

**BACTERIOLOGICAL ANALYSES OF MUNICIPAL SOLID WASTE LEACHATE IN
(IGUOMO AND ST SAVIOUR DUMPSITES) BENIN CITY, EDO STATE, NIGERIA.**

BY

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DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER 2022

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**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF
MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN
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**IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE
OF B.Sc (HONS) IN MICROBIOLOGY.**

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CERTIFICATION

This is to certify that this project work was carried out by Ruth Akudo Okorie with the matriculation number LSC1705616 in the department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City.

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(Project supervisor)

Date

APPROVAL

This project work was carried out by Ruth Akudo Okorie under the supervision of Mr. Afamefuna Dunkwu-Okafor in partial fulfillment of the award of a Bachelor of science (B. Sc) degree in the Department of Microbiology, University of Benin, Benin City.

Prof. (Mrs.) F .I. Akinnibosun

(Head of Department)

Date

DEDICATION

This project is dedicated to God almighty for his strength, wisdom and understanding that brought this work to fruition.

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TABLE OF CONTENT

TITLE PAGE.....	ii
CERTIFICATION	iii
APPROVAL	iv
DEDICATION	v
ACKNOWLEDGEMENT	vi
TABLE OF CONTENT	vii
LIST OF TABLES	ix
LIST OF FIGURES	x
ABSTRACT	xi
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background of the study	1
1.2 Aim of the study	4
CHAPTER TWO	5
LITERATURE REVIEW	5
2.1 Solid waste	5
2.1.2 Solid waste management issues in Nigeria	5
2.2.3. Solid waste and Microorganism	6
2.2. Dumpsite leachates	8
2.3 Contamination Potential of Leachate from Municipal Solid Waste	9
2.3.1 Leachate characteristics	13
2.4 Microbial processes in landfills	14
2.5 Microbial characterization of leachate	16
2.6 Leachates and landfilling	18

2.7 Landfill management practices	19
2.8 Impact of Landfill Leachate on Soil Quality	19
2.9 Dumpsite Leachates and Effect	20
2.10 Pathogens in Leachates	21
CHAPTER THREE	24
MATERIALS AND METHOD	24
3.1 Study Area	24
3.2. Leachates and soil origin	24
3.3.1. Standard Inoculum	25
3.4 Characterization and Identification of Bacterial Isolates	25
3.5 Microbiological Analysis of leachate	25
3.6: Coliform and cultural characteristics	25
CHAPTER FOUR	27
RESULT	27
CHAPTER FIVE	31
DISCUSSION AND CONCLUSION	31
CONCLUSION	33
REFERENCES	34

LIST OF TABLES

Table 1	Cultural, morphological and biochemical characteristics of bacterial isolates	29
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LIST OF FIGURES

Figure 1	Heterotrophic bacteria count (\log_{10} cfu/g) at both Leachates sites (St. Saviour and Iguomo).	27
Figure 2	Coliform count (\log_{10} cfu/g) at both Leachates sites (St. Saviour and Iguomo)	28

ABSTRACT

Solid waste management is of global concern in both developing and developed countries. Despite much awareness aimed at reducing the waste generated due to anthropogenic activities, there has been an increase in solid waste generation throughout the world. Inefficient management of these dumpsites causes uncontrolled gas and liquid emissions such as leachates. The study focused on assessing the bacteriological analysis of municipal solid waste leachate in Benin city. The objectives included isolation and enumeration of heterotrophic bacteria and coliform present in MSW leachate. Raw landfill leachate samples were collected from landfill site (open dumpsite) having low lying land area located at St savior and Iguomo in Benin City and was analyzed for the cultural, morphological and biochemical characteristics of bacterial isolates. The result shows the four bacteria isolated from the leachate sites (St. Savior and Iguomo) which includes: *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens*. The bacterial isolates showed varying levels of which is higher at Iguomo leachate ($4.9 \log_{10}$ cfu/g) sites as compared to St. savior leachate sites ($4.6 \log_{10}$ cfu/g) and the Coliform count was also slightly higher at Iguomo leachate sites ($3.8 \log_{10}$ cfu/g) as compared to St. savior leachate sites ($3.3 \log_{10}$ cfu/g). The level of coliforms, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens* exceeded the EPA recommended limits for irrigation and for waste water discharged into the environment. Based on the findings of this study, there is the need to effectively review waste disposal system Nigeria. To forestall leaching of pollutants into water bodies, location of dumpsites should be at an appreciable distance to our water bodies. In addition, municipal dumpsites should be replaced with proper engineered landfills.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

The disposal of solid waste represents a global environmental problem that continues to grow as the world population and urban and industrial development increase. Inadequate waste management is a threat to sustainable development in a developing country like Nigeria (Ohimain and Jenakumo, 2013). Wastes are generated in nearly all sectors of the economy. In hospital, hazardous wastes are generated and they are discharged into the environment without treatment (Abah and Ohimain, 2011). These wastes include pathological, hazardous chemicals, radioactive, stock cultures, blood and blood products, animal carcasses, pharmaceutical, pressurized containers, batteries, plastics, low level radioactive materials, disposable needles, syringes, scalpels, clinical bandages, gauze, cotton and other sharp items (Al-Ghamdi, 2011).

Poor waste management poses a great challenge to the well-being of inhabitants of an area, particularly those living adjacent to the dumpsites (Laniyan, 2011). Typically, the types of wastes generated depend on the activities being carried out. The quantity and quality of wastes depend on population and size of the sector. Due to urbanization, industrialization, and population growth, rural-urban migration, unplanned development, industrial and technological expansion, energy utilization affluence and consumption pattern, the wastes generated in the country has been in increase (Ohimain and Jenakumo, 2013).

Landfilling remains one of the most commonly used methods for solid waste management in most parts of the world. Its efficiency and safety coupled with cost make it the preferred method (Al-Ghamdi, 2011). This method is superior to open dumping in terms of ecological

impact and sustainability because the waste is contained in landfilling cells and the often highly toxic leachate is collected and treated on-site instead of seeping into the groundwater (Laniyan, 2011).

Understanding microbial population development in landfills over a period of time is challenging due to the complexity of waste materials deposited and the spatial heterogeneity of landfills. Previous studies have focused on particular aspects of microbial populations in waste degradation processes. Group-specific primers were employed to detect cellulolytic clostridia (Van Dyke and McCarthy 2002) and fungi (Lockhart *et al.* 2006) in landfill leachate.

Each year, 1.3 billion tons of municipal solid waste (MSW) are produced on the planet, with a value of 2.2 billion projected for 2025 (Sulaimon *et al.*, 2017). According to Kassim *et al.* (2017), the current global scenario indicates that 94.5% of MSW is directed towards landfills. Therefore, it is important to understand that one of the main environmental impacts of MSW disposal are the leachates (Talalaj, 2015). This liquid without proper control has a negative and direct effect over the environment, reaching soil and water resources, the economy and society are also affected. It compromises the social aspects such as public health and water security (Odeyemi *et al.*, 2017).

