# EVALUATION OF THE BACTERIOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES OF ABATTOIR EFFLUENT-CONTAMINATED SOIL IN BENIN CITY

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BY

**Kelubia Uwabor MICHAEL**

**UNIVERSITY OF BENIN**
**BENIN CITY**

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**FEBRUARY, 2026**

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**A THESIS WRITTEN IN THE DEPARTMENT OF MICROBIOLOGY AND SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN ENVIRONMENTAL AND PUBLIC HEALTH MICROBIOLOGY**

**FEBRUARY, 2026**

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# CERTIFICATION

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We certify that this work was carried out by Kelubia Uwabor MICHAEL in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

**PROF. (MRS) F. I. AKINNIBOSUN** **Date**
Supervisor

**PROF. E. O. IGBINOSA** **Date**
Head of Department

# CERTIFICATION OF THESIS

We the undersigned attest and declare that the thesis of Kelubia Uwabor MICHAEL titled: “Evaluation of the Bacteriological and Physicochemical Properties of Abattoir Effluent Contaminated Soil in Benin City” has successfully passed the anti-plagiarism test and does not violate any copyright regulations.

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Supervisor

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**PROF. E. O. IGBINOSA** **Date**
Head of Department

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# DEDICATION

This work is dedicated to Almighty God for his unfailing love, care, divine guidance, and direction throughout my time of study. His name be praised. I also dedicate it to my wonderful family for their continual support.

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# ACKNOWLEDGEMENTS

My profound gratitude goes to God Almighty for the life, strength, inspiration, wisdom and understanding given to me that made this research a success.

I am indeed grateful to my supervisor Prof. (Mrs.) F. I. Akinnibosun for her support and encouragement. I want to thank the Head of Department of Microbiology, Prof. E. O. Igbinosa for his leadership role, the Post Graduate coordinator, Dr. (Mrs) O. B. Isichei-Ukah, her assistant, Dr. (Mrs) J. Z. Saidu and all other academic and laboratory staff of the department. I also appreciate the Management and staff of Lahor Centre for Molecular Diagnostics, Dr. P. O. Igbinomwahia, Mr. J. E. Ekundayo, and of course Miss J. George for their support and immense contributions to the success of this work.

Miss P. A. Ohioze, Mr. D. Itoro, my friends, course mates and church members are not also left out; thank you all for the role you played throughout the course of this study.

I also want to appreciate my Husband, Pastor V. E. Michael, my children, parents, and siblings; God bless you all.

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## TABLE OF CONTENTS

# LIST OF TABLES

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# ABSTRACT

Abattoirs play a vital role in environmental pollution and negatively affect the health of people living around, and even those who some distance stay away from them through air, water and food contamination. This study evaluated the bacteriological and physicochemical properties of abattoir effluent-contaminated soil in Benin City, Edo state with a view to highlighting the associated environmental and public health impacts.

Samples in this study were collected seasonally at different depths (2.5, 22.5 and 42.5cm) from four different abattoirs (Oluku, University of Benin, Ewah Road, and Dumez Road) in Benin City. Standard chemical analytical methods were used to evaluate the physicochemical parameters while standard bacteriological methods were used to determine the bacterial load and identity. Antibiotic susceptibility pattern was determined by disc diffusion method, and plasmid profiling (before and after curing) of selected resistant isolates were carried out using TENS method. .

The physico-chemical analysis of soil samples revealed a pH range of 6.25 - 6.76, electrical conductivity varied from 89 -161 µs/cm, organic matter (9.10-13.30%), iron (37.22-140.29 mg/kg), and phosphorus (4.91 – 12.82 mg/kg) for contaminated soil. For control samples, pH ranged between 6.28 and 6.90, electrical conductivity varied from 73 - 119 µs/cm, organic matter at 9.92 – 14.83%, iron (27.19 – 101.23 mg/kg), and phosphorus (5.22 – 12.88 mg/kg). Mean heterotrophic bacterial count (HBC) for contaminated soil in rainy season ranged $0.57\times10^8$ - $1.90\times10^8$ cfu/g, while that of dry season ranged $0.55\times10^7$ - $1.97\times10^7$ cfu/g. Topsoil layer consistently showed higher bacterial load across seasons. Isolates obtained in this study included *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Proteus vulgaris, Salmonella* spp., *Klebsiella* spp., *Enterococcus* spp. and *Alcaligenes* spp**.** There was a seasonal shift of *Proteus vulgaris* isolated in dry season to *P. mirabilis* in rainy season. *Staphylococcus aureus* was the most occurring isolate at 20.4% for dry season and 21.2% in rainy season. *Bacillus subtilis* and *Klebsiella* spp. exhibited the highest virulence. All isolates were sensitive to the Imipenem, but none was sensitive to Ceftriaxone and Ceftazidime.

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*Bacillus subtilis*, *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas* spp. had Multiple Antibiotics Resistance index (MAR) values of 0.6, 0.6, 0.5 and 0.4 respectively. *Klebsiella* spp., *Pseudomonas aeruginosa* and *Bacillus subtilis* were positive for Plasmid DNA while *Escherichia coli* was negative for Plasmid DNA. After curing, the MAR index values decreased to 0.3 for *Bacillus subtilis* and *Klebsiella* spp., while that of *Pseudomonas* aeruginosa decreased to 0.1. The presence of the pathogenic bacterial isolates in abattoir effluent-contaminated soil highlights significant environmental and public health concerns.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the Study

In recent times, cities in Nigeria have faced serious issues with waste disposal, especially in abattoirs, due to the unique health and environmental implications of disposing off animal by-products. Abattoirs are essential places of animal processing of meat and consumable-related products for human consumption. They are critical in food security as well as the meat supply industry. The operations of abattoirs nevertheless produce volumes of wastes that have become a gravely critical issue of environmental as well as public health concern, especially in underdeveloped nations like Nigeria. The wastes from slaughter operations are made up of animal blood, dung, fat, undigested feedstuffs, urine, bones, as well as wastewater collectively known as abattoir effluents. When such wastes are not well managed and dumped directly in the environment, these become great contributors to pollution to soil, atmosphere, as well as neighboring water bodies, thus posing risks to human, animal, as well as ecosystem health (Shima *et al*., 2015).

Abattoir effluent is typically high in organic matter, nutrients, and microbes. Some of these microbes are pathogenic bacteria, viruses, and parasites, some of which are zoonotic in character and can be passed on to humans directly or through contaminated water, foodstuffs, or soil. Some of the diseases associated with poor sanitation of abattoirs and inadequate effluent treatment are brucellosis, campylobacteriosis, tuberculosis, and fascioliasis (Ibrahim *et al*., 2021). Most abattoirs in Nigeria lack adequate proper waste disposal facilities, and effluent is typically discharged untreated in open drains, streams within abattoirs' proximity, or on landscape surfaces.

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The practice not only spawns foul odour release but also provides breeding niches for agents of diseases such as flies, rodents, and mosquitoes .

Environmental effect of abattoir effluents is two-pronged. When effluents permeate into the soil, its physicochemical content and biotic balance are modified. High organic content creates oxygen deficiency as well as shifts in pH levels, conductivity, as well as nutrient levels in soil, leading to impairment of healthy microbial action in soil as well as soil fertility (Omole and Ogbiye, 2013). Furthermore, effluents possess certain heavy metals like lead, zinc, chromium, iron, as well as copper that are deposited within soil as well as gain access to food chains through vegetation as well as groundwater. With time, such bioaccumulation can produce toxicities within humans as well as livestock in the form of neurological disorders along with damage to kidneys as well as lowered agricultural yield (Ubwa *et al*., 2013).

The aquatic ecosystems are also impacted negatively as a result of abattoir effluent releases. Research identified that abattoir effluents commonly possess elevated biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids content, as well as nutrient content in terms of nitrogen  and phosphorus (Bustillo-Lecompte and Mehrvar, 2017). Upon entrance in watercourses, these elements lead to eutrophication, elevated algal increase, as well as dissolved oxygen depletion that is toxic to aquatic organisms. The occurrence of enteric organisms such as *Escherichia coli, Salmonella spp., Campylobacter spp*., as well as *Listeria monocytogenes* in abattoir effluent worsens the affliction through contaminating surface as well as groundwaters that are both used for domestic  and agricultural uses (Kaakoush *et al*., 2015). Human ingestion of such contaminated waters fosters outbreak of diarrhoea, typhoid fever, cholera,  and other  waterborne infections.

In Benin City, Edo State, Nigeria, abattoir waste disposal is a growing environmental issue. The constant pollution of soils is evidence of a strong demand for proper monitoring as well as regulatory as well as remediation measures.

Additionally, abattoir effluents possess long-term ecological consequences. Their multi-component composition of organic matter, blood, fat, detergents, and heavy metals can blur the natural constitution of soil microorganisms, causing lower biodiversity levels as well as damaged functionality of soil (Rabah *et al*., 2010). Soil microbes are essential for nutrient cycling, organic matter decay, as well as detoxification of pollutants. Nevertheless, abundance of effluent pollutants can shift microbial population structures, facilitate the advent of resistant strains, as well as lower self-purification capacity of soil (Rabah *et al*., 2010). Particularly worrisome is the spread of antibiotic-resistant bacteria within abattoir premises. Most livestock receive antibiotics prior to slaughter due to veterinary practice, while traces of these drugs within effluents exert selective pressure that favours survival of resistant bacteria (Olarewaju and Olufayo, 2014). Spread of these resistant microbes through water as well as soil represents a serious international public health risk (Sadowy and Luczkiewicz, 2014).

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Environmental pollution from abattoir operations is not only an environmental issue but also a social and economic concern. Substandard soil lower agricultural output, impact fishery production, and escalate waterborne as well as foodborne diseases-related expenditure for the public. The World Health Organization (WHO, 2017) has stressed the enforceable protection of the environment as well as safe water standard as part of sustainable development requirements. Yet enforcement of environmental policies on abattoir operations within most Nigerian cities is weak. The lack of proper wastewater treatment facilities as well as limited technical capacity and inadequate sensitization of people on the importance of this aspect of sanitation administration has made indiscriminate release of abattoir effluent continue unchecked (Falodun and Rabiu, 2017).

In addition, climate and seasonal variations influence the extent of abattoir pollution. During the rainy season, effluents are washed into nearby water bodies and farmlands, increasing the spatial extent of contamination. Conversely, in the dry season, effluents accumulate and decompose, emitting foul odours and releasing volatile compounds that contribute to air pollution (supply reference). These seasonal dynamics affect both the physicochemical and bacteriological properties of soils and surrounding water sources (Olaiya *et al*., 2016). Understanding these variations is crucial for assessing the true impact of abattoir effluents and for designing effective management and control measures.

In sustainability terms, abattoir pollution is a quintessential example of the confluence of environment management, public health, as well as city planning. Sustainable waste control within abattoirs features prominently in United Nations Sustainable Development Goals (SDGs), namely Goal 3 (Good Health and Well-being), Goal 6 (Clean Water and Sanitation), as well as Goal 15 (Life on Land). This challenge can be managed through concerted action from government ministries, public health officials, as well as local populations. It further entails environmental education, compliance with waste disposal regulations, as well as adoption of suitable treatment technologies for waste management. Thus, it is critical to evaluate abattoir effluent-polluted soil's bacteriological and physicochemical characteristics in Benin City to estimate the degree of environmental pollution, determine significant contributors to pollution, and estimate their possible adverse health as well as ecological impacts. The results from such research would serve as scientific evidence to inform policy development, environmental governance, as well as better waste control measures. It would also help in protecting public health, ensuring soil fertility, as well as encouraging Benin City's sustainable urban environmental practice as well as those in other parts of Nigeria.

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## 1.2 Scope of Study

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The scope of this study involved evaluating the bacterial content and physiochemical properties of abattoir effluent contaminated soil The investigation encompassed the collection of soil samples at discharge points within the abattoir varying depths; the topsoil at a depth of 0-5cm, the mid-soil at a depth of 20 - 25cm and the core soil at a depth of 40 - 45cm. The samples were analyzed for the presence and concentration of potentially harmful bacteria and physicochemical properties, with a focus on specific strains and elements known to pose risks to human health.

## 1.3 Problem Statement

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Nigeria, as a developing nation, has been characterised by inadequate waste disposal, treatment, and management technologies, leading to pollution. The high production of meat and meat products for human consumption has led to high waste generation from abattoirs. The fast rate at which the environment is being degraded due to the unwholesome disposal of the effluents from abattoirs has been a serious public health and environmental concern in Nigeria. In Benin City, among other cities, effluents from abattoirs are easily discharged directly onto open areas or neighboring water bodies without any form of treatment. The trend has resulted in the degradation of the soils and water sources around the areas and also affected negatively the usage and quality (Atuanya *et al*., 2015). Despite the critical role the sub-sector of abattoir plays to the production of foods and the economy, not so much attention has been accorded the environmental status, particularly the bacteriological and physicochemical impacts of effluents from abattoirs on the ecosystem of soils (Omole and Ogbiye, 2013).

Physicochemical attributes of soils such as pH, electrical conductivity, carbon, and nutrient levels, act as indicative parameters for the fertility and health status of soils (supply reference). Effluents from abattoirs that are highly concentrated with suspended solids, nutrient, and heavy metals, disrupt microbial balance , decrease fertility , and agricultural productivity of affected soils (Ubwa *et al*., 2013). The presence of bacterial pathogens such as Escherichia *coli, Salmonella* spp., and *Staphylococcus aureus* in abattoir effluent discharged onto soils creates opportunity for the possible contamination of groundwater through percolation, thereby providing avenue for disease transmission (Kaakoush *et al*., 2015). The presence of the antibiotic-resistant bacteria also creates an emerging risk for both human and animal welfare. While some studies have analyzed the physicochemical and microbiological properties of abattoir wastewater in Nigeria (Shima *et al*., 2015), not many studies have addressed specifically how these effluents interact with and impact soils in Benin City, Nigeria . Even fewer studies exist that discuss how depth and seasonal changes affect the distribution and survival of pollutants (including bacteria) within effluent-polluted soils (Olaiya *et al*., 2016). The absence of localized empirical data complicates effective environmental monitoring and policy making. Hence, it is imperative to analyze both the bacteriological and physicochemical attributes of abattoir effluent-polluted soils in Benin City to determine the level of contamination, the extent to which it poses health and ecological hazards, and to generate a baseline data that will serve to develop sustainable waste disposal and environmental protection strategies.

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## 1.4 Justification of Study

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Soils contaminated with abattoir effluent can pose significant health risks to both workers and consumers. Evaluating the bacterial composition is vital to ensuring the safety of the soil and consequently the safety of the meat products processed within the abattoir. This study also will shed light on the environmental implications of abattoir effluent discharge in the area of environmental pollution by heavy metals, nutrients and pathogenic bacteria; health risk to human, animals and plants; soil fertility alteration, surface and groundwater contamination and adherence to environmental regulations. This research would in turn guide in effective waste management and food safety practices, public health protection, soil fertility and crop safety, prevention of surface and groundwater contamination and adherence to environmental regulations, recommendations for process improvements within the abattoir, enhancing overall hygiene and minimizing contamination risks. This study can contribute to optimizing water treatment processes and facility layouts.

## 1.5 Aim and Objectives of the Study

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The aim of this research was to evaluate the physicochemical and bacteriological properties of abattoir effluent contaminated soil in Benin City, Edo state with a view to highlighting the associated environmental and public health impacts.

The specific objectives of this research were to:

1. determine the physico-chemical parameters of the abattoir effluent-contaminated soil and uncontaminated soil;
2. isolate, enumerate and identify the bacterial isolates from abattoir contaminated soil in Benin City;

3. determine the variations of bacterial species along the depth of the contaminated soil;
4. ascertain the effect of seasonal variations on the physico-chemical properties of contaminated soil;
5. determine the virulence factors of the bacterial isolates;
6. evaluate the antibiotic susceptibility pattern of selected isolates ; and
7. determine the plasmid profile of selected multidrug resistant isolates.

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# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Abattoir

Abattoir, also known as slaughterhouse; has been defined as a premises approved and registered by the controlling authority for hygienic slaughtering and inspection of animals, processing, effective preservation and storage of meat produced for human consumption (Omole, 2008). While slaughtering these animals results in significant meat supply, a good source of protein and production of useful by-product such as leather, skin and bones, the processing activities involved sometimes results in environmental pollution and other health challenges that may threaten animal and human health (Alfonso-Muniozguren *et al.,* 2018). The US Environmental Protection Agency (USEPA,1999) defined meat hygiene as a system of principle designed to ensure that meat product is safe, wholesome and processed in a hygienic manner and are fit for human consumption. Previous studies have shown that the characteristics of abattoir wastes and effluents vary from day to day depending on the number of stocks being processed (Badejo *et al.,* 2017). These wastes from abattoir operation can also be separated into solid, liquid and fat. The wastes are highly organic. The solid waste includes condensed meat, undigested feed, bones, horns, hair, and aborted fetus. The liquid waste is usually composed of dissolved solids, blood, gut content, urine, and water; while the fatty waste consists of fat oil, grease which have high organic matter content (Umaru *et al.,* 2018). Animal waste is usually microbiologically contaminated by micro-organism living naturally or entering it from the surrounding such as those resulting from processing operations. The killing of animals for community consumption is inevitable in most nations of the world and dated back to antiquity which result in in the pollution of the underground aquifer (Zahedi *et al.,* 2018).

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Public slaughterhouses became a  well known in the 1400s and 1500s when France started providing them as part of community facilities (supply reference). In the 1600s and 1700s, Italy, Norway, Sweden, Denmark, the Netherlands, and Romania legislated laws that required all towns above a certain population limit to have public slaughterhouses (Tyagi *et al*., 2013). Currently, both publicly and privately run slaughterhouses have become a norm, with a single or multiple such facilities in towns depending on their population. While essential for food processing, slaughterhouses present severe environmental risks in areas surrounding their location (Zahedi *et al*., 2018). The generation of large volumes of solid and liquid waste at slaughterhouses including  rumen contents, blood, and vast volumes of wastewater from cleaning activities. For instance, it is reported that a cow brought for slaughtering generates 328.4 kg of waste in form of dung, bone, blood, horn and hoof (Tyagi *et al*., 2013). Moreover, on the average, about 45 percent of each live beef animal, 53 per cent of each sheep, and 34 per cent of each pig consists of non-meat materials (Maxwell *et al*., 2019).

Slaughterhouses abound in most Nigerian towns and are grossly inadequately supervised and controlled. Most of them are located along rivers, canals, or running streams. Their generic Yoruba name, *Odo Eran*, is a direct translation for "Abattoir River." The generated wastewater from this operation is thus discharged directly or indirectly into the receiving water bodies with little or no treatment.

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## 2.2 Classification and Types of Abattoirs

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The Food and Agriculture Organization (FAO, 1985) suggests that abattoirs within developing countries can broadly be classified within three main categories: modern abattoirs, older abattoirs, and slaughter slabs or makeshift installations. Whilst modern abattoirs are well-built and operate similarly to those in industrialized nations with upmarket technology and compliance with regulations in place, even within developing regions these are scarce due to immense investment requirements in terms of design as well as maintenance. In direct comparison with older abattoirs as well as slaughter slabs that are of a more ad-hoc and informal nature with pay-as-you-need operations offered with minimal installations as well as sanitary coverage.

Building on this categorization, Ayoade and Olayioye (2016) identified three main abattoir systems commonly used across sub-Saharan Africa: the line slaughter system, the slaughter slab system, and the batch system. The line slaughter system is defined by a well-organized facility that is equipped with up-to-date machinery and is constructed according to guidelines developed by the World Health Organization (WHO), as explained by Eriksen (1999). The slaughter slab system is on the other hand the most prevalent in most developing countries such as Nigeria and is usually operated through municipal authorities or local governments (Nwanta *et al*., 2008). Under the 1999 Constitution of Nigeria (4th Schedule), local governments are tasked with licensing and maintenance of these slaughter slabs that provide butchers with the necessary facilities for slaughter as well as processing and packaging meat. In contrast, the batch method is the most unsanitary and informal method of the three. It usually entails slaughter in rundown structures or makeshift pens that are characterised with weak or no flooring, occasionally topped with corrugated metal sheets. They are often run without necessary licenses and are in compliance with minimal to no sanitation or regulatory guidelines (supply reference). In this sense, such abattoirs are a significant contributor to environmental degradation as well as presenting critical threats to human health.

Additional abattoir classification as discussed by Cook *et al*. (2017) is according to processing capacity, geographical location, as well as scale of distribution. Category A abattoirs handle more than 40 livestock per day with country-wide distribution of meat. Category B abattoirs deal with 6 to 39 livestock per day with supply within local-government-areas or regional markets, whereas Category C abattoirs deal with five or fewer livestock per day with supply within local communities as well as households. This classification is indicated in regulatory policies like the Meat Control Act of Kenya, which underlines abattoir capacity's relationship with regulatory control levels (Cook *et al*., 2017).

While some abattoirs, particularly slaughter slabs, might at initial inspection satisfy requirements comparable to those of the World Health Organization, many of these abattoirs degenerate over time due to poor maintenance, overcrowding, and lack of attention from relevant authorities (Nwanta *et al*., 2008). Field observations combined with related research (Timothy, 2020) reveal that many abattoirs in developing countries exhibit features of systemic failing, such as dilapidated buildings, unhygienic processing environments, and inadequate sanitation disposal systems. All these unfavorable conditions compounded with poor regulatory enforcement as well as inadequate infrastructure usually lead to meat that is unsafe for human consumption (Gurmu and Gebretinsae, 2013).

In terms of construction, abattoirs across sub-Saharan Africa are haphazardly constructed with minimal thought for human or environmental cleanliness (Ayoade and Olayioye, 2016). Roofs are sometimes constructed from zinc or asbestos materials, with floors made from bare soil, concrete, or even vegetation materials. Some abattoirs are without walls, electricity supply, gates, nor proper drains (Timothy, 2020). In addition, most of these abattoirs do not possess standard waste-disposal systems; blood, bones, hides, and other wastes are usually dumped outside open arenas or adjacent water channels, which become breeding grounds for agents of diseases and cause odour as well as groundwater pollution (Seiyaboh and Izah, 2017). In general terms, slaughterhouse classification in low-income countries shows a marked dichotomy between modern well-equipped facilities and traditional or makeshift types that dominate the region. While such traditional arrangements are essential to regional meat supply chains, they are compromised through poor hygiene controls, inadequate regulatory jurisdiction, and unsound waste disposal, factors that make them relevant to research on bacteriological as well as physicochemical contamination that ensues from abattoir effluents.

## 2.3 Abattoirs Processes and Operations

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The slaughtering process in abattoirs is a process involving sequential operations from the arrival of animals to the last processing of by-products. Animals upon arrival are housed in holding pens where they are examined and sorted for slaughtering and the generated waste at this level, like dung, is disposed of through burial or other sanitary disposal methods to reduce environmental pollution . This is followed by the immobilization or stunning phase in which animals are made insensible before slaughter through the use of electric shock, mechanical restraint, or gas. Immobilization hinders movement but does not always ensure complete unconsciousness, and this phase produces no waste under regular conditions. Slaughtering and bleeding include cutting the animal's main blood vessels to ensure good exsanguination so that partial bleeding will not lead to contamination and microbial development. Blood handling and disposal are hence crucial to ensure cleanliness. Bleeding is followed by skinning of the carcass either manually or mechanically; the big animals are open-skinned and small animals are case skinned . Evisceration and splitting of the carcass is the subsequent operation where the belly is slit open and the interior organs are removed. The solid wastes like bones, entrails, and unused feed and liquid wastes like blood and wash water are produced in this process. Roasting of skin in

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Nigerian abattoirs is normally carried out using firewood or burning of car tires to depilate and dress  the carcass, prior to a thorough washing. Operations in the abattoir in most cases generate huge biological and organic wastes that require effective management and handled hygienically in order to help promote public health safety and avoid pollution of the environment (Mamhobu *et al*., 2019).

## 2.4 Abattoir waste contribution to Municipal Waste Generation

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Ever since humans tied down their health and well-being to the quality of their environment, sanitation which ought to have been one of the determinants of the quality of life has been neglected, (Burmamu *et*. *al*., 2014). A decade ago, 2.9 billion urban residents generated about 0.64 kg of municipal solid waste (MSW) per person per day, with an estimated future amount of 0.68 billion tonnes per year due to urban lifestyle growing faster than the rate of urbanization, (Hoornweg and Bhada-Tata, 2015). As countries urbanize, their economic wealth increase with standards of living and disposable incomes increasing, consumption of goods and services increases, which results in a corresponding increase in the amount of waste generated. The problem of waste disposal in the world continues to grow with industrialization and population growth (Bassis, 2009). Developed countries have an established infrastructure for waste management with systematic transportation of waste products, whereas developing nations face a lack of proper mechanisms or systems for waste handling and disposal along unorganized lines . According to Tettenborn *et al*. (2007) annual generation of waste by developed nations like the UK, France, and the USA  was around 695 kg per person, which accounts for a large proportion of pollution (Hoornweg and Bhada-Tata, 2015).

Slaughterhouse activities generate wastes that are characteristically high in organic composition, high levels of suspended solids, fluids, and grease elements. The recovered meat from slaughtered animals differs: about half of cattle, sheep, and goats yield edible meat, pigs give 60-62%, chickens yield 68-72%, and turkeys yield 78%, with much waste materials being generated . West Africa faces serious problems dealing with slaughterhouse wastes. With the implication of food safety right from the production line to consumer plates, coupled with increasing demands for proteins as a way of overcoming protein malnutrition, the need for adequate waste management arises.

Most West African abattoirs let waste stay in the open for days and thereby contaminate surface waters. Lack of conducive institutional frameworks and inadequate resources, human and financial, makes it impossible for the authorities to control abattoir waste collection and disposal. The challenge involves a series of connected public health risks, as well as environmental degradation issues that necessitate joint solutions involving governments, waste management agencies, private businesses, and the public. Since abattoir populations are rising, the need to quantify their contribution by waste categories is crucial to the enforcement of effective control measures. Indeed, landfills have evolved over modern times to safely handle several waste streams that are not only difficult to handle but also present unique challenges in order to minimize impact to the environment (Bogner *et al*., 2007).

These problems, however, also have provide opportunities for cities to find solutions that involve the community and the private sector, including innovative technologies, disposal methods, and behavior changes.

## 2.5 Abattoir Wastes Disposal and Treatment

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Waste disposal is a major issue for abattoir projects. There are two main component of waste disposal, including, one for the solid materials and one for liquids. The constraints relating to both have become more significant in recent times due to issue such as BSE (Bovine Spongiform Encephalopathy, also known as mad cow disease), water pollution, landfill restrictions and rendering industry charges (Badejo *et al.,* 2017).

The British Columbia Food Processors Association, in 2005, presented the following methods for slaughterhouse waste management.

**Composting**: The controlled biological breakdown of organic solid materials in an oxygen environment generates a humus-like product. In other words, compost is a result of the aerobic decomposition of organic matters. The most common method for composting condemned meat is to alternate the meat with materials high in carbon and chicken manure in a series of layers within a vessel, covering with a compost cap for insulation and odor suppression. The contents are turned once every six weeks, and the complete degradation of meat into a compost-like product takes about 6-12 weeks (Badejo *et al*., 2017).

**Incineration**: Under this method, slaughterhouse solid waste is burnt under controlled conditions at a temperature above 1000°C for an extended time. The process aims at sterilization and reduction of waste volume. However, this is extremely costly in terms of investment in equipment and running operation costs (Zahedi *et al*., 2018).

