrophs have been discovered (Davis et al. 2011); there is also evidence that these organisms breathe organo halide chemicals (Krzmarzick *et al*., 2011).

2.5. Human urine

Human urine is a liquid waste product of the human body that is ejected through the urethra after being secreted by the kidneys through a process of blood filtration known as urination. Because proteins are not filtered when the blood is filtered in the kidneys, urine is produced after the blood has been filtered.

2.6. Composition of human urine

The majority of the daily excretion of nitrogen (N), phosphorus (P), and potassium (K) from humans is found in urine, which contributes 88%, 67%, and 73% of these elements, respectively (Kirchmann and Pettersson, 1995; Maurer *et al*., 2003). However, depending on the individual's eating habits, the amount of water taken, physical activity, body size, and environmental factors, the composition of human urine differs from person to person and from region to region (Vinner's and Jönsson, 2002). The majority of the nitrogen fractions in urine are taken up by plants in the same way that urea or ammonium fertilizer is, with nitrogen efficiency being approximately 90% that of mineral fertilizer (Lentner, 1981). The remaining nitrogen fractions in urine are excreted in the form of creatine, amino acids, and uric acid. Urine has a low percentage of heavy metals.Additionally, it contains trace amounts of B, Cu, Zn, Mo, Fe, Co, and Mn (Rodushkin and Odman, 2001). Although human urine is not completely sterile, it can be easily disinfected by storage (Schöning *et al*., 2002) or by introducing urine to the soil (Heinonen-Tanski and van Wijk-Sijbesma, 2005). This is because human urine contains less of certain intestinal bacteria. In contrast, urine can be used as plant fertilizer without further processing, according to Höglund *et al*. (2002), unless cross-contamination with feces occurs, which could result in the survival of viruses.

2.7. Pathogens in urine.

2.7.1. *Staphylococcus saprophyticus*

Staphylococcus is a member of the Firmicutes subgroup of bacteria, which includes Gram positive bacteria with low GC content. There are three known Staphylococcus spp. That could cause disease in humans; *Staphylococcus aureus*, *Staphylococcus epidermidis*, and

Staphylococcus saprophyticus. In young and middle aged female outpatients, coagulase negative Staphylococcus is known to produce simple urinary tract infection while two additional bacteria are usually clinically isolated from hospitalized patients who have indwelling catheters rather than out patients, *Staphylococcus saprophyticus* cannot cause urinary tract infection without the participation of indwelling catheters (Kehlmeter, 2003; Torres Pereira, 1962; Meers *et al*., 1975; Wallmark *et al*., 1978). This finding suggests that, *Staphylococcus saprophyticus* may stick to and continue to proliferate in the urinary tract (Von Eiff *et al*., 2002). In addition, *Staphylococcus saprophyticus* is a common Gram positive uropathogen that causes simple urinary tract infection. Gram-negative bacteria, including *Escherichia coli*, *Proteus mirabilis*, and *Klebsiella* spp. is often observed to cause uncomplicated UTI (Kehlmeter, 2003). These bacteria's fimbriae or pili, which are surface features, are in charge of their adhesion to uroepithelial cells (Anderson, 2004). In this aspect, the majority of *Staphylococcus saprophyticus* strains exhibit significant adhesion to sheep erythrocytes and cells, resulting in hamagglutination (Hell *et al*., 1998). Urease has also been observed to play a significant role in the ongoing proliferation and invasiveness of the bladder (Gatermann and Marre, 1989). It has been hypothesized that *Staphylococcus saprophyticus* is more successful than other *Staphylococcus* and Gram positive bacteria at causing urinary tract infections because of uroepithelial adhesion and urease (Gatermann and Marre,1989; Gatermann *et al*., 1988).

The *Staphlococcus saprophyticus* genome is 2,516,575 BP in length and has 33.2% GC content, which is comparable to other Staphylococcus. The genome has tRNAs for all amino acids and six ribosomal RNA operons. Its chromosomal origin and terminal,which are located in positions comparable to those of *Staphlococcus aureus* and *Staphylococcus epidermidis*, are identified by GC-deviation. There are 2,446 ORFs and a variety of mobile genetic components in the genome,

including 39.3-kb prophage remnant, two IS431elements, nine putative transposases, two staphylococcal cassette chromosomes (SCCs, designated SCC15305RM and SCC15305cap), and one genomic island (designated Ss15305). The organism possesses two plasmids (designated pSSP1 and pSSP2), each measuring 38.4 and 22.9 kb in size (Hiramatsu *et al*., 2004; Ito *et al*., 2004). Instead of antibiotic resistance, *Staphylococcus saprophyticus* SCC15305cap possesses a locus for the synthesis of capsular polysaccharide that contains synthesis locus ORFs with similar to several different lineages organized as a mosaic , including three additional putative polysaccharide glycosyl transferase synthase, and some orthologs to of orthologs to *Staphylococcus aureus*-type I Cap enzymes (Naito and Kobayashi, 1995).

2.7.2. *Pseudomonas aeuginosa*

The opportunistic pathogen *Pseudomonas aeruginosa*, a Gram-negative bacterium, typically inhibits the soil and surfaces in watery environments. It can thrive in a variety of other natural and artificial contexts, including surfaces in health care facilities, because to its versatility and high intrinsic antibiotic resistance. Serious *Pseudomonas aeruginosa* infections frequently occur in hospitals, and almost all of them are accompanied by weakened host defences, such as neutropenia, serious burns, or cystic fibrosis (Lyczak *et al*., 2000). Due to the continuing evolution and disseminating of antibiotic resistant strains, therapeutic choices are becoming increasingly limited, and as a result, *Pseudomonas aeruginosa* infections exhibit substantial morbidity and mortality. *Pseudomonas aeruginosa* is the second most prevalent pathogen identified from patients with ventilator associated pneumonia in the United States and is one of the most common hospital related pathogen (VAP); (Hidron *et al*., 2008). Finding alternative prevention and treatment methods is a top priority due to the severity of *Pseudomonas aeruginosa* infections and the limited antibiotic arsenal available to treat them. *Pseudomonas*

aeruginosa is one of the most frequent pathogens to infect hospitalized patients with respiratory illness. Airway infections are frequently divided into two categories; acute and chronic. Transmission can occur in a hospital or in the community, though the latter is uncommon and virtually usually linked to an underlying immune problem (Arancibia *et al*., 2002). The most common cause of acute nosocomial pneumonia is direct trauma, such as injury to the epithelium from intubation or smoke inhalation. When a patient's underlying medical condition prevents an adequate immune response, such as in the elderly with CF, chronic infections can develop. The high carriage rate of frequently multidrug-resistant strains in hospital wards, the poor health status of the patients, and earlier usage of wide spectrum antibiotics all contribute to the high frequency of *Pseudomonas aeruginosa* in healthcare facilities. Even though rates differ between research and organizations, VAP typically exhibits the highest mortality, up to 30%. (Williams *et al*., 2010). The insertion of the endotracheal tube can cause a ruptured epithelium in patients with VAP, which can act as a reservoir for *Pseudomonas aeruginosa* developing as a biofilm on the plastic surface (Williams *et al*., 2010). Due to the bacteria's enhanced resistance to antibiotics and disinfectants, these biofilms are challenging to remove and treat. This helps to explain why *Pseudomonas aeruginosa* infections persist after the development of biofilms as opposed to the relative efficacy of antibiotic treatment regimens that are initiated before biofilm formation. Additionally, those who are unable to build a sufficient host response can get acute lung infections. Old age, neutropenia brought on by cancer chemotherapy, or immunosuppression brought on by organ transplant are a few underlying immunological weaknesses that can make a person more susceptible to *Pseudomonas* infection. Therefore, compared to patients who are generally healthy, community-acquired pneumonia affects these people more frequently (Williams *et al*., 2010). Because immune compromised patients are frequently admitted to

hospitals and are thus exposed to *Pseudomonas* reservoirs there, nosocomial infections are also very common. *Pseudomonas aeruginosa* can adapt to the lungs' environment and proliferate as a biofilm, leading to a chronic infection, if it is not eliminated during the acute infection phase. Most CF patients get a *Pseudomonas* lung infection by adolescence and can have one for 20 or more years, making them the most well-known cases of chronic pseudomonal lung infections. The airway surface liquid (ASL), which is dehydrated and thicker in CF patients due to a mutation in the cystic fibrosis transmembrane regulator (CFTR), a cAMP-dependent chloride channel, prevents mucociliary clearance from the conducting airways. Inhaled bacteria settle in the altered ASL, where they first cause an acute infection and a strong inflammatory response.

Chronic lung inflammation comes from the immune response being significantly compromised by the thicker ASL, ongoing immunological activation by the bacteria, and/or the host's incapacity to manage inflammation (Sadikot *et al*., 2005; Williams *et al*., 2010). Additionally, there is some evidence that the CFTR mutation itself affects the host's capacity to regulate inflammation brought on by bacteria (Blohmke *et al*., 2012). The hyperactive inflammatory response that results in the destruction of lung function, which may be aggravated by bacterial toxins, results in the progressive degradation of lung function and finally renders these lung infections lethal. Over many years, numerous investigations have tracked the development of *Pseudomonas* infections in CF patients. These investigations' findings showed that *Pseudomonas aeruginosa* undergoes both phenotypic and genotypic changes over time (Smith *et al*., 2006; Hogardt *et al*., 2007; Mena *et al*., 2008). The bacterium obtained from an established chronic infection is typically different from the strain that was isolated from the same patient years earlier during the first acute phase of the illness in that it is less inflammatory and less cytotoxic. The loss of the flagellum and pili, which are essential for motility and adherence and

consequently for the injection of type 3 secreted toxins (adherence is a prerequisite), is one example of these changes. Another is the mutation of mucA, mucB, or mucD, which causes the cells to form mucoid colonies that may protect them from the innate immune system. A third is the evolution of highly antibiotic-resistant small colony variants that are promoted by prolonged antibiotic therapy such as lasR's deactivation (Winstanley and Fothergill, 2009). The frequent appearance of mutator strains in the CF lung encourages such alterations (Oliver, 2010). When utilized to infect mice in models of acute lung infection, these changed strains are relatively avirulent, but are unhindered in their ability to form chronic infections (Bragonzi *et al*., 2009). People who have chronic bronchiectasis and chronic obstructive pulmonary disease are also at an increased risk of developing chronic pseudomonal lung infections (COPD). A severe childhood respiratory infection is typically the cause of chronic bronchiectasis, which is the irreversible dilatation of the bronchial airways brought on by the death of muscle and elastic tissue. The second infection does not spread, and the damage is typically contained to the lobe where the infection first occurred. Patients with non-CF bronchiectasis typically do not have genetic problems that result in immune system defects, therefore the disease is caused by decreased mechanical clearance as a result of the harm brought on by the original infection (Williams *et al*., 2010). Chronic lung tissue inflammation that results in airway constriction and airflow restriction is the root cause of COPD. Smoking cigarettes is thought to be the biggest risk factor for developing COPD because the unpleasant chemicals in smoke disrupt the innate immune system's natural responses in the lungs (Provinciali *et al*., 2011). As the complex process of aging adds to a general reduction in lung function and the changes caused by cigarette smoke often occur gradually over decades, patients with COPD are frequently elderly. Between 4% and 15% of COPD patients have *Pseudomonas* infections, and the clinical manifestations of these

infections blur the line between acute and persistent, with frequent sepsis along with both mild bronchitis and pneumonia (Williams et al., 2010). Most people with COPD are able to get rid of the infection, but roughly as many end up with a persistent infection that periodically worsens (Murphy *et al*., 2008).

2.7.3. *Salmonella* spp

Salmonella enterica is a Gram-negative facultative intracellular anaerobe of worldwide importance causing as many as 1.3 billion cases of disease annually. Over 2500 serovars of *Salmonella enterica* have been identified belonging to six subspecies (Ochman and Groisman, 1994; Fierer, 2001). Subspecies are further subdivided into serovars that are differentiated by their flagellar, carbohydrate and lipopolysaccharide (LPS) structures. *Salmonella enterica* species are typically orally acquired pathogens that cause one of four major syndromes: enteric fever (typhoid), enterocolitis/diarrhea, bacteremia and chronic asymptomatic carriage. The disease manifestation depends on both host susceptibility and the infectious *Salmonella enterica* serovar (Fierer, 2001). In humans, serovars Typhi, Paratyphi and Sendai cause enteric fever, while most serovars cause enterocolitis/diarrhea. Several serovars including Choleraesuis and Dublin are more commonly associated with bacteremia in humans (Fierer, 2001). While serovar Typhi is largely restricted to humans, other serovars are more broadly host adapted and cause natural animal infection. Serovars Dublin, Typhimurium and Choleraesuis cause disease in both humans and animals, but cause distinct syndromes in different hosts. Serovar Dublin causes intestinal inflammatory disease, bacteremia and abortion in cows; serovar Typhimurium causes a typhoid-like systemic illness in mice; and serovar Choleraesuis causes septicemia in pigs (Baumler *et al*., 1998) Human typhoid fever and intestinal/diarrheal disease represent the most common syndromes associated with *Salmonella enterica* infection and involve the pathogenic

processes of both bacteria and host most thoroughly investigated in infectious models of *Salmonella* pathogenesis. Significant inflammatory disease is a common feature of typhoid and enterocolitis. The various virulence programs employed by *Salmonella* species interact with host defense mechanisms at various tissues in different stages of infection resulting in significant host immunopathology, morbidity and mortality.