The generation of leachate occurs due to biodegradation of MSW provided by anaerobic and/or aerobic microorganism combined with residues characteristics and precipitation (Obayagbona, 2012). It is important to elucidate that organic and inorganic recycling process reduce the amount of leachate produced due to the deviation of litter from landfills. Leachate has a dark color and contains inorganic salts, possibly heavy metals, ammoniacal nitrogen and refractory and biodegradable organic matter (Obasi., 2012). Its composition has a vast physicochemical and biological variability, depending on factors such as residues type, climate, hydrology and landfill physical characteristics. Due to leachate high pollution potential,

environmental control agencies were pressured by society to implement more rigid leachate discharge parameters (Naveen, 2016).

Leachates are the aqueous effluent generated from solid waste owing to their physical, chemical, and biological alteration in landfills (Youcai, 2018) and is considered as a chemical soup of dissolved organic matter (DOM), xenobiotic organic compounds, different anions and cations, and heavy metals (Christensen *et al.*, 2001). Among the different component of landfill leachate, the heavy metals are non-biodegradable, able to deteriorate the surface and groundwater quality and toxic even at low level to biological system (Akpor, 2014). Heavy metals are also persistent, bio-accumulative, and toxic as well as endocrine disrupting and carcinogenic (Kibria *et al.*, 2016).

The principal concern about municipal landfill is focused on the pollution potential due to mobilization of the generated leachate through the subsoil into the surface and groundwater (Mishra *et al.*, 2019). Further, during the wet season, water containing leachate from landfill site drains into the nearby lowlands and surface water bodies and pollutes the local environments (Hossain *et al.*, 2018). Hence, this toxic aqueous effluent from landfill site can causes potential risks to surface and groundwater (Vaccari *et al.*, 2019) and eventually found to poses a threat for aquatic biota, plant and public health (Ideriah *et al.*, 2013). A post-closure care of uncontrolled landfills is essential for risk assessment over a long time of period which is based on the threat to human and environmental receptors. These threats are based on leachate quality and quantity, landfill gas production, system integrity and groundwater quality (Ibiene *et al.*, 2014).

1.2 Aim of the study

The aim of this work was to determine the bacteriological analysis of municipal solid waste leachates in Benin city.

The specific objectives are to:

- i. isolate and enumerate the heterotrophic bacteria present in solid waste leachates
- ii. isolate and enumerate the coliforms present in solid waste leachates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Solid waste

Solid waste refers to any type of garbage, trash, refuse or discarded material. It can be categorized according to where the waste is generated, for example as municipal solid waste, health care waste and e-waste (Kjeldsen *et al.* 2002). Over 2 billion tons of municipal solid waste are produced annually. Improper disposal can lead to adverse health outcomes, for example through water, soil and air contamination (Huang *et al.* 2005). Hazardous waste or unsafe waste treatment such as open burning can directly harm waste workers or other people involved in waste burning and neighbouring communities (Manimekalai and Vijayalakshmi, 2012). Vulnerable groups such as children are at increased risk of adverse health outcomes. Poor waste collection leads to environmental and marine pollution and can block water drains. Resulting flooding and other standing waters in waste items favours cholera and vector-borne diseases such as malaria and dengue (Aziz *et al.*, 2010).

About 54 million tons of e-waste, such as TVs, computers and phones, are created annually (2019 data) with an expected increase to 75 million tons by 2030. In 2019 only 17% of e-waste was documented as being properly collected and recycled (Tariwari *et al.*, 2021)

2.1.2 Solid waste management issues in Nigeria

Solid waste management is the most pressing environmental challenge faced by urban and rural areas of Nigeria. Nigeria, with population exceeding 170 million, is one of the largest producers of solid waste in Africa (Osazee *et al.*, 2013). Despite a host of policies and regulations, solid waste management in the country is assuming alarming proportions with each passing day (Ikpe and owunna,2016).

Nigeria generates more than 32 million tons of solid waste annually, out of which only 20-30% is collected. Reckless disposal of MSW has led to blockage of sewers and drainage networks, and choking of water bodies. Most of the wastes is generated by households and in some cases, by local industries, artisans and traders which litters the immediate surroundings (Ikpe and Owunna,2017).

Improper collection and disposal of municipal wastes is leading to an environmental catastrophe as the country currently lack adequate budgetary provisions for the implementation of integrated waste management programmes across the States (Joseph *et al.*,2016).

According to the United Nations Habitat Watch, African city populations will more than triple over the next 40 years. African cities are already inundated with slums; a phenomenon that could triple urban populations and spell disaster, unless urgent actions are initiated (Ochuko,2014). Out of the 36 states and a federal capital in the country, only a few have shown a considerable level of resolve to take proactive steps in fighting this scourge, while the rest have merely paid lip services to issues of waste management indicating a huge lack of interest to develop the waste sector (Emankhu *et al.*, 2017).

2.2.3. Solid waste and Microorganism

Wastes are discharged into the environment especially in unapproved dumpsites including markets, drainage channels, undeveloped land (Ikpe *et al.*, 2018), and surface water. This is associated to inadequate and irregular evacuation practices by the government waste disposal authority (Samuel *et al.*,2016). Due to inadequate management practice, solid wastes often block part of the major high way. Worst still, during the raining season, the waste are deposited into nearby surface water (river, stream, creek) via runoff (Agbozu *et al.*, 2015). These often led to pollution of the water body. In Nigeria, some inhabitants of the coastal

areas obtain their drinking water from the surface water (Kurniawan et al.,2006). Some of the microbial species found in the surface water are of medical importance. Microbes found in surface water include *Staphylococcus aureus*, *Escherichia coli*, *Bacillus*, *Pseudomonas*, *Proteus*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Streptococcus*, *Salmonella*, *Shigella* and *Vibrio species* (Agbozu et al.,2015).

Similarly, in leachates, bacteria species of the genera *Escherichia*, *Salmonella*, *Shigella* and *Vibrio* have been identified (Nwaka et al.,2018). Other microbes isolated from leachate includes *Pseudomonas*, *Proteus*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Arthrobacter*, *Klebsiella pneumonia* and *Staphylococcus species Enterobacter cloacae* and *Escherichia coli* (bacteria), *Fusarium oxysporum*, *Trichoderma harzianum*, *Aspergillus*, *Penicillium species* (fungi) (Nwaka et al., 2018). Egharevba et al. (2016) reported *staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella aerogenes*, *Serratia marcescens* and *Alcaligenes species* (bacteria), *Aspergillus*, *Rhizopus species* and yeast as microbes found in leachate in Benin City, Nigeria.

In Ekiti state, *Escherichia*, *Enterobacter*, *Klebsiella*, *Bacillus*, *Enterococcus*, *Salmonella*, *Pseudomonas* and *Staphylococcus species* have been isolated from leachate in dumpsite (Odeyemi et al., 2011). Some of these microbes are linked to borne diseases such as cholera, typhoid fever, diarrhoea etc when they find their way to drinking water sources. In addition, dumpsites also serve as media for transmission of infectious and non-infectious diseases (Sabejeje et al., 2014). Owing to the different chemical composition of these wastes discharged into the environment, it could contain some recalcitrant and toxic materials including heavy metals. On aquatic ecosystem, these wastes could influence the water quality (i.e. physico-chemical and microbial characteristics) due to the presence of heavy metals, ions, oil and grease, organic compound (Ohimain and Jenakumo, 2013)

Heavy metals cannot be degraded hence they leach into the environment i.e food chain (Abah and Ohimain, 2011) which could affect aquatic life forms. Heavy metals pose a risk to human health on exposure via water consumption especially the once not needed by the body such as mercury. Organism contamination is largely linked to industrial solid waste such as mercury amalgam, dental fillings, lead in paints, chemical residues in processed foods and personal care products such as cosmetics, shampoo and other hair products, mouth wash, toothpaste, soap (Abah and Ohimain, 2011). These are also part of the wastes deposited dumpsite. Leachates are formed when precipitation enter wastes in a dumpsite.