**Burial:** This method is employed in the disposal of carcasses and other meat wastes by producers, slaughterhouses, and dead animal collectors. It is required by regulations that dead animals or condemned meat should be buried under at least two feet of soil (Addy *et al*., 2015).

**Rendering**: This process takes the materials from slaughtering, packaging, processing, meal preparation, and dead animals and puts them through heat, moisture removal, and separation into sterilized animal protein powder and fatty by-products such as tallow, meat meal, blood meal, and feather meal . Animal tissues-muscle, fat, and bones-are converted to a uniform granular protein product that looks a great deal like sand or dirt, making it a much safer, more storable, and less objectionable product. Rendered products do not spoil after prolonged storage, unlike the untreated waste product. The high temperatures and prolonged time of rendering destroys typical disease-causing microorganisms, so instead of waste, something quite useful is generated . The rendering plant products can be used as animal feed additives; they are also used in oil lamps, candles and in the manufacture of soap and biofuels (Addy *et al.,* 2015). These methods, however, have their merits and demerits.

## 2.6 Animal Slaughtering Practices

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Animal slaughtering techniques in abattoirs are a significant factor in determining the safety and healthiness of meat produced (Adeyemo, 2002). Hygiene is of utmost importance, with aspects being environmental, personal, and co-workers. Slaughtering procedures rely on equipment and training that abattoir workers receive, and this determines the quality of meat (Ayoade and Olayioye, 2016). The general principle of hygiene in abattoirs is the segregation of "clean" and "dirty" operations to prevent cross-contamination (FAO, 1985).

The design of abattoirs should be such that all operations proceed in a specific direction without cross-flow of products (Cook *et al*., 2017). The "dirty" sector comprises livestock entrance, lairage for holding animals' antemortem inspection, stunning, bleeding area, by-products storage, condemned products, store rooms, and washing facilities (WHO, 2004). The "clean" section, or post-evisceration section, contains space for the separation of portions of an animal under sanitary conditions to avoid cross-contamination, inspect after death, and chilling for product quality and shelf-life optimization (Eriksen, 1999).

The kind of practice followed in abattoirs in the developing world varies with the animal type (domestic or wild), ethical and religious aspects (halal or non-halal), legislative, material requirements, where meat is to be recovered, meat products and parts of meat needed, and available facilities (such as water and electricity) (Ahouandjnou *et al*., 2015). Not every meat animal eaten or in retail outlets or on dinner tables was killed in developing nations' abattoirs, but every food animal can be killed. Lairage, stunning, and other operations to improve safety of animals and ensure good health are some of the common practices in slaughtering (Kyayesimira *et al*., 2020). Slaughtering and bleeding are quite important operations in animal handling, improving the safety of the animal and preventing pollution (Lawan *et al*., 2013). Slaughtering is carried out as soon as possible after stunning, with a sterile knife, and animals need to be hoisted vertically head downwards to bleed more quickly (Adeyemo, 2002). Blood should be released separately and should not be permitted to flow into wastewater because it contributes the highest chemical oxygen demand (COD) as one of the main dissolved pollutants in abattoir wastewater (Seiyaboh and Izah, 2017). In the developing world, this is not usually considered, and the normal practice is to cut the throat of the animal and collect the blood in a bucket (Timothy, 2020). Skinning and dehairing should be done in separate compartments, and unskinned carcasses should be hygienically moved to a clean compartment for evisceration (Ayoade and Olayioye, 2016). Hoists should be used for better cleanliness, but carcasses should be hung off the floor level (FAO, 1985). Evisceration is the most dangerous section since it is the period of greatest contamination if stringent hygiene practices are not followed (Fasanmi *et al*., 2010). The

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chief objective of decent slaughter is to avoid the contamination of the sterile muscles of the carcass with the gastrointestinal tract (Omotosho *et al*., 2016). Edible organs should be hygienically removed and stored, and trash should be quickly cleared from the floor (Timothy, 2020).

Chilling and packaging are significant activities in the meat sector (Cook *et al*., 2017). Chilling is to be initiated within 1 hour of bleeding of the carcass or quartered/halved and sold immediately (FAO, 1985). There should be a capacity and space for chilling and there should be a maintained constant temperature (WHO, 2004). In Nigeria, Togo, and Burkina Faso, as developing countries, there is no public abattoir that is registered with chilling facilities, as carcasses are sold immediately after they are butchered without any preservative procedure (Timothy, 2020). Cutting and deboning should be performed on surfaces cleaned regularly or, even better, hung, and the meat should be placed in clean, meat-only containers (Nwanta *et al*., 2008). Nigerian, Angola, and Benin abattoir cutting and deboning are performed along with other procedures, i.e., the evisceration process (Ayoade and Olayioye, 2016). Packaging should be clean and approved for packaging food to avoid contamination (WHO, 2004). But the practice is not followed in the majority of the developing nations under sub-Saharan Africa, as it is in South Asia (Mpundu *et al*., 2019). The dressed meat is carried in big sacks or plastic containers where flies settle on them and cross-contaminate them (Timothy, 2020).

## 2.7 Sustainable management strategies of slaughterhouses practices in developing countries

Sustainable management of slaughterhouses is important in developing countries due to their far-reaching environmental and public health effects. Slaughterhouses must be located on urban fringes or countryside, away from residential communities and water bodies, in an effort to

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Formatted[DELL]: Normal (Web), Justified, Space Before: 0 pt, After: 0 pt, Line spacing: Double, Tab stops: Not at 44.57 ch minimize the possibility of contamination. However, in the majority of developing nations, unsound waste disposal and sanitation in abattoirs are causes of disease outbreaks related to food and water contamination, vector proliferation, and pollution (Adonu *et al*., 2017). Abattoirs generate solid, liquid, and gaseous wastes, but the majority are run in unhygienic circumstances, draining animal blood on open spaces, stocking intestinal materials inside buildings, and burning bones and tissues, all which discharge dangerous pollutants and pathogens into the environment (Oruonye, 2015). These operations induce microbial contamination of meat and are of serious public health risk (Thomas *et al*., 2016).

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In pursuit of achieving sustainability, slaughterhouses should be renovated with improved facilities in order to allow hygienic slaughtering, meat handling, and storage. Infrastructural upgrading like drainage, interlocked floors, and underground water reservoirs can aid in wastewater management and reduce pollution. Tire burning in singeing animals should be banned due to its health and environmental hazards (Ekpo, 2019). Waste treatment before disposal is imperative, and modern waste management techniques, reduction, reuse, and recycling, should be adopted to take advantage of by-products like bones, hides, and blood. Local authorities should ensure regular inspection and enforcement of sanitary conditions, as well as stakeholder sensitization on the environmental and health implications of abattoir hygiene (Ekpo, 2019).

In addition, slaughterhouses also need safe water, electricity, and transportation systems to allow for smooth operations (Adonu *et al*., 2017). Good personal and environmental cleanliness of the workers is critical in the avoidance of foodborne pathogen transmission (Okoli *et al*., 2018). Poorer institutional regulatory control by authorities such as the National Food Safety Management Committee and the Inter-Ministerial Committee on Food Safety must be complemented with powerful regulation to determine compliance through physical, chemical, and biological testing (Diyantoro and Wardhana, 2019). As foodborne diseases remain an important public health problem in developing countries ( Ishola *et al*., 2016; Odeyemi and Bamidele, 2016; Ajuwon *et al*., 2021), the application of sustainable management systems in abattoirs is of importance for environmental conservation and public health security.

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## 2.8 Overview of Waste Management Issues in Urban Abattoirs

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Research conducted by Fearon *et al*. (2014) concerning the management of abattoir waste indicates that approximately 141.255 tons of blood, 77.562 tons of gut contents, and 78.475 tons of waste tissues are produced and disposed of each year as a result of slaughtering activities. The substantial quantity of waste, when appropriately managed, has the potential to markedly alleviate the sanitation and health issues frequently associated with abattoir facilities. Beyond mitigating pollution, such waste materials offer considerable opportunities for resource recovery. For example, they can be repurposed for the production of organic manure for agricultural applications and biogas intended for both domestic and industrial energy consumption. Additionally, Fearon *et al*. (2014) approximated that one kilogram of fresh animal waste can yield approximately 0.03 $m^3$ of methane gas daily, suggesting that nearly 2,326.86 $m^3$ of biogas could theoretically be produced each year from gut contents alone. This discovery highlights the economic and environmental prospects inherent in effective abattoir waste management.

Despite these prospects, the vast majority of abattoir plants in Nigeria do not seize this opportunity. The biodegradable and organic character of the waste is also disregarded, with the waste often left to rot in open spaces, producing offensive odours and serving as breeding grounds for flies and other disease-carrying agents (Fearon *et al*., 2014). The buildup of putrefying gut contents and waste tissues around abattoirs not only causes environmental degradation but also escalates the risk of pathogen spread to employees, neighbourhood persons, and animals around the premise. Though abattoir waste contains a large microbial load that is a potential risk to human health, it also presents a very favourable substrate to produce biogas, an angle that has not been adequately recognised under local waste disposal planning (Rabah *et al.*, 2010). Conversely, bone waste raises relatively minimal disposal problems since it is frequently traded with meat or is processed to animal feeds, thus reducing the environmental footprint it otherwise creates. Therefore, the lack of structured waste disposal systems at abattoirs around Benin City represents a missed opportunity to convert biological wastes to useful resources that can promote sanitation, produce clean energy, and promote sustainable environmental practices.

## 2.9 Challenges of Abattoir Management

Obsolete infrastructure, inadequate equipment, poor facility design, and inappropriate siting within residential areas are some of the challenges facing abattoir waste management in developing regions, particularly Nigeria. Most abattoirs operate with obsolete layouts characterized by a lack of drainage, ventilation, and waste segregation systems; hence, the accumulation of organic waste promotes environmental contamination. Infrastructure is further deteriorated by years of neglect and exposure to corrosive materials, which has caused floors to crack, drains to block, and storage units to deteriorate, making it difficult to store waste properly . The shortage of important equipment such as covered bins, effluent treatment systems, and protective gear has made workers resort to unsafe, rudimentary methods of waste disposal . The location of these slaughterhouses near residential neighborhoods heightens the exposure to odors, disease vectors, and contamination of water sources, hence posing severe risks to public health .

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These are all very serious shortcomings with far-reaching consequences. Environmental pollution is caused by improper waste disposal, where leachates and runoff contaminate soil, groundwater, and surface water bodies serving domestic and agricultural purposes. Decomposition of waste yields offensive odors and air pollutants like ammonia and hydrogen sulfide, which cause respiratory irritation and reduced quality of life for nearby residents. Abattoir effluents have high BOD and nutrient loads that deplete dissolved oxygen in aquatic systems, disrupt ecosystems, and promote eutrophication by excessive algal growth. Accumulation of filth serves as ideal breeding sites for flies, mosquitoes, and rodents, enhancing the spread of diseases such as malaria, diarrhea, and leptospirosis. Overall, poor management of wastes at abattoirs contributes to gross environmental degradation, ecosystem disequilibrium, and rampant public health hazards (Adeyemi and Adeyemo, 2007).

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## 2.10 Health Hazards of Abattoir Effluents

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The contamination of surface and underground water sources is a critical challenge in most developing countries. Traditionally, water pollution is defined as the introduction of undesirable substances into water bodies, changing the quality and its physical-chemical properties, and may lead to environmental damages and health hazards (Haseena *et al*., 2017). Water pollutants are usually toxic to the aquatic organisms, especially because their continued presence in water causes chronic damaging effects (Soldán, 2003). Significantly, the risks of long-term surface and groundwater contamination are generally underestimated because of the poor visibility of their long-term or delayed consequences (Dellasala *et al*., 2018). Unlike acute effects, these do not lead to immediate or evident deaths among the affected organisms.

Historically, groundwater was regarded as pure and free from contamination. However, increasing pollution levels coupled with rapid industrialization and higher chemical applications have allowed many contaminants to reach groundwater systems. Key sources of groundwater contamination include agricultural chemicals, sewage from septic tanks, waste disposal facilities, poorly disposed hazardous waste, underground storage tanks, and atmospheric pollutants. Apart from these inorganic pollutants, biological contaminants from human and animal faeces, like bacteria, viruses, and parasites, are responsible for waterborne diseases like typhoid, cholera, and dysentery (Dellasala *et al*., 2018). Similarly, pollution of surface water which is the natural habitat of aquatic animals could produce a consequential impact on man, directly or indirectly since it's the most common source of water for human use both domestic and industrial. Hence, the pollution of surface water in any form is a critical issue in water resource management. In Nigeria, like most African countries, access to clean and safe drinking water is a constitutional right for all citizens (United Nations, 2014).

Unfortunately, sustainable access to a potable water supply for the millions of its citizens is lacking. This has made community members who lack adequate water infrastructure rely on other drinking water sources such as wells, ponds, springs, lakes, rivers, and harvested rainwater to meet their household water needs (Edokpayi *et al*., 2018). Water from these sources is also often consumed without treatment, posing severe risks to public health (Edokpayi *et al*., 2015). In addition, the available literature indicates that widespread contamination of the country's river systems originates from multiple sources, such as the discharge of industrial wastewater, disposal of sewage, and agricultural runoff (Elemile *et al*., 2019). Meat processing plants produce high volumes of wastewater containing blood, stomach contents, and solid waste materials generated by animal slaughter and facility cleaning activities, which, when disposed of improperly, can create environmental contamination (Bustillo and Mehrvar, 2015).

### 2.11 Types of Abattoir Waste

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Abattoir wastes are commonly classified into solid and liquid

**Solid waste:** Solid waste is classified as garbage and rubbish. Garbage is putrefied waste from food processing industries, while rubbish is a nonperishable waste that is either combustible or non-combustible. Abattoir solid wastes are made of carcasses, bones, scraps of inedible tissue, horns, hooves, and feathers (Nwanta *et al.,* 2010).

**Liquid waste:** Abattoir liquid waste usually composed of dissolved solids, blood; gut content, urine and fat, waste water, suspended fat/oil (supply reference). The meat processing industries generate large quantities of effluents rich in organic compounds and nutrient and also require the tools available to manage waste water effectively (Eryuruk *et al.,* 2017).

### 2.12 Sources and Nature of Contaminants in Abattoirs

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Abattoirs generate various contaminants during animal slaughter and meat processing that can compromise food safety and product quality. These contaminants are categorized as biological, chemical, and physical, with biological contaminants being the most significant concern for food safety (Sofos, 2008). Understanding these contamination sources is essential for implementing effective control measures in meat processing facilities.

#### 2.12.1 Biological Contaminants

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Biological contamination constitutes the most significant threat to meat safety in abattoirs. The primary sources include animal feces, hides, gastrointestinal contents, blood and body fluids, air, personnel, pests, and water. Among these, fecal material is the most critical because it contains large concentrations of pathogenic bacteria ranging from $10^9$ to $10^{11}$ cfu/g. Common organisms include *Salmonella* spp., *Escherichia coli, Campylobacter* spp., and *Clostridium perfringens* (Jay *et al*., 2005). During evisceration, intestinal rupture or leakage allows these microbes to contact carcass surfaces, resulting in direct contamination (Bacon *et al*., 2000). Even small quantities of fecal matter, less than two grams, can contain millions of bacteria capable of causing foodborne disease (Zweifel *et al*., 2008).

Microbial contamination is a significant concern in the meat industry, particularly in abattoirs where animals are slaughtered, and carcasses are processed. Abattoirs play a crucial role in the meat supply chain, ensuring that meat products are safe and suitable for consumption. However, the presence of microbial pathogens in abattoirs can pose serious risks to public health, leading to foodborne illnesses and outbreaks. Therefore, understanding and effectively managing microbial contamination in abattoirs is essential to ensure food safety and protect consumer health (Badejo *et al.,* 2017)

External surfaces of animals also harbour vast microbial loads. Cattle hides are particularly contaminated, often carrying between $10^7$ and $10^9$ cfu/cm$^2$, with pathogens such as *E. coli* and *Salmonella* frequently isolated (Nou *et al*., 2003). The prevalence of *E. coli* on hides varies from 10% to 90% depending on environmental conditions and season, peaking during warmer months (Barkocy-Gallagher *et al*., 2003). During hide removal, microbes can be transferred to carcasses through direct contact, contaminated equipment, or workers' hands (Bacon *et al*., 2000). Pigs and poultry present similar risks; pig skin may carry *Salmonella* and *Yersinia enterocolitica* (Davies *et al*., 1999), while poultry feathers and skin often harbour *Campylobacter and Salmonella* from litter and fecal exposure (Berrang *et al*., 2001). Improper scalding or defeathering processes can

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exacerbate contamination by spreading aerosols or maintaining biofilms on processing equipment (Berrang and Dickens, 2000).

The gastrointestinal tract of food animals acts as a major reservoir for foodborne pathogens. In cattle, the intestines may contain *E. coli, Salmonella* spp*, Campylobacter* spp., and *Clostridium perfringens* at varying prevalence rates (Elder *et al*., 2000). Swine carry *Salmonella* and *Yersinia enterocolitica*, particularly in the tonsils, while poultry intestines are frequently colonized by *Campylobacter jejuni* and *Salmonella* spp. (Newell and Fearnley, 2003). The rumen also contains about $10^{10}$ to $10^{11}$ bacteria per gram, primarily anaerobes that, while not typically growing on meat, contribute to contamination when rumen contents spill onto carcass surfaces (Gill, 2007). Improper sealing of the esophagus or accidental puncture of filled stomachs during evisceration are common routes of contamination (Bacon *et al*., 2000). Blood and other body fluids released during slaughter can act as secondary contamination sources. Approximately 3–4% of live weight in cattle is released as blood during exsanguination, which can become contaminated by contact with knives, hides, or equipment used on previous animals (Gill, 2007). Splashes or aerosols spread bacteria to nearby carcasses, and stagnant blood on floors or equipment supports microbial proliferation. Furthermore, lymph nodes embedded within carcasses may contain *Salmonella*, and cutting through these tissues releases contaminated lymph fluid (Gragg *et al*., 2013).

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Another persistent biological threat comes from environmental biofilms, structured microbial communities attached to surfaces within the abattoir. Biofilms form on stainless steel equipment, conveyor belts, drains, and walls, particularly in moist environments (Tompkin, 2002). Bacteria such as *Listeria monocytogenes, Pseudomonas* spp. and *Staphylococcus* spp. can persist for extended periods, resisting cleaning and sanitizing chemical. Biofilm-associated bacteria are up

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to a thousand times more resistant to sanitizers than free-living cells, enabling continuous contamination through direct contact or water drips. Airborne microorganisms further contribute to contamination during operations such as carcass splitting, defeathering, or high-pressure washing. Studies have shown that when airborne bacterial counts exceed 500 cfu/$m^3$, surface contamination levels on carcasses also increase (Whyte *et al*., 2001). Common airborne species include *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp. and *Listeria monocytogenes* in cold, humid environments.

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Other sources of biological contamination include pests, workers, waste, and water. Rodents and insects such as flies and cockroaches are notorious for mechanically transmitting pathogens between waste areas and processing zones (Meerburg *et al*., 2009). Birds entering abattoirs can spread *Salmonella* spp. and *Campylobacter* through droppings and feathers (Gaukler *et al*., 2009). Workers also represent critical contamination vectors; hands, nasal passages, and clothing often harbour *Staphylococcus aureus*, *E. coli*, and *Salmonella* spp. (Todd *et al*., 2008). Poor personal hygiene or illness significantly heightens contamination risk, as even clean hands can retain $10^3$ to $10^6$ bacteria cells if improperly washed (Hanson *et al*., 2011). In addition, contaminated water used for carcass washing, equipment cleaning, or chilling can transfer pathogens when sourced from unsafe supplies or inadequately treated storage tanks (WHO, 2011). Maintaining potable water standards and frequent monitoring for coliforms and total bacterial counts are essential (Gill and Jones, 2000).

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### 2.12.2 Chemical Contaminants

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Chemical contamination in abattoirs primarily arises from processing chemicals, veterinary drug residues, and environmental pollutants. Cleaning and sanitizing agents, such as chlorine compounds, quaternary ammonium compounds, peracetic acid, and organic acids, are critical for hygiene but can leave residues if equipment is not properly rinsed (Sofos, 2008). Chlorine-based disinfectants may react with organic matter to form trihalomethanes, which are potentially harmful to human health (Tsai, 2005). Similarly, antimicrobial agents like lactic and acetic acid are often sprayed directly on carcasses to reduce bacterial load, but concentrations exceeding approved limits can lead to accumulation of chemical residues (Bacon *et al*., 2000).

Veterinary drug residues constitute another major category of chemical contaminants. Animals treated with antibiotics such as beta-lactams, tetracyclines, and sulfonamides may retain residues in tissues if slaughter occurs before completion of withdrawal periods (Beyene, 2016). These residues often result from noncompliance, misuse of drugs, or poor record-keeping (Moreno *et al*., 2008). Consumption of such meat poses several health hazards, including allergic reactions, disruption of gut microbiota, and the development of antimicrobial resistance in humans (supply reference). Routine screening using ELISA, high-performance liquid chromatography (HPLC), and mass spectrometry ensures compliance with safety limits and prevents contaminated products from entering the market (Beyene, 2016).

Environmental contaminants also play a role in chemical pollution. Animals can accumulate heavy metals such as lead, cadmium, mercury, and arsenic from polluted feed, water, or soil (Brito *et al*., 2005). These metals tend to concentrate in the liver, kidneys, and other organs. Additionally, pesticide residues from treated crops may enter the food chain through contaminated feed, especially organochlorine, organophosphate, and pyrethroid compounds (Darko and Akoto, 2008). Lipid-soluble pesticides accumulate in animal fat and may persist even after rendering or cooking. Persistent organic pollutants, including polychlorinated biphenyls (PCBs) and dioxins, have also been detected in animal tissues despite bans in many countries

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(Bernard *et al.*, 2002). Continuous surveillance, environmental monitoring, and strict enforcement of feed safety regulations are crucial for minimizing such contamination risks.

### 2.12.3 Physical Contaminants

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In abattoirs, the most common examples include animal hair, bristles, feathers, metal fragments, bone splinters, and pieces of plastic or wood (Buncic and Sofos, 2012). Cattle hair and pig bristles often remain on carcasses after hide removal or dehairing, while poultry feathers may persist after defeathering operations. Though these materials may not pose direct toxicological hazards, their presence is aesthetically unacceptable and signals inadequate hygienic control during processing.

Metal fragments represent more serious hazards. They may originate from broken injection needles, damaged knives, or machine components such as bolts and washers (Barbut and Tataroff, 2010). Ingestion of such particles can cause choking, dental damage, or gastrointestinal injury. To prevent these incidents, abattoirs employ metal detectors and X-ray scanners before final packaging. Other potential physical pollutants include plastic and rubber fragments from equipment, wood splinters from pallets, glass shards from light fixtures, stones or grit from the gastrointestinal tract, and bone fragments in mechanically separated meat (Barbut and Tataroff, 2010). Preventive measures include the use of food-grade materials, proper equipment maintenance, and visual inspections.

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Waste management practices also influence physical and biological contamination levels. Solid waste such as condemned organs, stomach contents, and inedible offal, if not promptly removed, provides breeding grounds for pests and microbial growth (Jayathilakan *et al.*, 2012). Liquid waste including blood and wash water can spread bacteria when allowed to stagnate (Mittal, 2006). Effective waste control requires segregation systems, covered containers, and timely disposal through rendering, incineration, or wastewater treatment.

Personnel hygiene represents an equally important preventive factor for both physical and microbial contamination. Workers' personal items such as jewelry, hairpins, or buttons can accidentally fall into meat products. Inadequate protective clothing or failure to maintain cleanliness contributes to both physical debris and microbial transfer. Control strategies include strict dress codes, frequent handwashing, use of gloves, regular replacement of aprons, and exclusion of sick employees from handling operations (Hanson *et al*., 2011).

## 2.13 Bacterial Contaminants of Abattoirs and Processing Equipment

Bacterial diversity and microbial load in sub-Saharan Africa abattoirs have been deeply researched to determine the sanitary conditions and handling practices employed in the slaughtering and meat processing exercise. Meat microbial quality is an important indicator of abattoir hygiene status and effectiveness of measures to control contamination in slaughtering and animal processing (Bersisa *et al*., 2019). High bacterial density in meat and slaughter areas are often a reflection of inadequate abattoir facilities, filthy handling of carcasses, and substandard sanitary procedures. Oluwafemi *et al*. (2013) had observed that microbiological meat quality is largely influenced by the health status of the animal prior to slaughter, handling method during processing, and environmental factors generally within the abattoir.

Empirical research in sub-Saharan Africa, including Nigeria, Ethiopia, Ghana, Rwanda, Uganda, and Mali, has revealed a great variety of bacterial contaminants in abattoir environments and meat samples. Such common isolates are *Escherichia coli, Salmonella* spp.*, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus* spp.*, Clostridium* spp.*, and Vibrio* spp (supply

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reference). These bacteria can be isolated from abattoir wastewater, slaughter floor, equipment, meat samples, and hands or clothing of the workers. For instance, Akinnibosun and Ayejuyoni (2015) isolated *Pseudomonas aeruginosa, Bacillus* spp*., Staphylococcus aureus* and *Klebsiella* spp. from abattoir wastewater in Benin City, Nigeria, whereas Azuonwu *et al*. (2019) isolated *Clostridium* spp*. and Bacillus* spp. from tabletop abattoir swabs and knives in Port Harcourt.

Research has shown that improper hygiene procedures in slaughterhouses are the primary reason for meat contamination and foodborne disease. In Mali, Sacko *et al*. (2022) established that non-compliance with minimum hygiene standards in abattoirs caused high *Salmonella* contamination rates of 15.97% in unsanitized facilities, whereas only 7.29% was registered in sanitized facilities. A number of studies have attested that *Listeria monocytogenes* and other meat pathogens are everywhere in unhygienic abattoir surroundings due to poor hygiene and unclean surfaces (Huang *et al*., 2021). Chiaramonte *et al*. (2009) noted that contamination between gut microbiota like *E. coli* and meat-associated bacteria like *Lactobacillus sakei* can enhance bacterial survival, colonization, and virulence. Contamination sources are not only carcasses but also contact surfaces and tools like knives, tables, scales, clothing, buckets and meat carriers (Faleke *et al*., 2017). Bersisa *et al*. (2019) reported that without sanitation, the abattoir environment is a huge reservoir for microbial growth with serious human health impact through direct and indirect contact.

Nigerian abattoir reports have shown high bacterial loads including *E. coli, S. aureus, and Salmonella* spp. These bacteria have been recovered in different concentrations in different states, as revealed in studies conducted by Azuonwu *et al*. (2019), and Iroha *et al*. (2011). Other pathogenic bacteria such as *Bacillus anthracis, Klebsiella pneumoniae, Proteus* spp. and *Yersinia enterocolitica* were also recovered from abattoir environments and animal feces (Schlech *et al*.,

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2005). These bacteria, with the ability to double fast in faeces, pose a serious public health risk through direct contact with infected water, environmental surfaces, and meat, as well as contact with vectors such as rodents and flies. These observations have been corroborated internationally by reports from Indonesia, Ethiopia and Morocco (supply reference). Soepranianondo *et al*. (2019) isolated *E. coli, S. aureus* and *Salmonella* spp. from beef samples collected from abattoirs in ratios of 32.5%, 20.0%, and 2.5%, respectively, with low levels of antibiotic residues. Bersisa *et al*. (2019) also isolated *Klebsiella* spp., *Proteus* spp. and *Shigella* spp in Ethiopian abattoirs.. Bahir *et al*. (2022) documented similar observations in Morocco. In all these studies, the most common isolate was *E. coli* in all instances. These pathogens, along with *Campylobacter* spp., *Clostridium perfringens, Listeria monocytogenes, and Shigella* spp., are prominent foodborne pathogens associated the meat- industry throughout the globe.