Human typhoid occurs following the ingestion of *Salmonella enterica* serovar Typhi bacteria, usually from contaminated water or animal products or close contact with an infected individual or carrier (Hornick, 1970). Much of the understanding of typhoid pathogenesis has arisen from the study of infection of susceptible mice with *Salmonella enterica* serovar Typhimurium. In this model, following oral inoculation, virulent serovar Typhimurium survives gastric acidity and colonizes the ileum and cecum, likely by out-competing the resident microflora (Bonhoff *et al*., 1954; Stecher *et al*., 2005). Via invasion of the phagocytic epithelial M-cells covering Peyer's patches (PP), as well as through uptake by dendritic cells (DCs), bacteria are translocated across the intestinal epithelium and gain access to the host circulation or are carried from the gut within CD18 expressing phagocytes (Jones *et al*., 1994; Rescigno *et al*., 2001; Vazquez-Torres *et al*., 1999). Upon extraintestinal infection, bacteria disseminate via the reticuloendothelial system (RES) and take up residence in granulomatous foci within various splenocytes, predominantly macrophages, DCs and polymorphonuclear leukocytes (PMNs), as well as hepatocytes and other non-professional phagocytes in the liver (Richter *et al*., 1997; Yrlid *et al*., 2001; Nakoneczna, 1980). In the absence of intestinal infection, intracellular replication and survival may be considered the central virulence features of typhoid. Fields *et al*., (1986), demonstrated that bacterial survival within phagocytes was essential for virulence. *Salmonella* is capable of infecting a wide variety of cells including DCs, macrophages, hepatocytes, neutrophils,

colonocytes and other epithelial cells. In vitro, within minutes of contact with cells, *Salmonella* are internalized and take up residence in a unique membrane-bound compartment distinct from a phagosome or lysosome, termed the *Salmonella* containing vacuole (SCV) (Gorvel and Meresse, 2001; Meresse *et al*., 1999; Steele-Mortimer *et al*., 1999). Within phagocytes, *Salmonella* SCV formation has the important function of evading endosomal fusion with the phagocyte oxidase complex (Vazquez-Torres *et al*., 2000). In humans, typhoid disease manifests one to 2 weeks following bacterial inoculation with generalized fever and malaise, abdominal pain with or without other symptoms including headache, myalgias, nausea, anorexia and constipation. Diarrhea occurs occasionally but is typical only of infection in the immunocompromised. Hepatosplenomegaly is common but not present in all cases and diffuse abdominal tenderness is usual. Fever is typically mild at first and worsening as disease progresses (Parry *et al*., 2002). In the absence of complications, disease resolves following varied periods of infection although carriage of the bacteria can continue in post-symptomatic patients for months or years and relapse occurs in a minority of patients. The primary treatment for serovar Typhi infection is fluoroquinolones, although nalidixic acid and other antimicrobial agents are also used. Treatment is effective in the vast majority of cases and decreases time to bacterial clearance, carriage rates and infection associated morbidity and mortality (Parry *et al*., 2002). Although estimates vary greatly due to a lack of consistent diagnosis and reporting, between 200 million and 1.3 billion cases of intestinal disease including 3 million deaths due to non-typhoidal *Salmonella* are estimated to occur each year worldwide (WHO, 2005). Like typhoid, the incidence of intestinal disease caused by non-typhoidal *Salmonella* species is highest in the developing world, but is also of considerable importance in developed countries. Until the development of a new murine model of *Salmonella* enteropathogenesis (Barthel *et al*., 2003).

2.7.4. *Escherichia coli*

Escherichia coli is a Gram-negative, adaptable bacterium that is simple to find and susceptible to random and natural genetic mutation. There is a sizable collection of *Escherichia coli* genomes that have been sequenced, and these genomes show diverse sizes and genetic diversity across commensal and pathogenic, demonstrating a wide range within the same bacterial species. They are made up of non-pathogenic bacteria from the common gut microbiota of humans and many other animals, which may function as commensals. There are additional pathogenic variants, which are separated into extraintestinal and diarrheagenic pathogens, with distinct pathotypes and natural hybrid strains. These subtypes might be obligate or facultative pathogens (Nataro and Kaper, 1998).

Enteroinvasive *Escherichia coli* (EIEC), enteroaggregative *Esherichia coli* (EAEC), diffusely adhering *Escherichia coli* (DAEC), adherent invasive *Escherichia coli* (AIEC), and cell detaching *Escherichia coli* (CDEC) are some examples of pathotypes proposed by their differential features and the essential virulence genes defining each subgroup (Kaper *et al*., 2004). Sometimes only a few unique virulence factors, as several previously reported in ExPEC strains, can distinguish pathogenic *Escherichia coli* from commensal *Escherichia coli* (Köhler and Dobrindt, 2011). However, the sophistication and accessibility of molecular typing technologies are changing this situation. Numerous crucial details on the interactions between hosts and pathogens, reservoirs, clinical diagnoses, and novel ExPEC transmission mechanisms are now available because to new computational techniques (Johnson and Russo, 2018). Since virulence genes are frequently found in transmissible genetic elements such genomic islands, bacteriophages, insertion sequences (ISs), integrons, plasmids, and transposons, they are simple for bacteria to trade (Hacker *et al*., 2003; Dobrindt *et al*., 2010). Additionally, they have

numerous genes for antibiotic resistance that have developed under heavy selective pressure due to the widespread use of antibiotics (Brzuszkiewicz *et al*., 2009).

Due to frequent genetic processes that result in gene acquisition and loss, the *Escherichia coli* genomes have a high degree of genetic diversity. Numerous ExPEC strains are part of the intestinal microbiota and frequently populate the gut asymptomatically. However, just a small portion of ExPEC, such as UPEC, SEPEC, and NMEC, are accountable for the great majority of illnesses, including sepsis, meningitis, and urinary tract infections (Kaper *et al*., 2004). ExPEC strains have a wide range of virulence factors, including adhesins (fimbrial and non-fimbrial), siderophores, toxins, invasins, and the capacity to persist in serum. Additionally, many of these virulence factors may coexist and work in concert within the same strain. The septic strains always have at least an iron absorption system, an adherence system, and genes for serum survival despite additional variables (Biran and Ron, 2018; Johnson and Russo, 2018). In the pathogenesis of *Escherichia coli*, horizontal transmission mechanisms are used within and across related species. As a result, the IS, transposons, and integrons may promote novel genomic rearrangements like gene duplication, gene suppression, and gene capture. According to several studies (Frost *et al*., 2005; Brigulla and Wackernagel, 2010; Dobrindt *et al*., 2010; Jackson *et al*., 2011; Sheppard *et al*., 2018), this genetic material transit can lead to greater flexibility regarding various features, including the transition of pathogenic bacteria between humans and animals, resistance to antimicrobials, the appearance of emerging pathogens due to the gain of virulence genes, increased pathogenicity All of these factors, including the role of bacteriophages in disease, may increase the virulence of these bacteria. With differences in the bacteriophage repertoire directly affecting their virulence, new harmful strains are more likely to evolve as a

result of horizontal transfer between different strains (Manning *et al*., 2008; Ogura *et al*., 2009; Dobrindt *et al*., 2010; Jackson *et al*., 2011).

Beyond possible genetic and phenotypic benefits, the co-evolution of bacterial genomes with plasmids may have an impact on cellular metabolism to maintain and stabilize the plasmid (Jackson *et al*., 2011). Plasmids frequently including the ColV family of genes, which code for colicin, serum survival factors, and iron absorption systems, contain several ExPEC virulence genes (Biran and Ron, 2018). Similar to this, plasmids associated with virulence, mostly those belonging to the incompatibility group IncF and possessing transfer activities, are carried by intestinal pathogens (Carattoli, 2009). According to the characteristics of each individual group, certain pathotypes of *Escherichia coli* require virulence plasmids, such as pINV and pAA in EIEC and EAEC, respectively (Kaper *et al*., 2004).

Despite this, because ExPEC and DEC pathotypes are so changeable and allow the constant emergence of distinctive hybrid-formed strains within this dynamic bacterial species, they are insufficient to fully categorize all pathogenic *Escherichia coli* strains. The ability to adapt to various environments and the presence of virulence genes crucial to the pathogenesis of each pathotype allow the creation of hybrid pathogenic *Escherichia coli* (HyPEC). The ability of *Escherichia coli* to replicate, modify, and spread is astounding. These characteristics enabled the development of new HyPEC. Mutation, recombination, and other genetic alterations result in the appearance of acquired virulence genes and unique activities. The prevalence of novel hybrid and antibiotic resistance among DEC and ExPEC has increased as a result of all these genetic variations (Dobrindt *et al*., 2003; Bielaszewska *et al*., 2007; Khan *et al*., 2018).

After an outbreak of foodborne bloody diarrhea and hemorrhagic uremic syndrome (HUS) in Germany, a HyPEC strain recently attracted a lot of attention. Consumption of raw fenugreek

sprouts was linked to this E. coli O1O4:H4 outbreak because it was a hybrid EAEC strain with STEC characteristics, such as the presence of Shiga toxin. Even with a prompt response and discovery, this HyPEC's complicated nature could not be immediately unraveled by sequencing, and 3,842 hospitalizations throughout Europe and North Africa with numerous fatalities resulted as a result (Bielaszewska *et al*., 2011; Rasko *et al*., 2011). The HyPEC events are caused by emerging mechanisms. Here, the combination of enteroaggregative characteristics in a rare serotype led to significant cell attachment and the creation of a biofilm (Navarro-Garcia, 2014). Additionally, this strain's genome now has a lambdoid phage with the stx2 gene, which means it might release the Shiga toxin. When compared to STEC, these characteristics have increased the occurrence of HUS during the outbreak on this HyPEC (Muniesa *et al*., 2012). There are numerous examples of distinct genetic hybrids in E. coli, including the STEC/ExPEC O80:H2 serotype, which caused HUS and bacteremia due to the presence of stx2 and eae genes from STECs and pS88-like plasmid, described in meningitis, urosepsis, and avian pathogenic ExPEC strains (Peigne *et al*., 2009).

The STEC/UPEC strain O2:H6 serotype has the ability to produce diarrhea and urinary tract infections due to the presence of the virulence genes a-hlyA, cnf1, and clb from UPEC (Bielaszewska *et al*., 2014). The LEE Island was obtained by the EPEC/ETEC strain, which also carries the LT toxin gene (Dutta *et al*., 2015). E. coli ST131, which has been widely documented to be multidrug resistant, is an example of a very virulent ExPEC linked to bloodstream and urine infections. Due to the presence of the pAA plasmid, it has also developed the enteroaggregative diarrheagenic phenotype (Boll *et al*., 2018). However, they are not properly characterized, and many more HyPEC are described as case reports. Here, we've quickly examined a few of these strains' acquired genes, their direct connection. The recently formed

names hybrid- and hetero-pathogenic *Escherichia coli* have been characterized as new combinations of virulence factors among classic *Escherichia coli* groups, similar to these HyPEC. They demonstrate distinctions between common and uncommon subgroups of the EAEC and EPEC pathotypes, as well as hybrids like EPEC/STEC, ExPEC/EPEC, and ExPEC/EAEC (Santos *et al*., 2020). This study demonstrates how important this subject is in the area using a similar strategy as ours here.

Due to comparable genetic pathways, which also allow bacteria to tolerate the presence of various antimicrobials, the high frequency of traditional pathogenic *Escherichia coli* and the emergence of HyPEC both occur. The complicated combination of intrinsic and acquired resistance genes, which may collaborate, is linked to bacteria's resistance to different classes of antibiotics (Cag *et al*., 2016; Khan *et al*., 2018). Together, these factors contribute to the alarming global trend of multi resistant bacteria in various bacterial species.