A consequence of growing populations in urban areas is the increased production of wastewater, coupled with a decrease in land availability for land application of associated biosolids. Treated and dewatered biosolids are used as fertilizers and soil amendments. The unutilized biosolids are disposed of in landfills (Reinhart, 2003). Biosolids are rich in moisture and nutrients and contain diverse microbial populations that can provide supplemental inocula for waste degradation reactions (Harley and Prescott, 2002). Waste degradation in landfills is a slow, ongoing process occurring over decades and characterized by physicochemical reactions and microbial interactions.

2.2. Dumpsite leachates

Leachate is the liquid that results from the passage of water through the waste at a dumpsite (Englehardt *et al.*, 2007). The chemistry of landfill leachate is intricate, and it can harm the quality of groundwater. The first coordinated publication on leachate from home landfill sites was made by Calvert (2014). According to his study, groundwater pollution occurred 500 meters from groundwater wells as a result of leachate migration from waste slurry. The amount of precipitation determines the specific water flux into the landfill system, which in turn controls the quality of leachate. Leachates exhibit superior qualities than sewage.

A typical early leachate may have a chemical oxygen demand (COD) 36 times higher than raw sewage, while a mature leachate may have a COD that is equivalent to that of raw sewage but include a larger concentration of refractory organic elements than household sewage. Municipal landfill leachate's composition varies noticeably over time and depending on the location. This variation in chemical and microbiological properties is attributed to a variety of elements, such as the age of the landfill, the type of trash, the availability of moisture, the temperature, the pH, the depth of the fill, and the degree of compaction (Bossi *et al.*, 2008; Busch *et al.*, 2010). Leachate toxicity often reduced over time according to the correlation between landfill age and leachate composition (Möder *et al.*, 2007). On the other hand, (Salvito, 2005) proposed that infiltration, which in turn is regulated by water circulation through the fill, determines the rate at which waste stabilizes. Therefore, the leachate stability won't always be directly proportional to chronological age. Natural leachates were highly toxic within the confines of landfills and usually remained so even at the point of receiving stream discharge, in spite of being attenuated somewhat by time because of considerable dilution afforded surface drainage and groundwater.

Temporal changes in toxicity paralleled the documented patterns of variation that characterized other leachate parameters i.e. an apparent logarithmic decay over time as the landfill ages (McDonald *et al.* 2008). The leachate components which dissolve into water percolating through the waste reach a solubility limit. Further release of these components can then only be achieved by the passage through the waste of additional less contaminated water (Mogensen *et al.*, 2004).

2.3 Contamination Potential of Leachate from Municipal Solid Waste

In recent decades the municipal solid waste (MSW) has received huge attention due to the social and environmental impacts. Various policies, strategies, plans and methods have been evolved for MSW management. These include various components such as waste reduction

and waste recovery for reuse, recycling, composting and incineration for energy generation in addition to landfilling of final rejects. Landfilling is one of the simple options for MSW disposal all over the world because they offer dumping high quantities of MSW at economical costs in comparison to other waste disposal methods (Mohajeri *et al.*, 2010; Palaniandy *et al.*, 2009; Foul *et al.*, 2009; Daud *et al.*, 2009).

MSW landfills are significant sources of wide range of pollutants of environmental concern. Majority of MSW disposal sites are still open dumps especially in under developed and developing countries. The generation of leachate is a result of percolation of precipitation through open landfill or through cap of the completed site (Susu and Salami, 2011). Landfill leachate is characterized by high concentration of organic and inorganic matters and heavy metals (Renou *et al.*, 2008). A leachate characteristic is highly variable and depends on the waste composition, amount of precipitation, site hydrology, waste compaction, cover design, sampling procedures, interaction of leachate with the environmental and landfill design and operation (Reinhart and Grosh, 1998). Properly designed and operated MSW landfills eliminate adverse environmental impacts that result from MSW disposal alternatives like waste burning in open-air burning sites and illegal /open-pit dumping. Other adverse impacts may arise from landfills i.e. gas and leachate formation if not well managed. These environmental impacts also include fires and explosions, vegetation damage, unpleasant odors, landfill settlement/failures, contamination of soil, water and air pollution and global warming.

Currently in developing countries, MSW landfills have been largely unsuccessful due to improper site selection which have limited time frame of usage. These are also receiving all of the waste materials such as MSW, commercial and industrial as well construction and demolition wastes which may contain hazardous substances leading to health risks emanating from the landfill leachate and methane gas. MSW landfill leachate is the main source of

contamination of waterbodies (i.e. ground and surface) if it is not properly collected and treated and safely disposed off as it may percolate through the soil layers and reaching to the aquifers. Once groundwater is contaminated and making it clean enough to drink can be extremely difficult and expensive for several reasons. In developing nations especially, the problem is more serious where the landfills do not have any leachate containment i.e. collection and treatment systems. Thus, it is very essential to adopt appropriate treatment/remedial measures to avoid contamination of the underlying soils and groundwater aquifers from the leachate generated from the landfills.

Generally, the older landfills do not have barrier systems or leachate collection systems to restrict the migration of leachate to the surrounding soil and groundwater. When the landfills are in permeable soils with shallow water table, the potential of the leachate to contaminate the groundwater increases. Closed landfills can continue to pose threat to the groundwater contamination if they are not capped properly before closure to prevent the leaching of contaminants by percolating rain water. According to an estimate all the cities and towns in India generate about 52 million tonnes of solid waste every year and this requires 1,250 hectares of landfill for every year of dumping. In India, unscientific disposal of MSW is adopted in every city, town and villages. MSW generated is usually disposed directly on low lying areas. Almost no urban local bodies (ULBs) have adequate engineering landfilling facility and MSW is disposed off in the outskirts of city along the roads. Unscientific disposal of MSW is prone to flooding and the main source of contamination of ground and surface water bodies are due to percolation of leachate. Leachate contains high toxic effluents with a complex matrix of organic and inorganic pollutants.

Leachate pollutants consists of mainly: dissolved organic matter; inorganic macro components; heavy metals and xenobiotic organic compounds. Among the various pollutant's heavy metals, organic substances and ammoniacal nitrogen are of significant concern to the

environment (Yusof *et al.*, 2009). A dissolved organic effluent imparts a significant effect on other pollutant's characteristics due to redox reactions and hydrophobic /hydrophilic sorption (Seo *et al.*, 2007). Ammonia is the odorous substances which are emitted from the MSW landfill sites (Fang *et al.*, 2012). Heavy metals are highly toxic, persistent, capable to bioaccumulate and able to pollute the surrounding waterbodies to a landfill site and thus lead to ecological risk (Karbassi *et al.*, 2015). So, leachate has the possibility of contaminating the adjoining aquatic and lithosphere system unless remedial measures are implemented. To prioritize the actions of the landfill remediation works, Kumar and Alappat (2003) developed a leachate pollution index [LPI] and the dominant group of leachate pollutants is analyzed by calculating the sub-leachate pollution indices: LPI organic, LPI inorganic and LPI heavy metals depending on the leachate characteristics. Umaer *et al.*, (2010) reported that LPI values of four landfills in Malaysia were observed to be very high indicating that the immediate remediation works should be encouraged.