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Sanitation facilities and abattoir activities in Nigeria are not effectively monitored, and this results in environmental pollution and widespread contamination (Eze *et al*., 2013). Borch and Arinder (2002) found that microbial contamination of abattoirs may come from the live animals, environment, or through cross-contamination by means of hands and equipment of the workers. The primary sources of bacterial contaminants of abattoirs belong to some categories, such as:

**Live Animals**: Skin, fleece, and hooves of animals become charged with bacteria due to direct contact with faeces or contaminated environments like lairages The gut of the animals also has high bacterial counts, most of which are non-pathogenic but may carry pathogenic ones that infect meat during slaughter (Meat Industry Guide, 2015).

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**Water**: Water is at the center of abattoir processes and may prevent or lead to contamination based on its quality. Adetunji and Awosanya (2011) indicated that contaminated water used in cleaning the carcasses, surfaces, and hands of workers raises the risk of microbial contamination at every meat processing stage.

**Unhygienic Practices**: Unhealthy habits in sanitation and hygiene, including improper handwashing and use of dirty utensils, contribute to a  large extent to risk of contamination. Gurmu and Gebretinsae (2013) clarified that any slaughtering process offers a chance for bacterial transmission from the surface, equipment, or gastrointestinal tract of the animal to the meat.

**Poor Sanitation**: The quality of sanitation directly impacts the microbial load in abattoirs. Dirty facilities  are more susceptible to  contamination, while cleaning greatly decreases bacterial density  (Gurmu and Gebretinsae, 2013).

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**Carcasses and Other Factors**: Cross-contamination among carcasses during slaughtering is prevalent, usually as a result of improper handling and contact with contaminated surfaces, water, or aerosols (Meat Industry Guide, 2015). Moreover, Adeyemo *et al*. (2009) noted that butchers slaughter infected animals for fear of losing money, consequently contaminating healthy carcasses, workers, and equipment and resulting in extensive microbial transmission. Contamination of abattoir meat results from synergy between poor sanitation, poor infrastructure, and inadequate handling procedures. The predominance of pathogenic bacteria including *E. coli, S. aureus, Salmonella* spp., *and Listeria monocytogenes* in most areas highlights the importance of having rigorous standards of hygiene, tight surveillance systems, and enhanced abattoir management regulations to protect public health.

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## 2.14 Physicochemical Properties of Abattoir Effluents and Contaminated Soils

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Important physicochemical parameters help provide key measures to assess the state and quality of natural resources such as water bodies and soil biota. Acidity/alkalinity ratio, ionic conductivity, temperature ranges, suspended/soluble matter, clarity, nutrient content, metal ions, and carbon from organic matter pertain to this category. The assessment of these parameters provides vital insight into the nature and intensity of contamination and the status of the biotic component dwelling in these soils. For the waste generated in meat processing industries, assessment of these parameters becomes imperative in gaining insights into the effect of meat processing on the environment and in shaping efficient methods to manage the wastes properly (Ogbuene *et al*., 2024).

### 2.14.1 pH (Potential of Hydrogen)

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The pH level shows whether it is an acid, base, or somewhere in between, and it has very significant parts in controlling natural processes and reactions in nature. pH level range goes from 0 to 14, where 7 is neutral, lower values are acids, while higher values are bases or alkaline in nature. The pH level shows the ease with which nutrients and toxins can be dissolved and transported through nature and made available to plants and animals (Ogbuene *et al*., 2024).

Slaughterhouse waste can affect the pH level of waters and soil in different ways depending on the nature of the waste matter. The pH values in waters influenced by abattoirs were recorded to range from 5.79±0.66 to 6.58±0.46 units by Isoken and Ita (2018). On the contrary, Ebong *et al*., in 2020, indicated that pH values in soils contaminated with slaughterhouse activities' were between 5.74 and 6.69 with an average of 6.25 units, which was higher than the average in the reference site, standing at 4.77 units. The higher pH values in abattoir soils can be attributed to basic substances in abattoir wastes such as ammonia and decomposed biological matter . The

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acceptable range of pH values in waters is between 6.5 to 8.5 units, according to the World Health Organization (2011).

### 2.14.2 Electrical Conductivity and Total Dissolved Solids

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The capability of waters or soil to facilitate the flow of electric current shows the concentration of the dissolved charged ions , while total dissolved solids measure the total mass of substances dissolved in unit volume. These values are directly proportional to each other, in that an increase in one aspect shows an escalation in the other. The influxes created by abattoirs enhance these values through the addition of mineral solutes and organic/inorganic matter in the waters. Isoken and Ita (2018) demonstrated the range of electrical conductivity of abattoir wastewaters to be between 23.00±13.89 to 305.33±147.05 µS/cm with values of dissolved solids varying between 11.33±6.65 to 153.00±73.72 mg/L.

Dan *et al*. (2018) showed that the conductivity in soils influenced by the operation of slaughterhouses was higher at 40-46 µS/cm, compared to pristine soils with conductivity of 19.56-20.86 µS/cm. High conductivity can lower crop-yielding capability through the alteration of osmotic pressure, hindrances in plant nutrients uptake, and ionic poisoning. The conductivity level in water recommended by the World Health Organisation should not exceed 1000 µS/cm (WHO, 2011).

### 2.14.3 Total Suspended Solids (TSS)

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The determination of suspended solids provides information on the quantity of particulate matter in the water, including sediments, biological matters, and processing waste. Slaughterhouse effluent has high suspended substances resulting from meat processing activities. The concentration of suspended solids determined by Isoken and Ita (2018) was 7.50 ± 0.78 to 30.93 ± 5.08 mg/L in receiver waters, while Ansari *et al*. (2022) revealed higher values of 1722 ± 159 mg/L for undertreated abattoir effluent. The values recorded by Omole and Longe (2008) were higher at 1026 mg/L at the point of discharge, well above the recommended 500 mg/L by the World Health Organization. The reduction in water clarity, known as turbidity, is attributed to the presence of suspended materials in the water. The turbidity recorded was between 8.66±1.35 and 63.12±12.43 NTU in waters where the slaughterhouses were affecting the waters, according to Isoken and Ita (2018), with values between 227 and 647 NTU recorded in the Nassarawa River by Alabi *et al*. (2020), well beyond the acceptable limit of 25 NTU set by the World Health Organization. High suspended substances and turbidity in the waters reduce light penetration, inhibit photosynthesis, and thereby disrupt the aquatic life.

### 2.14.4 Organic matter

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Organic matter content is a critical physicochemical parameter in assessing soil quality and levels of contamination in abattoir-impacted environments. Recent studies have reported significantly high concentrations of organic matter in soils proximal to abattoir facilities, with high organic matter content attributed to the continuous discharge of organic-rich waste materials, which include blood, animal tissues, fats, and other biodegradable residues emanating from slaughtering operations. Fatunsin *et al*. (2023) reported organic matter content in ranges of 0.2494 - 4.7128 in abattoir contaminated soils in Asaba, Nigeria. The study further indicated a higher organic matter content in soil contaminated with effluent with ranges from 1.858-1.943. The elevated organic matter directly affects other physicochemical parameters. The incidence of microbes, especially bacteria, coliforms, and fungi species, in abattoir waste and its effluents can be related to its high organic matter content, as organic matter acts as nutrients in the growth and development of these microbes. High organic loads mean the availability of adequate carbon and

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energy sources that support a high microbial proliferation of heterotrophs, including potential pathogens, and provide suitable conditions that will favor pathogen survival and dissemination.

### 2.14.5 Nutrients

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Abattoir effluents are wastewater streams rich in nutrients emanating from various activities associated with a slaughterhouse operation, including washing of carcasses, cleaning of floors, processing of organs, and handling of blood and fat. Commonly, they contain several macronutrients at high concentrations that may affect soil fertility, water quality, and air emissions according to a given management practice. The major nutrients commonly reported in abattoir effluents include nitrogen, phosphorus, calcium, magnesium, sodium, and potassium. Understanding the behavior of such nutrients is essential for environmental monitoring, agricultural reuse, and public health protection.

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**Nitrogen**: Nitrogen is one of the most abundant nutrients in abattoir wastewater. A study by Matheyarasu *et al.* (2016) recorded the concentration of nitrogen as around 168 mg/l in wastewater used for irrigation purposes. When this kind of wastewater with a high concentration of nitrogen is continuously applied to soils, nitrogen accumulates in those soils. For example, nitrogen content was measured as high as two thousand and eighty mg of nitrogen per kilogram of soil in soils irrigated continuously with abattoir wastewater . Apart from nutrient accumulation, nitrogen-rich effluent can lead to gaseous emissions. A study by Seshadri *et al.* (2016) showed that soils irrigated with abattoir wastewater emitted significant amounts of nitrous oxide, especially when maintained at moisture levels close to field capacity. Nitrous oxide is a potent greenhouse gas with strong implications in terms of climate change, thus showing the necessity for proper management of nitrogen from abattoir wastewater. Recent studies have focused on nutrient recovery from slaughterhouse waste. One such publication by Soja *et al.*

(2023), showed how bone chars derived from abattoir waste can adsorb ammonium from digestates. The ammonium-enriched bone char is a slow-release nitrogen fertilizer and can enhance plant growth while providing effective nutrient cycling.

**Phosphorus**: The concentration of phosphorus in abattoir wastewater is also relatively high. According to Matheyarasu *et al.* (2016), phosphorus concentrations are around 30.4 mg/l in abattoir effluent. Long-term irrigation with such wastewater leads to the accumulation of phosphorus in soils, increasing total phosphorus levels and influencing soil fertility. Efforts have also been made towards the recovery of phosphorus directly from wastewater. A study by Ramaswamy *et al*. (2022), optimized the precipitation of struvite, magnesium ammonium phosphate, from slaughterhouse wastewater and succeeded in recovering about 85% phosphorus. The struvite crystals formed act as slow-release fertilizers, reducing environmental loading while providing a valuable agricultural product.

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**Calcium**: Another important nutrient that grows considerably in soils contaminated by abattoir wastewater is calcium. Ediene *et al.* (2016) discovered during their research in Calabar, Nigeria, that contaminated soils possessed higher exchangeable calcium compared to other surrounding control soils. Calcium is vital in improving soil structure through flocculation, in which clay particles bind together to form stable aggregates. These aggregates enhance water infiltration, improve soil aeration, and reduce erosion. Research by Pereira *et al*. (2025) on tropical soils that were exposed to cattle slaughterhouse effluent for various lengths of time showed increases in calcium levels, as well as increases in a number of other nutrients. It also showed improved soil pH, proving that long-term exposure to abattoir effluent can decrease soil acidity.

**Magnesium**: Magnesium is one of the most widely distributed cations, acting similarly to calcium in soil systems. Ediene *et al.* (2016) recorded high levels of magnesium in soils near abattoir facilities. Being the central part of the chlorophyll molecule, magnesium is an extremely important nutrient for plant growth due to the process of photosynthesis. However, outcomes do differ according to region. For instance, Abubakar and Tukur (2014) did find that abattoir effluent from Yola, Nigeria, was not significantly increasing magnesium levels in certain types of soil. Such anomalies could be related to soil texture, composition of effluent, or period of exposure.

**Potassium**: Other nutrients which occur in high amounts in abattoir-polluted soils include potassium. Exchangeable potassium was indeed higher in the soil surrounding the slaughterhouse than in the control environment according to studies by Ediene *et al.* (2016). Potassium is essential in plants for regulating water, activating enzymes, and maintaining tolerance against stress. In addition, Matheyarasu *et al.* (2016), showed that plants irrigated with abattoir wastewater had increased uptake of potassium. However, being a monovalent cation, meaning not strongly held on soil exchange sites, the lability of potassium often leads to leaching. Continuous irrigation or high rainfall can result in potential environmental hazards due to the leaching of potassium into the groundwater.

**Sodium**: Of all the nutrients associated with abattoir wastewater, the nutrient of greatest concern is sodium. Sodium levels are usually high in soils contaminated by effluent. Ediene *et al.* (2016), measured significantly higher levels of sodium in affected soils. In contrast to calcium and magnesium, sodium acts to deflocculate and destabilize soil aggregates. The outcome is soil crusting, reduced porosity, lowered water infiltration, and eventual formation of sodic soil conditions. High levels of sodium also have very harmful effects on the growth of plants, especially sensitive crops.

### 2.14.6 Organic Carbon Content

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The organic carbon content shows the level of carbon in the soil organic matter, thereby affecting soil productivity. The carbon content in soils is greatly enhanced by the waste products from slaughterhouses. Ediene *et al*. (2016), explained that the reference soils were characterized by low values of organic carbon content less than the 1.5% threshold in productive soils, while soils under the influence of slaughterhouses showed high values of organic carbon content. This was because the effluent was being processed to decompose animal waste in it. Abhanzioya and James (2013), showed that there was an increase in soil organic carbon content from 8.5% to 10.2–12.4% upon the application of abattoir effluent, which was useful in crop production.

### 2.14.7 Cation Exchange Capacity (CEC)

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This property of soil defines the capability to bind positively charged ions and can be related to the soil acidity, texture, and accessibility of nutrients in soil. Dan *et al*. (2018) showed that in soils influenced by the slaughterhouse, the soils' CEC range was 25.11–28.63 Cmol/kg, higher than in control soils with values ranging from 20.84 to 21.67 Cmol/kg. The higher CEC values improve the soils' fertility and usability to grow plants.

### 2.14.8 Heavy Metal

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Slaughterhouse waste regularly has metal pollutants such as iron, copper, chromium, nickel, magnesium, cadmium, and cobalt, in that order, with iron being the highest concentration of metal in the waste, coming from cleaning agents used to wash livestock, the burning of animal hide with tires, high organic content, and metal content in livestock feeds (Ogun *et al*., 2023). The concentration of metals in the soil that has been influenced by abattoirs was recognized by Ebong *et al*. (2020), to include iron at 877.8 mg/kg, copper at 18.2 mg/kg, zinc at 24.04 mg/kg, and lead at 0.99 mg/kg. The presence of metals in soils reduces the microorganisms in substantial amounts, thereby resulting in the loss of fertility and plant yield. The human inhabitants can be affected by consumption of the contaminated vegetation growing in the soil and the utilization of the waters for household

### 2.14.9 Chloride ion

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The measurement of chloride ion concentration is critical in identifying sewage contamination of underground water resources. High chloride ion concentration causes harm to plants upon irrigation and makes drinking water unpalatable. The major source of chloride ion in abattoirs can be traced to the cleaning agent that uses soluble salt solutions such as KCl and NaCl in washing animal hide skins (Osinbajo and Adie, 2007). Adeyemo *et al*. (2019) illustrated that wells located within abattoirs recorded a concentration of chloride ions of 748.8 mg/L, which exceeded the WHO standard of 250 mg/L.

# CHAPTER THREE

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# MATERIALS AND METHODS

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## 3.1 Study Area

This study was conducted in four selected abattoirs located in the University of Benin, Oluku, dumez Road and Ewah Road across Ovia, Oredo and Ikpoba-Okha local government areas of Benin City, Edo State, Nigeria. These Abattoirs were selected for the purpose of this study because they are strategically located and highly patronized by people in Benin City.

## 3.2 Sample Collection

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Soil samples used were collected seasonally over a period of three months in dry season and three months in rainy season . At every location, samples were taken in triplicates every two weeks for both dry and rainy season at varying depths; the topsoil at a depth of 0-5cm which is most impacted by the abattoir effluent; the mid-soil at a depth of 20 -25cm and the core soil at a depth of 40 -45cm. Correspondingly, samples were also collected from environs around the abattoir not impacted by the effluent, 36 meters away from areas impacted by the effluent to serve as control. A total of 288 samples were collected; 144 samples were collected each during the rainy season and dry season. Collections were done twice a month for the months of November, December and January for dry season and May, June and July for rainy season. Soil samples were aseptically collected using soil auger and were placed in sterile pet bottles and delivered within four hours of collection to the Laboratory for microbiological and physico-chemical analysis.

## 3.3 Sterilization of Media and Materials

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All laboratory materials were sterilized by appropriate methods before beginning any experimental procedure. Glassware, like test tubes, conical flasks, and pipettes,  were washed with a detergent solution,  rinsed with clean water and allowed to complete drain. Items were then wrapped with aluminum foil and subjected to dry heat sterilization in a hot air oven at 170°C for one hour. Metallic instruments like inoculating loops were sterilized by heating them till red hot in a Bunsen burner flame before and after each use. Work surfaces in the laboratory were disinfected by wiping with 70% ethanol solution before commencing any analytical work. In order to avoid microbial contamination during the sample isolation and inoculation operations, all manipulations were performed closer to a flame source for maintaining aseptic conditions of agar plates and culture tubes.

## 3.4 Determination of Physical Properties of Effluent Contaminated Soil

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Determination of pH and electrical conductivity (EC), were done using a hand-held Extik EC 600 multimeter, macronutrients were determined by standard chemical analytical methods, while heavy metals were determined using Atomic Absorption Spectrophotometer.

### 3.4.1 Determination of Hydrogen Ion Concentration (pH)

An air-dried soil sample weighing 20 grams was sieved and combined with 20 mL of distilled water and was left to for 30 minutes. The soil-water mixture was stirred occasionally during this period with a glass rod to ensure proper mixing. After equilibration time had passed, the pH value was measured by placing the pH meter probe into the resulting suspension.

### 3.4.2 Determination of Electrical Conductivity

This was determined with a HACH CO150 TDS/Conductivity/Salinity meter. The probe was then dipped in the soil sample mixed with distilled water and the values displayed were recorded (Mustapha and Halimoon, 2015).

### 3.4.3 Determination of Organic matter

Half a gram (0.5 g) of each air-dried soil sample was put into a conical flask and 2.5 ml of 1N potassium dichromate solution $K_2Cr_2O_7$ was added and swirled gently to disperse the sample in the solution. Five millilitres (5 mL) of concentrated tetraoxosulphate (VI) acid was added rapidly into the flask and swirled gently until the sample and reagents were mixed and finally swirled vigorously for about a minute. The flask was allowed to stand in a fume cupboard for 30 minutes. Five (5) drops of the diphenylamine indicator were added and the solution titrated with 0.5N FeSO4 to maroon colour). A blank determination was carried out to standardize the dichromate and calculated as:

$$\text{Organic matter } \% = 1 + \frac{(B-S)\times N\times 3\times 100\times 100\times 100}{weight\ of\ soil\times 1000\times 77\times 8} = \frac{(B-S)\times N}{weight\ of\ soil}\,0.67$$

B is the volume of ferrous solution used in the blank titration;

S is the volume of ferrous solution used in the sample titration;

N is the normality of Ferric Ammonium Sulfate (FAS)solution

### 3.4.4 Determination of Organic Carbon

Soil sample (0.5) grams passed through a 0.2 mm sieve was weighed, after which 10 mL of 1N K2Cr2O7 solution was added to the sample in a 250 mL conical flask. Subsequently, 20 mL of concentrated sulfuric acid was added and mixed for one minute before allowing the mixture to cool for thirty minutes. A blank, which does not include soil, was prepared using the method as

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mentioned above. Following the cooling period, approximately 200 mL of distilled water was added along with 10 mL of orthophosphoric acid and 1 mL of diphenylamine indicator. Fifty (50) mL of 0.5N ferrous ammonium sulfate (FAS) solution was titrated against the prepared solution until a dark green colour change occurred, indicating the presence of organic carbon

Organic carbon content in the sample was calculated as:

$$\text{Organic carbon (\%)} = \frac{(B-S)\times N\times \ 0.003}{m} \text{ x } 100\%$$

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where

B is the volume of ferrous solution used in the blank titration;

S is the volume of ferrous solution used in the sample titration;

N is the normality of FAS solution;

m is the mass of the sample in g used in the analysis

### 3.4.5 Determination of Potassium

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Five grams (5g) of each air-dried soil sample was weighed into a digestion vessel and then a 10 ml of a mixture of HCl and $HNO_3$ was added. The mixture was heated in an Analytik Jena's microwave digester, following the manufacturer's guidelines until the sample was fully digested. The digestate was allowed to cool, transferred to a volumetric flask and then diluted to 50 ml. The potassium concentration was then measured using flame photometry. Potassium was calculated using the formula:

Total Potassium (mg/kg) = c(mg/L) × v (L)//w (kg)

Where:

C is the concentration of potassium in the extract (mg/L)

V is the volume of the extract (L)

W is the weight of the soil sample (kg)

### 3.4.6 Determination of Calcium

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One (1) mL of 0.2N KOH buffer solution was added to 50 mL of soil sample followed by the addition of a pinch of CalVer 2 calcium indicator. The resulting mixture was titrated against 0.8M EDTA solution till a blue end point was observed. Calcium hardness values were first calculated by multiplying the number of digits on the digital titrator with the digits multiplier (2.0) (Fan *et al*., 2014). Calcium was then calculated using the formula:

Calcium (mg/L) = Ca hardness mg/L as $CaCO_3$ × 0.40

### 3.4.7 Determination of Magnesium

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Ten (10) grams of soil was weighed into a 250 mL flask and diluted with 30 mL of distilled water. Then, 5 mL buffer solution was added, and a few drops of Eriochrome black indicator. This was titrated with 0.01N EDTA until the colour changed from red to blue . Magnesium was estimated using the formula:

magnesium hardness (Mg/L) = total hardness (mg/L) – calcium hardness mg/L MgCO3

= mg/L Mg hardness as CaCO3 X 0.842 MG/L Mg = mg/L MgCO3 x 0.29

or

Mg (ppm) = (Titre value × 12.15) / Sample volume

### 3.4.8 Heavy Metal Analysis

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Mortar and pestle was used to grind the dried soil samples into a fine powder with particle sizes of 80 microns. A one-gram portion of the pulverized sample was transferred into a digestion tube, to which 10 mL of 98% concentrated nitric acid was added. The tube was subsequently placed in a water bath and heated to boiling for a duration of 72 hours. The resulting pale yellow digest was then diluted to a final volume of 25 mL using deionized water for each sample and preserved for analysis. The prepared solutions were analyzed for zinc, copper, and iron concentrations using an Atomic Absorption Spectrophotometer (AAS, Perkin Elmer model 2130). Quality assurance was maintained through the use of certified standard reference materials to verify analytical accuracy, with the obtained values falling within the acceptable range of the certified concentrations.

### 3.4.9 Determination of Nitrogen

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Nitrogen was estimated in the sampled effluent through the employment of the procedure reported by the American Water Works Association (AWWA) (2017). This was done as follows: 1g of the ground soil was weighed into the Kjeldahl flask (digestion tube). A few drops of water were added and allowed to stand for about 30 minutes. 5g of the Kjeldahl catalyst mixture (500g of $Na_2SO_4$ + 50g of selenium catalyst grinded to fine powder) was added. Twenty millimeters of concentrated sulphuric acid was added and heated on a digestion block until frothing ceases, and the temperature was increased until the digest cleared. The flask was allowed to cool, and a little water with care was added before washing the content into 199ml volumetric flask. 10ml was pipetted from the digest into a distillation flask. Twenty millimeters of Boric acid was measured into the 100ml conical flask and was introduced to the bottom of the condenser with 3 drops of the Boric acid indicator. Twenty millimeters of NaOH was added to the 10ml of the digest in the distillation flask, and was immediately introduced in the distillation unit to distil off. The trapped ammonia was then titrated with standard acid ($H_2SO_4$) to quantify the nitrogen content.

### 3.4.10 Determination of Phosphorus

The soil contaminated effluent was weighed using the electrical weighing balance. 7ml of the extraction solution (0.5M HCl + 0.5M NH4F) was added. It was shaken for 1 minute and centrifuged to about 5 minutes in order to obtain clear supernatant. 2ml of the extract was taken into another clean vial. Then 4ml of the ascorbic acid (molybdate solution) was added and diluted with distilled water to the mark of 25ml. The colour was allowed for about 30 minutes to develop. It was thereafter taken to the calorimeter to detect the colour intensity or concentration of phosphorus.

### 3.4.11 Determination of Sodium

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One gram (1g) of soil was weighed, air-dried and ground to fine powder. The soil was treated with ammonium acetate to leach out the exchangeable sodium. Then it was shaken, centrifuged and filtered to get a clear extract. The extract was diluted with deionized water to bring it into the linear range of the flame photometer. A series of standard sodium solution was then prepared and were ran through the flame photometer to plot a calibration curve. The prepared soil extract was aspirated into the flame photometer, and then the emission intensity at 589 nm was recorded and compared to the calibration curve to get the sodium concentration. The extract concentration was converted back to the soil's sodium content (mg kg-$^{1}$) using the dilution factor and original soil weight.

## 3.5 Preparation of Media

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Media such as Nutrients agar (NA), Eosin methylene blue agar (EMB), Blood agar, DNase agar, Simmon citrate agar (SCA), Triple sugar iron (TSI), Spirit blue agar, Nutrient gelatin medium, Muller Hinton broth (MHB) were prepared according to manufacturer's instructions using distilled water and autoclaved at 121°C for 15 minutes.

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## 3.6 Microbiological Analysis

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Total heterotrophic and total coliform count was carried out on the samples using standard pour plate technique on Nutrient agar and MacConkey agar respectively, where 0.5ml of the sample was pour plated into Nutrient agar and 1 ml of the sample was pour plated into MacConkey agar. The Petri dishes were rotated gently for proper homogenization. The contents were allowed to set and the plates were incubated at 37°C for 24 hours Bacterial colonies appearing on the plates after the incubation period was enumerated to determine soil samples with significant bacterium. The colonies were isolated using inoculating loop and subsequently sub-cultured.

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### 3.6.1 Enumeration of Bacterial Isolates

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Following the completion of the incubation period, the culture plates were examined and assessed for microbial growth. The bacterial population density was determined by enumerating the distinct colonies present on each plate. Morphological features and cultural characteristics of the colonies were carefully observed and recorded. Individual discrete colonies were isolated and purified using the streak plate technique to establish pure cultures. Stock cultures were then prepared from each isolated colony by inoculating agar slants, which were incubated for 24 hours to facilitate growth. After the incubation period, these stock cultures were preserved at 4°C in a refrigerator for subsequent experimental procedures

### 3.6.2 Sub-culturing

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The prepared media was poured into Petri-dishes. Bacteria with different characteristics were picked from the previously cultured plates and sub-cultured on plates containing the same medium from which they were isolated by using a wire loop to pick a particular colony and was incubated again for 24 hours at an environmental temperature (28±2). The process was carried out very close to the flame for proper sterility.

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### 3.6.3 Morphological Analysis

#### 3.6.3.1 Gram staining reaction

The Gram staining process differentiates the bacteria into two groups based on their cell wall composition. Bacteria that retain the primary stain through the decolorization process are termed Gram positive, whereas bacteria that lose the primary stain during decolorization are called Gram negative. This difference in retention of the stain is due to the structure of the cell wall. Gram staining reagents include crystal violet (primary stain), iodine solution (mordant), 70% ethanol (decolorizing agent), and safranin (counterstain).