2.7.5. *Proteus* spp

Proteus spp. are common commensals of the gut microbiota and are Gram-negative bacteria from the Enterobacteriaceae family (Penner, 2005). Hauser documented and described the first isolates in the late 19th century (Manos and Belas, 2006). Proteus mirabilis, *Proteus vulgaris*, *Proteus penneri*, *Proteus hauseri*, *Proteus terrae*, and *Proteus cibraius*, as well as the unnamed genomospecies 4, 5, and 6, currently make up the genus (Hara *et al*., 2000; Hyun *et al*., 2016; Behrendt *et al*., 2015). *Proteus vulgaris*, *Proteus mirabilis* and *Proteus penneri* typically populate the human gut in different combinations, but they make up less than 0.05% of the gut microbiota of healthy patients (Yatsunenko *et al*., 2012).

Although *Proteus* species are well-known pathobionts and the gut is where they are found, research on this genus has focused more on their role in urinary tract infections than on its intestinal symptoms (Chow *et al*., 1979; Mobley and Belas, 1995; Coker *et al*., 2000). Recent research on the gut "microbiome" in health and disease has shown that there are significant changes in the relative proportions of important bacterial taxa linked to active disease, a condition known as "dysbiosis." The growth in the population of the phylum Proteobacteria, notably the Enterobacteriaceae, is one of the characteristics of dysbiosis in inflammatory bowel disorders (IBDs) (Mukhopadhya *et al*., 2012). While *Proteus* hasn't been thoroughly studied, other genera in the Enterobacteriaceae family, such *Escherichia*, *Shigella*, *Salmonella*, and *Klebsiella*, have gotten the proper attention in this area.

Short (1.5 to 2 m) straight rods *Proteus* species exhibit dimorphism in the form of "swimming" and "swarming" forms, as do several other Enterobacteriaceae family members (Armbruster and Mobley, 2012). In liquid habitats, solitary cells with 4 to 10 peritrichous flagella are most common (Mobley and Belas, 1995). A cyclic sequence of swarming and consolidation phases causes the swarming habit of *Proteus* species to culminate in a distinctive bull's-eye pattern on a plate culture (Pearson *et al*., 2010). *Proteus* cells differentiate into filamentous, multinucleated, highly flagellated swarmer cells when they are put in a viscous environment or on a solid surface. After this differentiation, the cells return to a shorter morphotype during the consolidation phase, and metabolic preparation takes place before the subsequent swarming cycle (Pearson *et al*., 2010). Compared to other swarming bacteria, *Proteus mirabilis* passes through swarming differentiation at substantially higher agar concentrations (1.5 to 2%) (Rather, 2005).The synthesis of secreted proteins, including virulence factors like the protease ZapA, increases dramatically when *Proteus* spp. swarm (Armbruster and Mobley, 2012; Pearson *et al*., 2010 17,

Walker *et al*., 1999; Belas *et al*., 2004). Only occasionally have swarmer cells been observed in ascending urinary tract infection animal models in vivo, with the odd swarmer cell identified from infected mice's kidneys and bladder stones (Allison *et al*., 1994; Li *et al*., 2002). The swarming phenotype can be produced by the quantity of amino acids, in particular, and can occur under both aerobic and anaerobic conditions (Ambruster *et al*., 2013). Additionally, it has been shown that a more acidic pH, similar to what would be seen in the proximal small bowel and cecum, significantly increased swarming behavior (Nugent *et al*., 2001; Fujihara *et al*., 2011; Pickard *et al*., 2017). Swarming has been demonstrated to play a significant role in intracellular invasion and persistence, with uroepithelial cells able to invade intracellularly with 15–20 times more swarmer cells than swimmer cells (Allison *et al*., 1992). There is some evidence that swarming *Proteus* strains are more invasive than swarm-defective mutant strains in urinary tract mice models (Allison *et al*., 1994). Additionally, it has been demonstrated that a number of intestinal tract metabolites, such as choline, glutamine, and putrescine, the most prevalent polyamine in the gut, encourage swarming (Milovic, 2001; Jameson *et al*., 2016; Sturgill, 2004).

Although we cannot say for sure that swarming behavior occurs in the gut in vivo just yet, it is likely that the environment in the gut may be favorable for swarming given the presence of a viscous surface (such as the gut mucosa), high glutamine and polyamine availability, including putrescine and electron acceptors for anaerobic respiration such as choline ((Sturgill and Rather, 2004). Attachment to the mucosa and adhesion. The pathogenesis of *Proteus* infections in the urinary and gastrointestinal systems depends on adhesion to epithelial surfaces. More fimbrial gene sets (operons) than any other characterized bacterial genome were discovered after *Proteus mirabilis* strain HI4320's genome was sequenced (Schaffer and Pearson, 2015; Pearson *et al*., 2008; Kuan *et al*., 2014).

There are six distinct fimbrial types that *Proteus mirabilis* can produce including mannose-resistant *Proteus*-like fimbriae (MR/P fimbriae), mannose-resistant *Klebsiella*-like fimbriae (MR/K fimbriae), non-agglutinating fimbriae (NAF), also known as uroepithelial cell adhesin (UCA), ambient-temperature (Kuan *et al*., 2014). These fimbriae and adhesins significantly contribute to the development of bacterial biofilms, a frequent issue with urinary and gastrointestinal instrumentation.

Due to their role in epithelial adherence, it is expected that the MR/P and NAF/UCA fimbrial types are crucial in the development of gastrointestinal pathophysiology (Jansen *et al*., 2004; Rocha *et al*., 2007; Lee *et al*., 2000). A comparison of the 7 chaperone-usher fimbrial operons and their 17 individual 58 clinical isolates and sequenced *Proteus mirabilis* strains both displayed 99% conservation inAmong the 17 fimbrial operons, 13 are present, indicating that these genes are highly conserved strains gathered from a range of clinical locations (Kuan *et al*., 2014). At least two and possibly as many as six of the characterized fimbriae can likely be assembled on the cell surface at any given time (Kuan *et al*., 2014; Adegbola *et al* 1983). There is a close relationship between the regulation of motility and the expression of adhesion factors. Of the 17 fimbrial operons, at least 10 gene clusters contain a homolog of the mrpJ gene, which inhibits motility. By interacting with the promoter sequence, MrpJ reduces the activity of the flagellar master regulator flhDC (Pearson and Mobley, 2008). When these cells switch back to their swimming shape, MR/P and NAF fimbria expression is once again present (Latta *et al*., 1999).

2.8. Urine effect on soil

2.8.1 pH and soil moisture

According to Bol *et al*. (2004), urine deposition enhances soil moisture content (Williams and Haynes, 1994). The addition of urine may also stop the soil from losing moisture (Shand *et al*., 2000). According to several studies, the pH of the top 20–40 mm of soil often rises by 1-3 pH units after urine deposition (Doak, 1952; Sherlock and Goh, 1984; Shand *et al*., 2002. This happens as a result of the majority of urine urea being kept in the upper soil layers, where it is hydrolyzed, leading to the development of fundamental conditions.

2.8.2. Availability of nitrogen

The mineral N concentration in the soil may rise to nearly 400 mg N kg-1 after urine deposition (Williams and Haynes, 1994). The majority of the N initially exists as urea-N, which quickly hydrolyzes to ammonium (NH4+-N), which can then be volatilized as NH3-N. The remaining NH4+-N is then converted to nitrate (NO3—N) via nitrification, after which denitrification may take place. Nitrous oxide (N2O-N) and dinitrogen gas (N2-N) may be lost to the environment as a result, although urea and nitrate (NO3—N) may travel down the soil profile due to leaching and/or macropore flow (Haynes and Williams, 1993; Clough *et al*., 1998). Plants may utilize both NH4 +-N and NO3 -N, and isotopic studies have shown that living plant biomass retains 22-78% of the urea-N in urine, whereas soil organic matter (SOM) retains 20-30% (Clough *et al*., 1998; Leterme *et al*., 2003). A rise in dissolved organic N (DON) concentrations was only linked to sources in the soil in investigations utilizing urine (Shand *et al*., 2000). The recovery of urine-15N is higher in a sandy loam soil than in a sandy soil, and the fate of urinary-N is partially dependent on soil parameters (Sørensen and Jensen, 1996). According to Sørensen and Jensen (1996), this larger loss of 15N from the sandy soil was associated with a higher net rate of urine-N mineralization and less N uptake by plants. In a different study, clay had the highest urine-15N recovery (81%) followed by silty loam (77%) and sandy loam (71%).

(Clough *et al*., 1998). Therefore, in soils with significant quantities of sand, supplemented urine-N is probably less accessible to plants or soil bacteria.

2.8.3. Availability of carbon and soil organic matter

The release of organic intracellular solutes by soil microorganisms as they try to maintain cell integrity or as microbial cells lyse, the addition of urinary compounds, the release of C from SOM due to the increased soil pH, and other factors can all cause an increase in the C content of a soil (Kieft *et al*., 1987; Halverson *et al*., 2000; Although urine deposition has been associated with an increase in the soil's soluble C content (Monaghan and Barraclough, 1993; Shand *et al*., 2000), the persistent rise in dissolved organic C (DOC), as shown by Petersen *et al*.(2004) suggested that microorganisms might not have easy access to this C. Several days after urine application, studies have indicated that 20–50% of the urea-derived C may be accounted for in the top 200 mm of soil, in above-ground plant matter, and as CO2-C that is released (Bol *et al*., 2004; Petersen *et al*., 2004).

When excess urine was applied to soil, the pH rose along with the concentrations of DOC (as well as dissolved organic N and P) and the dark color of the soil solution indicated that humic matter had been solubilized (Shand *et al*., 2000). There aren't enough studies, though, linking changes in the soil microbial ecology to nutrient release in urine patches.

2.8.4. Nutrients in the soil solution

Both directly from the urine and indirectly through soil changes brought on by pee deposition, nutrients are delivered to the soil solution (Monaghan and Barraclough, 1993; Shand *et al*., 2000). Bromine tracer tests have shown that the bulk of the urine is retained in the top 100 mm of the soil (Williams and Haynes, 1994). The top 25 mm of urine-affected soil has the largest increase

in electrical conductivity (EC), and the ionic strength of the soil solution in this zone may increase to be 6–8 times that of untreated soil (Haynes and Williams, 1992).

The ions measured in the urine and those that were immediately found in the soil solution following pee deposition are consistent. The ions found in the soil solution right after urine deposition match those found in the urine, (Haynes and Williams, 1992). As nitrification progresses, HCO3-, Cl-, and SO4 2-concentrations decrease over time, and NO3 - emerges as the major anion (Williams and Haynes, 1994). The concentration of NH4+, which results from the hydrolysis of urea, likewise decreases as a result of nitrification (Williams and Haynes, 1994). Increase in the concentrations of the counter-ions Ca2+ and Mg2+ occur at the same time as increases in NO3-. These counter-ions are primarily obtained from soil exchange sites through displacement of urinary K+, Na+, and NH4+ as well as hydrogen ions (H+) released during nitrification (Haynes and Williams, 1992; Williams and Haynes, 1994; Early *et al*., 1998). Leaching with NO3- across the soil profile redistributes Ca2+, Mg2+, some K+, and Na+ over time (Holland). However, because the soil's cation exchange complex (CEC) strongly adsorbs it, exchangeable K+ concentrations may continue to be high (Haynes and Williams, 1992; Early *et al*., 1998). The capacity of the CEC to retain K+ is increased in soils with a high concentration of pH-dependent negative charge sites by the rise in pH following urine deposition (Williams *et al*., 1988; Haynes and Williams, 1993). Although it depends on how the P is bound in the soil, PO4 3--P concentrations in soil solution typically range between 0.1 and 1.0 mg kg-1 (Plante, 2007). However, they may rise with an increase in pH, as seen in a urine patch (Gahoonia *et al*., 1992; Hartikainen). Higher amounts of dissolved organic P have been seen after sheep urine was added to pasture soil, and it has been hypothesized that this is due to the humic substance becoming more soluble due to an elevated pH (Shand *et al*., 2000; Shand *et al*., 2002). Additionally, as they

are bound in the SOM, this might lead to higher concentrations of trace elements and other metals.

CHAPTER 3

METHOLODOLOGY

3.1 Sample collection

Soil sample was collected using sterile polythene bags. The sites used are the undesignated areas for urine discharge. The experimental site is the soil area noted for regular urine discharge. While the control soil was collected from areas not contaminated with urine. A total of three (03) soil samples were collected from urine contaminated site and control soil samples were collected from two (02) sites. The soil samples were collected from the top soil (5-20cm) respectively. Sample collection sites were Hall 3, Life science complex, Library extension for urine contaminated soil and Hall 2 car park, Hall 3 for control soil. Samples from urine contaminated and control soil were sealed respectively and were appropriately labelled before they were transferred to Microbiology Laboratory of University of Benin for immediate analysis.