Similarly, in another research by Kale *et al.*, (2010) seasonal variation of LPI values were analyzed and they recommended that the landfill leachate has less pollution potential during post monsoon in comparison to pre-monsoon. It is essential to assess the leachate pollution potential of a MSW landfill site to suggest the remedial measures for leachate treatment. Therefore, this research focuses on the assessment of the contamination potential threat from the leachate from the landfill sites located in the major metropolitan cities (Bangalore, Delhi, Mumbai, Kolkata, Chennai) in India by using the LPI. This index is mainly used to report the leachate pollution changes in a landfill over a period of time.

The leachate trend analysis developed for the landfill can be used to assess the post closure monitoring periods. Based on the leachate trend at a given landfill site, the index facilitates the design of leachate treatment units for other landfills in the same region. The other potential application of LPI is to assess, monitor and compare leachate contamination

potential of different landfills in a given geographical area. Further this will help in assessing and identifying whether the solid waste leachates are hazardous or not and also develop a sustainable leachate treatment process besides to foresee the impacts of leachate on groundwater.

2.3.1 Leachate characteristics

The liquid that percolates through the waste matrix is termed leachate, which solubilizes and mobilizes minerals associated with the waste (Kjeldsen *et al.* 2002), and also promotes microbially-mediated degradation of waste (El-Fadel 1999). The composition of leachate is related to the moisture content within landfills and biogeochemical reactions that occur within the waste matrix. In general, the amount of moisture available to support microbial activity depends on local climatic conditions, e.g. rainfall and temperature, the specific materials deposited in the landfill, and the frequency of leachate removal and/or recirculation (El-Fadel 1999). Among the dominant components of municipal waste are cellulose, hemicellulose, lignin and protein (Barlaz *et al.* 1989).

Leachates have been analyzed to determine the amount of dissolved organic matter (recorded as chemical oxygen demand or COD), volatile fatty acids (VFAs), inorganic components such as calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), ammonium (NH_4^+), iron (Fe^{2+}), sulfate (SO_4^{2-}) and hydrogen carbonate (HCO_3^-), heavy metals such as copper, lead nickel and zinc and trace amounts of arsenate, mercury, lithium, etc (Kjeldsen *et al.* 2002). Due to the differences in waste composition, age and landfilling practices the composition of leachate varies between landfills. The pH of leachate can vary from 4.5 to 9 depending on the stage of waste decomposition.

Landfill leachate can carry toxic metabolic products, heavy metals, pharmaceuticals and human pathogens (Mose and Reinthaler 1985; Gerba *et al.* 1995). Leachate contamination of

groundwater due to improper waste management practices poses a threat to public health (Roling *et al.* 2001). Leachate pooling at the bottom of the landfill is collected in a network of pipes called the leachate collection system and pumped to a storage tank. The collected leachate is then transported in tankers to a neighboring wastewater treatment plant where it is treated and disposed.

Leachate recirculation is practiced in many landfills because it provides an additional inoculum of established microbial flora that can boost the rate of the degradation process (Chan 2002). It also reduces the amount of leachate requiring treatment and increases gas production (Warith, 1999). In contrast, excessive leachate recirculation can result in over-saturation of waste and have a toxic effect on sensitive organisms such as methanogens (Reinhart, 1996). The frequency and amount of leachate recirculation also plays an important role in the development of certain microbial groups at different time points during waste degradation (Shen *et al.*, 2001). Leachates collected from landfills carry a representative sample of the numerous microorganisms involved in degradation of that waste (Pohland and Kim, 2000). Studying the microbial community profile of this leachate over a period of time could provide interesting insights into the physical, chemical and biological processes occurring within the landfill.

2.4 Microbial processes in landfills

Landfills provide excellent environments for the development of diverse microbial populations due to the wide variety of substrates available to support the physiological requirements of microorganisms. The complexity and composition of microbial communities in any particular landfill depends on several parameters such as the types of wastes deposited, moisture availability, landfill age and operating practices, toxicity of waste components and their breakdown products, and leachate management practices (Kjeldsen *et al.*, 2002). These

parameters have a direct or indirect effect on the pH, dissolved organic and inorganic carbon content, oxidation-reduction potential and temperature of the leachate.

Waste deposited in landfills is at different stages of degradation depending on the age and depth of the waste. In general, the process of waste degradation is presumed to progress in four stages from the time of deposition till maturation: aerobic, anaerobic, accelerated methane production and decelerated methane production (Barlaz *et al.* 1989). The first stage is dominated by aerobic heterotrophic bacteria that decompose cellulose and consume oxygen and nitrate present in the waste (Pourcher *et al.* 2001). This results in the production of carbon dioxide and possibly an increase in temperature. After oxygen is consumed, anaerobic cellulolytic bacteria, i.e., *Clostridium* spp. and *Eubacterium* spp., hydrolyze cellulose and hemicellulose into monosaccharides that are further fermented to produce alcohols and carboxylic acids (Burrell *et al.*, 2004). Acetogenic bacteria convert these organic acids and alcohols to acetate, carbon dioxide and hydrogen (Mackie and Bryant, 1981). Acetogens can also act as hydrogen oxidizers by reducing carbon dioxide to produce acetate and other low molecular weight organic compounds.

Hydrogen has an important role in waste decomposition (Mormile, 1996). Hydrogenoxidizing methanogenic *Archaea* oxidize hydrogen and reduce carbon dioxide to methane (Griffin *et al.* 1997). Sulfate reducing bacteria (SRB), on the other hand, use hydrogen as the electron donor and sulfate as the terminal electron acceptor (Daly *et al.* 2000). These two groups of microorganisms compete for available hydrogen in the landfill waste, which sometimes leads to the inhibition of methanogenesis by the SRBs (Mormile 1996; Raskin 1996). Carbon mineralization by methanogenesis is preferred for landfill functioning because sulfate reducers produce hydrogen sulfide, causing the phenomenon of “souring” (Gurijala 1993). Souring of waste reduces the pH and inhibits the activity of methanogens.

Methane production and recovery is environmentally and economically desirable due to its usefulness as a biogas for the production of energy in the form of heat and electricity. Unfortunately, methane recovery from landfills is affected by waste composition, microbial degradation dynamics and more importantly cost effectiveness. Methane is a potent greenhouse gas and the alternative to recovering methane, controlling methane emissions, is equally expensive. Methanotrophic bacteria present in the upper oxic region of the landfill use methane as their carbon and energy source and convert it to carbon dioxide (Wise *et al.* 1999). Therefore, in landfills where methane recovery is not an option, methanotrophs play an important role in controlling the emission of methane from landfill sites.