**Methodology**: A drop of sterile distilled water was placed on the center of a clean, grease-free glass slide. The inoculating loop was sterilized by heating it in the flame until glowing red hot and then allowed to cool. Then, pick up a small volume of bacterial culture and emulsify in the water droplet on the slide. Allow the slide to air dry completely, followed by a heat fixation by gently passing the slide several times through a flame. The fixed smear was then flooded with 1% crystal violet solution for one minute and washed with distilled water. Iodine solution was applied as a mordant to the smear and remained there for 30 seconds before being drained from the slide.

The smear was decolorized with 70% ethanol for 20 seconds on the slide; this was the primary staining step of the Gram stain. The slide was rinsed well with distilled water after decolorizing. Safranin counterstain was added the smear for one minute and then washed off with distilled water; the slide was then air dried. The microorganisms were observed under the oil immersion objective lens at 100× magnification. Gram positive bacteria appeared purple or violet under the microscope, and Gram negative bacteria appeared red or pink in color.

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### 3.6.4 Biochemical Test

#### 3.6.4.1 Oxidase test

This test was carried out to check if the isolated bacteria produce the oxidase enzyme. A part of the filter paper was impregnated with a few drops of oxidase reagent, which consisted of Tetramethyl-p-phenyl-diamine-dihydrochloride. One colony of the test bacteria was streaked onto the impregnated part of the filter paper. The oxidase-producing bacteria will bring about oxidation of the phenyl-diamine compound, which is present in the reagent, resulting in a deep purple coloration. The emergence of the purple color within 10 seconds indicates a positive result (Cheesbrough, 2006).

#### 3.6.4.2 Indole test

This test detects bacterial isolates with the ability to break down the amino acid tryptophan, using the enzyme tryptophanase. The indole test uses Kovac’s reagent, which has yellow coloration. The bacterial culture was placed in a test tube with 3 mL of peptone water and incubated at 37°C for 24 hours. After that, about 0.5 mL of Kovac’s reagent, consisting of 1-p dimethylaminebenzaldehyde, was added, and the test tube was agitated gently. The presence of rose-pink or purple-colored growth at the surface of the medium within 10 minutes marked a positive test for indole production, and if there was no color change, then it was negative (Cheesbrough, 2004).

#### 3.6.4.3 Citrate test

This test assesses the organism's ability to metabolize citrate as its sole carbon and energy source, and ammonium compounds as its sole nitrogen source. Simmons citrate agar is used as the growth medium in assessing the above-mentioned ability. The test has been widely used as a diagnostic method to distinguish between members of the family Enterobacteriaceae and various other bacterial species. Bijou bottles with slanted Simmons citrate agar were each inoculated with 24-hour bacteria culture and incubated at 37°C for approximately 24 hours. The change in color from green to blue after incubation indicated a positive result.

### 3.6.4.4 Coagulase test

This test is used to determine the enzyme coagulase that differentiates between pathogenic strains of *Staphylococcus*. A colony of the test organisms was emulsified with sterile normal saline solution on a clean slide using a sterile wire loop. A drop of human plasma was added and mixed . Coagulase positive organisms showed clumping while coagulase negative organisms showed no clumping (Cheesbrough, 2006).

### 3.6.4.5 Catalase test

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This test is used to identify the presence of the catalase enzyme, which catalyzes the breakdown of hydrogen peroxide, producing oxygen gas. The bacterial culture was placed on a clean surface and mixed with a drop of hydrogen peroxide solution of 3% concentration. The presence of gas bubble formation at the surface was an indication of a positive result (Cheesbrough, 2006).

### 3.6.4.6 Urease test

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This was used to demonstrate the ability of the isolates to produce the enzyme urease which splits urea to form ammonia. A loop full of the bacterial isolates were inoculated into slants of sterile urea medium and incubated at 37 $^{o}$C for 24-48 hours. Urease positive cultures produced a red-pink colour due to changes in the colour of the Phenol Red indicator (Cheesebrough, 2006).

$$NH_2CO.NH_2 + H_2O \rightarrow 2NH_3 + CO_2$$

### 3.6.4.7 Sugar fermentation test using triple sugar iron (TSI) agar

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The preparation of the TSI medium was according to the manufacturer's instruction, and then it was placed in test tubes at an slant position to solidify. The slanted surface and basal part of the solidified medium were then inoculated with the bacterial sample using a sterilized inoculation loop and incubated for 18-24 hours. The results were noted depending on whether there was acidic or basic metabolism present in either the slanted or basal parts of the test tube, with gas production confirmed by checking if there were crevices or air bubbles in either of these parts. The production of hydrogen sulfide was also confirmed by checking if the growth medium turned black. Red slant / yellow bottom indicate that only Glucose was fermented, yellow slant / yellow bottom indicate that Glucose, Lactose and Sucrose were fermented, while red slant / red bottom indicate that there was no fermentation, The results were analyzed using a standard table designed by the laboratory, based on set neurological standards along with biochemical tests carried out on the isolates to confirm or determine its taxonomy.

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### 3.7 Antibiotics Susceptibility Test

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The bacterial isolates were tested for resistance and sensitivity to antibiotics using the Kirby Bauer disc diffusion method. The commercially prepared antibiotic discs used for susceptibility testing included Imipenem (IMI) 10µg, Cefixime (CFM) 5µg, Levofloxacin (LEV) 5µg, Gentamycin (GM) 10µg, Azithromycin (ATH) 15µg, Ceftriaxone (CRO) 30µg, Ceftazidime (CAZ) 10µg, Cefuroxime (CXM) 30µg, Erythromycin (E) 15µg. Five (5) colonies of the test bacteria from standardized culture medium were inoculated into Mueller Hinton broth and incubated at 37$^{o}$ C for 24 hours. Using sterile syringe and needle 0.5ml of the culture was transferred on to the surfaces of sterile Mueller Hinton agar and spread evenly by gently rotating the plates. Using sterile forceps, the antibiotic discs were placed appropriately and evenly on the inoculated plates. The plates were incubated at 37 °C overnight. After incubation, the plates were observed for zones of inhibition around each of the antibiotics. The diameter of the zone of

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inhibition was then measured in millimeter (mm) and the results were interpreted using the CLSI (Clinical and Laboratory Standards Institute) breakpoints (Bauer *et al.,*1966; CLSI, 2018).

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## 3.8 Multiple antibiotic resistance (MAR) index

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The MAR index is a good tool for health risk assessment which identifies if isolates are from a region of high or low antibiotic use. An MAR index ≥ 0.2 indicates a high-risk source of contamination.

$$\text{MAR Index was calculated as follows: MAR} = \frac{a}{b}$$

Where: a, is number of antibiotics to which isolate is resistant; b, total number of antibiotics tested.

## 3.9 Detection of Phenotypic Virulence Factors

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### 3.9.1 Hemolysin production

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Bacteria was suspended in 3mL of Mueller Hinton broth. The density of this suspension was adjusted to 0.5 McFarland standards by weighing 0.1g $BaSO_4$, then 99.9ml distilled water was added, it was thoroughly mixed and autoclaved. Turbidity was verified by comparing with 1% HCl solution. A 5ml sample of this suspension was inoculated onto sterile sheep blood agar plate and incubated at 37 °C for 24 to 48 hours. Thereafter, beta hemolysis is indicated by clear colourless zones surrounding the colonies indicating that there has been total lyses of the red blood cells. Alpha hemolysis is indicated by a small zone of greenish to brownish decolourization of the media. This is caused by the reduction of haemoglobin to methemoglobin and its subsequent diffusion into the surrounding medium. Gamma hemolysis is indicated by no change in the media.

### 3.9.2 Gelatinase Production

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The gelatinase production of the isolates was assayed in a nutrient gelatin medium. Bacteria culture was suspended in 3mL of Mueller Hinton broth. The density of this suspension was adjusted to 0.5 McFarland standard, which is the equivalent of 1.5 x $10^8$ cells/mL. A 1 mL sample of this suspension was inoculated onto gelatin medium (3%) and incubated at 37°C for 24 to 48 hours. Zones of clearance due to gelatin hydrolysis by the enzyme gelatinase in the media indicated the presence of gelatin-liquefying microorganisms. No zone of clearance indicates a negative result.

### 3.9.3 DNA Degrading Activity

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To test for DNA degrading activity, bacteria was cultured on DNase agar plates. Bacteria suspended in 3mL of Mueller Hinton broth. The density of this suspension was adjusted to 0.5 McFarland standards, which is the equivalent of 1.5 x $10^8$ cells/mL. A 1 mL sample of this suspension was inoculated on DNase agar plates and incubated in triplicate at 37°C for 24 to 48 hours. When DNA is hydrolysed, methyl green dye is decolourized turning the medium colourless around the test organism. Where there is no degradation of DNA, the medium remains green (supply reference).

### 3.9.4 Lipase Test

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Lipase test was done as described by Cheeseborough (2006). The lipase activity of the isolates was assayed on tryptone soy agar (TSA) plates supplemented with 1% Tween 80 (v/v). Colonies grown on tryptone soy broth (TSB) agar were suspended in 3mL of Mueller Hinton broth. The density of this suspension was adjusted to 0. 5 McFarland standards, which is the equivalent of 1. 5 x $10^8$ cells/mL.1ml sample of this suspension was inoculated on tryptic soya agar (TSA) and incubated at 37°C for 24 to 48h.

## 3.10 Plasmid profiling (TENS method)

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Isolation of bacterial plasmid DNA was carried out to determine the molecular weight of an unknown plasmid on the basis of its mobility through agarose gel in comparison with a molecular maker. 1.5ml Overnight bacterial culture was spun at top speed for 1mins, the supernatant was discarded and ~150 µl of media was left. The cells were then resuspended by vortexing and 300 µl of TENS (Total DNA digestion, Endonuclease digestion, Number of plasmid, Size of plasmids)buffer was added, gently inverted 3-4 times to lyse cells. The solution was washed by adding 70% Ethanol, dried and dissolved in Tris buffer (pH8) before use.

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## 3.11 Agarose Gel Electrophoresis

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DNA gel electrophoresis is a laboratory technique that separates DNA fragments according to the size of the molecules. The process works by using an electric current that pulls negatively charged DNA molecules through a gel-like substance. Smaller fragments travel faster and farther compared to larger ones.

To make the gel, 0.8 grams of agarose powder was added to 100 ml of TBE buffer solution and heated on a magnetic stirrer until completely dissolved. After cooling the mixture down to about 60°C, 10 µl of ethidium bromide (a fluorescent dye) was stirred into the liquid with gentle care. This liquid gel was then poured into an electrophoresis tray with a comb-like insert, which creates sample loading wells. The gel was left to solidify for 20 minutes without being disturbed, after which the comb was carefully extracted.

The set gel was then placed in the electrophoresis chamber filled with TBE buffer, making sure that the surface of the gel was covered by the liquid. To prepare each sample of DNA, 15 µl of a DNA sample was mixed with 2 µl of loading dye and carefully pipetted into the wells. The power supply was attached, with the negative electrode at the sample end, as DNA would naturally migrate to the positive charge.

The electric field was applied at 60-100 volts and was kept on until the visible loading dye had migrated approximately three-quarters down the gel length. Following this, the power was turned off and the electrodes disconnected. Next, the gel was observed under UV light, whereby the DNA-stained ethidium bromide is excited to fluoresce and becomes visible (Voytas et al., 2001).

## 3.12 Plasmid curing and subsequent evaluation of antibiotic susceptibility testing of the Multi Drug resistance bacterial isolates

Plasmid curing refers to the loss of plasmid; it usually occurs spontaneously or can be induced by treatments that inhibit plasmid replication but not host cell reproduction. Some commonly used curing treatments are ion and ionizing radiation, antibiotics, acridine, mutagens, targeting growth above optimum temperature and pH and using other extreme environmental conditions (Zaman *et al.*, 2011). The characterized bacterial isolates were inoculated into 10 ml of nutrient broth containing 100µg/mL of the mutagen (acridine orange) as described by the modified methods of Sheikh *et al*. (2003) and Yah *et al*. (2007). The mixture was incubated overnight at 37 $^{o}$C for 24hours. Upon incubation, each mutagen-exposed culture was plated on nutrient agar medium and incubated at 37 $^{o}$C for 24 hours. Colonies were randomly selected from each of the plate per isolates, for sensitivity testing. Upon incubation overnight in 5 ml nutrient broth and diluted to 10$^{-2}$ in sterile distilled water, 0.1 ml of the diluted (10$^{-2}$) inoculum was seeded onto solidified nutrient agar. Thereafter, multi antibiotic discs containing different antibiotics were firmly placed onto the surface of the agar plates using forceps. The plates were incubated at 37 $^{o}$C for 24 hours and visible zones of inhibition were measured. Inhibition zone diameters (IZD) less

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3.12 Plasmid curing and subsequent evaluation of antibiotic susceptibility testing of the Multi Drug resistance bacterial isolates

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than 14 mm were recorded as resistant (R), while IZD ranging from 14 mm to 17 mm were reported as intermediate (I). Zones of inhibition greater than 17 mm were recorded as susceptible (S) for the respective isolates (Harley and Prescott, 2002).

## 3.13 Data Analysis

The data were analysed using IBM SPSS package version 20.0 statistical package for Duncan ANOVA and significant order difference evaluation. All data are mean of three replicates. The mean, range and standard deviation of each parameter was determined. Differences in mean were compared using Duncan's Multiple Range test (Ogbeibu, 2015).

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# CHAPTER FOUR

# RESULTS

Table 4.1 shows the physico-chemical properties of soil samples taken from four abattoir locations in both dry and rainy seasons. From Table 4.1, the pH values for all the sites and seasons were between 6.25 and 6.90. Electrical conductivity ranged from 73-161 µS/cm, with generally higher values from contaminated topsoil during the dry season. The organic matter content was between 9.10% and 14.83%, and organic carbon between 5.28% and 8.90%. there was statistical difference for Electrical conductivity, organic carbon and organic matter, but there was no statistical difference across sites for pH.

Table 4.2 shows the analysis of heavy metals, such as zinc, iron, and copper, in the soil samples. As described in Table 4.2, iron had the highest concentration among all assayed metals, ranging from 27.19 to 140.29 mg/kg, with the highest value obtained in Oluku Top during the rainy season. Zinc values ranged from 16.42 to 48.69 mg/kg, exhibiting considerable enrichment in the topsoil during the dry season, especially in Oluku Top. Copper varied from 4.95 to 22.57 mg/kg; contaminated sites were consistently higher compared with controls.

Table 4.3 shows the concentrations of the macronutrient elements in all the sampling sites. Calcium ranged between 2.13 and 4.77 mg/kg, with the highest value of 4.77 mg/kg obtained at Oluku Top contaminated soil during the dry season and the lowest value of 2.17 mg/kg obtained at Uniben bottom (Table 4.3). Concentrations of magnesium were from 5.24 to 15.76 mg/kg; potassium showed the highest degree of enrichment in contaminated sites, ranging between 13.82 and 52.48 mg/kg. Nitrogen varied between 3.17 and 4.80 mg/kg, while phosphorus fluctuated between 4.91 and 12.88 mg/kg. Values did not show consistent enrichment for phosphorus in contaminated areas.

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**Table 4.1: Physicochemical Properties of Soil Samples for Both Dry and Rainy Seasons**

| | **pH** | | **EC (µs/cm)** | | **OC (%)** | | **OM (%)** | |
|---|---|---|---|---|---|---|---|---|
| | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** |
| Oluku Top | 6.41±1.00 | 6.53±1.55 | 161±12.50 | 154±15.00 | 6.89±1.33 | 7.65±1.3 | 11.88±0.50 | 12.51±2.5 |
| Oluku Top Control | 6.55±2.62 | 6.63±1.25 | 116±6.0 | 119±20.00 | 8.06±0.98 | 8.90±.33 | 12.90±2.6 | 14.83±2.0 |
| Oluku Middle | 6.44±1.22 | 6.61±1.00 | 124±3.44 | 97±14.0 | 6.85±0.5 | 6.93±1.5 | 11.81±0.0 | 12.15±3.0 |
| Oluku Middle Control | 6.53±0.5 | 6.90±2.00 | 92±7.0 | 81±10.8 | 7.85±1.0 | 8.06±1.0 | 11.83±1.0 | 13.90±2.0 |
| Oluku Bottom | 6.48±0.0 | 6.54±1.88 | 144±12.5 | 125±17.5 | 5.28±0.88 | 6.12±0.5 | 9.10±0.0 | 11.60±2.0 |
| Oluku Bottom Control | 6.28±0.12 | 6.43±0.98 | 107±22.0 | 96±10.0 | 6.44±0.6 | 7.44±0.0 | 10.22±0.0 | 11.81±0.5 |
| **P value** | 0.018 | | 0.035 | | 0.010 | | | 0.007 |
| Uniben Top | 6.44±1.33 | 6.48±0.77 | 147±15.6 | 144±12.8 | 7.65±1.5 | 7.78±0.0 | 12.38±1.0 | 13.14±1.5 |
| Uniben Top Control | 6.51±0.60 | 6.74±2.00 | 131±10.8 | 116±13.0 | 7.76±1.55 | 8.06±1.0 | 13.61±1.0 | 13.90±2.5 |
| Uniben Middle | 6.52±1.33 | 6.76±1.5 | 111±5.5 | 89±22.0 | 7.18±1.0 | 7.24±0.5 | 12.15±2.5 | 12.38±2.5 |
| Uniben Middle Control | 6.64±0.0 | 6.80±0.5 | 103±12.5 | 74±10.0 | 7.15±0.5 | 7.44±0.5 | 12.83±10.0 | 13.15±1.0 |
| Uniben Bottom | 6.54±1.0 | 6.62±0.0 | 125±20.0 | 107±18.5 | 6.89±1.0 | 7.04±1.0 | 11.88±1.0 | 12.14±0.5 |
| Uniben Bottom Control | 6.32±0.5 | 6.55±0.0 | 123±22.0 | 96±20.0 | 5.62±0.0 | 6.73±0.5 | 9.92±1.5 | 11.60±0.5 |
| **Pvalue** | 0.006 | | 0.004 | | 0.085 | | | 0.052 |

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Keys: EC=electrical conductivity, OC= organic carbon, OM= organic matter

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## Table 4.1 (continued): Physicochemical Properties of Soil Samples for Both Dry and Rainy Seasons

| | pH | | EC (µs/cm) | | OC (%) | | OM (%) | |
|---|---|---|---|---|---|---|---|---|
| | DS | RS | DS | RS | DS | RS | DS | RS |
| Ewah Top | 6.25±0.5 | 6.50±1.0 | 145±18.0 | 144±13.8 | 7.65±1.22 | 7.96±0.5 | 11.83±1.0 | 13.30±1.0 |
| Ewah Top Control | 6.54±0.5 | 6.57±0.5 | 120±20.0 | 97±10.0 | 7.78±1.5 | 8.49±1.0 | 12.14±1.0 | 13.88±1.0 |
| Ewah Middle | 6.53±1.0 | 6.55±0.5 | 118±15.5 | 110±10.0 | 7.54±2.0 | 7.84±1.5 | 11.81±2.0 | 12.83±0.5 |
| Ewah Middle Control | 6.56±1.0 | 6.62±2.5 | 101±10.8 | 73±12.0 | 7.18±0.5 | 7.84±1.5 | 12.12±2.5 | 12.38±1.5 |
| Ewah Bottom | 6.57±2.5 | 6.57±2.0 | 122±10.0 | 121±22.0 | 7.44±1.5 | 7.65±1.2 | 11.15±1.0 | 11.95±1.5 |
| Ewah Bottom Control | 6.54±1.0 | 6.60±1.0 | 106±22.0 | 85±15.0 | 7.04±1.0 | 7.46±1.0 | 11.90±1.5 | 12.08±0.5 |
| **Pvalue** | | 0.119 | | 0.037 | | 0.004 | | 0.016 |
| Dumez Top | 6.51±1.0 | 6.54±1.0 | 135±13.5 | 125±13.0 | 7.24±0.5 | 7.73±1.0 | 12.41±0.5 | 12.64±0.0 |
| Dumez Top Control | 6.53±1.0 | 6.56±2.5 | 110±20.0 | 107±10.0 | 7.10±0.5 | 8.06±1.0 | 12.38±0.5 | 13.90±0.0 |
| Dumez Middle | 6.57±0.5 | 6.59±1.0 | 114±22.0 | 92±12.0 | 7.18±1.0 | 7.65±2.0 | 12.15±1.5 | 12.21±1.5 |
| Dumez Middle Control | 6.59±0.75 | 6.74±0.0 | 105±15.5 | 80±10.0 | 7.04±1.5 | 7.90±1.0 | 12.14±1.0 | 12.81±1.0 |
| Dumez Bottom | 6.61±0.0 | 6.60±0.0 | 120±18.5 | 118±12.0 | 6.89±1.5 | 7.28±0.0 | 11.88±0.0 | 12.18±1.0 |
| Dumez Bottom Control | 6.57±0.0 | 6.58±1.5 | 108±20.8 | 96±9.0 | 6.84±2.0 | 6.86±1.5 | 11.80±0.0 | 11.93±1.5 |
| **Pvalue** | | 0.159 | | 0.025 | | 0.012 | | 0.083 |

**Keys: EC=electrical conductivity, OC= organic carbon, OM= organic matter**

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**Table 4.2: Heavy Metals of Abattoir Effluent Contaminated Soil Samples**

| **Samples** | **Zn(mg/kg)** | | **Fe(mg/kg)** | | **Cu(mg/kg)** | |
|---|---|---|---|---|---|---|
| | **DS** | **RS** | **DS** | **RS** | **DS** | **R** |
| Oluku Top | 48.69±6.8 | 34.72±9.0 | 134.94±12.0 | 140.29±20.0 | 14.26±5.0 | 22.57 |
| Oluku Top Control | 33.23±3.0 | 18.69±5.5 | 101.23±13.5 | 98.57±12.0 | 9.37±4.5 | 17.38 |
| Oluku Middle | 34.72±2.5 | 24.52±7.0 | 94.33±7.0 | 123.51±15.0 | 13.91±3.6 | 14.26 |
| Oluku Middle Control | 28.69±7.0 | 17.23±5.0 | 85.57±9.5 | 89.48±9.5 | 7.38±4.0 | 12.57 |
| Oluku Bottom | 26.94±3.0 | 21.98±3.5 | 63.19±8.0 | 81.44±4.0 | 9.43±1.5 | 13.63 |
| Oluku Bottom Control | 18.28±2.2 | 11.28±2.2 | 56.69±9.0 | 72.93±7.0 | 4.95±0.0 | 10.40 |
| **pvalue** | | 0.031 | | 0.063 | | 0.0 |
| Uniben Top | 36.52±4.5 | 22.42±3.0 | 97.92±15.0 | 111.51±21.0 | 12.22±2.0 | 13.94 |
| Uniben Top Control | 22.98±4.0 | 19.92±2.0 | 78.90±3.9 | 82.74±8.5 | 11.63±3.2 | 11.90 |
| Uniben Middle | 22.38±3.0 | 20.81±1.5 | 49.15±5.6 | 62.28±9.0 | 8.94±3.5 | 10.52 |
| Uniben Middle Control | 19.72±2.2 | 16.10±2.8 | 34.33±2.8 | 59.10±5.0 | 8.30±3.0 | 9.57 |
| Uniben Bottom | 16.42±2.0 | 16.94±2.2 | 37.22±5.5 | 49.38±4.8 | 5.91±1.5 | 9.71 |
| Uniben Bottom Control | 17.19±1.5 | 12.73±1.5 | 27.19±3.8 | 37.29±4.5 | 5.82±1.5 | 8.21 |
| **Pvalue** | | 0.062 | | 0.031 | | 0.03 |
| Ewah Top | 36.52±1.2 | 25.23±3.2 | 97.57±5.0 | 102.56±20.0 | 12.57±1.8 | 18.22 |
| Ewah Top Control | 30.28±10.0 | 22.38±7.0 | 62.22±3.0 | 78.68±4.0 | 10.12±4.0 | 16.83 |
| Ewah Middle | 25.33±1.9 | 24.69±3.0 | 70.68±0.9 | 86.43±3.7 | 11.89±3.9 | 15.38 |
| Ewah Middle Control | 25.23±5.5 | 16.42±4.0 | 57.92±1.8 | 47.92±4.0 | 9.74±2.8 | 10.67 |
| Ewah Bottom | 21.95±6.0 | 18.28±3.0 | 68.69±2.0 | 77.18±4.0 | 8.42±4.0 | 13.57 |
| Ewah Bottom Control | 18.70±3.0 | 13.08±2.5 | 41.15±4.0 | 49.15±2.0 | 6.68±3.8 | 9.94 |
| **Pvalue** | | 0.031 | | 0.219 | | 0.0 |

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Keys: Zn= Zinc, Fe= Iron, Cu= Copper

**Table 4.2 (Continued): Heavy Metals of Abattoir Effluent Contaminated Soil Samples**

| **Samples** | **Zn(mg/kg)** | | **Fe(mg/kg)** | | **Cu(mg/kg)** | |
|---|---|---|---|---|---|---|
| | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** |
| Dumez Top | 23.11±2.0 | 24.72±4.0 | 77.22±6.0 | 98.73±5.0 | 13.26±5.0 | 13.63 |
| Dumez Top Control | 26.94±1.6 | 20.23±2.0 | 53.29±3.8 | 71.59±2.8 | 8.57±3.0 | 13.24±2.2 |
| Dumez Middle | 20.57±5.0 | 21.98±3.3 | 57.92±0.0 | 66.41±3.0 | 9.43±1.0 | 10.67±1.1 |
| Dumez Middle Control | 22.38±3.0 | 18.69±2.0 | 31.43±1.0 | 57.99±1.5 | 8.38±1.0 | 10.23±1.6 |
| Dumez Bottom | 17.42±5.0 | 16.52±2.5 | 39.15±1.0 | 49.48±0.5 | 6.22±2.5 | 8.29±1.0 |
| Dumez Bottom Control | 16.42±4.0 | 13.28±2.0 | 28.11±0.0 | 44.20±0.5 | 5.48±0.5 | 6.94±0.5 |
| **Pvalue** | | 0.312 | | 0.031 | | 0.031 |