3.2. Sample preparation

Soil samples were subjected to microbiological analysis using the method of Olutotiola *et al*., (2001). Pour plating was done using nutrient agar (NA-oxoid). Standard microbiological techniques were employed to reduce bacteria load and prevent overcrowding of petri dishes are the soil dilution for bacteria. Culture media used was prepared according to manufacturer's specification and sterilization of materials was done in an autoclave at 121℃ for 15minutes.

Ten grams (10g) of each soil sample was diluted in 90ml of sterile distilled water, followed by ten -fold serial dilution with sterilized saline. After which 1ml of the dilution was plated out into Nutrient agar in duplicates using pour plate method and allowed to solidify. Nutrient agar plate

for bacteria were incubated at 35℃ for 24hr. Bacteria counts were recorded in colony forming units per ml (cfu/ml).

3.3. Isolation and identification of bacteria

Distinct colonies of bacteria were purified by repeated subculture on the respective isolation media and preserved at 4℃. Morphological and biochemical tests to identify bacteria isolates were carried out using the methods of Cheesebrough (2006).

3.3.1Morphological test

3.3.1.1 Gram stain

A thin smear of the test isolates was made on different slides with the aid of a sterile wire loop and aallowed to dry, after which they were heat fixed and allowed to cool. Each fixed smear was covered with crystal violet stain for 60 seconds. Then the stain was rapidly washed off with clean running water. All the water was tipped off. After which the smear was covered with Lugol's iodine for 60 seconds. The iodine was washed off with clean running water. Then the smear was covered with acetone-alcoholfor 10 seconds. Then washed off immediately with clean water. Then the smear was covered with neutral red stain for 2 minutes and washed off with clean water. The slide was placed on a draining rack for the smear to air-dry. After which a few drop of immersion oil was dropped on the stained smear and examined microscopically with the 100x objective. Then the result was documented (Cheesebrough, 2006).

3.3.2. Biochemical test

3.3.2.1 Indole test

Indole test was carried out with the use of tryptone water and kovac's reagent. The test organism was inoculated in a bijou bottle containing 3ml of sterile tryptone water. Then incubated at 35-37℃ for up to 24hrs. Test for indole was carried out by adding 0.5ml of kovac's reagent, then gently shook. After which it was examined for a red color in the surface layer within 10mins (Cheesebrough, 2006).

3.3.2.2. Citrate test

Citrate test was performed using Simmon's citrate agar. Slopes of the medium was prepared in bijou bottles as recommended by the manufacturer. Using a sterile straight loop saline suspension of the test organism was streaked in the slope and then the butt was stabbed. Then it was incubated at 35℃ for 24hrs. Then the medium was observed for a bright blue color (Cheesebrough, 2006).

3.3.2.3. Oxidase test

A piece of filter paper was soaked with a few drops of oxidase reagent. Then a colony of the test organism was smeared on the filter paper (Cheesebrough, 2006).

3.3.2.4. TSIA (triple sugar iron agar)

The TSIA media was prepared according to manufacturer's recommendation. With a sterile loop the test organism was inoculated into the prepared TSIA by first stabbing through the center of the tube and then streaked on the surface of the agar slant. The cap was left on loosely and

then the tube was incubated at 35℃ for 24hrs. After incubation the tube was examined for color change in the slant and butt, blackening, cracks and gas formation in the medium.

3.4. Antibacterial susceptibility test

With the use of a sterile wire loop the test isolates were emulsified in 3-4ml of sterile physiological saline. In a good light the turbidity of the suspension was matched with the turbidity standard. With the use of a sterile swab Mueller Hinton agar was inoculated with the well emulsified isolates. Excess fluid was removed by pressing the swab against the side of the tube above the level of the suspension. The petri dish was streaked evenly over the surface of the medium in three directions, rotating the plate approximately 60° to ensure even distribution. With the petri dish lid in place the surface of the agar was allowed to dry for 3-5 minutes. With the use of a sterile forceps the antibiotic discs were placed evenly on the inoculated plate. The disc were placed about 15mm away from the edge of the plate and not closer than about 25mm from disc to disc. Within 30 minutes when the discs were placed, the plate was inverted and incubatedat 35℃ for 24 hours. After which the plate was observed for growth then with the use of a ruler the area of growth was measured from the underside of the plate and documented (Cheesebrough, 2006)**.**

CHAPTER FOUR

RESULTS

Table 4.1 shows the cultural, morphological and biochemical characteristics of bacteria isolates from urine contaminated soil and control soil in University of Benin, Ugbowo Campus.

Table 4.2 shows the percentage prevalence of bacteria isolates from urine contaminated soil and control soil sample from University of Benin. *Bacillus subtilis* being the highest (80%) and *Escherichia coli* the lowest (20%).

Table 4.3 and figure 4.1 shows the total heterotrophic bacteria count in cfu/ml of urine contaminated soil and control soil samples from University of Benin, Ugbowo Campus. It was observed that the mean heterotrophic bacteria count range from 19.5± 0.5 to 83.5± 6.5cfu/ml.

Table 4.4 shows antibiotic susceptibility test of bacteria isolates. All bacteria isolates were susceptible to Ciprofloxacin and resistant to Colistin.

Table 4.1: Cultural, morphological, and biochemical characteristics of isolates

Colony	1	2	3	4	5	6
Elevation	Convex	Convex	Convex	Undulate	Raised	Unbonate
Margin	Entire	Entire	Unbonate	Unbonate	Entire	Wavy
Color	Golden yellow	Yellow	Cream	Cream	Cream	Bluish green
Shape	Circular	Circular	Irregular	Circular	Circular	Circular
Size	Pin head	Pin head	Large	Large	Small	Medium
Morphology						
Gram stain	+	+	+	-	-	-
Cell type	Cocci	Cocci	Rod	Rod	Rod	Rod
Arrangement	Cluster	Cluster	Pairs	Dispersed	SIINGLY	Dispersed
Color	Purple	Purple	Purple	Pink	Pink	Pink
Biochemical						
KOH	-	-	-	+	+	+
Indole	+	-	-	-	+	-
Citrate	-	+	+	+	-	+
Oxidase	-	+	-	-	-	+
Glucose	+	+	+	+	+	+
Sucrose	+	+	+	-	+	-
Lactose	+	-	-	+	+	-
Mannitol	+	+	+	-	-	+
Gas formation	+	+	-	+	-	+
H2S formation	+	-	-	-	-	-
TSIA	A/A	K/A	A/A	A/A	A/A	K/K
Identity	*Staphylococcus aureus*	*Micrococcus luteus*	*Bacillus subtilis*	*Klebsiella pneuminia*	*Escherichia coli*	*Pseudomonas aeruginosa*

Table 4.2: Percentage prevalence of bacteria isolate

Bacteria	Hall 2 car park CS	Hall 3 CS	Life science complex UCS	Library extension CS	Hall 3 UCS	Percentage prevalence %
Staphylococcus aureus	-	_	_	+	+	40
Micrococcus luteus	+	+	-	-	+	60
Bacillus subtilis	-	+	+	+	+	80
Klebsiella pneumonia	-	-	+	_	+	40
Escherichia coli	-	_	_	-	+	20
Pseudomonas aeruginosa	+	-	-	+	+	60

LEGEND: UCS = Urine control soil, CS = Control soil

Table 4.3: Total heterotrophic bacteria count of urine contaminated soil and control soil samples in University of Benin, Ugbowo Campus in cfu/ml.

Location	THBC 1	THBC 2	THBC 1 Cfu/ml	THBC 2 Cfu/ml	THBC 1	THBC 2	Mean±SE	SD	Variance	THBC 1 Log 10cfu	THBC 2 Log 10cfu	Average THBC 1/THBC 2
Hall 2 Car park control soil	42	47	4200	4700	$4.2x10^3$	$4.7x10^3$	44.5±2.5	3.56	12.5	3.6721	3.672098	3.6721
Hall 3 control soil	77	90	7700	9000	$7.7x10^3$	$9x10^3$	83.5±6.5	9.19	84.5	3.88649	3.954243	3.92037
Life science complex	19	20	1900	2000	$1.9x10^3$	$2x10^3$	19.5±0.5	0.71	0.5	3.27875	3.30103	3.28989
Library extension	35	48	3500	4800	$3.5x10^3$	$4.8x10^3$	41.5±6.5	9.19	84.5	3.54407	3.681241	3.61265
Hall 3 urine contaminated	40	32	4000	3200	$4.0x10^3$	$3.2x10^3$	36.0±4	5.66	32	3.60206	3.50515	3.5536

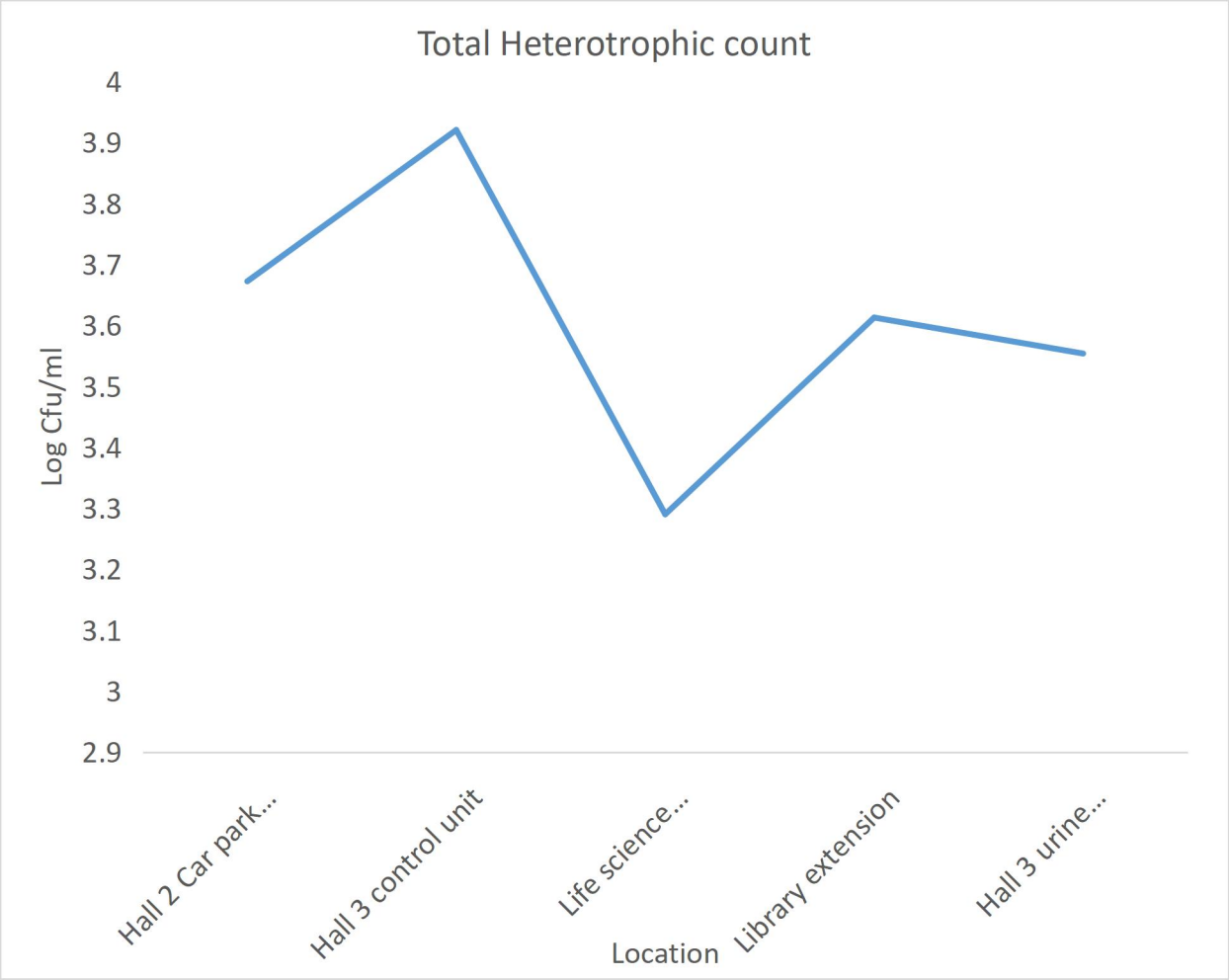

Figure 4.1: total heterotrophic bacteria count

Table 4.4: Antibacterial susceptibility test of isolates from urine contaminated soil and control soil samples from University of Benin, Ugbowo Campus

Bacteria	CIP	GEN	CB	TE	CS
Staphylococcus aureus	17(S)	14(S)	0(R)	15(S)	1(R)
Miocrococcus leteus	17.5(S)	16(S)	2(R)	14.5(S)	2(R)
Bacillus subtilis	15.5(S)	14(S)	1(R)	0(R)	1(R)
Klebsiella specie	16.0(S)	15.5(S)	0(R)	0(R)	0(R)
Escherichia coli	17(S)	15(S)	14(S)	14(S)	10.5(R)
Pseudomonas aeruginosa	17.5(S)	10.5(R)	0(R)	1(R)	2(R)

LEGEND: 1-12mm= Resistant, 13mm and above=Susceptibility, CIP = Ciprofloxacin, GEN = Gentamacin, CB = Carbenicillin, TE = Tetracycline, CS = Colistin

CHAPTER FIVE

DISCUSSION

According to the study, soils from public lavatories include opportunistic microbial species that are significant for both individual and societal health. In this investigation, bacterial isolates from studied soils concur with findings from Cheesebrough (2006). Despite being present in the soil samples, *Micrococcus luteus* is also a natural component of mammalian skin flora. In immunocompromised patients and individuals, this organism, which is often non-pathogenic and is generally classified as a contaminant, may be an emerging nosocomial pathogen.