2.5 Microbial characterization of leachate

In several studies, culture-based methods such as heterotrophic plate counts have been applied to evaluate microbial numbers in leachates (Boothe *et al.* 2001). However, the utility of culture-based methods to enumerate and characterize environmental microorganisms is limited due to the highly specific and stringent growth requirements of many microorganisms, coupled with the need of many organisms to function as a consortium (Amann *et al.* 1995). Results from culture-based methods yield a biased subset of the total population and are not effective for assessing the microbial community structure (Hugenholtz and Pace 1996). Molecular methods such as fluorescence in-situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), and sequencing of cloned DNA (clone libraries) have proven to be useful in identifying microbial species and community diversity in landfill waste (Roling *et al.* 2001; Huang *et al.* 2002; Burrell *et al.* 2004; Huang *et al.* 2005). Complex community profiles can be statistically analyzed to determine the degree of diversity and levels of similarity among microbial populations in any given microbial ecosystem (Fromin *et al.* 2002).

Microbial ecosystem studies focus on microbial interactions with their environments and changes occurring in the community structures in response to shifts in environmental parameters. The extent of microbial diversity can be effectively captured by molecular biological techniques like genetic fingerprinting, temperature gradient gel electrophoresis (TGGE) and denaturing gradient gel electrophoresis (DGGE) (Muyzer and Smalla 1998). Bands from DGGE and TGGE gels can be excised and used for subsequent sequencing reactions. Complex community profiles obtained by using these techniques can be analyzed using statistical programs to determine the degree of diversity and levels of similarity between microbial populations in any given microbial ecosystem (Demba Diallo, 2004).

Huang *et al.*, used cloning and restriction fragment length polymorphism (RFLP) analysis to outline the archaeal community structure in landfill leachate (Huang *et al.* 2002). The cloning and sequencing approach was also used to study the bacterial diversity in landfill leachate by Huang *et al.* in another experiment (Huang *et al.* 2005). Roling *et al.* performed cloning and DGGE profiling of landfill leachate polluted aquifers to determine the correlation between the microbial community structure and hydrochemistry in the aquifers (Roling *et al.* 2001). To the best of our knowledge, no previous study has followed changes in archaeal and bacterial DGGE profiles over an extended period as attempted in this study. When the landfill does not impose a threat to human health and environment in the absence of active controls, it is considered as functional stable. The risk of pollution potential of any MSW landfills can also be determined by leachate pollution index.

Leachate pollution index (LPI) is treated as an important parameter used to quantify the landfill leachate pollution potential which is based on Rand Corporation Delphi technique (Kumar and Alappat, 2005a). Manimekalai and Vijayalakshmi (2012) and Aziz *et al.* (2010) have also used LPI value as a tool to assess the leachate pollution potential from landfill sites particularly at places where there is at high risk of leachate migration and pollution of

groundwater. LPI is used to monitor the leachate trends over the lifetime of the site and thus suitable to take essential decisions (Esakku *et al.*, 2007).

Talalaj (2014) has used modified water quality index known as landfill water quality index (LWPI) near the landfill site, which quantifies overall water quality in single index value. LPI and its sub-indices is a significant tool in planning and designing of forecasting actions for environmental pollution by landfill leachate.

2.6 Leachates and landfilling

The survival and composition of microbial communities in landfills depends on several factors such as waste composition, pH, temperature, toxic metal levels and moisture availability (Boothe *et al.* 2001). The amount of moisture available to support microbial activity depends on local climatic conditions, i.e. rainfall and temperature, the composition of waste deposited in the landfill, and the frequency of leachate removal and/or recirculation (El-Fadel 1999). The majority of the waste deposited in landfills comprises municipal solid waste (MSW) which includes household waste, newspapers, glass, and metals (Kjeldsen *et al.* 2002). Co-disposal of ash from waste-to-energy (WTE) processes and biosolids from wastewater treatment is also practiced in Class I landfills (Reinhart 2003).

Landfills undergo periods of moisture abundance during rainy periods and moisture deprivation in most climates. Compaction of refuse reduces infiltration of water through the waste layers, thereby pooling less leachate in the collection systems (Tatsi and Zouboulis 2002) and minimizing the probability of leachate contaminating the groundwater. In contrast, heavy rainfall dilutes toxic compounds in leachate and accelerates refuse decomposition (Wreford *et al.* 2000) but increases the potential for groundwater contamination.

2.7 Landfill management practices

Typically, leachate that is formed in landfills flows into leachate collection systems that are installed beneath a drainage layer, and is subsequently either recirculated through the landfill, treated on-site, or treated at a wastewater treatment facility. In some cases, clogging of leachate collection systems can occur and prevent drainage of the landfill, resulting in waste submergence (Fleming *et al.* 1999). Waste submergence causes accumulation of toxic compounds, inhibiting microbial activity and slowing waste degradation. Leachate may also leak through compromised pipes and contaminate the groundwater (Fleming *et al.* 1999).

A host of factors have been implicated in the clogging of landfill leachate collection systems including waste characteristics (Cardoso *et al.* 2006), physical-chemical parameters (Ledakowicz and Kaczorek 2004; VanGulck 2004), and drainage system design (Rowe 2000b), however, limited information has been reported on the temporal variations of the microbial community structure associated with the clogging phenomenon.

The deposition of inorganic chemicals such as calcium carbonate and hydroxyapatite, as well as the presence of biofilms presumably result in the phenomenon of clogging of leachate collection systems, thereby reducing the life of the landfill (Rowe, 2002). The “life” of a landfill is directly related to its capacity (area available for waste disposal) and the amount of waste deposited over a period of time. Upon “closure”, the landfill must be overlaid with a flexible membrane liner and earthen material and monitored for the next thirty years.

2.8 Impact of Landfill Leachate on Soil Quality

A municipal solid waste (MSW) landfill is not a benign repository of discarded material; it is in fact a biochemically active unit where toxic substances are leached or created from combinations of non-toxic precursors and gradually released into the surrounding environment over a period of decades (Carlig and Macoveanu, 2009; Josimović and Crnčević;

2009). Biological, chemical and physical processes within the landfill promote the degradation of wastes and result in the production of leachate and gases (Draghici *et al.*, 2007). Municipal landfill leachates are considered one of the types of wastewater with the greatest environmental impact since its quality and quantity are modified with time in the same landfill (Tengrui *et al.*, 2007).

Landfill leachate results from the decomposition of waste and infiltrates owing to rainwater in the landfill soil. Depending on the composition of landfilled waste and the extent of degradation and decomposition in relation with hydrological factors, leachate may become highly contaminated. It dissolves the contaminants into liquid phase and becomes sufficient to initiate a liquid flow (Vishvanatan, 2007). The downward transfer of leachate contaminates groundwater resources, whereas the outward flow causes leachate springs at the periphery of the landfill that may affect surface water bodies. Hence, leachate seepage is a long-term phenomenon that must be prevented in order to protect natural water resource (Kulikowska and Klimiuk, 2008).

2.9 Dumpsite Leachates and Effect

The developing trend of industrialization and modernization is generating tons of solid waste of different categories posing severe environmental threat. The activities of both the developed and developing countries are equally affecting the soil fertility. Though there are many factors affecting the soil characteristics the unscientific disposal and dumping of solid waste has become one of the major concerns due to the irreversible leachate effect (Siva and Prasada, 2016).