Keys: Zn= Zinc, Fe= Iron, Cu= Copper

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## Table 4.3: Physico-chemical properties (macronutrients) of Soil Samples for Both Dry and Rainy Seasons

| **Samples** | **Ca(mg/kg)** | | **Mg(mg/kg)** | | **K(mg/kg)** | | **Na(mg/kg)** | | **N(mg/kg)** | | **P(mg/kg)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** |
| Oluku Top | 4.77±0.0 | 3.21±1.0 | 15.76±1.0 | 10.76±0.5 | 52.48±1.0 | 49.48±2.0 | 1.73±0.0 | 0.94±0.0 | 4.69±1.0 | 4.50±0.0 | 12.73±0.5 | 12.82± |
| Oluku Top Control | 3.46±1.0 | 3.32±1.0 | 10.22±1.3 | 8.82±1.1 | 32.33±1.0 | 26.33±2.0 | 1.05±0.0 | 0.73±0.0 | 4.12±1.0 | 3.98±0.6 | 12.88±0.3 | 12.31± |
| Oluku Middle | 3.86±0.0 | 3.54±0.0 | 11.59±0.3 | 11.51±0.0 | 38.74±0.5 | 38.74±3.5 | 1.12±0.0 | 1.41±0.0 | 4.78±0.5 | 4.69±0.4 | 8.11±0.3 | 8.28± |
| Oluku Middle Control | 2.18±1.5 | 3.37±0.0 | 9.76±0.0 | 8.98±0.2 | 28.48±0.3 | 21.40±1.0 | 0.87±0.0 | 1.01±0.0 | 4.80±0.5 | 4.01±0.0 | 8.55±0.2 | 11.29± |
| Oluku Bottom | 3.87±1.0 | 3.61±0.0 | 11.46±0.0 | 12.16±0.8 | 39.68±0.4 | 25.32±1.0 | 1.59±0.0 | 1.22±0.0 | 3.63±0.8 | 3.63±0.2 | 7.65±0.1 | 8.64± |
| Oluku Bottom Control | 2.13±1.5 | 3.53±1.5 | 9.72±1.0 | 9.27±1.0 | 29.29±1.0 | 13.82±2.0 | 1.02±0.0 | 0.88±0.0 | 3.95±1.0 | 3.57±0.1 | 7.71±0.0 | 7.97± |
| Pvalue | | 0.461 | | 0.031 | | 0.218 | | 0.312 | | 0.031 | | 0.21 |
| Uniben Top | 3.75±0.0 | 2.75±1.0 | 12.93±1.0 | 9.93±1.5 | 47.19±1.1 | 47.19±1.3 | 1.22±0.0 | 0.87±0.0 | 3.56±1.0 | 3.44±0.5 | 8.96±0.3 | 9.21± |
| Uniben Top Control | 2.98±0.0 | 2.60±0.5 | 7.63±1.5 | 6.94±1.0 | 23.74±2.0 | 35.73±2.8 | 0.93±0.0 | 0.67±0.0 | 3.54±1.0 | 3.21±0.0 | 11.30±2.0 | 8.85± |
| Uniben Middle | 3.12±1.0 | 3.12±1.0 | 9.52±0.0 | 9.52±1.0 | 28.52±2.5 | 28.52±1.2 | 1.15±0.0 | 0.93±0.0 | 3.89±0.6 | 3.56±0.4 | 5.22±2.5 | 7.96± |
| Uniben Middle Control | 2.08±1.5 | 3.00±0.5 | 7.82±2.0 | 7.32±0.0 | 21.46±3.0 | 29.17±2.0 | 0.61±0.0 | 0.86±0.0 | 4.67±0.4 | 3.38±0.3 | 7.79±1.3 | 8.99± |
| Uniben Bottom | 2.17±0.5 | 3.17±0.0 | 8.26±1.2 | 8.26±0.3 | 24.06±3.6 | 24.06±1.1 | 1.18±0.0 | 1.13±0.0 | 3.35±0.4 | 3.31±0.5 | 4.91±1.3 | 5.24± |
| Uniben Bottom Control | 2.46±0.3 | 2.92±0.0 | 7.13±1.0 | 8.32±0.2 | 20.33±2.0 | 26.92±1.0 | 0.87±0.0 | 0.85±0.0 | 4.12±0.0 | 3.35±0.5 | 5.23±1.4 | 6.64± |
| Ewah Top | 3.67±0.0 | 3.34±0.0 | 12.72±1.0 | 9.82±0.0 | 40.33±2.9 | 47.19±1.0 | 1.75±0.0 | 0.95±0.0 | 4.11±0.0 | 3.77±0.3 | 8.84±1.2 | 11.23± |
| Ewah Top Control | 3.29±0.0 | 2.52±0.1 | 7.52±1.0 | 6.28±0.3 | 22.53±3.3 | 34.82±3.0 | 1.21±0.0 | 0.76±0.0 | 3.76±0.3 | 3.45±0.0 | 11.25±1.0 | 11.03± |

Key: Ca= Calcium, Mg= Magnesium, K= Potassium, Na= Sodium, N= Nitrogen, P= Phosphorus

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**Table 4.3 (continued): Physico-chemical properties (macronutrients) of Soil Samples for Both Dry and Rainy Seasons**

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| **Samples** | **Ca(mg/kg)** | | **Mg(mg/kg)** | | **K(mg/kg)** | | **Na(mg/kg)** | | **N(mg/kg)** | | **P(mg/kg)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| _ | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** | **DS** | **RS** |
| Ewah Middle | 3.22±0.4 | 3.58±0.0 | 9.38±1.0 | 9.98±0.0 | 20.78±4.0 | 20.33±5.5 | 1.29±0.0 | 1.06±0.0 | 4.40±0.6 | 4.12±0.0 | 5.32±2.0 | 10.21±1.5 |
| Ewah Middle Control | 3.10±0.0 | 3.09±0.2 | 6.69±0.8 | 7.99±0.0 | 20.06±2.0 | 28.52±3.0 | 0.88±0.0 | 0.92±0.0 | 4.58±0.3 | 4.34±0.2 | 7.96±2.0 | 9.57±1.0 |
| Ewah Bottom | 2.74±0.0 | 3.67±0.1 | 8.82±2.0 | 10.76±1.0 | 19.74±2.5 | 17.93±2.8 | 1.31±0.0 | 1.04±0.0 | 4.02±0.2 | 3.82±0.3 | 5.12±1.6 | 8.73±1.1 |
| Ewah Bottom Control | 2.70±0.0 | 2.88±0.8 | 5.24±0.3 | 8.52±0.5 | 19.68±2.0 | 24.06±2.0 | 0.86±0.0 | 1.15±0.0 | 4.22±1.2 | 3.88±0.0 | 6.24±0.0 | 5.22±1.0 |
| Dumez Top | 4.21±0.4 | 3.12±0.4 | 13.59±0.5 | 9.46±0.5 | 38.74±1.2 | 39.68±0.9 | 1.18±0.0 | 0.99±0.0 | 4.29±1.0 | 3.82±0.0 | 9.60±0.0 | 9.11±1.0 |
| Dumez Top Control | 3.91±0.3 | 2.72±0.2 | 7.82±0.5 | 6.36±0.3 | 20.33±2.4 | 21.88±3.0 | 0.72±0.0 | 0.87±0.0 | 4.12±1.0 | 3.95±0.6 | 10.34±0.0 | 8.25±0.t |
| Dumez Middle | 3.72±0.4 | 3.17±0.2 | 10.63±0.4 | 9.52±0.6 | 21.46±2.2 | 28.53±1.2 | 1.04±0.0 | 1.13±0.0 | 4.52±1.2 | 4.33±0.5 | 5.96±0.7 | 8.28±0.0 |
| Dumez Middle Control | 3.32±0.3 | 3.13±0.2 | 7.76±0.3 | 7.52±0.5 | 20.48±2.0 | 20.51±1.0 | 0.61±0.0 | 1.23±0.0 | 4.48±1.0 | 4.29±0.4 | 6.12±0.2 | 8.17±0.5 |
| Dumez Bottom | 3.78±0.0 | 3.83±0.0 | 9.83±0.4 | 10.26±0.5 | 21.49±2.5 | 24.06±1.0 | 0.98±0.0 | 1.24±0.0 | 4.57±.06 | 4.18±0.8 | 5.27±0.0 | 7.64±0.3 |
| Dumez Bottom Control | 3.18±0.4 | 3.10±0.0 | 7.73±0.6 | 8.36±0.5 | 19.82±1.6 | 19.37±2.8 | 0.66±0.0 | 0.95±0.0 | 3.78±0.5 | 3.60±0.7 | 5.91±0.0 | 6.89±0.2 |
| Pvalue | | 0.461 | | 0.031 | | 0.461 | | 0.461 | | 0.031 | | 0.031 |

Key: Ca= Calcium, Mg= Magnesium, K= Potassium, Na= Sodium, N= Nitrogen, P= Phosphorus

Table 4.4 presents the mean heterotrophic bacterial counts in soil samples from the rainy season. Values ranged from 0.27 $\times 10^8$ to 1.90 $\times 10^8$ cfu/g across the months of May, June, and July, 2024. Contaminated soils showed higher counts than control sites, but there was no significant statistical difference in all the sites. Table 4.4 also shows that topsoil generally had the highest population, with counts ranging from 0.81 $\times 10^8$ to 1.90 $\times 10^8$ cfu/g, while generally lower bacterial densities were recorded among the control soil samples.

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Table 4.5 shows the heterotrophic bacterial counts for the dry season months of November, December, and January. As evident from Table 4.5, bacterial populations were drastically reduced during this period compared with the rainy season. Contaminated topsoil samples gave counts that ranged between 1.60 $\times 10^7$ and 1.97 $\times 10^7$ cfu/g; this is about a 10-fold decrease over the values obtained during the rainy season. Table 4.5 reveals that Ewah Top had the highest count value during the dry season, with 1.97 $\times 10^7$ cfu/g obtained in January, while bottom layers gave as low counts as 0.55 $\times 10^7$ cfu/g at Ewah Bottom in December. Control samples maintained relatively low levels of bacteria throughout the period of the dry season, often less than 1.18 $\times 10^7$ cfu/g, as depicted in Table 4.5.

Table 4.6 shows the distribution of bacterial isolates during the dry season across the four abattoir locations. As indicated, Table 8 had a total bacterial frequency of 93, with the most prevalent organism being *Staphylococcus aureus* with 19 isolates (20.4% of the total), followed by *Bacillus subtilis* with 18 isolates (19.4%). As revealed in Table 8, Dumez Road had the highest total number of isolates with 27, followed by Oluku (24), Uniben (23), and Ewah Road (19).

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## Table 4.4: Heterotrophic bacteria count of samples in rainy season

| **Sample** | **May** | **June** | **July** | **P value** |
|---|---|---|---|---|
| Oluku TOP | 1.05x$10^8$ ± 10.30 | 1.26 x$10^8$ ± 11.37 | 1.86 x$10^8$ ± 5.13 | |
| Oluku MIDDLE | 1.06 x$10^8$ ± 6.51 | 0.77 x$10^8$ ± 6.51 | 1.26 x$10^8$±2.00 | |
| Oluku BOTTOM | 0.57 x$10^8$ ± 4.44 | 0.85 x$10^8$ ± 8.74 | 0.77 x$10^8$±2.50 | 0.440 |
| Oluku Control TOP | 0.85 x$10^8$ ± 3.33 | 0.85 x$10^8$±1.75 | 1.75 x$10^8$±3.33 | |
| Oluku Control MIDDLE | 0.50 x$10^8$ ± 1.77 | 0.50 x$10^8$±2.30 | 0.5 x$10^8$ ± 6.66 | |
| Oluku Control BOTTOM | 0.44 x$10^8$ ± 3.10 | 0.34 x$10^8$±0.96 | 0.34 x$10^8$ ± 1.15 | 0.724 |
| Uniben TOP | 1.90 x$10^8$ ± 4.00 | 1.49 x$10^8$ ± 4.16 | 1.89 x$10^8$ ± 1.73 | |
| Uniben MIDDLE | 1.51 x$10^8$ ± 2.00 | 0.94 x$10^8$ ± 2.08 | 1.49 x$10^8$±4.10 | |
| Uniben BOTTOM | 0.94 x$10^8$ ± 3.00 | 1.06 x$10^8$ ± 3.06 | 0.94 x$10^8$±3.54 | 0.665 |
| Uniben Contro TOP | 1.06 x$10^8$ ± 5.20 | 1.06 x$10^8$±3.33 | 1.86 x$10^8$±2.30 | |
| Uniben Control MIDDLE | 0.68 x$10^8$ ± 4.5 | 0.61 x$10^8$±2.40 | 0.61 x$10^8$ ± 8.33 | |
| Uniben Control BOTTOM | 0.31 x$10^8$ ± 1.73 | 0.31 x$10^8$±2.30 | 0.31 x$10^8$ ± 4.73 | 0.820 |
| Ewah TOP | 1.89 x$10^8$ ± 3.61 | 1.75 x$10^8$ ± 3.61 | 1.89 x$10^8$±2.11 | |
| Ewah MIDDLE | 1.75 x$10^8$ ± 9.29 | 0.84 x$10^8$ ± 9.29 | 1.75 x$10^8$±6.42 | |
| Ewah BOTTOM | 0.84 x$10^8$ ± 4.0 | 0.81 x$10^8$ ± 4.00 | 0.84 x$10^8$±2.33 | 0.678 |
| Ewah Control TOP | 0.91 x$10^8$ ± 2.31 | 0.81 x$10^8$±3.33 | 1.73 x$10^8$ ± 2.08 | |
| Ewah Control MIDDLE | 0.51 x$10^8$ ± 1.15 | 0.50 x$10^8$±1.95 | 0.5 x$10^8$ ± 2.31 | |
| Ewah Control BOTTOM | 0.30 x$10^8$ ± 2.08 | 0.27 x$10^8$±4.00 | 0.27 x$10^8$ ± 1.15 | 0.743 |
| Dumez TOP | 1.83 x$10^8$ ± 3.61 | 1.53 x$10^8$ ± 3.61 | 1.73 x$10^8$±2.00 | |
| Dumez MIDDLE | 1.53 x$10^8$ ± 2.0 | 0.82 x$10^8$ ± 2.00 | 1.53 x$10^8$±3.65 | |
| Dumez BOTTOM | 0.82 x$10^8$ ± 4.60 | 1.22 x$10^8$ ± 4.62 | 0.82 x$10^8$±4.55 | 0.846 |
| Dumez Control TOP | 1.20 x$10^8$ ± 2.50 | 1.22 x$10^8$±5.00 | 1.26 x$10^8$ ±2.00 | |
| Dumez Control MIDDLE | 1.18 x$10^8$ ± 4.21 | 1.08 x$10^8$±4.62 | 1.08 x$10^8$ ± 2.52 | |
| Dumez Control BOTTOM | 0.71 x$10^8$ ± 2.0 | 0.81 x$10^8$±4.00 | 0.81 x$10^8$ ± 7.21 | 0.995 |
| *p* value | 0.039 | 0.107 | 0.763 | |

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## Table 4.5: Heterotrophic Bacteria Count in Dry Season

| Sample | November<br>(Mean ± SD) | December<br>(Mean ± SD) | January<br>(Mean ± SD) | P value |
|---|---|---|---|---|
| Oluku TOP | 1.72x$10^7$ ± 2.52 | 1.63 x$10^7$ ± 11.27 | 1.60 x$10^7$ ± 11.50 | |
| Oluku MIDDLE | 1.13 x$10^7$ ± 5.86 | 1.14 x$10^7$ ± 3.51 | 1.13 x$10^7$ ± 3.21 | |
| Oluku BOTTOM | 0.81 x$10^7$ ± 6.51 | 0.76 x$10^7$ ± 5.57 | 0.75 x$10^7$ ± 5.00 | 0.945 |
| Oluku Control TOP | 0.85 x$10^7$± 6.66 | 0.89 x$10^7$ ± 5.03 | 0.85 x$10^7$ ± 6.66 | |
| Oluku Control MIDDLE | 0.50 x$10^7$ ± 1.15 | 0.57 x$10^7$ ± 3.00 | 0.50 x$10^7$ ± 1.15 | |
| Oluku Control BOTTOM | 0.34 x$10^7$ ± 5.13 | 0.36 x$10^7$ ± 3.51 | 0.34 x$10^7$± 5.13 | 0.602 |
| Uniben TOP | 1.87 x$10^7$ ± 5.51 | 1.81 x$10^7$ ± 10.41 | 1.81 x$10^7$ ± 9.87 | |
| Uniben MIDDLE | 1.47 x$10^7$ ± 4.51 | 1.45 x$10^7$ ± 14.11 | 1.31 x$10^7$ ± 1.73 | |
| Uniben BOTTOM | 0.92 x$10^7$ ± 3.79 | 0.91 x$10^7$ ± 7.02 | 0.81 x$10^7$ ± 2.89 | 0.124 |
| Uniben Contro TOP | 1.06 x$10^7$ ± 8.33 | 0.85 x$10^7$ ± 17.90 | 1.06 x$10^7$ ± 8.33 | |
| Uniben Control MIDDLE | 0.61 x$10^7$± 4.73 | 0.42 x$10^7$ ± 11.02 | 0.61 x$10^7$ ± 4.73 | |
| Uniben Control BOTTOM | 0.31 x$10^7$ ± 1.73 | 0.28 x$10^7$ ± 1.73 | 0.31 x$10^7$ ± 1.73 | 0.484 |
| Ewah TOP | 1.90 x$10^7$ ± 2.00 | 1.90 x$10^7$ ± 1.53 | 1.97 x$10^7$ ± 11.02 | |
| Ewah MIDDLE | 1.78 x$10^7$ ± 15.62 | 1.77 x$10^7$ ± 8.02 | 1.72 x$10^7$ ± 10.79 | |
| Ewah BOTTOM | 0.83 x$10^7$ ± 4.58 | 0.55 x$10^7$ ± 41.97 | 0.70 x$10^7$ ± 6.43 | 0.078 |
| Ewah Control TOP | 0.81 x$10^7$ ± 2.31 | 0.84 x$10^7$ ± 7.51 | 0.81 x$10^7$ ± 2.31 | |
| Ewah Control MIDDLE | 0.50 x$10^7$ ± 1.15 | 0.53 x$10^7$ ± 3.51 | 0.50 x$10^7$ ± 1.15 | |
| Ewah Control BOTTOM | 0.27 x$10^7$ ± 2.08 | 0.30 x$10^7$ ± 4.04 | 0.27 x$10^7$ ± 2.08 | 0.721 |
| Dumez TOP | 1.73 x$10^7$ ± 3.51 | 1.75 x$10^7$ ± 5.00 | 1.72 x$10^7$ ± 3.46 | |
| Dumez MIDDLE | 1.52 x$10^7$ ± 2.52 | 1.55 x$10^7$ ± 4.04 | 1.56 x$10^7$ ± 5.77 | |
| Dumez BOTTOM | 0.84 x$10^7$± 7.23 | 0.74 x$10^7$ ± 12.66 | 0.68 x$10^7$ ± 8.54 | 0.287 |
| Dumez Control TOP | 1.18 x$10^7$± 7.02 | 1.16 x$10^7$ ± 4.58 | 1.17 x$10^7$ ± 5.29 | |
| Dumez Control MIDDLE | 1.01 x$10^7$± 7.94 | 1.01 x$10^7$ ± 7.94 | 0.98 x$10^7$ ± 2.00 | |
| Dumez Control BOTTOM | 0.78 x$10^7$ ± 3.61 | 0.76 x$10^7$ ± 5.86 | 0.73 x$10^7$ ± 4.93 | 0.778 |
| p value | 0.313 | 0.226 | 0.404 | |

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## Table 4.6: Frequency and Percentage Occurrence of Bacterial Isolates in Contaminated Samples during Dry Season

| | UNIBEN | | OLUKU | | EWAH RD | | DUMEZ RD | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Frequency | % occurrence | Frequency | % occurrence | Frequency | % occurrence | Frequency | % occurrence | Total | Total % occurren |
| *Staphylococcus aureus* | 6 | 26.1 | 5 | 20.8 | 4 | 21.1 | 4 | 14.8 | 19 | 20.4 |
| *Alcaligenes* spp | 4 | 17.4 | 4 | 16.7 | 2 | 10.5 | 2 | 7.4 | 12 | 12.9 |
| *Pseudomonas aeruginosa* | 2 | 8.7 | 1 | 4.2 | 2 | 10.5 | 4 | 14.8 | 9 | 9.7 |
| *Salmonella* spp | 2 | 8.7 | 2 | 8.3 | 1 | 5.3 | 1 | 3.7 | 6 | 6.5 |
| *Bacillus subtilis* | 2 | 8.7 | 5 | 20.8 | 6 | 31.6 | 5 | 18.5 | 18 | 19.4 |
| *Escherichia coli* | 2 | 8.7 | 0 | 0 | 0 | 0 | 3 | 11.1 | 5 | 5.4 |
| *Klebsiella* spp | 0 | 0 | 2 | 8.3 | 0 | 0 | 2 | 7.4 | 4 | 4.3 |
| *Proteus vulgaris* | 0 | 0 | 4 | 16.7 | 0 | 0 | 2 | 7.4 | 6 | 6.5 |
| *Enterococcus* spp | 5 | 21.7 | 1 | 4.2 | 4 | 21.1 | 4 | 14.8 | 14 | 15.0 |
| **Total** | **23** | | **24** | | **19** | | **27** | | **93** | **100** |

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The total number of bacterial frequency increased to 118 in rainy season (Table 4.7), representing a 26.9% increase compared to the dry season. *Staphylococcus aureus* remained the most prevalent with 25 isolates (21.2%), followed *by Bacillus subtilis* with 21 isolates (17.8%) and *Enterococcus* spp. with 19 isolates (16.1%). Table 4.9 also shows a shift within the *Proteus* species, as *Proteus mirabilis* was isolated in the rainy season with 12 isolates (10.2%), replacing *Proteus vulgaris* which was isolated during the dry season.

Table 4.8 shows the distribution of bacterial isolates at three different soil depths within the dry season. As observed from Table 4.10, in general, bacterial isolates were more dominant in topsoil and middle layers, while there were minimal isolates from the bottom layers. The topsoil layers had the highest diversity and number, with Dumez Top producing 17 isolates, Uniben Top 15, and Oluku Top and Ewah Top each producing 12.

Table 4.9 presents the depth distribution for the rainy season. From Table 4.11, increase in bacterial isolates from middle and bottom layers was recorded compared to the dry season. The topsoil layers produced high numbers of isolates, with Ewah Top and Uniben Top having 17 isolates each and Oluku Top and Dumez Top coming in with 16 and 15 isolates, respectively.

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**Table 4.7: Frequency and Percentage Occurrence of Bacterial Isolates in Contaminated Samples during Rainy Season**

| | UNIBEN | | OLUKU | | EWAH RD | | DUMEZ RD | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Frequency | % occurrence | Frequency | % occurrence | Frequency | % occurrence | Frequency | % occurrence | Total | Total occur |
| *Staphylococcus aureus* | 8 | 25.8 | 6 | 20.7 | 6 | 20.7 | 5 | 17.2 | 25 | 21.2 |
| *Alcaligenes* spp | 5 | 16.1 | 4 | 13.8 | 4 | 13.8 | 2 | 6.9 | 15 | 12.7 |
| *Pseudomonas aeruginosa* | 3 | 9.7 | 1 | 3.4 | 2 | 6.7 | 4 | 13.8 | 10 | 8.5 |
| *Salmonella* spp | 2 | 6.5 | 2 | 6.9 | 2 | 6.7 | 1 | 3.4 | 7 | 5.9 |
| *Bacillus subtilis* | 4 | 12.9 | 7 | 24.1 | 6 | 20.7 | 4 | 13.8 | 21 | 17.8 |
| *Escherichia coli* | 2 | 6.5 | 0 | 0.0 | 0 | 0.0 | 2 | 6.9 | 4 | 3.4 |
| *Klebsiella* spp | 0 | 0.0 | 3 | 10.3 | 0 | 0.0 | 2 | 6.9 | 5 | 4.2 |
| *Proteus mirabilis* | 0 | 0.0 | 5 | 17.2 | 3 | 10.3 | 4 | 13.8 | 12 | 10.2 |
| *Enterococcus* spp | 7 | 22.6 | 1 | 3.4 | 6 | 20.7 | 5 | 17.2 | 19 | 16.1 |
| **Total** | 31 | | 29 | | 29 | | 29 | | **118** | **100** |

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**Table 4.8: Frequency and Percentage Occurrence of Bacterial Isolates for Contaminated Samples Across Different Depths in Dry Season**

| | OT | | OM | | OB | | ET | | EM | | EB | | DT | | DM | | DB | | UT | | UM | | UB | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % |
| *Proteus vulgaris* | 2 | 33.3 | 2 | 33.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 33.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0 |
| *Salmonella* spp | 0 | 0.0 | 2 | 33.3 | 0.0 | 0.0 | 1 | 16.7 | 0 | 0.0 | 0 | 0.0 | 1 | 16.6 | 0 | 0.0 | 0 | 0.0 | 2 | 33.3 | 0 | 0.0 | 0 | 0 |
| *Klebsiella* spp | 0 | 0.0 | 2 | 50 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 50 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0 |
| *Staphylococcus aureus* | 3 | 15.8 | 2 | 10.5 | 0 | 0.0 | 2 | 10.5 | 2 | 10.5 | 0 | 0.0 | 4 | 21.1 | 0 | 0.0 | 0 | 0.0 | 4 | 21.1 | 2 | 10.5 | 0 | 0 |
| *Pseudomonas aeruginosa* | 1 | 11.1 | 0 | 0.0 | 0 | 0.0 | 2 | 22.2 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 44.4 | 0 | 0.0 | 2 | 22.2 | 0 | 0.0 | 0 | 0 |
| *Escherichia coli* | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 3 | 60 | 0 | 0.0 | 0 | 0.0 | 2 | 40 | 0 | 0.0 | 0 | 0 |
| *Enterococcus* spp | 0 | 0.0 | 1 | 7.1 | 0 | 0.0 | 3 | 21.4 | 1 | 7.1 | 0 | 0.0 | 2 | 14.3 | 2 | 14.3 | 0 | 0.0 | 3 | 21.4 | 2 | 14.3 | 0 | 0 |
| *Bacillus subtilis* | 4 | 22.2 | 1 | 5.6 | 0 | 0.0 | 4 | 22.2 | 1 | 5.6 | 1 | 5.6 | 3 | 16.7 | 2 | 11.1 | 0 | 0.0 | 0 | 0.0 | 2 | 11.1 | 0 | 0 |
| *Alcaligenes* spp | 0 | 0.0 | 2 | 16.7 | 2 | 16.7 | 0 | 0.0 | 0 | 0.0 | 2 | 16.6 | 2 | 16.6 | 0 | 0.0 | 0 | 0.0 | 2 | 16.6 | 2 | 16.6 | 0 | 0 |
| **Total** | **10** | | **12** | | **2** | | **12** | | **4** | | **3** | | **17** | | **10** | | **0** | | **15** | | **8** | | **0** | |

Key: OT – Oluku Top, OM – Oluku Middle, OB – Oluku Bottom, ET – Ewah Top, EM – Ewah Middle, EB – Ewah Bottom, DT – Dumez Top, DM – Dumez Middle,
DB – Dumez Bottom, UT – Uniben Top, UM – Uniben Middle, UB – Uniben Bottom, F-Frequency

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## Table 4.9: Frequency and Percentage Occurrence of Bacterial Isolates for Contaminated Samples Across Different Depths in Rainy Season

| | OT | | OM | | OB | | ET | | EM | | EB | | DT | | DM | | DB | | UT | | UM | | UB | | To |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % | F | % | |
| *Proteus mirabilis* | 3 | 25 | 2 | 16.7 | 0 | 0.0 | 1 | 8.3 | 2 | 16.7 | 0 | 0.0 | 0 | 0.0 | 4 | 33.3 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 12 |
| *Salmonella* spp | 0 | 0.0 | 2 | 28.6 | 0 | 0.0 | 2 | 28.6 | 0 | 0.0 | 0 | 0.0 | 1 | 14.3 | 0 | 0.0 | 0 | 0.0 | 1 | 14.3 | 1 | 14.3 | 0 | 0.0 | 7 |
| *Klebsiella* spp | 2 | 40 | 1 | 20 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 40 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 5 |
| *Staphylococcus aureus* | 4 | 16 | 2 | 8.0 | 0 | 0.0 | 2 | 8.0 | 4 | 16 | 0 | 0.0 | 4 | 16 | 1 | 4.0 | 0 | 0.0 | 4 | 16 | 4 | 16 | 0 | 0.0 | 25 |
| *Pseudomonas aeruginosa* | 1 | 10 | 0 | 0.0 | 0 | 0.0 | 2 | 20 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 4 | 40 | 0 | 0.0 | 2 | 20 | 1 | 10 | 0 | 0.0 | 10 |
| *Escherichia coli* | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 2 | 50 | 0 | 0.0 | 0 | 0.0 | 2 | 50 | 0 | 0.0 | 0 | 0.0 | 4 |
| *Enterococcus* spp | 0 | 0.0 | 1 | 5.3 | 0 | 0.0 | 4 | 21 | 1 | 5.3 | 1 | 5.3 | 2 | 10.5 | 2 | 10.5 | 1 | 5.3 | 4 | 21 | 2 | 10.5 | 1 | 5.3 | 19 |
| *Bacillus subtilis* | 4 | 19 | 2 | 9.5 | 1 | 4.8 | 4 | 19 | 1 | 4.8 | 1 | 4.8 | 2 | 9.5 | 1 | 4.8 | 1 | 4.8 | 2 | 9.5 | 1 | 4.8 | 1 | 4.8 | 21 |
| *Alcaligenes* spp | 2 | 13.3 | 2 | 13.3 | 0 | 0.0 | 2 | 13.3 | 1 | 6.7 | 1 | 6.7 | 2 | 13.3 | 0 | 0.0 | 0 | 0.0 | 2 | 13.3 | 2 | 13.3 | 1 | 6.7 | 15 |
| Total | 16 | | 12 | | 1 | | 17 | | 9 | | 3 | | 15 | | 12 | | 2 | | 17 | | 11 | | 3 | | 11 |

Key: OT – Oluku Top, OM – Oluku Middle, OB – Oluku Bottom, ET – Ewah Top, EM – Ewah Middle, EB – Ewah Bottom, DT – Dumez Top, DM – Dumez Middle, DB – Dumez Bottom, UT – Uniben Top, UM – Uniben Middle, UB – Uniben Bottom, F – Frequency

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Table 4.10 shows the results of phenotypic virulence test for the selected bacterial isolates . From the Table , the production of DNase was positive for six species: *Klebsiella* spp., *Bacillus subtilis*, *Alcaligenes* spp., *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Escherichia coli*, whereas three species, including *Enterococcus* spp, *Staphylococcus aureus*, and *Salmonella* spp., were negative. Upon hemolysis testing, beta-hemolysis (the total lysis of RBCs) occurred in all the tested isolates except *Escherichia coli*, where gamma-hemolysis (no hemolysis) was observed. Gelatinase production was observed in seven species: *Enterococcus* spp, *Klebsiella* spp., *Bacillus subtilis*, *Alcaligenes* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli;* whereas *Salmonella* spp., *Proteus mirabilis*, and *Proteus vulgaris* showed negativity. The least virulence factor detected among the test organisms was lipase production, which occurred only among three species: *Enterococcus* spp, *Klebsiella* spp., and *Bacillus subtilis*. As shown in Table 4.10, *Klebsiella* spp. and *Bacillus subtilis* were positive for all (4/4) the virulence factors;

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Table 4.11 shows the details of the antibiotic susceptibility patterns of ten bacterial isolates in relation to the nine antibiotics tested. All bacterial isolates showed universal susceptibility to imipenem with zone diameters in a range from 23mm (*Klebsiella* spp.) to 36mm (*Salmonella* spp.). Table 4.12 indicates that *Escherichia coli* had the highest resistance profile with a MAR index of 0.6, which was susceptible only to imipenem (32mm, S) while it exhibited resistance to cefixime (11mm, R), levofloxacin (11mm, R), gentamycin (11mm, R), ceftriaxone (10mm, R), and ceftazidime (11mm, R).