Bacteria belonging to the genus *Micrococcus* can be found in a variety of settings, such as dirt, dust, and water. Beer, animal and dairy products, as well as human skin, have all been found to contain micrococci. They can also be present in water, dust, and soil, among other elements of the environment. In both natural settings and clinical specimens, *Micrococcus luteus* is the most prevalent species. In situations with little water or lots of salt, micrococci can thrive. Micrococcus cells can endure for a long time while not producing spores (Greenblat *et al*., 2004). Although it can be an opportunistic pathogen, *Micrococcus* is typically considered of as a saprotrophic or commensal organism, especially in hosts with weakened immune systems, such as HIV patients (Smith *et al*., 1999). Since the organism typically exists in skin microflora and the genus is rarely associated with disease, it might be challenging to pinpoint *Micrococcus* as the source of an infection. Infrequently, deaths utilized to treat these bacteria-related illnesses (Bannerman and Peacock, 2007). Although there is a low chance of infection, it is crucial to prevent unintentional parenteral inoculation, ingestion, and inhalation of infectious droplets (Lieb *et al*., 2002).

The diverse chemoheterotrophic species of the genus *Bacillus* that generate aerobic spores are able to respire using a range of simple organic molecules (sugars, amino acids, organic acids). B. cereus is a pathogen that affects humans (as well as other animals), causing opportunistic infections like endophtalmia, keratitis, septicemia, meningitis, endocarditis, pneumonia, osteomielitis, urinary infections, and cutaneous infections as well as food-borne illnesses like diarrheal-type and emetic-type syndromes. Additionally, it causes mastitis and abortion in cattle, among other illnesses in domestic animals (Logan, 2005).

Round, parasitic bacteria belonging to the *Staphylococcus* genus are frequently found in air, water, on human skin, and in the upper region of the throat. These bacteria are known to cause kidney and wound infections, boils, pneumonia, and septicemia. *Staphylococcus epidermidis* occurs everywhere in a benign symbiotic relationship, and it typically does not cause infection. The majority of the skin, as well as the mouth, external ear, urethra, and nostrils, typically have it.

Staphylococcus epidermidis can, however, exploit a host with a compromised immune system and exacerbate an already present illness. *Staphylococcus epidermidis* may induce endocarditis after heart surgery. A urinary tract abnormality may already present and develop into cystitis as a result of *Staphylococcus epidermidis* (Cheesebrough, 2006).

Urine has a pH of 5.6- 6.8. The pH of the urine-contaminated soil may have been lowered, turning it acidic, which favored fungus development over bacterial growth (Drangert, 2000). Particularly where sufficient sanitary facilities are lacking, the sediments from public urinals may play a significant role in the transmission of infection. Public urine-contaminated soil microbes have the ability to act as either primary or opportunistic pathogens, resulting in disease. These urine-contaminated soils have an unpleasant smell as well. People with UTIs excrete more microflora than people who appear to be in good health, which may explain why organisms

discovered in urine-contaminated soils. The microbial load within the bodily systems may rise above threshold levels as a result of frequent use of public restrooms. This supports the findings of the Hoglund *et al*. (2002). This would frequently lead to a diseased or infectious state. Visitors to these urinals therefore run the danger of getting opportunistic illnesses or disorders. In undeveloped nations, it's typical for people to urinate anywhere in public. The microbial load within the bodily systems may rise above threshold levels as a result of frequent use of public restrooms. This supports the findings of the Hoglund *et al*. (2002). This would frequently lead to a diseased or infectious state. Visitors to these urinals therefore run the danger of getting opportunistic illnesses or disorders. In undeveloped nations, it's typical for people to urinate anywhere in public. There has been a lot of research on the microflora of soil and urine, but not much on the microbiota of pee-contaminated soils. The main goals of this research were to increase understanding, spread awareness of the technique, and provide information.

5. 1. CONCLUSION AND RECOMMENDATION

Urine-contaminated soils are a threat to the public's health since they can be used as routes for the spread of infection. Lack of hygiene, population growth, and overcrowded facilities encourage indiscriminate incontinence in public areas. This method has the potential to alter the soil microbiota in urine-contaminated areas. In such soil settings, an increase in microbial load may raise the risk of getting opportunistic illnesses. The number of restrooms at the university is significantly below what is needed to accommodate the staff and the constantly increasing number of admitted students. Hostels are also overcrowded, which has the accompanying effect

of causing damage to the available restrooms and indiscriminate peeing surrounding the hostels. One cannot emphasize enough how important it is for the institution to provide more and better restrooms. Students' restrooms could be isolated from staff restrooms due to the generally poor sanitary habits and practices of students and to prevent overstretching of restroom facilities. Additionally, portable or water closet toilets might be made available. However, it is necessary to regularly dispose of the contents of the portable restrooms. Unquestionably, public spending on fundamental sanitation is important. WHO/UNICEF, (2000) found that following and enforcing basic sanitation laws would help prevent needless deaths and safeguard the health of millions of people.

REFERENCES

Adegbola, R.A., Old, D. C., Senior, B.W. (1983). The adhesins and fimbriae of *Proteus mirabilis* strains associated with high and low affinity for the urinary tract. *Journal of Medical Microbiology* **169**(1):427–431.

Allison, C., Lai, H-C., Gygi, D., Hughes, C. (1993). Cell differentiation of *Proteus mirabilis* is initiated by glutamine, a specific chemo attractant for swarming cells. *Journal of Molecular Microbiology* **8**:53–60.

Allison, C., Coleman, N., Jones, P.L. and Hughes C. (1992). Ability of *Proteus mirabilis* to invade human urothelial cells is coupled to motility and swarming differentiation. *Journal of Infection and Immunity* **60**:4740–4746.

Anderson, G. G., Dodson, K. W., Hooton, T. M., Hultgren, S. J. (2004). *Trends in Microbiolology* **12**(1):424–430.

Armbruster, C. E. and Mobley, H. L. T. (2012). Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. *Nature Review Microbiology* **10**:743–754.

Armbruster, C. E. Hodges, S. A. and Mobley, H. L. T. (2013). Initiation of swarming motility by *Proteus mirabilis* occurs in response to specific cues present in urine and requires excess L-glutamine. *Journal of Bacteriology* **195**:1305–131.

Arancibia, F., Bauer, T. T., Ewig, S., Mensa, J., Gonzalez, J., Niederman, M. S. and Torres, A. (2002). Community-acquired pneumonia due to Gram-negative bacteria and *Pseudomonas aeruginosa*: incidence, risk, and prognosis. *Archives of Internal Medicine* **162**: 1849–1858.

Barthel, M., Hapfelmeier, S., Quintanilla-Martinez, L., Kremer, M., Rohde, M., Hogardt, M., Pfeffer, K., Russmann, H. and Hardt, W.D. (2003). Pretreatment of mice with streptomycin provides a *Salmonella enterica* serovar Typhimurium colitis model that allows analysis of both pathogen and host. *Infections and Immunity* **71**: 2839–2858.

Baumler, A.J., Tsolis, R. M, Ficht T. A., Adams, L. G. (1998). Evolution of host adaptation in *Salmonella enterica*. *Infection and Immunity*; **66**: 4579–4587.

Belas, R, Manos, J., Suvanasuthi, R. (2004). *Proteus mirabilis* ZapA metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides. *Infections and Immunity* **72**:5159–5167.

Behrendt, U., Augustin, J., Sproer C, Gelbrecht, J., Schumann, P. and Ulrich, A. (2015). Taxonomic characterisation of *Proteus terrae* sp. nov., a N2O producing, nitrate-ammonifying soil bacterium. *Antonie Van Leeuwenhoek* **108**:1457–1468.

Bergmann, G. T, Bates, S. T, Eilers K.G., Lauber CL, Caporaso, J. G, Walters, W. A., Knight, R. and Fierer, N. (2011). The under-recognized dominance of Verrucomicrobia in soil bacterial communities. *Soil Biology & Biochemistry* **43**: 1450–1455.

Blohmke, C.J., Mayer, M.L., Tang, A.C., Hirschfeld, A.F, Fjell, C.D., Sze, M.A., Falsafi, R., Wang, S., Hsu, K., Chilvers, M.A., Hogg, J.C., Hancock, R.E. and Turvey, S.E. (2012). Atypical activation of the unfolded protein response in cystic fibrosis airway cells contributes to p38 MAPK-mediated innate immune responses. *Journal of Immunology* **189**: 5467–5475.

Biran, D., and Ron, E. Z. (2018). Extra intestinal Pathogenic *Escherichia coli*. *Current Topics in Microbiology and Immunology* **416**: 149–161.

Bridge, P. and Spooner, B. (2001). Soil fungi: Diversity and detection. *Plant and Soil* **232**(2):147-154.

Bonhoff, M, Drake, B. L., Miller, C.P. (1954). Effect of streptomycin on susceptibility of intestinal tract to experimental *Salmonella* infection. *Proceedings of the Society for Experimental Biology and Medicine* **86**: 132–137.

Brzuszkiewicz, E., Gottschalk, G., Ron, E., Hacker, J. and Dobrindt, U. (2009). Adaptation of Pathogenic *Escherichia coli* to Various Niches: Genome Flexibility is the Key. *Genome Dynamics* **6**: 110–125.

Bragonzi, A., Paroni, M., Nonis, A., Cramer, N., Montanari, S., Rejman, J., Di Serio, C., Doring, G. and Tummler, B. (2009). *Pseudomonas aeruginosa* microevolution during cystic fibrosis lung infection establishes clones with adapted virulence. *American Journal of Respiratory and Critical Care Medicine* **180**: 138–145.

Boll, E. J., Overballe-Petersen, S., Hasman, H., Roer, L., Ng, K., Scheutz, F., Hammerum, A.M., Dungu, A., Hansen, D.S., Krogfelt, K.A., Johnson, T.J., Price, L.B., Johnson, J.R., Struve, C., Olesen, B and Stegger, M. (2020). Emergence of enteroaggregative *Escherichia coli* within the ST131 lineage as a cause of extraintestinal infections. *Millenium Bio Therapeutics* **11**(3): e00353–e00420.

Bol, R., S.O. Petersen, C., Christofides, K., Dittert, G., and Hansen, M. N. (2004). Short-term N2O, CO2, NH3 fluxes, and N/C transfers in a Danish grass-clover pasture after simulated urine deposition in autumn. *Journal of Plant Nutrition and Soil Science / Zeitschrift für Pflanzenernährung und Bodenkunde* **167**:568-576.

Cag, Y., Caskurlu, H., Fan, Y., Cao, B., and Vahaboglu, H. (2016). Resistance mechanisms. *Annals of Translational Medicine* **4** (17): 326.

Carter, M. R. and Gregorich, E. G. (2007). Soil sampling and methods of analysis. CRC press.

Cheesebrough, M. (2006). District laboratory practice in tropical countries. Cambridge University press, UK. Second low price edition. p. 434.

Chukwu, V. A., Nnadozie, T. N. and Nsemoh, H. E. (2018). Physicochemical and Microbiological Studies of Urine Contaminated Soil in Abia State University, Uturu Campus. *Current Trends Biomedical Engineering & Bioscience* **14**(1): 555-877.