Dumpsite leachate produced as a result of organic dissolution from dumpsites is generally heavily contaminated and consists of complex waste water that is very difficult to deal with (Longe and Enekwechi, 2007). Most waste landfills and dumpsites in Nigeria have not been

designed to protect the environment from pollution. They are unlined and are located in public places surrounded by residential quarters and in wetlands or other areas with seasonally high-water tables. Classically unlined sanitary landfills and open dump are all known to release large amounts of hazardous and otherwise deleterious chemicals into nearby groundwater, surface water and soil as well to the air, via leachate and landfill gas (Allen, 2001).

Following the unrelenting urbanization and largely unimpressive performance of the public sector in the provision of infrastructure in many cities in developing countries, the search for alternative strategies for urban environmental services became inevitable. One obvious consequence of rapid urbanization is the growing generation of solid wastes, and many city authorities face unprecedented challenges in managing these, including problems coping with their collection and disposal (Ogu, 2000). This has led to the excessive production of leachates. Earthworms play an important role in the soil by providing suitable aeration which allows for water percolation and plant growth. On the other hand, *Nitrosomonas* sp plays a critical role as an ammonia oxidizing bacteria by converting ammonia to nitrite.

2.10 Pathogens in Leachates

The technological advances and population growth have contributed to an increase in the formation of municipal solid waste (MSW). This production may significantly increase environmental and public health risks worldwide due to the presence of toxic compounds and potentially pathogenic microorganisms found in these residues (Alfaia *et al.*, 2017, Almeida *et al.*, 2018). MSW from landfills releases a liquid residue of dark color and nauseating odor. This liquid (leachate) is originated from biological, chemical, and physical processes from the organic matter decomposition, may contain organic pollutants, inorganic salts, and heavy metals (Mavakala *et al.* 2016). municipal landfill can be considered sources of bioaerosol as

well as habitat for insects and rodents responsible for transporting potentially pathogenic microorganisms (Kalwasińska and Burkowska 2013).

Works have emphasized the presence of pathogenic bacteria associated to residues generated in municipal landfills. Kalwasińska & Burkowska (2013) reported the presence of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Salmonella*, *Clostridium perfringens* and coliform in air and soil samples obtained from municipal landfill in Torun, Poland. According to Grisey *et al.* (2010), total coliforms, *Escherichia coli*, *Enterococci*, *Pseudomonas aeruginosa*, *Salmonella* and *Staphylococcus aureus* were found in groundwater and leachate from aquifer beneath the Etueffont landfill, France. In the same way, pathogenic fungi have been found in the same sampling sites, such as *Aspergillus fumigatus*, *Cladosporium herbarium*, *Alternaria alternata* and other airborne microorganisms including *Aspergillus* and *Penicillium* species (Breza-Boruta, 2012). Microbial cells may trigger the development of several diseases including allergies, infectious diseases, lung damage, and epidemics (Falencka-Jabłońska and Skorupa, 2014).

Microbial contamination observed near municipal landfill can be caused by the spread of bioaerosol, birds, rodents, insects, and leachate leaking, mainly in controlled dumps and sanitary landfills, which do not have a leachate waterproofing system (Gouveia 2012, Kalwasińska and Burkowska 2013).

However, microorganisms that thrived in toxic environments can develop a unique metabolic capacity to process these xenobiotic compounds and transformed them into metabolically assimilable and/or less toxic forms. This ability comes from genetic and biochemical adaptation by microbial communities to different toxic chemicals (Bernal *et al.* 2021). The use of microbial communities recovered from landfill leachate can be considered a promising strategy for application as environmental bioremediation of xenobiotic compounds. The

understanding of the pathogenic microbial community associated to leachate from landfills is particularly important for the environment and human health.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Study Area

Benin city, the capital city of Edo state in the South-South of Nigeria lies in the equatorial climate region between latitude $6^{\circ} 47'$ and $7^{\circ} 15'$ and longitude $5^{\circ} 49'$ and $6^{\circ} 14'$ (Ministry of Lands and Survey, Edo State, Nigeria). The city experiences mainly tropical climate. There are about seven (7) registered legal solid waste dumpsite that serve Benin city and all located in the outskirts of the city but two was chosen for this study. They are generally un-hired and have no leachate drains.

3.2. Leachates and soil origin

The soil was surface sampled from leachate contaminated area around the dumpsite. The field-moist surface soil samples were collected using sterile soil auger at a depth of between 0-15cm and delivered in sealed plastic bag to the laboratory immediately for analysis.

Raw landfill leachate samples were collected from landfill site [open dumpsite] having low lying land area located at St savior and Iguomo in Benin City. These selected dumpsites are all in active operation. Raw leachate samples were collected from two (2) randomly selected leachate wells in the landfill sites very well labeled clean bottles (Aluko *et al.*, 2003). Leachate were collected once during the rainy season in the month of July

Raw leachate from each of the dumpsites were mixed together to form composite samples. Leachate. simulation was done using American Society of Testing and Material (ASTM) method (Perk *et al.*, 1982) with slight modifications. Two hundred and fifty grams (250g) for the waste samples collected at depth of 6 to 12 inches were shredded and packed in 2L flat bottom flasks. A litre of distilled sterile water was added for extraction, the waste mixture was thoroughly mixed and allowed to stand for 4hours at room temperature with continuous

stirring and allowed to stand for 4hours. This was done manually at regular intervals of 2 hours. After 4 hours, the solid and liquid portions thoroughly mixed and filtered to remove debris.

3.3.1. Standard Inoculum

Discrete colonies from each of the different plates were subculture from fresh media. These were transferred into slants and stored at 4°C. The slant culture serves as stock culture. The standard culture were prepared from the stock culture. Some of the isolate was picked from the stock cultures and incubated at 28°C for 24hrs. The plates were incubated at room temperature for 48hrs.

3.4 Characterization and Identification of Bacterial Isolates

All bacterial isolates were characterized and identified based on their cultural, morphological and biochemical characteristics.

3.5 Microbiological Analysis of leachate

Aerobic and anaerobic investigations were carried out as reported by Naubia and Okpokwasili (1993) for the study, isolation of heterotrophic bacterial and fungi was performed by adding 20gml of leachate sample aseptically to 180ml of sterile mineral salt medium. Tenfold dilution of the leachate were prepared and carried out accordingly. 1ml of the stock solution and that of the dilution were pour plated on nutrient agar and potato dextrose agar for bacterial and fungi, the other set were incubated in an aerobic jar with an oxygen removing system [oxid gas pack] to create an anaerobic system.

3.6: Coliform and cultural characteristics

The method employed was the standard plate count where 1 ml of the samples were cultured in duplicates onto MacConkey agar for total coliform and Eosine methylene blue agar for faecal coliform count using pour-plate technique. The plates were incubated at 37°C and 44°C

respectively for total and faecal coliform count. Colonies were counted after 24 hours of incubation and the count in colony forming unit per ml was calculated using the formula below

$$\frac{cfu}{ml} = \frac{\text{number of colonies}}{\text{volume of inoculum}} \quad (1)$$

Following pour plate culture of bacterial isolates from the samples, single colonies were subcultured on tryptone soya agar and incubated for 24 h.