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**Table 4.10: Phenotypic Virulence Test**

| | DNAse | HEMOLYSIS | Gelatin | Lipase |
|---|---|---|---|---|
| *Enterococcus faecalis* | - | Beta | + | + |
| *Klebsiella* spp | + | Beta | + | + |
| *Bacillus subtilis* | + | Beta | + | + |
| *Alcaligenes* spp | + | Beta | + | - |
| *Staphylococcus aureus* | - | Beta | + | - |
| *Salmonella* spp | - | Beta | - | - |
| *Proteus mirabilis* | + | Beta | - | - |
| *Proteus vulgaris* | + | Beta | - | - |
| *Pseudomonas aeruginosa* | + | Beta | + | - |
| *Escherichia coli* | + | Gama | + | - |

Key:

+: positive

-: negative

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**Table 4.11: Antibiotic Susceptibility Profiling of Bacterial Isolates**

| ISOLATES | IMI (10µg) | CFM (5µg) | LEV (5µg) | GM (10µg) | ATH (15µg) | CRO (30µg) | CAZ (10µg) | CXM (30µg) | E (15µg) | MA Ind |
|---|---|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* | 32(S) | 11(R) | 11(R) | 11(R) | 16(I) | 10(R) | 11(R) | - | 14(I) | 0.6 |
| *Klebsiella* spp. | 23(S) | 16(I) | 15(I) | 13(I) | 10(R) | 11(R) | 10(R) | - | 13(R) | 0.5 |
| *Pseudomonas aeruginosa* | 32(S) | 12(R) | 14(I) | 10(R) | 18(S) | 21(I) | 11(R) | - | 15(I) | 0.4 |
| *Salmonella* spp. | 36(S) | 10(R) | 20(S) | 13(I) | 14(I) | 21(I) | 16(I) | - | 14(I) | 0.1 |
| *Alcaligenes* spp. | 30(S) | 9(R) | 18(S) | 14(I) | 16(I) | 22(I) | 15(I) | - | 23(S) | 0.1 |
| *Proteus vulgaris* | 30(S) | 10(R) | 20(S) | 16(S) | 14(I) | 21(I) | 16(I) | - | 24(S) | 0.1 |
| *Proteus mirabilis* | 28(S) | 16(I) | 16(I) | 20(S) | 18(S) | 20(I) | 12(R) | - | 26(S) | 0.1 |
| *Staphylococcus aureus* | 30(S) | 20(S) | 18(S) | 15(S) | 14(I) | 20(I) | 14(R) | 24(S) | - | 0.1 |
| *Enterococcus* spp. | 30(S) | 16(I) | 21(S) | 18(S) | 14(I) | 21(I) | 10(R) | 16(I) | - | 0.1 |
| *Bacillus subtilis* | 32(S) | 13(R) | 14(I) | 16(S) | 10(R) | 13(R) | 10(R) | 11(R) | - | 0.6 |

KEY: Imipenem (IMI), Cefixime (CFM), Levofloxacin (LEV), Gentamycin (GM), Azithromycin (ATH), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefuroxime (CXM), Erythromycin (E)

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Deleted[DELL]: Plate 4.1 shows the plasmid DNA extraction for multi-drug resistance (MDR) isolates. Plasmids were detected in *Klebsiella* spp., *Pseudomonas aeruginosa, and Bacillus subtilis*, while no detectable plasmid bands were seen in *Escherichia coli*.

Plates 4.2 and 4.3 present *Klebsiella* spp. pre-curing and post-curing antibiograms, respectively, which visually demonstrate increased antibiotic susceptibility upon removal of the plasmid.

Table 4.12 shows the antibiotic susceptibility pattern of *Klebsiella* spp. before and after curing of plasmids. The results from the pre-curing test indicated susceptibility to imipenem (23mm, S), intermediately resistant to cefixime (16mm, I), levofloxacin (15mm, I), and gentamycin (13mm, I), with absolute resistance to azithromycin (10mm, R), ceftriaxone (11mm, R), ceftazidime (10mm, R), and erythromycin (13mm, R). As revealed in Table 4.12, changes were observed in post-curing; imipenem increased to 36mm (S), cefixime became susceptible at 19mm, while levofloxacin remarkably increased to 28mm (S), gentamycin increased to 28mm (S), azithromycin improved to become intermediately resistant at 16mm, I, and ceftazidime became susceptible at 18mm, and some level of resistance to ceftriaxone (17mm, R) and erythromycin (13mm, R) persisted after curing was observed. The antibiotic susceptibility profile of *Pseudomonas aeruginosa* pre-curing show susceptibility to imipenem (32mm, S) and azithromycin (18mm, S), intermediate resistance to levofloxacin (14mm, I), ceftriaxone (21mm, I), and erythromycin (15mm, I), and complete resistance to cefixime (12mm, R), gentamycin (10mm, R), and ceftazidime (11mm, R). Remarkable improvements in the antibiotic susceptibilities post-plasmid curing was also observed. The antibiotic susceptibility profile for *Escherichia coli* indicates that the organism demonstrated high levels of multi-drug resistance with a MAR index of 0.6, displaying only susceptibility to imipenem (32mm, S), intermediate resistance to azithromycin (16mm, I) and erythromycin (14mm, I) and total resistance to cefixime (11mm, R), levofloxacin (11mm, R), gentamycin (11mm, R), ceftriaxone (10mm, R) and ceftazidime (11mm, R). No post-curing data was available since no plasmid DNA was detected upon extraction. Antibiotic susceptibility pattern of *Bacillus subtilis* pre-curing stage showed susceptibility only to imipenem and gentamicin; had an intermediate response to levofloxacin, while it was resistant to the rest of the antibiotics tested. However, in the post-

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## Table 4.12: Antibiogram for Multi-Drug Resistance Isolates. (Pre and Post-Curing)

| Isolate | Gram Reaction | Condition | IMI | CFM | LEV | GM | ATH | CRO | CAZ | CXM / E | MA Ind |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Klebsiella* spp.** | – | Pre-curing | 23(S) | 16(I) | 15(I) | 13(I) | 10(R) | 11(R) | 10(R) | 13(R) | 0.5 |
| | | Post-curing | 36(S) | 19(S) | 28(S) | 28(S) | 16(I) | 17(R) | 18(S) | 13(R) | 0.3 |
| ***Pseudomonas aeruginosa*** | – | Pre-curing | 32(S) | 12(R) | 14(I) | 10(R) | 18(S) | 21(I) | 11(R) | 15(I) | 0.4 |
| | | Post-curing | 36(S) | 20(S) | 18(S) | 18(S) | 20(S) | 23(S) | 14(R) | 20(I) | 0.1 |
| ***Escherichia coli*** | – | Pre-curing | 32(S) | 11(R) | 11(R) | 11(R) | 16(I) | 10(R) | 11(R) | 14(I) | 0.6 |
| | | Post-curing | – | – | – | – | – | – | – | – | |
| ***Bacillus subtilis*** | + | Pre-curing | 32(S) | 13(R) | 14(I) | 16(S) | 10(R) | 13(R) | 10(R) | 11(R) | 0.6 |
| | | Post-curing | 36(S) | 15(R) | 20(S) | 20(S) | 18(S) | 20(I) | 12(R) | 23(S) | 0.3 |

KEY:
PEF: Imipenem (IMI), Cefixime (CFM), Levofloxacin (LEV), Gentamycin (GM), Azithromycin (ATH), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefuroxime (CXM), Erythromycin (E)
S: Sensitive, R: Resistant, I: Intermediate, MAR Index: Multiple Antibiotic Resistance index
MAR Index > or = 0.2 (Public Health significance)

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# CHAPTER FIVE

## DISCUSSION

The pH levels recorded in all sampling points varied between 6.25 and 6.90 during both dry and rainy seasons. These levels indicate slightly acidic to neutral soil types, which are expected in tropical regions such as Nigeria. The levels for both contaminated and control sites showed minimal difference. For example, Oluku contaminated site had a pvalue of 0.945 during dry season and 0.440 during rainy season, compared to 0.602 and 0.724 for control site. Uniben contaminated site also had a pvalue of 0.124 during dry season and 0.665 during rainy season, compared to 0.484 and 0.820 for control site. These levels can be considered ideal for agricultural purposes and suggests that industrial effluents have not had adverse effect on soil acidity or alkalinity. This is in agreement with the report of Brady and Weil (2016), which showed that soil samples with a pH ranging between 6.0 and 7.0 can easily sustain plant life and microbial growth. The small variations observed could be explained by the diluting effect caused by rainwater and exhaustion caused by leaching out acidic substances (Nelson *et al.*, 2010). Also, soil buffering capacity cannot be entirely ruled out (Rowell, 1994), which resists any change in soil pH levels due to waste effluent.

Electrical conductivity shows the concentration of dissolved ions in soil solution, including cations such as calcium, magnesium, potassium, sodium, and ammonium, and anions such as chloride, sulfate, nitrate, and phosphate. The electrical conductivity  observed showed variations between contaminated and control areas. During the dry season, it was noted that the electrical conductivity values for contaminated areas were higher compared to those of control areas. This is because Oluku Top contaminated areas registered 161 µs/cm compared to 116 µs/cm for the control area. Uniben Top contaminated areas also registered 147 µs/cm compared to 131 µs/cm

Deleted[DELL]: **The antibiotic susceptibility profile of *Pseudomonas aeruginosa* before and after plasmid curing is shown in Table 4.14. The pre-curing results indicate**

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for the control area. The same trend was noted for all areas. During the rainy season, it was noted that this difference is not significant (p=0.159), with some areas registering similar or lower values for contaminated areas compared to control areas. This variation can be attributed to the process that favors concentration during the dry season due to a high rate of evapotranspiration compared to the amount of precipitation. This causes a build-up effect due to the accumulation of salts in the soil. During the rainy season, these salts are leached out or dissolved by excess water, thus reducing the value for electrical conductivity (Akan *et al*., 2013). The high values for electrical conductivity in contaminated areas depict high ion concentrations caused by the breakdown of organic components such as blood, tissue, and other matter coming from abattoirs. These components translate to the formation of compounds such as sodium, potassium, calcium, magnesium, nitrates, and chlorides, which increase the ion concentration and thus increase the value for electrical conductivity (Adelegan, 2002). The value for electrical conductivity, which was between 73-161 µs/cm, is below that which was found by similar studies conducted on several abattoirs in Nigeria (Sangodoyin and Agbawhe, 1992).

The contaminated sites did not exhibit higher organic carbon or organic matter levels compared to the control sites. In fact, the control areas recorded slightly elevated values relative to the contaminated areas. For example, Oluku Top control, showed organic carbon content values of 8.06% in the dry season and 8.90% in the rainy season, compared to 6.89% and 7.65% for the contaminated site. Similarly, organic carbon content values for Oluku Middle control areas showed 7.85% in the dry season compared to 6.85% for the contaminated site.

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The observed variations in organic carbon and organic matter are better explained by differences in vegetation cover, land disturbance, and seasonal conditions rather than rapid microbial depletion. Organic carbon in soil is primarily derived from plant inputs such as litter fall and root

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biomass. In this study, the control sites were observed to have greater vegetation cover than the abattoir-contaminated sites, which likely contributed more organic residues to the soil and resulted in higher organic carbon and organic matter levels (Brady and Weil, 2016). Although contaminated sites recorded higher heterotrophic bacterial counts due to the availability of readily degradable organic substrates from abattoir wastes, this does not necessarily translate into higher stable soil organic matter. Abattoir wastes mainly supply labile organic materials that stimulate microbial activity but contribute less to long-term organic matter accumulation. In addition, routine abattoir activities such as livestock trampling and vehicular movement, such as trucks used for transporting the livestock can compact the soil and disturb the topsoil, thereby reducing organic matter incorporation.

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The generally higher organic carbon and organic matter levels observed during the rainy season in both contaminated and control sites may be attributed to increased plant growth and organic inputs, as well as reduced decomposition rates under temporarily waterlogged conditions (Obi, 2000).

Heavy metal analysis revealed consistent contamination patterns across all sites, indicating a high degree of zinc accumulation in contaminated soil compared to control soil samples across all sampling points. Zinc levels in contaminated soil samples were between 16.42 mg/kg at Uniben Bottom during the dry season and 48.69 mg/kg at Oluku Top during the dry season. However, zinc levels in control soil samples were between 11.28 mg/kg and 33.23 mg/kg. Oluku Top was the highest with zinc concentration of 48.69 mg/kg during the dry season and 34.72 mg/kg during the rainy season, clearly establishing a high degree of contamination. The high levels of zinc found in contaminated soil can be attributed to the decomposition of animal body tissues, blood, and other organs, which have been established to have high zinc concentrations

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(Adesodum *et al*., 2006). Zinc is a trace element with high dietary requirements for normal animal metabolism. It is found in high levels in several animal body tissues such as liver, kidneys, and muscles. Upon decomposition, these body components in soil increase zinc levels, which can thus build up. Higher levels of zinc in contaminated soil can be attributed to a concentration effect due to low leaching and evapotranspiration during dry seasons. Although zinc is considered a trace element playing a significant role in plant and animal nutrition, it can be toxic if taken in excess.

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Iron had the highest levels among the three heavy metals investigated, with levels between 37.22 mg/kg (Oluku Top dry season), and 140.29 mg/kg (Oluku Top) in the rainy season. The control areas had iron levels between 27.19 mg/kg (Uniben Bottom dry season), and 101.23 mg/kg, (Uniben Top dry season). Notably, this is contrary to zinc and copper, which showed higher levels in the dry season. Notably, iron levels rose during the rainy season with areas such as Oluku Top registering an increase from 134.94 mg/kg in the dry season to 140.29 mg/kg in the rainy season (pvalue 0.031). High levels of iron in contaminated soil are not surprising since blood, which constitutes a significant portion of waste delivered by slaughter houses, is very iron-rich due to the high levels of hemoglobin. The breakdown and putridity of blood and other iron-laden animal matter result in iron diffusing extensively in soil (Adelegan, 2002). The increase in iron levels with the onset of the rainy season compared to zinc and copper, which rose with the dry season, can be explained by several reasons. Firstly, iron can diffuse upward via capillary activities in drier soil masses (Sposito, 2008). Iron levels would thus increase on soil surfaces with lower moisture. Similarly, iron can involve redox reactions with soil components, which would increase iron levels. (Sposito, 2008). Thirdly, iron in sedimentary matter could wash up on soil surfaces (Alloway, 2013). Iron levels would thus increase with the rainy season due to sedimentation on soil surfaces. The levels observed in this investigation are relatively high but fall well within levels required for tropical soil. They would not have significant toxic effects on plant and animal life due to lower iron mobility and non-carcinogenesis compared to other heavy metals with similar levels (Kabata-Pendias, 2011).

The copper levels in contaminated soil samples, varied between 5.91 mg/kg and 22.57 mg/kg. These levels are compared to 4.95 mg/kg and 17.38 mg/kg for control soil samples. Thus, contaminated soil samples generally have higher levels of copper compared to control soil samples, with this difference clearly demonstrated during the rainy seasons (pvalue 0.031). For instance, Oluku Top contaminated soil samples have levels of 14.26 mg/kg compared to 9.37 mg/kg for control soil samples during the dry season. During the rainy season, these samples have levels of 22.57 mg/kg compared to 17.38 mg/kg for control samples. The accumulation of copper in contaminated soil samples due to abattoirs can be attributed to the decomposition process of animal tissues, which includes organs like liver and kidneys. These organs have high accumulations of copper (Odeyemi *et al*., 2012). Copper is a trace element with high importance to animals due to involvement in animal enzymatic activities. Its high levels in animal tissues cause it to leach out to soil components during decomposition. The levels revealed in this research fall within levels reported by Mgbemena *et al*. (2016) for abattoir contaminated soils in Nigeria and tropical regions. Although it is imperative to note that copper is an essential element for plant growth and development, high levels (>100 mg/kg) can affect plant growth and development. Due to this effect, it leads to bio-accumulations in food chains. On this note, levels revealed in this research for other sites, especially Oluku Top, can affect plant growth and development due to phytotoxicity (Kabata-Pendias, 2011). It can also be noted that the heavy metal concentration distribution follows a distinct trend with increasing soil depth. Top soil

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samples were found to have higher heavy metal concentrations than those in mid and bottom soils. This soil depth trend can thus be related to the subsequent application and infiltration process of effluents emanating from abattoirs, with minimal heavy metal migration to lower soil layers because heavy metals tend to adsorb on soil particles and organic matter, which are dominant in top soil layers (Sposito, 2008). Of all four sampling sites considered for this research, Oluku had higher heavy metal concentrations than others, followed by Ewah, Dumez, and Uniben. This can be thus related to variability in amounts and types of effluents discharged by each sampling site, duration of operations, soil characteristics, and geological characteristics (Adelegan, 2002). Higher heavy metal concentrations in the mid and bottom soil layers at the Uniben site may indicate relatively recent or intermittent contamination rather than long-term surface accumulation. In well-drained soils with relatively higher moisture content, dissolved metals introduced through effluent discharges can be transported downward from the surface via percolating water. As infiltration progresses, metals migrate through the soil profile and may accumulate in deeper layers where finer soil particles and higher adsorption capacity retard further movement. This downward translocation results in greater metal concentrations in mid and bottom soils compared to the topsoil.

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The macronutrient distribution across the sampling sites, showed the presence of calcium, magnesium, potassium, sodium, nitrogen, and phosphorus. Calcium levels were generally similar between contaminated sites, ranging from 2.13 mg/kg to 4.77 mg/kg, and control sites, ranging from 2.08 mg/kg to 3.91 mg/kg. Oluku Top contaminated site showed the highest calcium concentration of 4.77 mg/kg during the dry season, compared to 3.46 mg/kg in the control site.. Magnesium concentrations showed slight elevation in contaminated samples, particularly at top layers. For instance, Oluku Top contaminated site had magnesium concentrations of 15.76 mg/kg during the dry season and 10.76 mg/kg during the rainy season, compared to control values of 10.22 mg/kg and 8.82 mg/kg respectively. Similarly, Dumez Top contaminated site showed magnesium values of 13.59 mg/kg during the dry season compared to 7.82 mg/kg in the control. The elevated magnesium levels in contaminated sites can be attributed to the decomposition of animal tissues and bones, which contain significant amounts of magnesium (Ogunwande and Osunade, 2011).

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Potassium exhibited notable enrichment in contaminated sites compared to controls across most sampling locations. Oluku Top contaminated site showed potassium concentration of 52.48 mg/kg during the dry season compared to 32.33 mg/kg in the control site. Similarly, Uniben Top contaminated site had potassium values of 47.19 mg/kg during both dry and rainy seasons, compared to control values of 23.74 mg/kg and 35.73 mg/kg respectively. Ewah Top contaminated site showed potassium concentrations of 40.33 mg/kg during the dry season and 47.19 mg/kg during the rainy season, compared to control values of 22.53 mg/kg and 34.82 mg/kg. This potassium enrichment could be as a result of blood and tissue decomposition, as these materials are rich in potassium, which is a major intracellular cation in animal tissues (Adelegan, 2002). The release of potassium from decomposing organic matter makes it readily available in the soil solution, contributing to the elevated levels observed in contaminated sites. Potassium is an essential macronutrient for plant growth, and the enrichment observed in contaminated sites could potentially benefit crop production if other contamination issues such as heavy metals and pathogens are addressed (Brady and Weil, 2016).

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Sodium concentrations showed variable patterns between contaminated and control sites, with no consistent trend of enrichment. Values ranged from 0.61 mg/kg to 1.75 mg/kg across all sites and seasons. For example, Oluku Top contaminated site had sodium values of 1.73 mg/kg during the dry season and 0.94 mg/kg during the rainy season, compared to control values of 1.05 mg/kg and 0.73 mg/kg. The relatively low sodium concentrations and lack of consistent enrichment in contaminated sites suggest that sodium is not a major component of abattoir waste or that it is readily leached from the soil profile due to its high solubility and mobility (Sposito, 2008). Nitrogen levels showed minimal differences between contaminated and control sites, with values ranging from 3.31 mg/kg to 4.80 mg/kg across all locations. This observation is somewhat surprising given that animal tissues and blood are rich sources of nitrogen. The lack of significant nitrogen enrichment in contaminated sites may be explained by several factors. First, the rapid uptake of nitrogen by the high microbial populations in contaminated soils, as evidenced by the elevated heterotrophic bacterial counts, could be consuming available nitrogen for biomass production (Ogunwande and Osunade, 2011). Second, nitrogen losses through volatilization of ammonia, particularly under the slightly acidic to neutral pH conditions observed in this study, could be significant. Third, denitrification under anaerobic conditions, especially during the rainy season, could convert nitrate to gaseous nitrogen forms that are lost to the atmosphere (Brady and Weil, 2016).

Phosphorus levels fluctuated greatly between sampling points and seasons, ranging between 4.91 mg/kg and 12.88 mg/kg. At different contaminated sites, phosphorus levels were higher than control levels. At Oluku Top Contaminated site, phosphorus levels were 12.73 mg/kg during the dry season and 12.82 mg/kg during the rainy season. However, for Oluku Middle Contaminated site, phosphorus level was 8.11 mg/kg during the dry season, lower than 8.55 mg/kg for control sites. The irregular distribution observed in this study may be due to uneven waste disposal, differences in microbial or plant uptake, or localized leaching. These site-specific factors likely

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influenced phosphorus accumulation, resulting in the variability observed across the samples." (Brady and Weil, 2016).

The mean heterotrophic bacterial counts in soil samples showed that across all locations and seasons, contaminated soils recorded higher bacterial loads than their corresponding control sites, but there was no significant statistical difference in all the sites for both seasons. For example though in Oluku Top contaminated areas, bacterial load increased from 1.05 x $10^8$ cfu/g in May to 1.86 x $10^8$ in July, compared to control areas which was from 0.85 x $10^8$ to 1.75 x $10^8$, there was no statistical difference between contaminated soil ( pvalue 0.440) and control (pvalue 0.724) There was significant difference in the bacterial load increase from May to July in rainy season (p= 0.039) It was also evident during the dry season, with Ewah top ranging from 1.90 x $10^7$ to 1.97 x$10^7$ compared to the control sample which ranged from 0.81 x $10^7$ to 0.84 x $10^7$. This trend held true for all four areas surveyed. The reason for these high levels in contaminated areas is due to the levels of nutrients found in these areas due to abattoir effluent-containing organic matter. These matters amount to carbon and nitrogen sources necessary for growth (Adelegan, 2002).

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Data from this study showed that bacterial counts in topsoil layers always recorded the highest bacterial counts compared to mid and bottom layers. This variation can be attributed to the amount of organic matter and nutrients distributed across the soil layers, with top soil layers receiving wastes in higher amounts due to their closeness to the source (Brady and Weil, 2016). Also, this variation can be attributed to the low supply of oxygen in mid and bottom soil layers, which inhibits aerobic bacterial growth (Obi, 2000). Samples from Uniben and Ewah generally contained higher bacterial counts compared to the other four sampling sites for contaminated soil samples. This implies that these sampling sites have higher amounts of waste or that these environments favor bacterial growth.The isolation and identification of bacterial species illustrate the varieties of bacterial species detected in the abattoir effluent contaminated soil samples. During the dry season, a cumulative bacterial frequency occurrence of 93 were recorded in contaminated soil samples across four sites. *Staphylococcus aureus* was the most frequently isolated organism, accounting for 19 isolates representing 20.4% of the total. *Bacillus subtilis* was the second most common isolate with 18 occurrence representing 19.4%, followed by *Enterococcus* species with 14 isolates representing 15.0%. *Alcaligenes* species accounted for 12 isolates representing 12.9%, while *Pseudomonas aeruginosa* had 9 isolates representing 9.7%. The less frequently isolated organisms included *Salmonella* species and *Proteus vulgaris*, each with 6 isolates representing 6.5%, *Klebsiella* species with 4 isolates representing 4.3%, and *Escherichia coli* with 5 isolates representing 5.4%. The distribution of these isolates varied across the four locations, with Dumez site yielding the highest number of isolates (27), followed by Oluku (24), Uniben (23), and Ewah (19).