Chow, A. W., Taylor, P. R., Yoshikawa, T. T. and Guze, L. B. (1979). A nosocomial outbreak of infections due to multiply resistant *Proteus mirabilis*: role of intestinal colonization as a major reservoir. *Journal of Infectious Disease* **139**:621–627.

Clunes, J., and Pinochet, D. (2021). Leucine retention by the clay-sized mineral fraction. An indicator of C storage. *Agronomy Survey* **48**(3):37-46.

Clough, T.J., Ledgard, S.F., Sproson, M.S. and Kear, M.J. (1998). Fate of 15N labelled urine on four soil types. *Plant and Soil* **199**:195-203.

Coker, C., Poore, C.A., Li, X., Mobley, H. L. T. (2000). Pathogenesis of *Proteus mirabilis* urinary tract infection. *Microbes Infections* **2**:1497–1505.

Dada, E.O. and Aruwa, C. E. (2014). Microorganisms associated with urine contaminated soils around lecture theatres in Federal University of Technology, Akure, Nigeria. *International Journal of Applied Microbiology and Biotechnology Research* **2**(6): 79-85.

Davis, K. E., Sangwan, P. and Janssen, P. H. (2011). Acidobacteria, Rubrobacteridae and Chloroflexi are abundant among very slow-growing and mini-colony forming soil bacteria. *Environmental Microbiology* **13**: 798–805.

Dexter, A.R. (2002). Soil structure: the key to soil function. In: Sustainable land management – environmental protection, a soil physical approach. Eds. M. Pagliai and R. Jones. *Advances in GeoEcology* **35**: 57–69.

De Boer, W., Leveau, J. H. J., Kowalchuk, G. A., Klein Gunnewiek, P. J. A., Abeln, E. C. A., Figgge, M. J., Sjollema, K., Janse, D.J. and Veen van, A.J. (2004). *Collimonas fungivorans* gen. nov., sp. nov., a chitinolytic soil bacterium with the ability to grow on living fungal hyphae. *International Journal of Systematic and Evolutionary Microbiology* **54**: 857–864.

Di Stefano, C., Ferro, V. and Mirabile, S. (2010). Comparison between grain-size analyses using laser diffraction and sedimentation methods. *Biosystem Engineering*. **106**(2):205-215.

Dobrindt, U., Chowdary, M.G., Krumbholz, G. and Hacker, J. (2010). Genome dynamics and its impact on evolution of *Escherichia coli*. *Medical Microbiology and Immunology* **199** (3): 145–154.

Drangert, J. O. (2000). Resuse– The ultimate sink: Urine-diverting toilets to protect groundwater quality and fertilise urban agriculture. In: Chorus, I., Ringelband, G. Schla, G. and Schmll, O. (eds), Water, sanitation and health.

Dutta, S., Pazhani, G.P., Nataro, J.P., and Ramamurthy, T. (2015). Heterogenic virulence in a diarrheagenic *Escherichia coli*: evidence for an EPEC expressing heat-labile toxin of ETEC. *International Journal Medical Microbiology* **305**: 47–54.

Dwivedi, D.J., Tang, N., Bouskill, K., Georgiou, S.S., Chacon, and Riley, W.J. (2019). Abiotic and biotic controls on soil organo–mineral interactions: developing model structures to analyze why soil organic matter persists. *Reviews in Mineralogy and Geochemistry* **85**(1):329-348.

Early, M.S.B., Cameron, K.C. and Fraser, P.M. (1998). The fate of potassium, calcium, and magnesium in simulated urine patches on irrigated dairy pasture soil. *New Zealand Journal of Agricultural Research* **41**:117-124.

Eilers, K.G., Lauber, CL., Knight, R. and Fierer, N. (2010). Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biology and Biochemistry* **42**: 896–903.

Elaine, I. R. (2009). The soil quality concept. United State Department of Agriculture (USDA). National Resource Conservation Services (NRCS). Oregon State University.

Fierer, J. and Guiney, D.G. (2001). Diverse virulence traits underlying different clinical outcomes of *Salmonella* infection. *Journal of Clinical Investigation* **107**: 775–780.

Fierer, N, Bradford, M.A. and Jackson, R.B. (2007). Towards an ecological classification of soil bacteria. *Ecology* **88**: 1354–1364.

Fields, P.I, Swanson, R.V., Haidaris, C.G. and Heffron, F. (1986). Mutants of *Salmonella* Typhimurium that cannot survive within the macrophage are avirulent. *Proceeding of the National Academy of Sciences United State of America* **83**: 5189–5193.

Fujihara, M., Obara, H., Watanabe, Y., Ono, H.K., Sasaki, J., Goryo, M., Harasawa, R. (2011). Acidic environments induce differentiation of *Proteus mirabilis* into swarmer

morphotypes. *Microbiology and Immunology* **55**:489–493. Gatermann, S., Marre, R., Heesemann, J. and Henkel, W. (1988). *FEMS Microbiology and Immunology* **1**:179-185.

Gatermann, S., John, J. and Marre, R. (1989). *Infections Immunity* **57**: 110–116.

Glover, J.D., Reganold, J.P. and Andrews, P.K. (2000). Systematic method for rating soil quality of conventional, organic and integrated apple orchards in Washington State. *Agriculture, Ecosystems and Environment* **80**(2):29–45.

Greenblat, C. L., Baum, J., Klein, B. Y., Nachshon S., Koltunov V. and Cano, R. J. (2004). *Micrococcus luteus* – Survival in amber. *Microbial Ecology* **48**(1):120–127.

Gorvel, J.P. and Meresse, S. (2001) Maturation steps of the *Salmonella*-containing vacuole. *Microbes Infection* **3**(1): 1299–1303.

Gorde, S.P. (2013). *International Journal of Engineering Research and Applications*; **3**(6):2029-2035.

Gugino, B.K., Idowu, O.J., Schindelbeck, R.R., van Es, H.M., Wolfe, D.W., Moebius-Clune, B.N., Theis, J.E. and Abawi, G.S. (2009). Cornell soil health assessment training manual, 2nd edn (Cornell University: Geneva, NY).

Halverson, L.J., Jones, T.M. and Firestone, M.K. (2000). Release of intracellular solutes by four soil bacteria exposed to dilution stress. *Soil Science Society of America Journal* **64** (1):1630-1637.

Hacker, J., Hentschel, U. and Dobrindt, U. (2003). Prokaryotic chromosomes and disease. *Science* **301** (5634), 790–793.

Hell, W., Meyer, H.G. and Gatermann, S. G. (1998). *Molecular Microbiology* **29**: 871–881.

Hiramatsu, K., Watanabe, S., Takeuchi, F., Ito, T. and Baba, T. (2004).Structural comparison of Ten Serotypes of Staphylocoagulases in *Staphlococcus aureus*. *Journal of Bacteriology* **22**(1): 5–8.

Hidron, A.I., Edwards, J.R., Patel, J., Horan, T.C., Sievert, D.M., Pollock, D.A. and Fridkin, S.K. (2008). National Healthcare Safety Network Team & Participating National Healthcare Safety Network Facilities NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infection Control and Hospital Epidemiology* **29**: 996–1011.

Hornick, R.B. (1970). Pathogenesis of typhoid fever. *Journal of Egypt Public Health Association* **45**: 247–259.

Hoglund, C., Ashbolt, N. and Strenstrom, T. A. (2002). Microbial risk assessment of source-separated urine used in agriculture. *Waste Management and Research* **20**:162-171.

Höglund, C., Ashbolt, N., Stenström T.A. and Svensson L. (2002). Viral persistence in source separated human urine. *Advances in Environment Research* **6**:265–75.

Heinonen-Tanski, H., van Wijk-Sijbesma, C. (2005). Human excreta for plant production. *Bioresource Technology* **96**:403–11.

Hogardt, M. and Heesemann J. (2010). Adaptation of *Pseudomonas aeruginosa* during persistence in the cystic fibrosis lung. *International Journal of Medical Microbiology* **300**: 557– 562.

Hyun, D.W., Jung, M.J, Kim, M.S., Shin, N.R., Kim, P.S., Whon, T.W. and Bae, J.W. (2016). *Proteus cibarius* sp. nov., a swarming bacterium from Jeotgal, a traditional Korean fermented seafood, and emended description of the genus *Proteus*. *International Journal Systematic and Evolutionary Microbiology* **66**:2158–2164.

Ito, T., Ma, X.X., Takeuchi, F., Okuma, K., Yuzawa, H. and Hiramatsu, K. (2004). *Antimicrobial Agents Chemotherapy* **48**: 2637–2651.

Jameson, E., Fu, T., Brown, I.R., Paszkiewicz, K., Purdy, K.J., Frank,S. and Chen, Y. (2016). Anaerobic choline metabolism in micro compartments promotes growth and swarming of *Proteus mirabilis*. *Environmental Microbiology* **18**:2886–2898.

Janssen, P.H. (2006). Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. *Applied and Environmental Microbiology* **72**(1): 1719–1728.

Jansen, A.M., Lockatell, V., Johnson. D.E. and Mobley, H.L.T. (2004). Mannose resistant *Proteus*-like fimbriae are produced by most *Proteus mirabilis* strains infecting the urinary tract, dictate the invivolocalization of bacteria, and contribute to biofilm formation. *Infectious Immunity* **72**(1):7294–7305.

Jones, R.T., Robeson, M.S., Lauber, C.L., Hamady, M., Knight, R. and Fierer, N. (2009). A comprehensive survey of soil Acidobacterial diversity using pyrosequencing and clone library analyses. *The ISME Journal* **3**(1): 442–453.

Jones, B.D., Ghori, N. and Falkow, S. (1994). *Salmonella* Typhimurium initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. *Journal of Experimental Medicine* **180**: 15–23.

Joanne, M. W., Linda, M. S., and Christopher J. W. (2008). Prescott harley and Klein`s microbiology. McGraw Hill. 7th edition. p. 1088.

Johnson, J. R., and Russo, T. A. (2018). Molecular Epidemiology of Extraintestinal Pathogenic *Escherichia coli*. *EcoSal Plus* **8** (1): 4–22.

Kant, R., van Passel, M.W.J., Palva, A., Lucas, S., Lapidus, A., Del Rio, T. G., Dalin, E. Tice, H., Bruce, D., Goodwin, L., Pitluck, S., Larimer, W. F., Land, L.M., Hauser, L., Sangwan, P., de Vos, M.W., Janssen, H.P. and Smidt, H. (2011). Genome sequence of *Chthoniobacter flavus* Ellin 428, an aerobic heterotrophic soil bacterium. *Journal of Bacteriology* **193**(1): 2902–2903.

Kahlmeter, G. (2003). *Journal of Antimicrobial Chemotherapy* **51**: 69–76.

Kekane, S.S R.P., Chavan, D.N. Shinde, C.L. and Patil, S.S. (2015) *Sagar International Journal of Chemical Studies* **3**(4): 29-32.,

Knuttson, M. and Kidd-Ljunggen, K. (2000). Urine from chronic hepatitis B virus carriers: Implications for infectivity. *Journal of Medical Virology* **60**(1):17-20

Kleber, M., K. Eusterhues, M. Keiluweit, C. Mikutta, R. Mikutta, and Nico, P.S. (2015). Mineral–organic associations: formation, properties, and relevance in soil environments. *Advances in Agronomy* **130**:1-140

Kettler, T.A., Doran, J.W. and Gilbert, T.L. (2001). Simplified method for soil particle-size determination to accompany soil-quality analyses. *Soil Science Society of America Journal* **65**(3):849-852.

Keesstra,S., Pereira, P., Novara, A., Brevik, E. C., Azorin-Molina, C., Parras-Alc´antara, L, Jord´an, A., Cerd`a A. (2016). Effects of soil management techniques on soil water erosion in apricot orchards. *Science Total Environment* **551**: 357–366.

Kieft, T.L., Soroker, E. and Firestone, M.K. (1987). Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology & Biochemistry* **19**:119-126.

Kirchhof, G. and Daniells, I. (2001). Soil structure: the key to sustainable agro-ecosystem management. In: Soil health – the foundation of sustainable agriculture. Proceedings of a Workshop on the Importance of Soil Health in Agriculture, Wollongbar, NSW, Australia, 20–21 June 2001. Ed. R. Lines-Kelly (NSW Agriculture: Sydney, NSW) Pp. 81–87.

Kirchmann, H., and Peterson, S. (1995). Human urine-chemical composition and fertilizer efficiency. *Fertilizer Research* **40**:149–54.