CHAPTER FOUR

RESULT

The result shows the four bacteria isolated from the leachate sites (St. Savior and Iguomo) which includes: *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens* (Table 1). Cultural, morphological and biochemical characteristics of bacterial isolates were also determined. For the morphological characteristics of the bacterial isolates, it was observed that *Bacillus subtilis* and *Escherichia coli* had a flat elevation, an Undulate margin and an irregular shape while *Pseudomonas aeruginosa* and *Serratia marcescens* had a raised elevation, entire margin and circular. *Bacillus subtilis* was the only gram positive bacteria isolate and all other bacteria isolates were negative. All the bacteria isolates had a rod cell type and a disperse arrangement.

Figure 1 and 2 revealed that the Heterotrophic bacterial count (\log_{10} cfu/g) was higher at Iguomo leachate sites as compared to St. Savior leachate sites and the Coliform count (\log_{10} cfu/g) was also slightly higher at Iguomo leachate sites.

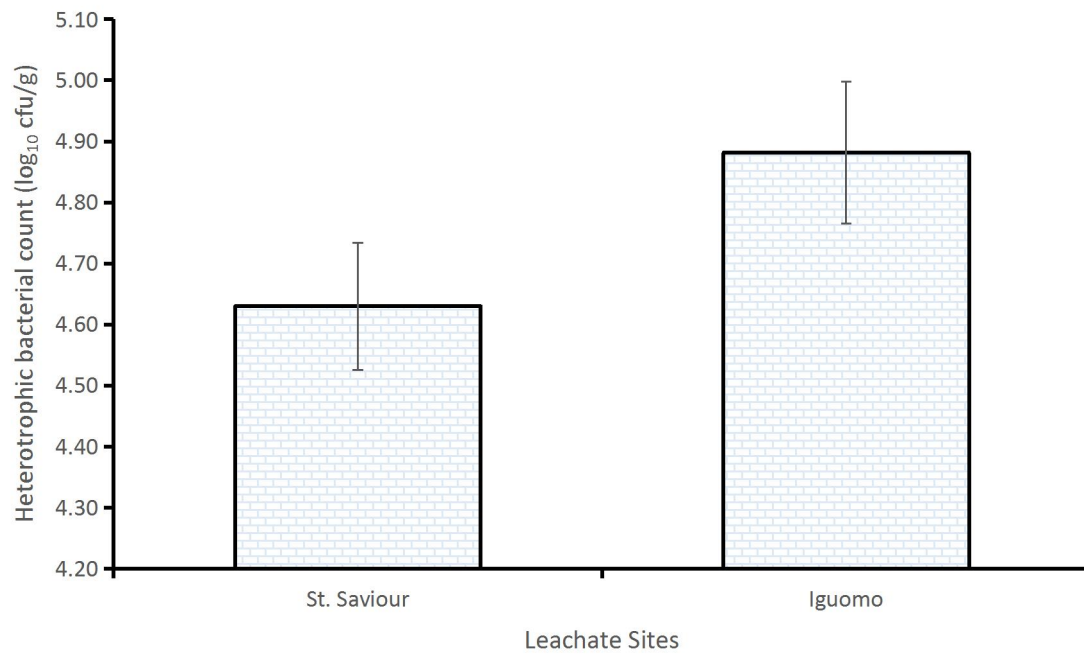


Figure 1: Heterotrophic bacterial count (log₁₀ cfu/g) at both Leachates sites (St. Saviour and Iguomo).

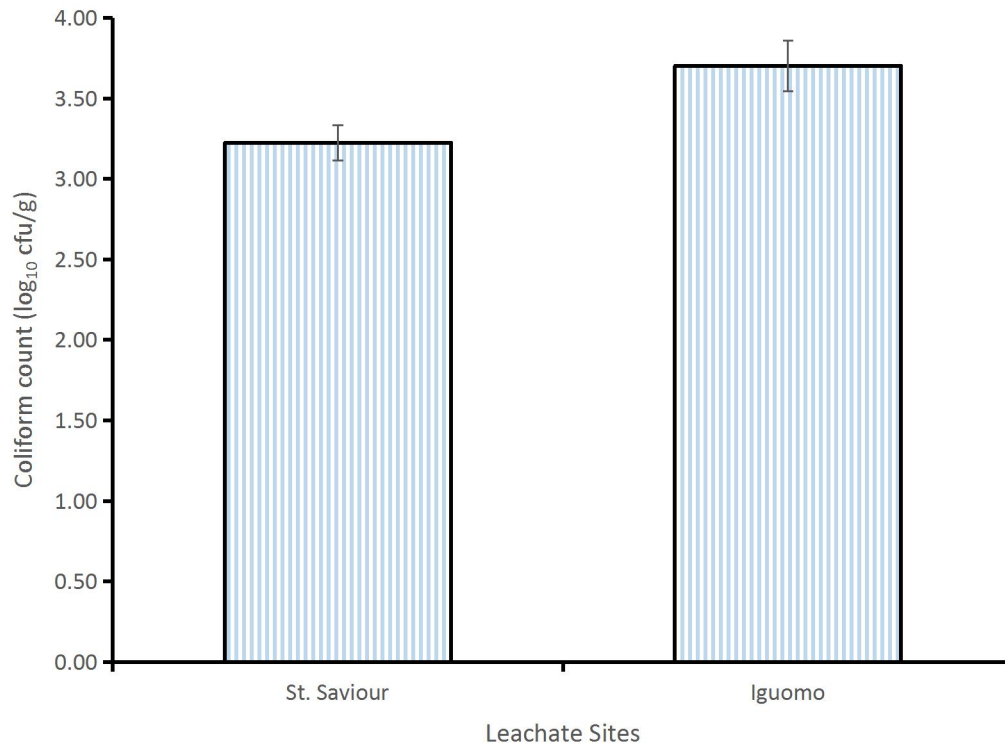


Figure 2: Coliform count (log₁₀ cfu/g) at both Leachates sites (St. Saviour and Iguomo).

Table 1. Cultural, morphological and biochemical characteristics of bacterial isolates

Morphological						
	Elevation	Flat	Raised	Flat	Raised	
	Margin	Undulate	Entire	Undulate	Entire	
	Color	Cream	Lemon	Cream	Cream	
	Shape	Irregular	Circular	Irregular	Circular	
	Size	large	Medium	Large	Medium	
	Gr. diff. agar	BCA	PCA	EMB	EMB	
	Colour	straw	Green	Green	Opaque	
Staining						
	Gram stain	+	-	-	-	
	Cell type	Rod	Rod	Rod	Rod	
	Arrangement	disperse	Disperse	disperse	Disperse	
	Colour	purple	Pink	Pink	Pink	
Biochemical						
	KOH test	-	+	+	+	
	Catalase	+	+	+	+	
	Indole	-	-	+	-	
	Citrate	+	-	-	+	
	Oxidase	-	+	-	-	
	Urease	-	+	-	-	
	Glucose	+	-	+	+	
	Sucrose	+	-	+	+	
	Lactose	+	-	+	-	
	Mannitol	-	-	-	+	
	Gas formation	-	-	+	-	
	H ₂ S formation	-	-	-	-	
	Identity	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>E. coli</i>	<i>Serratia marcescens</i>	

Key: Gr. diff. agar = Growth on differential agar, BCA= Bacillus cereus agar, EMB = Eosine methylene blue agar, SSA = Salmonella Shigella agar, PCA = Pseudomonas cetrimide agar, MSA= Mannitol salt agar

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1. Discussion

St. Savior and Iguomo leachate sites are unlined, uncontrolled planned and open dumpsite. This leachates harbors E-wastes, agricultural wastes, medical wastes and all forms of hazardous wastes. However, due to extreme variation of leachate composition and operating conditions in different landfills, no guideline or standard operating procedures for leachate treatment and disposal can be effectively chalked out (Ibiene *et al.*, 2012). Solid wastes are sorted out into biodegradable and non-biodegradable wastes as is the practice in developed countries due to the current management availed to the control of waste deposited in the environment (Enabulele and Obayagbona, 2013). Industrial waste which normally contains toxic chemicals and sometimes radio-active substances including electronic wastes, are dumped together with domestic, market and commercial wastes, when mashed together generates fluids known as leachate during the dry and wet season there is a constant run-off from this compost (Sabejeje *et al.*, 2014).