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During the rainy season, a total bacteria frequency of 118 were recorded, *Staphylococcus aureus* remained the most prevalent organism with 25 isolates representing 21.2% of the total. *Bacillus subtilis* had 21 isolates representing 17.8%, and *Enterococcus* species accounted for 19 isolates representing 16.1%. *Alcaligenes* species had 15 isolates representing 12.7%, while *Proteus mirabilis* showed a notable increase to 12 isolates representing 10.2% compared to its dry season occurrence. *Pseudomonas aeruginosa* had 10 isolates representing 8.5%, *Salmonella* species had 7 isolates representing 5.9%, *Klebsiella* species had 5 isolates representing 4.2%, and *Escherichia coli* had 4 isolates representing 3.4%. The distribution across locations during the rainy season was more uniform, with each of the four sites yielding between 29 and 31 isolates.

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The higher total number of isolates during the rainy season reflects increased microbial activity and diversity associated with favorable moisture conditions (Obi, 2000).

The seasonal shift from *Proteus vulgaris* in the dry season to *Proteus mirabilis* in the rainy season represents the only species-level change observed and most likely reflects the differences in environmental conditions. Soils of the rainy season are more saturated with water, with increased nutrient mobility, conditions that favor the highly motile *P. mirabilis* . In contrast, the dry season is characterized by drier, more concentrated organic waste deposits that support the survival of *P. vulgaris*, a species more tolerant of dehydrated and high–organic load environments .

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These bacteria pose important health and environmental hazards if present in soils contaminated with abattoirs. *Staphylococcus aureus* is the most predominantly isolated bacterium. Infections caused by *Staphylococcus aureus* range from mild to severe such as pneumonia, septicemia, and toxic-shock syndrome (Lowy, 1998). The prevalence of *Staphylococcus aureus* in abattoir effluent contaminated soils may have been from hides and handlers as well as during the handling process *subtilis* is generally non-pathogenic; this bacterium occurs ubiquitously in soil environments. They are opportunistic pathogens that can cause infection in immune-compromised individuals (Logan and De Vos, 2009). High prevalence was isolated due to the soil organism's universality and capacity to form heat-resistant endospores that protect them from environmental factors. *Enterococcus* spp. are fecal indicator bacteria; apart from that, they are linked to urinary tract infections, bacteremia, and endocarditis among hospitalized patients (Fisher and Phillips, 2009). These bacteria are linked to contamination with soil from animal intestines. *Pseudomonas aeruginosa* is an opportunistic bacterium that has been implicated in hospital infections particularly complicating individuals with wound resulting from fire burn,

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respiratory tracts infections, and urinary tracts infections (Lyczak *et al*., 2000). *Escherichia coli, Salmonella* spp.*, and Klebsiella* spp. are classified in the family Enterobacteriaceae; these bacteria are linked to contamination with soil from fecal matters (supply reference). These bacteria (*E. coli and Salmonella* spp.) are implicated in infections such as gastroenteritis and other serious systemic infections (Nataro and Kaper, 1998). The others (*P. vulgaris and P. mirabilis*) are linked to urinary tracts infections and wound infections (Schaffer and Pearson, 2015).

It can be observed that the bacterial isolates are mostly found in the upper and middle layers with few found in the lower layers. In the dry season, Oluku Top has 10 isolates, Oluku Middle has 12 isolates, but only 2 are found in Oluku Bottom. The same pattern is observed in Uniben Top with 15 isolates, Uniben Middle with 8 isolates, and no isolate found in Uniben Bottom. The impact of this contamination pattern is evident in the absence or low detection rates found in the lower layers (Brady and Weil 2016), since most bacteria are aerobic and facultative anaerobes that cannot thrive in the lower soil layers due to the anaerobic environment. The specific species of bacteria sampled across depths exhibited certain patterns. *Staphylococcus aureus* and *Bacillus subtilis* were consistently isolated from the top and middle layers in all locations, consistent with what would be expected of microorganisms associated with surface contamination from animal hides and meat processing.

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*Pseudomonas aeruginosa* was found uniformly from samples of the middle layer, but Dumez site showed a uniform dominance in the middle layer samples, where, during the dry season, 4 of the 9 *Pseudomonas* isolates were recovered from Dumez Middle. This distribution could reflect the microaerophilic preferences of *Pseudomonas* species, which are able to inhabit subsurface environments with different oxygen tensions (Lyczak *et al*., 2000). *Enterococcus* species showed

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a more uniform distribution across top and middle layers. Enteric bacteria such as *Escherichia coli*, *Salmonella* species, and *Klebsiella* species were primarily isolated from top layers, particularly at Dumez and Uniben sites, indicating recent fecal contamination at the soil surface.

The total number of isolates increased during the rainy season when compared with the dry season, and its distribution across depths became more varied. In the rainy season, enhanced moisture content promotes leaching and downward migration of bacteria through the soil profile, thus allowing the recovery of these organisms from middle and bottom layers (Obi, 2000). This trend is seen at a number of sites. At Dumez, the middle layer retrieved 12 isolates in the rainy season compared with 10 in the dry season, reflecting greater bacterial penetration into deeper layers. In the same light, Uniben Top had increased recovery with 17 isolates in the rainy season compared to 15 in the dry season. This same trend was found with Uniben Bottom where 3 isolates were recovered during the rainy season and none was recovered during the dry season. This clearly captures how water movement allows bacteria to migrate to lower layers (supply reference). Variations from this general trend were, however, observed for some locations. For example, Oluku Bottom had 1 isolate in the rainy season compared to 2 in the dry season. This seeming contradiction could be due to specific soil characteristics of the areas, like soil texture, organic matter content, or certain drainage patterns leading to preferential flow pathways or dilution effects (supply reference). In addition, the microbial community composition and competitive dynamics at the different sites can influence recovery patterns independently of moisture levels.

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The results of phenotypic virulence testing for the bacterial isolates, examined their ability to produce various enzymes and toxins that contributes to pathogenicity. DNase production was observed in *Klebsiella* species, *Bacillus subtilis*, *Alcaligenes* species, *Proteus vulgaris*, *Proteus* *mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*, but was absent in *Enterococcus faecalis*, *Staphylococcus aureus* and *Salmonella* species. DNase is an enzyme that degrades DNA and is considered a virulence factor as it can help bacteria evade the host immune system and facilitate tissue invasion (Pinchuk *et al*., 2004). Hemolysis testing revealed that all isolates except *Escherichia coli* produced beta-hemolysis, indicating their ability to completely lyse red blood cells. Beta-hemolytic bacteria can cause severe infections as hemolysis facilitates nutrient acquisition and tissue damage (Lowy, 1998). *Escherichia coli* produced gamma-hemolysis, meaning no hemolysis, which is typical for non-pathogenic environmental strains.

Gelatin liquefaction, which indicates the production of gelatinase enzyme, was positive for *Enterococcus faecalis*, *Klebsiella* species, *Bacillus subtilis*, *Alcaligenes* species, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. This enzyme can degrade collagen and gelatin in host tissues, facilitating bacterial spread and invasion (Miyoshi and Shinoda, 2000). The enzyme was absent in *Salmonella* species, *Proteus vulgaris*, and *Proteus mirabilis*. Lipase production, which enables bacteria to degrade lipids and facilitate tissue invasion and nutrient acquisition, was positive for *Enterococcus faecalis*, *Klebsiella* species, and *Bacillus subtilis*, but negative for all other isolates tested. The presence of these virulence factors in multiple bacterial isolates from abattoir-contaminated soils indicates that these environments harbor potentially pathogenic bacteria capable of causing infections in humans and animals (Balows *et al*., 1991). The combination of hemolytic activity, enzyme production, and the identity of the bacterial species suggests that abattoir-contaminated soils pose potential health risks, particularly for workers at these facilities and residents in surrounding areas.

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The antibiotic susceptibility profiles presented reveals concerning patterns of antimicrobial resistance among bacterial isolates from abattoir-contaminated soils. All isolates showed susceptibility to imipenem (IMI), a carbapenem antibiotic, with inhibition zone diameters ranging from 23 mm for *Klebsiella* species to 36 mm for *Salmonella* species. This universal susceptibility to imipenem is encouraging as carbapenems are often reserved as last-resort antibiotics for treating multidrug-resistant infections (Papp-Wallace *et al*., 2011). However, widespread resistance was observed against several other antibiotics tested. *Escherichia coli* showed resistance to cefixime (CFM), levofloxacin (LEV), gentamycin (GM), ceftriaxone (CRO), and ceftazidime (CAZ), with only intermediate resistance to azithromycin (ATH) and erythromycin (E). The multiple antibiotic resistance index (MARI) for *E. coli* was 0.6, indicating exposure to multiple antibiotics and high-risk contamination source (Krumperman, 1983). An MAR index value greater than 0.2 suggests that the bacterial isolate originated from an environment where antibiotics are frequently used, such as hospitals or livestock operation areas (Osundiya *et al*., 2013).

*Klebsiella* species exhibited a level of resistance to azithromycin, ceftriaxone, ceftazidime, and erythromycin; while being intermediate resistant to cefixime, levofloxacin, and gentamycin, producing a MARI of 0.5. *Pseudomonas aeruginosa* exhibited resistance to cefixime, gentamycin, and ceftazidime, but susceptibility was demonstrated for imipenem and azithromycin, while showing intermediate resistance to levofloxacin, ceftriaxone, and erythromycin, yielding a MARI of 0.4. The resistance patterns seen for these Gram-negative bacteria is worrisome as they are common pathogens causing nosocomial infections and the resistance to third-generation cephalosporins (ceftriaxone and ceftazidime) limits the ability to treat infections caused by them (Paterson and Bonomo, 2005). *Salmonella* spp.*, Alcaligenes* spp., *Proteus vulgaris* and *Proteus mirabilis* showed the lowest resistance with a MAR index of 0.1, indicating less exposure to antibiotics. These organisms had a mainly susceptible pattern of response to the antibiotics tested; with resistance mainly seen against cefixime, and some select resistance to ceftazidime. *Staphylococcus aureus* exhibited good susceptibility to most antibiotics tested except for ceftazidime, yielding a MAR index value of 0.1. This lower resistant pattern is somewhat surprising since *S. aureus* is known for developing antibiotic resistance, especially the methicillin-resistant strains (Lowy, 1998).

There were different patterns of resistance among the *Enterococcus* species and *Bacillus subtilis*. *Enterococcus* species were resistant to ceftazidime, had intermediate resistance to cefixime and cefuroxime, and had a MAR index value of 0.1. *Bacillus subtilis* was resistant to cefixime, azithromycin, ceftriaxone, ceftazidime, and cefuroxime, had intermediate resistance to levofloxacin, and had a MAR index value of 0.6. The *Bacillus subtilis* MAR index value is high since they are normally considered less clinically significant than other isolates. However, they were resistant to many classes of antibiotics, possibly in response to antibiotic exposure in animals (i.e., veterinary antibiotics in livestock) or from other sources of exposure (Marshall and Levy, 2011). The overall pattern of resistance observed in this study highlights the presence of antimicrobial resistance among some isolates, although several isolates remained sensitive to the tested antibiotics. This indicates that while resistance is a concern, it is not uniform across all bacterial populations studied (World Health Organization, 2014). The results also indicate that antibiotic-resistant bacteria are present in abattoir soils which indicates that these environments may harbor resistance genes that may be transferred to human pathogens via horizontal gene transfer routes.

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The plasmid DNA extraction results showed that Isolates 1 (*Klebsiella* species), 2 (*Pseudomonas aeruginosa*) and 4 (*Bacillus subtilis*) were positive for plasmid DNA . This provides molecular evidence to support the genetic basis for antibiotic resistance among the bacterial isolates. The large plasmids are typical of those carrying multiple antibiotic resistance genes, since resistance determinants are usually clustered on mobile genetic elements (Bennett, 2008). Isolate 3 (*Escherichia coli*) was negative for plasmid DNA, suggesting that its resistance could be chromosomally encoded . Plasmids detected in many bacterial genera constitute mobile genetic elements which may be transferred between bacteria through conjugation, transformation or transduction (Thomas and Nielsen, 2005); and enhances the spread of resistance genes in different bacterial species and genera (supply reference). This horizontal gene transfer is a major mechanism driving the dissemination of antibiotic resistance in environmental settings.

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The plasmid curing experiments reveals the involvement of plasmids in conferring antibiotic resistance. The antibiogram of *Klebsiella* species plasmids before curing, shows that the organism was susceptible to imipenem (23 mm), intermediately resistant to cefixime (16 mm), levofloxacin (15 mm), and gentamycin (13 mm), and resistant to azithromycin (10 mm), ceftriaxone (11 mm), ceftazidime (10 mm) and erythromycin (13 mm). After curing, there was a marked improvement in susceptibility profiles, with the organism becoming susceptible to imipenem (36 mm), cefixime (19 mm), levofloxacin (28 mm), gentamycin (28 mm) and ceftazidime (18 mm). However, resistance to ceftriaxone (17 mm) and erythromycin (13 mm) persisted, while there was no change in intermediate resistance to azithromycin at 16 mm. The antibiotic susceptibility testing before and after curing, showed visibly larger zones of inhibition after curing for several antibiotics, which confirm the restoration of susceptibility. The persistence of resistance to some antibiotics after plasmid curing indicates that *Klebsiella* species

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carries resistance genes on both plasmids and chromosomes, or that the curing process did not eliminate all plasmids (Courvalin, 1994). The significant increase in susceptibility to the fluoroquinolones (levofloxacin) and aminoglycosides (gentamycin) after curing indicates that resistance to these antibiotics was plasmid-mediated.

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The antibiogram for *Pseudomonas aeruginosa* showed that before curing, the organism was susceptible to imipenem (32 mm) and azithromycin (18 mm), showed intermediate resistance to levofloxacin (14 mm), ceftriaxone (21 mm), and erythromycin (15 mm), and was resistant to cefixime (12 mm), gentamycin (10 mm), and ceftazidime (11 mm). Curing dramatically improved susceptibility across most antibiotics, with the organism becoming susceptible to imipenem (36 mm), cefixime (20 mm), levofloxacin (18 mm), gentamycin (18 mm), azithromycin (20 mm), and ceftriaxone (23 mm). However, resistance to ceftazidime persisted at 14 mm, and erythromycin remained at an intermediate resistance of 20 mm. The restoration of susceptibility to multiple antibiotics upon plasmid curing confirms that *Pseudomonas aeruginosa* possessed plasmid-encoded beta-lactam, fluoroquinolone and aminoglycoside resistance genes. Continued resistance to ceftazidime points toward chromosomal resistance mechanisms involving mutations in porin genes or upregulation of efflux pumps as described by Poole (2011).

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The antibiogram of *Escherichia coli*, which is of particular interest since no plasmid DNA was detected for this isolate during extraction. Before curing, the organism was susceptible only to imipenem at 32 mm, showed intermediate resistance to azithromycin at 16 mm and erythromycin at 14 mm, and was resistant to cefixime at 11 mm, levofloxacin at 11 mm, gentamycin at 11 mm, ceftriaxone at 10 mm, and ceftazidime at 11 mm. Post-curing results were not presented because no plasmid was detected. The pre-curing antibiotic susceptibility profile with the observation that no plasmid was detected indicate that such an exceptionally high degree of antibiotic resistance was detected without the corresponding detection of plasmid DNA suggests several possibilities. Firstly, the genes coding for resistance may be chromosomally encoded through mutations in target genes or regulatory elements (Alekshun and Levy, 2007). Secondly, the resistance genes could have been located on integrons or transposons integrated into the chromosome (Hall and Collis, 1995). The MAR index of 0.6 for this *E. coli* isolate, depicted by its multi-drug resistance profile, indicates a high antibiotic exposure history and thus provides every reason to believe that resistance determinants could be present even when plasmids are not detected.

The antibiotic resistance profile of *Bacillus subtilis* pre- and post-plasmid curing was presented. Before curing, this organism showed susceptibility to imipenem (32 mm) and gentamycin (16 mm), intermediate resistance to levofloxacin (14 mm), while it was resistant to cefixime (13 mm), azithromycin (10 mm), ceftriaxone (13 mm), ceftazidime (10 mm) and cefuroxime (11 mm). After curing, susceptibility became remarkably improved as the organism expressed susceptibility to imipenem (36 mm), gentamycin (20 mm), levofloxacin (20 mm), azithromycin (18 mm), and cefuroxime (23 mm), an intermediate resistance to ceftriaxone (20 mm) while it remained resistant to cefixime (15 mm) and ceftazidime (12 mm). The pre- and post-curing antibiotic susceptibility pattern demonstrated a remarkable variation in the size of the inhibition zone for each antibiotic before and after curing. The acquisition of susceptibility from resistance for macrolides (azithromycin) and fluoroquinolones (levofloxacin) antibiotics after plasmid curing from the *Bacillus subtilis* demonstrates that such bacteria expressed a plasmid-encoded resistant gene(s).

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The results obtained from the plasmid curing experiments collectively point to a widespread distribution of plasmid-mediated antibiotic resistance among bacterial isolates from abattoir-

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contaminated soils. This is further verified by the successful elimination of resistance phenotypes after curing, showing that these mobile genetic elements play a major role in the dissemination of antibiotic resistance within environmental bacteria (Bennett, 2008). The persistence of certain resistance traits in some of the cured bacteria simply indicates that bacteria often develop multiple resistance mechanisms, combining both plasmid-encoded and chromosomal resistance genes. This provides a redundancy in resistance mechanisms that complicates the treatment of infections caused by such organisms as it limits treatment options, requires combination therapy, increases treatment duration and reflects the complexity of antibiotic resistance in environmental settings (Davies and Davies, 2010). The incidence of transferable antibiotic resistance genes in bacteria from abattoir soils has serious public health implications, as such genes can be transferred to human pathogens through direct contact, consumption of contaminated food products, or environmental dissemination (Marshall and Levy, 2011).

# 5.1 Contributions to Knowledge

The study has contributed to knowledge in the following ways:

1. Highlighted the chronic accumulation of toxic elements in abattoir-impacted soils.
2. Provided baseline data for assessing soil contamination thresholds, which can be used in future public health risk assessments and waste management planning.
3. Explained the prevalence of multidrug-resistant (MDR) bacterial strains in abattoir-impacted soils, revealing consistent resistance to β-lactams, macrolides, and aminoglycosides across diverse genera.

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## 5.2 Conclusion and Recommendations

The results from this work have shown that abattoir operations in Benin City impacts on soil physicochemical properties and microbial communities of its surroundings. The accumulation of heavy metals like zinc, iron, and copper in the soils presents an environmental risk that could potentially lead to health hazards through food chain contamination. The high counts of bacteria with the presence of multiple pathogenic species, including antibiotic-resistant strains, are serious public health concerns, especially to the abattoir workers and people who live nearby. The demonstration of plasmid-mediated antibiotic resistance highlights the role of abattoir environments as reservoirs and dissemination points for resistance genes. These findings point to the need to adopt better waste management practices at the abattoir facilities, ensuring proper treatment of blood and organic wastes before discharge, putting in place containment systems to prevent soil contamination, and regular monitoring of environmental quality. Public health interventions should involve educating workers about the infection risks and hygiene practices, restriction of access to contaminated areas, and a ban on the use of contaminated soils for crop production or animal grazing. Further studies will be required to ascertain the quality of groundwater in these areas, assess the bioaccumulation of heavy metals in plants and animals, and investigate the feasibility of bioremediation strategies to reduce the level of contamination in the affected soils.

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# APPENDIX I

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## CULTURE MEDIA

### SALMONELLA, SHIGELLA AGAR (SSA)

| | |
|---|---|
| Lab-lemco powder | 5.0g/L |
| Peptone | 5.0g/L |
| Lactose | 10.0g/L |
| Bile salts | 8.5g/l |
| Sodium citreate | 10.0g/L |
| Sodium thiosulphate | 8.5g/l |
| Ferric citrate | 1 .0g/L |
| Brillant gree | 0.00033g/L |
| Neutral red | 0.025g/L |
| Agar | 15.0g/L |

### NUTRIENT AGAR

| | |
|---|---|
| Yeast | 14.0g/L |
| Sugar | 15.0g/L |
| Ferric citrate | 0.5g/L |
| Aesculin | 1.0g/L |
| Agar | 15.0g/L |

### MUELLER-HINTON AGAR

| | |
|---|---|
| Dehydrated infusion from beef | 300.0g/L |
| Casein | 17.5g/L |
| Starch | 1.5g/L |
| Agar | 17.0g/L |

### MAC-CONKEY AGAR

***Formula gm/litre***

Peptone 20.0
Lactose 10.0
Bile salts 5.0
Sodium chloride 5.0
Neutral red 0.075
Agar 12.0
pH 7.4 ± 0.2

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# APPENDIX II

## GRAM STAINING AND BIOCHEMICAL REAGENTS

### GRAM STAINING REAGENTS

Gram crystal violet

**Solution A**

| | |
|---|---|
| Crystal violet | 2.0 g |
| Ethanol (95 ml) | 20.0 ml |

**Solution B**

| | |
|---|---|
| Ammonium oxalate | 0.8 g |
| Distilled water | 80.0 ml |

**Iodine**

| | |
|---|---|
| Iodine | 1.0 g |
| Potassium iodide | 2.0 g |
| Water | 300.0ml |

3.0 g of medium was dissolved in 300.0ml of distilled water.

**Gram's safranin**

| | |
|---|---|
| Safranin | 0.25 g |
| Ethanol | 10.0 ml |
| Distilled water | 100 ml |

**Gram staining technique**

The Gram staining reaction was carried out on 24h cultures. A smear of each of the bacterial isolates was made on clean glass slide and heat fixed using flame. Crystal violet stain (0.3%w/v) was added and allowed to stand for 1min. The stain was washed off with distilled water. Grams iodine (0.4% w/v) was added and allowed to stand for 1min before being rinsed off. Ethanol (95%v/v) was added and allowed to stand for about 30sec before being rinsed off with distilled water and then stained with secondary stain, safranin (0.4% v/v), for 1min. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective. Upon the experimentation, an inference of pink colouration and purple colouration was observed for Gram negative and Gram positive cells respectively, under the microscope.

**Biochemical reagents**

**Indole medium**

| | |
|---|---|
| Peptone | 20.0 g |
| Sodium chloride | 5.0 g |
| Distilled water | 1000 ml |
| pH | 7.4 |

15.0 g of indole medium (peptone water) was dissolved in 1000 ml of distilled water and autoclaved for 15 minutes at 121 $^0$C and dispensed aseptically into sterile bijou bottles.

**Simmon Citrate Agar**

| | |
|---|---|
| Sodium ammonium phosphate | 1.50 g |
| Potassium dihydrogen phosphate | 1.0 g |
| Magnesium sulphate | 0.2 g |
| Sodium citrate | 2.5 g |
| Bromothymol blue | 0.016 g |
| Distilled water | 1000 ml |
| pH | 7.0 |

22.5 g of Simmon’s citrate medium was dissolved in 1000ml of distilled water and autoclaved at

$121^0$C for 15 minutes and dispensed aseptically into sterile test tube.

**Sugar utilization medium**

| | |
|---|---|
| Peptone water | 2.0 g |
| Sodium chloride | 5.0 g |
| Water | 1000ml |
| Phenol red | 7.0 |

10% v/v of each of the sugar was dissolved in 1000 ml of distilled water and autoclaved at $121^0$C for 15 minutes. This was dispensed aseptically into sterile test tube containing Durham's tubes. The growth medium used was peptone water prepared in a conical flask and the indicator; phenol red was added. 1% solution of the sugar was prepared and sterilized separately at $115^0$c for 10 minutes. This was then ascetically dispensed in 5ml volume into the tubes containing the peptone water and indicator. The tubes were inoculated with young culture of the isolates and incubated at 37 $^0$C. Acid and gas production or acid only were observed after about 24 hours of incubation. Acid production was indicated by the change of the medium from light green to yellow colour while gas production was indicated by the presence of gas in the Durham's tubes.

**Kovac's reagent**

| | |
|---|---|
| Amyl-alcohol | 15.0 ml |
| p-dimethyl-aminobenzaldehyde | 0.5 ml |
| Concentrated HCl | 50.0 ml |

Small quantity of kovac's reagent were prepared by dissolving the aldehyde into alcohol and adding the acid slowly and then kept inside the refrigerator.