Köhler, C. D., and Dobrindt, U. (2011). What defines extra intestinal pathogenic *Escherichia coli*? *International Journal of Medical Microbiology* **301** (8): 642–647.

Khan, A., Miller, W. R., and Arias, C. A. (2018). Mechanisms of antimicrobial resistance among hospital-associated pathogens. *Expert Review of Anti Infective Therapy* **16** (4): 269–287.

Kuan, L., Schaffer, J.N., Zouzias, C.D. and Pearson, M.M. (2014). Characterization of 17 chaperone- usher fimbriae encoded by *Proteus mirabilis* reveals strong conservation. *Journal of Medical Microbiology* **63**(1):911–922.

Ku, S. T. and Sangita, I. (2015). A Review on Role of Physico-Chemical Properties in Soil Quality. *Chemical Science Review and Letters* **4**(13):57-66.

Larson, W.E. and Pierce, F.J. (1991) Conservation an enhancement of soil quality. In: Evaluation for sustainable land management in the developing world vol. 2. Technical Papers, Bangkok, Thailand. Eds J. Dumanski, E. Pushparajah, M. Latham and R. Myers (International Board for Soil Research and Management: Bangkok, Thailand) Proceedings No. 12(2).

Latta, R.K., Grondin, A., Jarrell, H.C., Nicholls, G.R. and Berube, L.R. (1999). Differential expression of non-agglutinating fimbriae and MR/P pili in swarming colonies of *Proteus mirabilis*. *Journal of Bacteriology* **181**:3220–3225.

Lane, M.C., Li, X., Pearson, M.M., Simms, A.N. and Mobley, H.L. (2009). Oxygenlimiting conditions enrich for fimbriate cells of uropathogenic *Proteus mirabilis* and *Escherichia coli*. *Journal of Bacteriology* **191**:1382–1392

Lauber, C.L, Hamady, M., Knight, R. and Fierer, N. (2009). Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* **75**(1) 5111–5120.

Lienert, J., Güdel, K. and Escher, B. (2003). Screening method for ecotoxicological hazard assessment of 42 pharmaceuticals considering human metabolism and excretory Technology **48**(1):47–56.

Lee, K.K, Harrison, B.A, Latta, R., Altman, E. (2000). The binding of *Proteus mirabilis* non agglutinating fimbriae to ganglio-series asialoglycolipids and lactosyl ceramide. *Journal of Clinical Microbiology* **46**(1):961–966.

Leterme, P., Barre, C. and Vertes, F. (2003). The fate of 15N from dairy cow urine under pasture receiving different rates of N fertiliser. *Agronomie* **23**:609-616.

Li, X, Zhao, H., Lockatell, C.V, Drachenberg, C.B., Johnson, D.E. and Mobley, H.L.T. (2002). Visualization of *Proteus mirabilis* within the matrix of urease induced bladder stones during experimental urinary tract infection. *Infectious diseases and Immunity* **70**:389–394.

Liebl, W., Kloos, W. E. and Ludwig, W. (2002). Plasmid-borne macrolide resistance in *Micrococcus luteus*. *Microbiology (Reading, England)* **148**(8):2479-2487.

Logan, N. A. (2010*). Bacillus anthracis*, *Bacillus cereus*, and other aerobic endospore-forming bacteria. In: Boriello, S. P., Murray, P. R.and Funke G. (Ed), Topley and Wilson's microbiology and microbial infections. *Bacteriology* **4**(2):922-952.

Lyczak, J.B, Cannon, C.L. and Pier, G. (2000). Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Journal of Microbiology and Infectious diseases* **2**(1): 1051–1060.

MacEwan, R.J. (2007). Soil health for Victoria's agriculture context, terminology and concepts. MIS07898 Final Report. Department of Primary Industries (Victorian Government: Melbourne,Vic.).

Manos, J. and Belas, R. (2006).The genera *Proteus, Providencia*, and *Morganella*. Pp 245–269.

Maurer, M., Schwegler, P., Larsen, T.A. (2003). Nutrients in urine: energetic aspects of removal and recovery. *Water Science and Technology* **48**(1):37–46.

Mena, A., Smith, E.E, Burns, J.L., Speert, D.P., Moskowitz, S.M., Perez, J.L. and Oliver, A. (2008). Genetic adaptation of *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients is catalyzed by hyper mutation. *Journal of Bacteriology* **190**(1): 7910–7917.

Meresse, S., Steele-Mortimer, O., Finlay, B.B. and Gorvel, J.P. (1999). The rab7 GTPase controls the maturation of *Salmonella typhimurium*-containing vacuoles in HeLa cells. *EMBO Journal* **18**(1): 4394–4403.

Milovic, V. (2001). Polyamines in the gut lumen: bioavailability and biodistribution. *European Journal of Gastroenterology and Hepatology* **13**(1):1021–1025.

Mobley, H.L and Belas, R. (1995). Swarming and pathogenicity of *Proteus mirabilis* in the urinary tract. *Trends in Microbiology* **3**:280–284.

Monaghan, R.M. and Barraclough, D. (1993). Nitrous oxide and dinitrogen emissions from urine-affected soil under controlled conditions. *Plant and Soil* **151**(1):127-138.

Mukhopadhya, I., Hansen, R., El-Omar, E.M. and Hold, G.L. (2012). IBD—what role do Proteobacteria play? *Nature Reviews Gastroenterology and Hepatology* **9**(1):219–230.

Murphy, T.F., Brauer A.L., Eschberger, K., Lobbins, P, Grove, L., Cai, X. and Sethi, S. (2008). *Pseudomonas aeruginosa* in chronic obstructive pulmonary disease. *America Journal of Respiratory and Critical Care Medicine* **177**(1): 853– 860.

Murray, R.S. and Grant, C.D. (2007). The impact of irrigation on soil structure. National Committee on Soil and Terrain (2009) Australian soil and land survey field handbook, Third edn (CSIRO Publishing: Collingwood, Vic.) Pp. 186–187.

Muniesa, M., Hammerl, J. A., Hertwig, S., Appel, B., and Brüssow, H. (2012). Shiga toxin-producing *Escherichia coli* O104: H4: a new challenge for microbiology. *Applied Environmental Microbiology* **78** (12): 4065–4073.

Nakoneczna, I. and Hsu, H.S. (1980). The comparative histopathology of primary and secondary lesions in murine salmonellosis. *British Journal of Experimental Pathology* **61**(1): 76–84.

Naito, T., Kusano, K. and Kobayashi, I. (1995). *Science* **267**: 897–899.

Navarro-Garcia, F. (2014). *Escherichia coli* O104:H4 Pathogenesis: an Enteroaggregative *Escherichia coli*/Shiga Toxin-Producing *Escherichia coli* Explosive Cocktail of High Virulence. *Microbiology Spectrum* **2** (6): 2–15.

Nataro, J.P. and Kaper, J.B. (1998). Diarrheagenic *Escherichia coli*. *Clinical Microbiology Review* **11**(1):142–201.

Nemergut, D.R., Costello, E.K., Hamady, M., Lozupone, C., Jiang, L., Schmidt, S *et al*. (2011). Global pattern in the biogeography of bacterial taxa. *Environmental Microbiology* **13**(1): 135–144.

Nugent, S.G., Kumar, D., Rampton, D.S. and Evans, D.F. (2001). Intestinal luminal pH in inflammatory bowel disease: possible determinants and implications for therapy with aminosalicylates and other drugs. *Gut* **48**(1): 571–577.

O'Hara, C.M., Brenner, F.W. and Miller, J.M. (2000). Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clininical Microbiology Review* **13**(1):534–546.

Ochman, H. and Groisman, E.A. (1994). The origin and evolution of species differences in *Escherichia coli* and *Salmonella* Typhimurium. *EXS* **69**: 479–493.

Osakwe, S.A. (2014). Evaluation of Physicochemical Characteristics of Soils in the Flood Disaster Affected Areas of Isoko Region of Delta State, Nigeria. *IOSR Journal of Applied Chemistry (IOSR-JAC)* **I7**(5):24-31.

Oliver, A. (2010). Mutators in cystic fibrosis chronic lung infection: prevalence, mechanisms, and consequences for antimicrobial therapy. *International Journal of Medical Microbiology* **300**(1): 563–572.

Olutiola, P. O., Famurewa, O. and Sonntag, H. G. (1991). An Introduction to general microbiology: A practical approach: Heidelberger Verlagsan salt und DruckereiGmbH. Heidelberg. p. 267.

Olutiola, P. O., Famurewa, O. and Sonntag, H. G. (2001). Biochemical reaction of microorganisms. In: An introduction to general microbiology: A practical approach. Bolabay Publications. Pp. 157-177.

Pattison, A.B., Moody, P.W., Badcock, K.A., Smith, L.J., Armour, J.A., Rasiah, V., Cobon, J.A., Gulino, L.M. and Mayer, R. (2008). Development of key soil health indicators for the Australian banana industry. *Applied Soil Ecology* **40**: 155–164.

Pagliai, P. and Vignozzi, N. (2002). The soil pore system as an indicator of soil quality. In: Sustainable land management – environmental protection, a soil physical approach. Eds. M. Pagliai and R. Jones. *Advances in GeoEcology* **35**: 71–82.

Parry, C.M., Hien, T.T., Dougan, G., White, N.J. and Farrar, J.J. (2002). Typhoid fever. *New England Journal of Medicine* **347**(1): 1770–1782.

Pearson, M.M, Rasko DA, Smith, S.N. and Mobley, H.L.T. (2010). Transcriptome of swarming *Proteus mirabilis*. *Infectious diseases and Immunity* **78**(1):2834–2845.

Pearson, M.M, Sebaihia, M., Churcher, C., Quail, M.A., Seshasayee, A.S., Luscombe, N.M, Abdellah,Z., Arrosmith, C., Atkin, B., Chillingworth ,T., Hauser, H., Jagels, K., Moule, S., Mungall, K., Norbertczak, H., Rabbinowitsch, E., Walker, D., Whithead, S., Thomson, N.R, Rather, P.N., Parkhill, J. and Mobley, H.L.T. (2008). Complete genome sequence of uropathogenic *Proteus mirabilis*, a master of both adherence and motility. *Journal of Bacteriology* **190**: 4027–4037.

Pearson, M.M. and Mobley, H.L.T. (2008). Repression of motility during fimbrial expression: identification of fourteen mrpJ gene paralogs in *Proteus mirabilis*. *Molecular Microbiology* **69**(1):548–558.

Paterson, E., and A. Sim. (1999). Rhizodeposition and C-partitioning of Lolium perenne in axenic culture affected by nitrogen supply and defoliation. *Plant and Soil* **216**:155-164.

Plante, A.F., R.T. Conant, C.E. Stewart, K. Paustian, and J. Six. (2006). Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. *Soil Science Society America Journal* **70**(1):287-296.

Penner, J.L. (2005). Genus XXIX. Proteus, In Brenner DJ, Krieg NR, Staley JT, Garrity GM (ed), Bergey's manual of systematic bacteriology, 2nd ed, vol 2. The Proteobacteria: part B, the Gammaproteobacteria. Lippincott Williams & Wilkins, Philadelphia, PA. Pp. 745–753.

Peigne, C., Bidet, P., Mahjoub-Messai, F., Plainvert, C., Barbe, V., Médigue, C., Frapy, E., Nassif, X., Denamur, E., Bingin, E. and Bonacorsi, S. (2009). The plasmid of *Escherichia coli* strain S88 (O45:K1:H7) that causes neonatal meningitis is closely related to avian pathogenic E. coli plasmids and is associated with high-level bacteremia in a neonatal rat meningitis model. *Infections and Immunity* **77** (6): 2272–2284.

Pickard, J.M., Zeng, M.Y., Caruso, R. and Nunez, G. (2017). Gut microbiota: role in pathogen colonization, immune responses, and inflammatory disease. *Immunology Reviews* **279**(1):70–89.

Provinciali, M., Cardelli, M. and Marchegiani, F. (2011). Inflammation, chronic obstructive pulmonary disease and aging. *Current Opinion in Pulmonary Medicine* **17**(1): 3–10.

Qi, F., Zhang, R., Liu, X., Niu, Y., Zhang, H., Li, H., Li, J., Wang, B. and Zhang, G. (2018). Soil particle size distribution characteristics of different land-use types in the Funiu mountainous region. *Soil and Tillage Research* **184**(1):45-51.