In these leachate sites four bacterial isolates were identified which include *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Serratia marcescens*. Agbor *et al.*, (2021) reported similar bacterial isolates including *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus* and *Bacillus subtilis* in a leachate sites in Calabar.

The presence of coliform bacteria in the water samples as observed in this study is a source of concern (Sulaimon *et al.*, 2014). These bacteria have been associated with a number of health problems such as cholera, vomiting and diarrhoea (Ideriah *et al.*, 2010). The use of leachate contaminated water for domestic purposes may cause several pathological diseases as indicated earlier (Faoro *et al.*, 2010). Presence of *E. coli* and total coliform bacteria indicates

microbial pollution of the groundwater by anthropogenic activities (Enabulele and Obayagbona, 2013). All bacterial isolates recovered from the waste dumpsite samples except *Serratia* spp are directly implicated in food-borne infections such as diarrhea, typhoid and gastroenteritis (Ekpo *et al.*, 2013). The usual disease pathway includes placing contaminated hands in the mouth or eating food, through vector insects such as cockroaches or mosquitoes or directly inhaling airborne dust particles contaminated with pollutants (Egharevba *et al.*, 2013).

The study further revealed that the concentration of waste materials in the leachate sites site had systematically polluted the soil and groundwater over time. The effect of such pollution as determined from the study declined away from the polluting source. This implied that the contamination of the surrounding water was more dependent on proximity to dump sites. The less dependency has been attributed to the influence of topography, type, state of waste disposal systems and to some extent, the hydrogeology of the area.

Also it was seen that the concentration of waste materials in the landfill site had systematically polluted the soil and groundwater over time. The effect of such pollution as determined from the study declined away from the polluting source. This implied that the contamination of the surrounding water was more dependent on proximity to dump sites.

The contamination of surface water by runoff of leachate could have adverse effects on the aquatic biodiversity including bio-accumulate in the body and organs of biological diversity, reduction in the rate of respiration by fishes due to precipitation of mucous secretion and reduction in oxygen level on long time exposure. It could also lead to changes in the physicochemistry of the water leading to increased cost of treatment.

Despite the positive impacts of the dumped municipal wastes on the microbial and organic properties of the analyzed soils, disposal of municipal wastes in open dump sites is an archaic and unsustainable option in the management of municipal wastes.

CONCLUSION

Leachates from solid waste dumpsites are linked to contamination of both surface and groundwater as a result of infiltration and runoffs. The microbial population is also high. Based on the findings of this study, there is the need to effectively review waste disposal system Nigeria. To forestall leaching of pollutants into water bodies, location of dumpsites should be at an appreciable distance to our water bodies. In addition, municipal dumpsites should be replaced with proper engineered landfills.

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APPENDIX

Table 1: Composition of *Bacillus cereus* Agar

Feature	
Gram reaction	+(a)
Catalase	+
Motility	+/- (b)
Reduction of nitrate	+
Tyrosine decomposed	+
Lysozyme-resistant	+
Egg yolk reaction	+
Anaerobic utilization of glucose	+
VP reaction	+
Acid produced from mannitol	-
Hemolysis (Sheep RBC)	+
Known pathogenicity ^c /characteristic	produces enterotoxins

^a +, 90-100% of strains are positive.

^b +/-, 50-50% of strains are positive. ^c -, 90-100% of strains are negative. ^d -, Most strains are negative

Preparation of *Bacillus cereus* Agar

Suspend 20.5 grams in 475ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes

Table 2: Composition of Eosine Methylene Blue Agar

The composition of EMB agar and modified EMB agar (Levine EMB) agar differs slightly. **Levine modification contains 10g of lactose (twice as in EMB agar) and no sucrose.**

Ingredients	EMB agar (gm/L)	Levine EMB agar (gm/L)
Peptone	10 g	10g
Lactose	5 g	10g
Sucrose	5g	–
Dipottasium,PO4	2g	2g
Agar	13.5g	13.5g
Eosin Y	0.4g	0.4g
Methylene blue	0.065g	0.065g

pH: 7.1 ± 0.2 at 25 °C.

Preparation of Eosine Methylene Blue Agar

Suspend 36 grams of EMB agar in 1000 mls of distilled water. Heat to dissolve the medium completely. Dispense and sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes.

Table 3: Composition of *Salmonella Shigella* Agar

Ingredients	Gms / Litre
Beef Extract	5.00
Enzymatic Digest of Casein	2.50
Enzymatic Digest of Animal Tissue	2.50
Lactose	10.00
Bile Salts	8.50
Sodium Citrate	8.50
Sodium Thiosulfate	8.50
Ferric Citrate	1.00
Brilliant Green	0.00033
Neutral Red	0.025
Agar	13.50
Distilled Water = 1000 ml	

pH (at 25°C) 7.0 ± 0.2

Preparation of *Salmonella Shigella* Agar

Suspend 60.0 grams of *Salmonella shigella* Agar in 1000ml distilled water. Heat to boiling to dissolve the medium completely. Do not autoclave.

Mix well and pour into sterile petri dish.

Table 4: Composition of *Pseudomonas cetrimide* Agar

Ingredients	In gm/Litre
Pancreatic Digest of Gelatin	20.0 gm
Potassium Sulfate	10.0 gm
Magnesium Chloride	1.4 gm
Cetyltrimethylammonium Bromide	0.3 gm
Glycerine	10.0 ml
Agar	13.6 gm
Distilled Water	1000 ml
Final pH 7.2 +/- 0.2 at 25 degrees °C	

Preparation of *Pseudomonas cetrimide* Agar

Add 45.3 grams of the medium in 1 litre of distilled water. Add 10 ml of glycerol and boil to dissolve completely. Sterilize by autoclaving at 121 °C for 15 minutes. Cool the medium to approximately 50°C and pour into sterile petri dish.

Table 5: Composition of Mannitol Salt Agar (MSA)

Ingredients	Gms/Litre
Proteose peptone	10.0
Sodium chloride	75.0
D- mannitol	10.0
Beef extract	1.0
Phenol red	0.025
Agar	15.0

Final pH 7.4 +/- 0.2 at 25°C

Preparation of Mannitol Salt Agar (MSA)

Suspend 111 grams of mannitol Salt agar in 1000mls of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs. pressure (121 °C) for 15 minutes.