Rasko, D. A., Moreira, C. G., de Li, R., Reading, N. C., Ritchie, J. M., Waldor, M. K., Williams, N., Taussig, R., Wei, S., Roth, M., Hughes, T.D., Huntley, F. J., Fina, W.M., Falck, R.J. and Sperandio, V. (2008). Targeting QseC signaling and virulence for antibiotic development. *Science* **321**(5892):1078–1080.

Rather, P.N. (2005). Swarmer cell differentiation in *Proteus mirabilis*. *Environmental Microbiology* **7**:1065–1073.

Rattan, L. (1991). Soil Structure and Sustainability. *Journal of Sustainable Agriculture* **1**(4): 67-92.

Rescigno, M., Urbano, M., Valzasina, B., Francolini, M., Rotta, G., Bonasio, R., Granucci, F., Kraehenbuhi, P.J. and Castagonoli, R.P. (2001) Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nature Immunol*ogy **2**(1): 361–367.

Ritz, K., McHugh, M. and Harris, J. (2003). Biological diversity and function in soils: contemporary perspectives and implications in relation to the formulation of effective indicators. OECD Expert Meeting on Soil Erosion and Soil Biodiversity Indicators, Rome, March 2003. Pp. 1–11.

Richter-Dahlfors, A., Buchan, A.M. and Finlay, B.B. (1997) Murine salmonellosis studied by confocal microscopy: *Salmonella* Typhimurium resides intracellularly inside macrophages and exerts a cytotoxic effect on phagocytes in vivo. *Journal of Experimental Medicine* **186**(1): 569–580.

Rocha, S.P., Pelayo, J.S. and Elias, W.P. (2007). Fimbriae of uropathogenic *Proteus mirabilis*. *FEMS Immunology and Medical Microbiology* **51**(1):1–7.

Rodushkin, I. and Odman, F. (2001). Application of inductively coupled mass spectrometry for elemental plasma sector field analysis of urine. *Trace Element Medical Biology* **14**(1):241–7.

Rudiyanto ., Minasny, B. and Setiawan, B. I. (2016). Further results on comparison of methods for quantifying soil carbon in tropical peats. *Geoderma* **269**: 108–111.

Russell, E. S., Liu, H. P., Gao, Z. M., Finn, D. and Lamb, B. (2015). Impacts of soil heat flux calculation methods on the surface energy balance closure. *Agriculture for Meteorology* **214-215**: 189–20.

Shand, C.A., Williams, B.L., Dawson, L.A., Smith, S. and Young, M.E. (2002). Sheep urine affects soil solution nutrient composition and roots: Differences between field and sward box soils and the effects of synthetic and natural sheep urine. *Soil Biology and Biochemistry* **34**(1):163-171.

Stecher, B., Macpherson, A.J, Hapfelmeier, S., Kremer, M., Stallmach, T. and Hardt, W.D. (2005). Comparison of *Salmonella enterica* serovar Typhimurium colitis in germfree mice and mice pretreated with streptomycin. *Infectious Diseases and Immun*ity **73**(7): 3228–3241.

Sandoval, M., J. Dörner, O. Seguel, J. Cuevas, D. Rivera. (2012). Métodos de análisis físicos de suelos. Chillan: Publicaciones - Departamento de Suelos y Recursos Naturales.

Santos, A. C. M., Santos, F. F., Silva, R. M., and Gomes, T. A. T. (2020). Diversity of Hybrid- and Hetero-Pathogenic *Escherichia coli* and Their Potential Implication in More Severe Diseases. Front. Cell. *Infectious Microbiology* **10** (1): 339.

Steele-Mortimer, O., Meresse, S., Gorvel, J.P., Toh, B.H., Finlay, B.B. (1999). Biogenesis of *Salmonella* Typhimurium-containing vacuoles in epithelial cells involves interactions with the early endocytic pathway. *Cell Microbiology* **1**: 33–4.

Saroj, M. and Dilip, B. (2014). Assessment of Physic and Paustian, K. (2002). Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. *Plant Soil* **241**(2):155-176.

Smith, K., Neafie, R., Yeager, J. and Skelton, H. (1999). *Micrococcus* folliculitis in HIV-1 disease. *British Journal of Dermatology* **141**(3):558–561.

Solanki, H.A. and Chavda, N.H. (2012). Physicochemical analysis with reference to seasonal changes in soils of Victoria park reserve forest, Bhavnagar (Gujarat). *Life sciences Leaflets* **8**(1):62-68.

Stecher, B., Macpherson, A.J., Hapfelmeier, S., Kremer, M., Stallmach, T. and Hardt, W.D. (2005). Comparison of *Salmonella enterica* serovar Typhimurium colitis in germfree mice and mice pretreated with streptomycin. *Infectious Diseases and Immun*ity **73**: 3228–3241.

Sturgill, G. and Rather, P.N. (2004). Evidence that putrescine acts as an extracellular signal required for swarming in *Proteus mirabilis*. *Molecular Microbiology* **51**(2)437–446.

Sparling, G.P., Schipper, L.A., Hill, R. and Bettjeman, W. (2004). Soil quality monitoring in New Zealand: practical lessons from a six-year trial. *Agricultural Ecosystems and Environment* **104**(2): 523–534.

Solanki, H.A and Chavda, N.H. (2012). Physicochemical analysis with reference to seasonal changes in soils of Victoria park reserve forest, Bhavnagar (Gujarat). *Life sciences Leaflets* **8**:62-68.

Sadikot, R.T, Blackwell, T.S, Christman, J.W. and Prince, A.S. (2005). Pathogen-host interactions in *Pseudomonas aeruginosa* pneumonia. *America Journal Respiratory and Critical* Care *Medicine* **171**(7): 1209–1223.

Smith, E.E, Buckley, D.G, Wu, Z., Saenphimmachak, C., Hoffman, R.L., D'Argenio, A.D., Miller, I.S., Ramsey, W.B., Speert. P.D., Moskowitz, M.S., Burns, L.J., Kaul, R. and Olson. V.M. (2006). Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proceedings of the National Academy of Sciences of the United States of America* 1**03**(6): 8487–8492.

Sørensen, P., and E.S. Jensen. (1996). The fate of fresh and stored 15N-labelled sheep urine and urea applied to a sandy and a sandy loam soil using different application strategies. *Plant and Soil* **183**(2):213-220.

Uroz, S., Calvaruso, C., Turpault, M.P., Pierrat, J.C., Mustin, C., Frey-Klett, P. (2007). Effect of the mycorrhizospere on the genotypic and metabolic diversity of the bacterial communities involved in mineral weathering in a forest soil. *Applied and Environmental Microbiology* **73**(2): 3019–3027.

Van Passel, W.J. M., Kant, R., Palva, A., Copeland, A., Lucas, S., Copeland, A., Lucas, S., Lapidus, A., del Rio, G. T., Pitluck, S., Schmutz, J., Larimer, W. F., Land, L. M., Hauser, L., Kyrpides, N., Mikhailavo, N., Richardson, P. P., Janssen, H. P., de Vos, M.W. and Smidt, H. (2011). Genome sequence of the Verrucomicrobium Opitutus terrae PB90-1, an abundant inhabitant of rice paddy soil ecosystems. *Journal of Bacteriology* **193**: 2367–2368.

Vazquez-Torres A., Jones-Carson, J., Baumler, A.J., Falkow, S., Valdivia R., Brown, W., Le, M., Berggren, R., Parks, W.T. and Fang, F.C. (1999). Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. *Nature* **401**: 804–808.

Velescu, A., T. Meßmer, T. Scholten, and P. Kühn. (2010). Removal of short-range-order minerals prior to grain-size analysis of volcanic ash soils. *Journal of Plant Nutrition and Soil Science* **173**(6):799-804.

Vinnerås, B. and Jönsson, H. (2002). The performance and potential of faecal separation and urine diversion to recycle plant nutrients in house hold wastewater. *Bioresource Technology* **84**:275–82.

Von Eiff, C., Peters, G. and Heilmann, C. (2002). *Lancet Infectious Diseases* **2**: 677–685.

Vazquez-Torres A, Xu Y.S., Jones-Carson, J, Holden, D.W., Lucia, S.M., Dinauer, M.C., Mastroeni, P., and Fang, F.C. (2000). *Salmonella* Pathogenicity Island 2-dependent evasion of the phagocyte NADPH oxidase. *Science* **287**: 1655–1658.

Ward, N.L., Challacombe, J.F., Janssen, P.H.., Henrissat, B., Coutinho, M.W., Wu, M., Xie, G., Haft, D.H., Sait, M., Badger, J., Barabote, R.D., Bradley, B., Brettin, T.S., Brinkac, L.M., Bruce, D., Creasy, T., Daugherty, S.C., Davidson, T.M., DeBoy, R.T., Detter, J.C., Dodson, R.J., Durkin, A.S., Ganapathy, A., Gwinn-Giglio, M., Han, C.S., Khouri, H., Kiss, H., Kothari, S.P., Madupu, R., Nelson, K.E., Nelson, W.C., Paulson, I., Penn, K., Ren, Q., Rosovitz, M.J., Selengut, J.D., Shrivastava, S., Sullivan, S.A., Tapia, R., Thompson, L.S., Watkins, K.L., Yang, Q., Yu, C., Zafar, N., Zhou, L. and Kuske, C.R. (2009). Three genomes from the phylum Acidobacteria provide insight into the

lifestyles of these microorganisms in soils. *Applied and Environmental Microbiology* **75**(1): 2046–2056.

Wallmark, G., Arremark, I. and Telander, B. (1978). *Journal of Infectious Diseases* **138**: 791–797.

Wagai, R., M. Kajiura, and M. Asano. (2020). Iron and aluminum association with microbially processed organic matter via meso-density aggregate formation across soils: organometallic glue hypothesis. *Soil* **6**(2):597-627.

Warner, B., Reeder, J., Kuczynski, J., Caporaso, J.G., Lozupone, C.A., Lauber, C., Clemente, J.C., Knights, D., Knight, R. and Gordon, J.I. (2012). Human gut microbiome viewed across age and geography. *Nature* **486**(4):222–227.

White, R.E. (2010). The status of soil health in the viticulture and wine industry: A review. GWRDC Report 0918.

Walker KE, Moghaddame-Jafari S, Lockatell CV, Johnson D, Belas R. (1999). ZapA, the IgA-degrading metalloprotease of *Proteus mirabilis*, is a virulence factor expressed specifically in swarmer cells. *Molecular Microbiology* **32**:825–836.

Wiesmeier, M., Urbanski, L., Hobley, E., Lang, B., von Lützow, M., Marin-Spiotta, E., Wesemael, B., Van, E., Rabot, M., Ließ, N., GarcíaFranco, U., Wollschläger, H-J. Vogel, N. and Kogej-Knabner, I. (2019). Soil organic carbon storage as a key function of soils- A review of drivers and indicators at various scales. *Geoderma* **333**(7):149-162. .

Williams, B.J, Dehnbostel, J. and Blackwell, T.S. (2010). *Pseudomonas aeruginosa*: host defence in lung diseases. *Respirology* **15**: 1037– 1056.

Winstanley, C. and Fothergill, J.L. (2009). The role of quorum sensing in chronic cystic fibrosis *Pseudomonas aeruginosa* infections. *FEMS Microbiology Letter* **290**(1):1–9.

Williams, P.H., and Haynes, R.J. (1994). Comparison of initial wetting pattern, nutrient concentrations in soil solution and the fate of 15N-labelled urine in sheep and cattle urine patch areas of pasture soil. *Plant and Soil* **162**(4):49-59.

World Health Organization (WHO/UNICEF), (2000). Global water supply and sanitation, Geneva; World Health organization with UNICEF. **40**: 82-89.

Yrlid, U., Svensson, M., Chambers A.B.J., Ljunggren, H.G. and Wick, M.J. (2001) In vivo activation of dendritic cells and T cells during *Salmonella enterica* serovar Typhimurium infection. *Infectious Diseases and Immunity* **69**(2):5726–5735.

Yi, X. S., Li, G. S. and Yin, Y. Y. (2016). Pedotransfer functions for estimating soil bulk density: A case study in the three-river headwater region of Qinghai Province, China. *Pedosphere* **26**: 362–373.

Yudina, A.V., Fomin, D.S., Kotelnikova, A.D. and Milanovskii, E.Y. (2018). From the notion of elementary soil particle to the particle-size and microaggregate-size distribution analyses: *A review. Eurasian* **51**(11):1326-1347.

Zilberstein, B, Quintanilha, A. G., Santos, M. A., Pajecki, D., Moura, E. G., Alves, P. R., Maluf, P., Filho, F., de Souza, J.A. and Gama-Rodrigues, J. (2007). Digestive tract microbiota in healthy volunteers. *Clinics* **62**(2):47–54.