## MONTHLY MEAN HETEROTROPHIC BACTERIA COUNT FOR CONTAMINATED AND UNCONTAMINATED SOIL IN DRY SEASON

| Sample type | November<br>(Mean ± SD) | December<br>(Mean ± SD) | January<br>(Mean ± SD) |
|---|---|---|---|
| contaminated soil | 1.37x$10^{7}$ ±0.42 | 1.33x$10^{7}$ ±0.46 | 1.31x$10^{7}$ ±0.45 |
| uncontaminated soil | 0.68x$10^{7}$ ± 0.29 | 0.66x$10^{7}$ ± 0.28 | 0.67x$10^{7}$ ± 0.29 |

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## MONTHLY MEAN HETEROTROPHIC BACTERIA COUNT FOR CONTAMINATED AND UNCONTAMINATED SOIL IN RAINY SEASON

| Sample type | May<br>(Mean ± SD) | June<br>(Mean ± SD) | July<br>(Mean ± SD) |
|---|---|---|---|
| Contaminated soil | 1.30x$10^{8}$ ± 0.45 | 1.11x$10^{8}$ ± 0.31 | 1.38x$10^{8}$ ± 0.41 |
| Uncontaminated soil | 0.72x$10^{8}$ ± 0.30 | 0.69x$10^{8}$ ± 0.30 | 0.93x$10^{8}$ ± 0.59 |

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# APPENDIX III

| **Season** | **Average heterotrophic bacteria count** |
|---|---|
| **Rainy** | $10.44 \times 10^8$ |
| **Dry** | $6.81 \times 10^7$ |

| | DRY SEASON COUNT (1ST) | | | | |
|---|---|---|---|---|---|
| O TOP | A | B | C | MEAN | STD |
| O MIDDLE | 175 | 172 | 170 | 172.3333 | 1.5 |
| O BOTTOM | 120 | 111 | 109 | 113.3333 | 4.5 |
| OCTOP | 75 | 81 | 88 | 81.33333 | 3 |
| OC MIDDLE | 93 | 84 | 80 | 85.66667 | 4.5 |
| OC BOTTOM | 52 | 50 | 50 | 50.66667 | 1 |
| U TOP | 33 | 30 | 40 | 34.33333 | 1.5 |
| U MIDDLE | 181 | 190 | 191 | 187.3333 | 4.5 |
| U BOTTOM | 152 | 147 | 143 | 147.3333 | 2.5 |
| UC TOP | 97 | 91 | 90 | 92.66667 | 3 |
| UC MIDDLE | 113 | 109 | 97 | 106.3333 | 2 |
| UC BOTTOM | 60 | 58 | 67 | 61.66667 | 1 |
| E TOP | 30 | 30 | 33 | 31 | 0 |
| E MIDDLE | 188 | 192 | 190 | 190 | 2 |
| E BOTTOM | 160 | 186 | 188 | 178 | 13 |
| EC TOP | 79 | 88 | 82 | 83 | 4.5 |
| EC MIDDLE | 84 | 80 | 80 | 81.33333 | 2 |
| EC BOTTOM | 50 | 52 | 50 | 50.66667 | 1 |
| D TOP | 30 | 26 | 27 | 27.66667 | 2 |
| D MIDDLE | 177 | 170 | 173 | 173.3333 | 3.5 |

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|  |  |  |  |  |  |
|---|---|---|---|---|---|
| D BOTTOM | 155 | 150 | 152 | 152.3333 | 2.5 |
| C TOP | 76 | 88 | 89 | 84.33333 | 6 |
| C MIDDLE | 125 | 119 | 111 | 118.3333 | 3 |
| C BOTTOM | 110 | 95 | 98 | 101 | 7.5 |

|  | DRY SEASON COUNT (2ND) |  |  |  |
|---|---|---|---|---|
|  | A | B | C | MEAN |
| O TOP | 150 | 169 | 170 | 163 |
| O MIDDLE | 118 | 111 | 115 | 114.6667 |
| O BOTTOM | 77 | 81 | 70 | 76 |
| OC TOP | 95 | 89 | 85 | 89.66667 |
| OC MIDDLE | 54 | 60 | 57 | 57 |
| OCBOTTOM | 33 | 37 | 40 | 36.66667 |
| U TOP | 185 | 190 | 170 | 181.6667 |
| U MIDDLE | 130 | 147 | 158 | 145 |
| U BOTTOM | 99 | 91 | 85 | 91.66667 |
| UC TOP | 105 | 70 | 81 | 85.33333 |
| UC MIDDLE | 50 | 48 | 30 | 42.66667 |
| UC BOTTOM | 30 | 27 | 27 | 28 |
| E TOP | 190 | 192 | 189 | 190.3333 |
| E MIDDLE | 170 | 186 | 177 | 177.6667 |
| E BOTTOM | 70 | 88 | 8 | 55.33333 |
| EC TOP | 93 | 80 | 80 | 84.33333 |
| EC MIDDLE | 54 | 57 | 50 | 53.66667 |
| EC BOTTOM | 35 | 30 | 27 | 30.66667 |
| D TOP | 180 | 170 | 175 | 175 |
| D MIDDLE | 155 | 160 | 152 | 155.6667 |
| D BOTTOM | 65 | 70 | 89 | 74.66667 |
| C TOP | 120 | 117 | 111 | 116 |
| C MIDDLE | 110 | 95 | 98 | 101 |
| C BOTTOM | 70 | 81 | 79 | 76.66667 |

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|  | DRY SEASON COUNT (3RD) |  |  |  |
|---|---|---|---|---|
|  | A | B | C | MEAN |
| O TOP | 149 | 160 | 172 | 160.3333 |
| O MIDDLE | 116 | 115 | 110 | 113.6667 |
| O BOTTOM | 80 | 75 | 70 | 75 |
| OC TOP | 93 | 84 | 80 | 85.66667 |
| OC MIDDLE | 52 | 50 | 50 | 50.66667 |
| OCBOTTOM | 33 | 30 | 40 | 34.33333 |
| U TOP | 186 | 188 | 170 | 181.3333 |
| U MIDDLE | 130 | 133 | 130 | 131 |
| U BOTTOM | 85 | 80 | 80 | 81.66667 |
| UC TOP | 113 | 109 | 97 | 106.3333 |
| UC MIDDLE | 60 | 58 | 67 | 61.66667 |
| UC BOTTOM | 30 | 30 | 33 | 31 |
| E TOP | 210 | 192 | 190 | 197.3333 |
| E MIDDLE | 160 | 180 | 177 | 172.3333 |
| E BOTTOM | 68 | 66 | 78 | 70.66667 |
| EC TOP | 84 | 80 | 80 | 81.33333 |
| EC MIDDLE | 50 | 52 | 50 | 50.66667 |
| EC BOTTOM | 30 | 26 | 27 | 27.66667 |
| D TOP | 176 | 170 | 170 | 172 |
| D MIDDLE | 160 | 160 | 150 | 156.6667 |
| D BOTTOM | 67 | 60 | 77 | 68 |
| C TOP | 121 | 119 | 111 | 117 |
| C MIDDLE | 100 | 96 | 98 | 98 |
| C BOTTOM | 71 | 70 | 79 | 73.33333 |

|  | RAINY SEASON COUNT 1ST |  |  |  |  |
|---|---|---|---|---|---|
|  | A | B | C | MEAN | STD |
| O TOP | 188 | 172 | 166 | 175.3333 | 11.3724814 |
| O MIDDLE | 120 | 133 | 127 | 126.6667 | 6.5064071 |
| O BOTTOM | 75 | 87 | 70 | 77.33333 | 8.73689495 |
| OC TOP | 93 | 84 | 80 | 85.66667 | 6.65832812 |
| OC MIDDLE | 52 | 50 | 50 | 50.66667 | 1.15470054 |
| OCBOTTOM | 33 | 30 | 40 | 34.33333 | 5.13160144 |
| U TOP | 188 | 190 | 182 | 186.6667 | 4.163332 |
| U MIDDLE | 151 | 147 | 150 | 149.3333 | 2.081666 |
| U BOTTOM | 97 | 91 | 95 | 94.33333 | 3.05505046 |
| UC TOP | 113 | 109 | 97 | 106.3333 | 8.326664 |
| UC MIDDLE | 60 | 58 | 67 | 61.66667 | 4.72581563 |

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| | | | | | |
|---|---|---|---|---|---|
| UC BOTTOM | 30 | 30 | 33 | 31 | 1.73205081 |
| E TOP | 190 | 192 | 185 | 189 | 3.60555128 |
| E MIDDLE | 169 | 186 | 171 | 175.3333 | 9.29157324 |
| E BOTTOM | 80 | 88 | 84 | 84 | 4 |
| EC TOP | 84 | 80 | 80 | 81.33333 | 2.30940108 |
| EC MIDDLE | 50 | 52 | 50 | 50.66667 | 1.15470054 |
| EC BOTTOM | 30 | 26 | 27 | 27.66667 | 2.081666 |
| D TOP | 177 | 172 | 170 | 173 | 3.60555128 |
| D MIDDLE | 155 | 153 | 151 | 153 | 2 |
| D BOTTOM | 80 | 88 | 80 | 82.66667 | 4.61880215 |
| C TOP | 125 | 120 | 122 | 122.3333 | 2.51661148 |
| C MIDDLE | 110 | 100 | 114 | 108 | 7.21110255 |
| C BOTTOM | 79 | 81 | 83 | 81 | 2 |

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# APPENDIX III

## CHARACTERIZATION OF BACTERIAL ISOLATES IN RAINY SEASON

| **Cultural** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Elevation | Flat | Raised | Raised | Flat | Flat | Flat | Raised | Flat | Flat |
| Margin | Entire | Entire | Entire | Undulate | Undulate | Undulate | Entire | Curled | Undulate |
| Color | Cream | Cream | Cream | Cream | Cream | Cream | Cream | Cream | Cream |
| Shape | Circular | Circular | Circular | Irregular | Irregular | Irregular | Circular | Irregular | Irregular |
| Size | Medium | Medium | Medium | Large | Large | Large | Medium | Medium | Large |
| Gr. diff. agar | MacConkey | SSA | MacConkey | PCA | Manitol | EMB | MacConkey | BCA | |
| Colour | opaque | Black | opaque | Green | golden yellow | Metallic Green sheen | opaque | Blue | pink |
| **Morphological** | | | | | | | | | |
| Gram stain | - | - | - | - | + | - | - | + | + |
| cell type | Rod | Rod | rod | rod | cocci | Rod | rod | rod | cocci |
| Arrangement | disperse | pair/chains | disperse | disperse | disperse | disperse | disperse | disperse | disperse |
| Color | Pink | Pink | pink | pink | purple | pink | pink | purple | purple |
| Spore staining | - | - | - | - | - | - | - | + | - |
| **Biochemical** | | | | | | | | | |
| KOH test | + | + | + | + | - | + | + | - | - |
| Catalase | + | + | + | + | + | + | + | + | + |
| Indole | - | - | - | - | - | + | - | + | - |
| Citrate | + | + | - | + | + | - | - | + | + |
| Oxidase | - | - | - | + | - | - | - | - | - |
| Urease | + | - | - | - | - | - | - | + | - |
| fermentation | | | | | | | | | |
| Glucose | + | + | + | - | + | + | + | + | + |
| Sucrose | - | - | - | - | + | + | - | + | + |
| Lactose | - | - | - | - | + | + | - | + | + |
| Mannitol | - | - | - | - | - | - | - | - | - |
| Gas formation | + | + | - | - | + | + | - | - | - |
| $H_2S$ formation | + | + | - | - | - | - | - | - | - |
| **Identity** | ***Proteus mirabilis*** | ***Salmonella* spp.** | ***Klebsiella* spp** | ***Pseudomonas aeruginosa*** | ***Staphylococcus aureus*** | ***Escherichia. coli*** | ***Alcaligenes* spp** | ***Bacillus subtilis*** | ***Enterococcus* sp** |

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# CHARACTERIZATION OF BACTERIAL ISOLATES IN DRY SEASON

| **Cultural** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Elevation | Flat | Raised | Raised | Flat | Flat | Flat | Raised | Flat | Flat |
| Margin | Entire | Entire | Entire | Undulate | Undulate | Undulate | Entire | Curled | Undulate |
| Color | Cream | Cream | Cream | Cream | Cream | Cream | Cream | Cream | Cream |
| Shape | Circular | Circular | Circular | Irregular | Irregular | Irregular | Circular | Irregular | Irregular |
| Size | Medium | Medium | Medium | Large | Large | Large | Medium | Medium | Large |
| Gr. diff. agar | MacConkey | SSA | MacConkey | PCA | Mannitol | EMB | MacConkey | BCA | |
| Colour | opaque | Black | opaque | green | golden yellow | Metallic Green sheen | opaque | Blue | pink |
| **Morphological** | | | | | | | | | |
| Gram stain | - | - | - | - | + | - | - | + | + |
| cell type | Rod | Rod | rod | rod | Cocci | Rod | rod | rod | cocci |
| Arrangement | disperse | pair/chains | disperse | disperse | disperse | disperse | disperse | disperse | disperse |
| Color | Pink | Pink | pink | pink | Purple | pink | pink | purple | purple |
| Spore staining | - | - | - | - | - | - | - | - | - |
| **Biochemical** | | | | | | | | | |
| KOH test | + | + | + | + | - | + | + | - | - |
| Catalase | + | + | + | + | + | + | + | + | + |
| Indole | + | - | - | - | - | + | - | + | - |
| Citrate | + | + | - | + | + | - | - | + | + |
| Oxidase | - | - | - | + | - | - | - | - | - |
| Urease | + | - | - | - | - | - | - | + | - |
| Fermentation | | | | | | | | | |
| Glucose | + | + | + | - | + | + | + | + | + |
| Sucrose | - | - | - | - | + | + | - | + | + |
| Lactose | - | - | - | - | + | + | - | + | + |
| Mannitol | - | - | - | - | - | - | - | - | - |
| Gas formation | + | + | - | - | + | + | - | - | - |
| H2S formation | + | + | - | - | - | - | - | - | - |
| **Identity** | ***Proteus vulgaris*** | ***Salmonella* spp** | ***Klebsiella* spp** | ***Pseudomonas aeruginosa*** | ***Staphylococcus aureus*** | ***Escherichia coli*** | ***Alcaligenes* spp** | ***Bacillus subtilis*** | ***Enterococcus* sp** |

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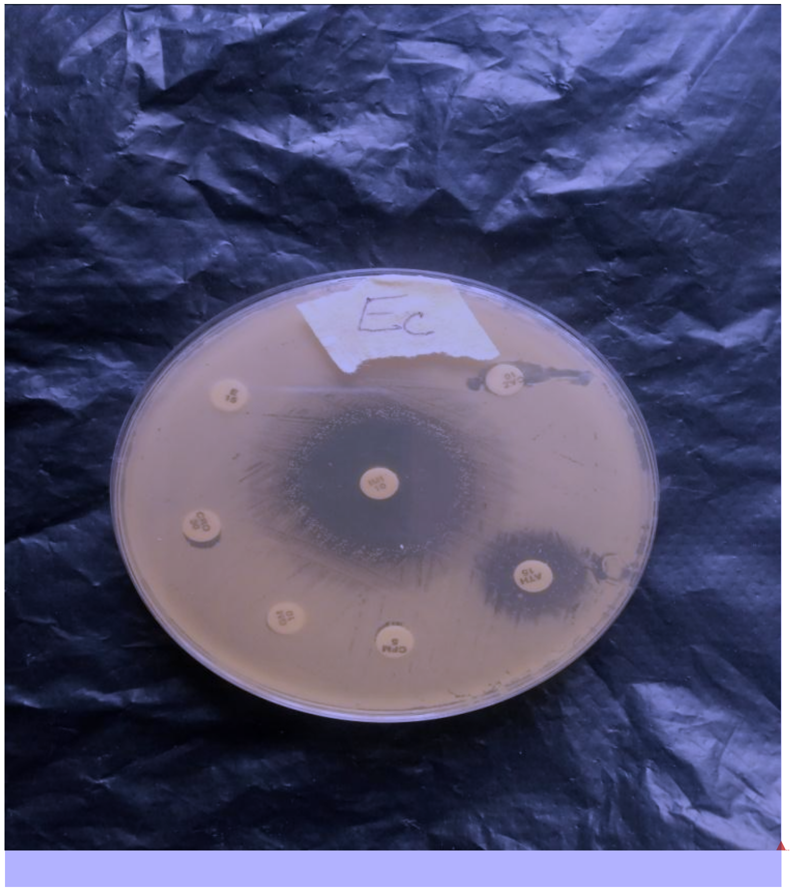

***Escherichia coli* PRE-CURING (NO PLASMID DETECTION)**

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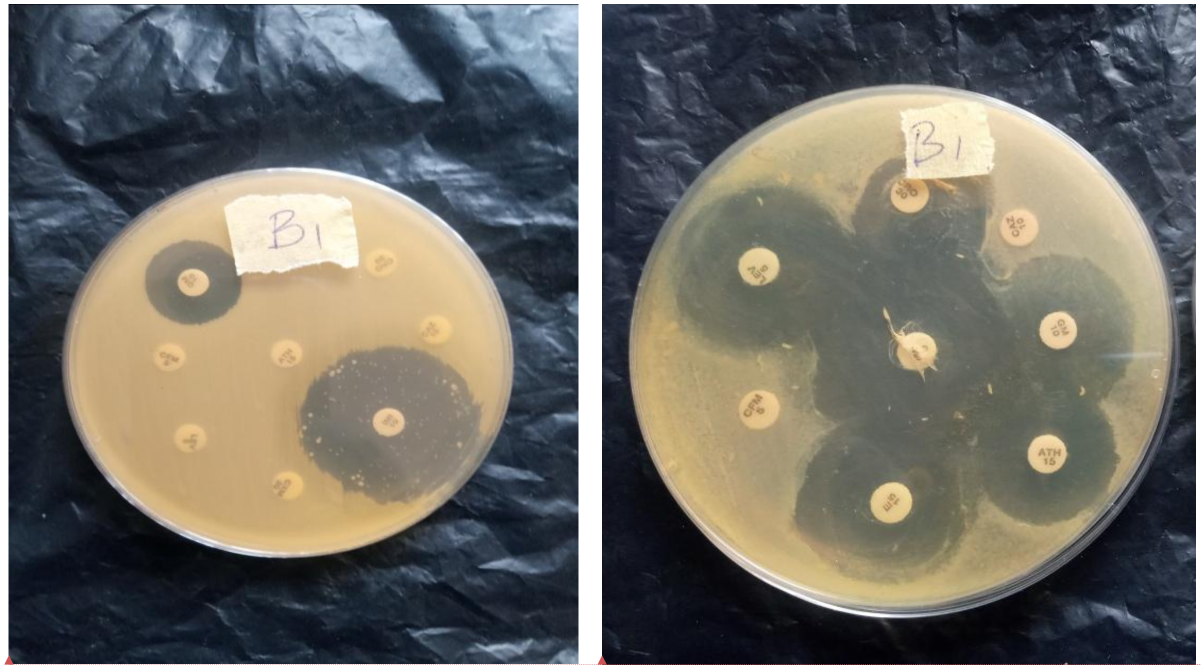
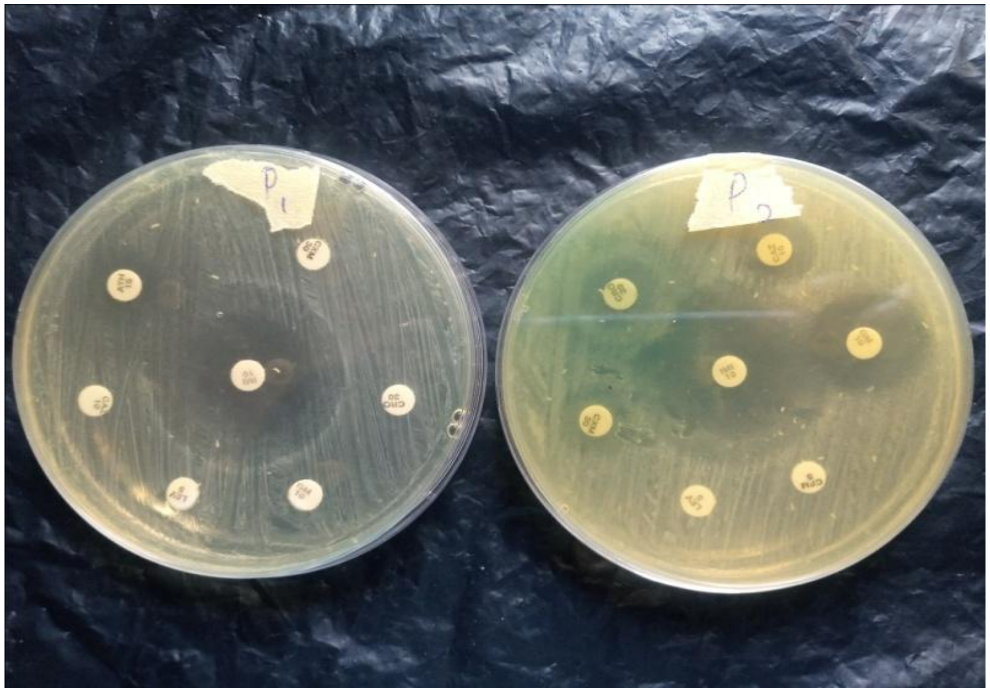


***Bacillus subtilis* PRE-CURING and *Bacillus subtilis* POST-CURING**



***Pseudomonas* spp. PRE and POST-CURING (P1 and P2)**

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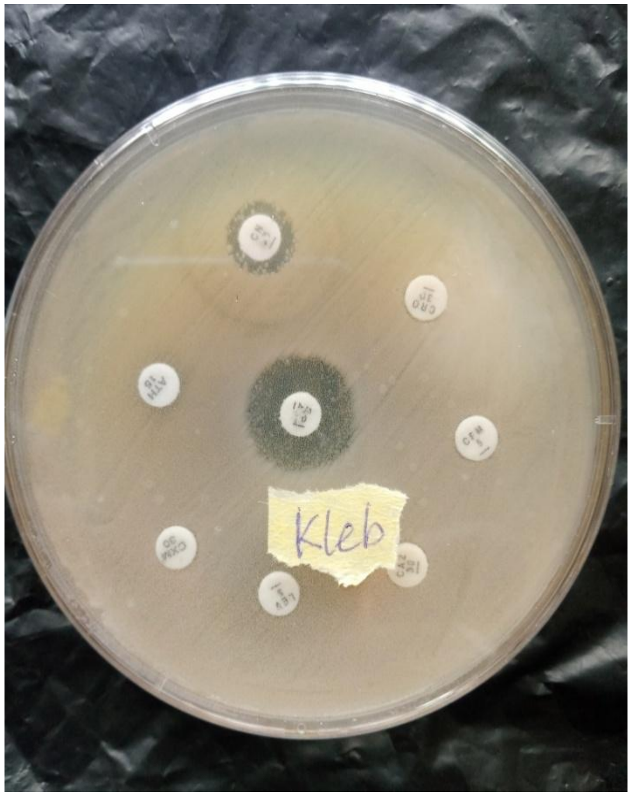

***Klebsiella* spp. PRE-CURING**

***Klebsiella* spp. POST-CURING**

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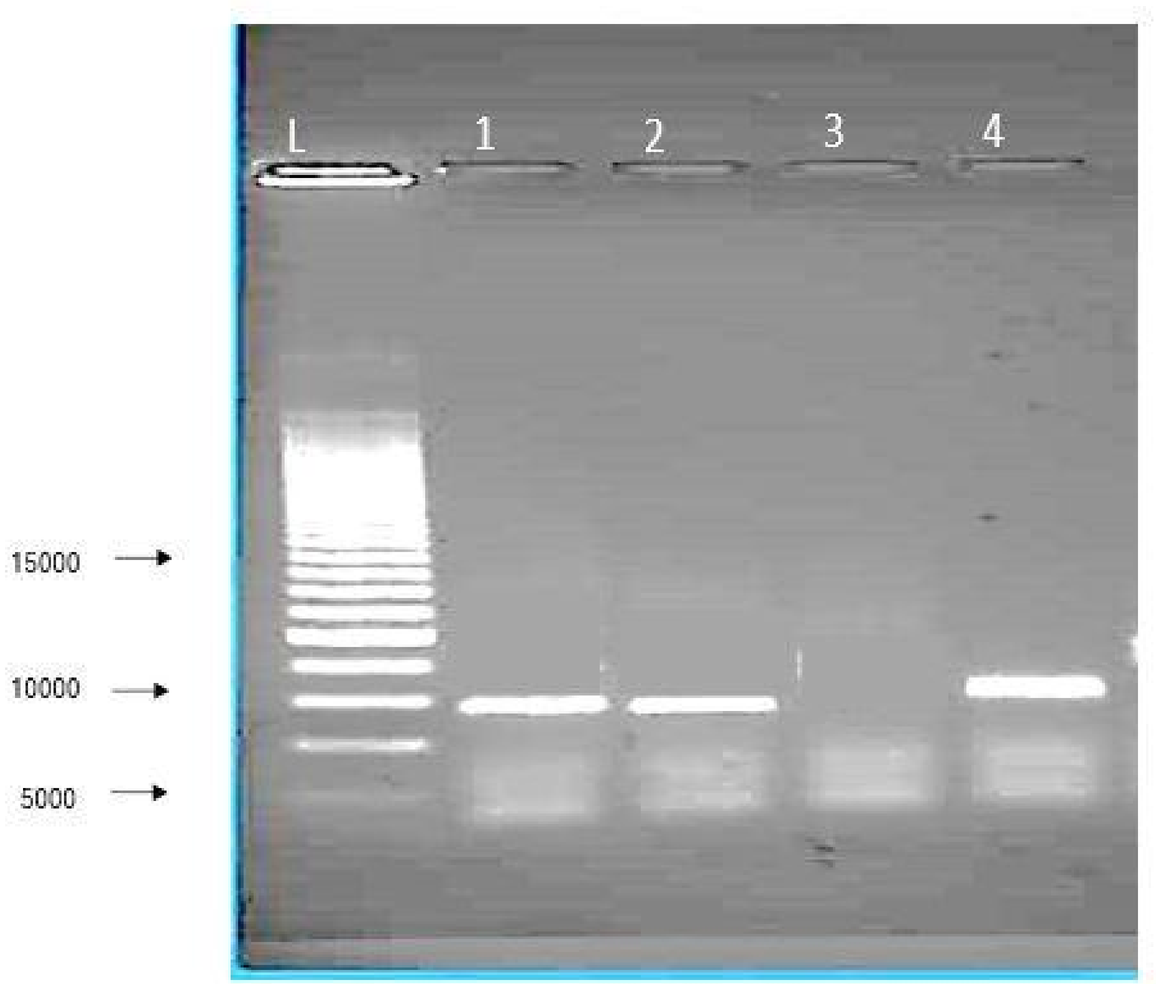

Plasmid DNA extraction results for multiple drug resistance bacterial isolates analyzed with 0.8% Agarose gel electrophoresis stained with ethidium bromide, L is 1kb plus ladder (100bp-10,000bp) DNA ladder (Molecular marker). Samples: Isolate 1, Isolate 2 and Isolate 4 are positive Plasmid DNA with bands ranging from 9000bp - 10000bp, while Isolate 3 is negative for Plasmid DNA. NC is no DNA template

Isolate 1: *Klebsiella* spp, Isolate 2: *Pseudomonas aeruginosa*, Isolate 3: *Escherichia coli*, Isolate 4: *Bacillus subtilis*

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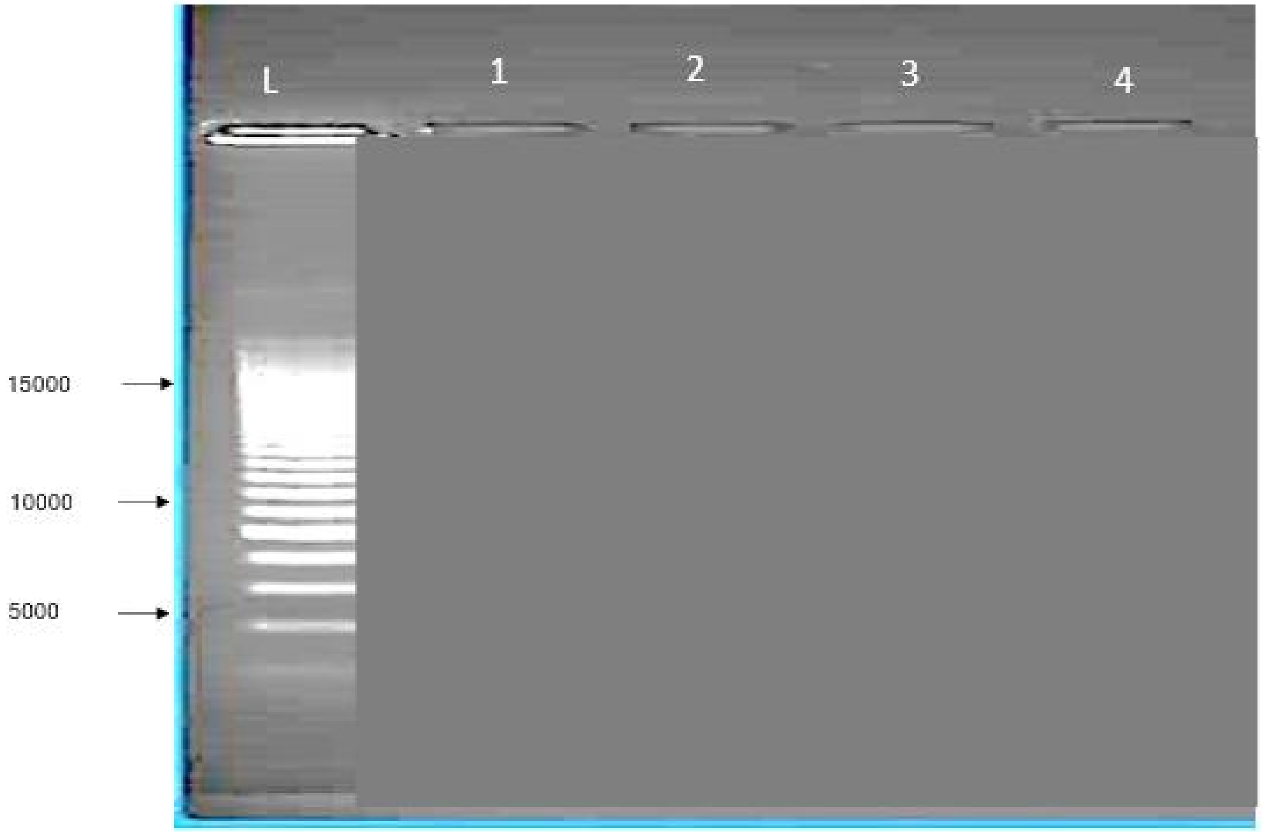

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Plasmid DNA extraction results for bacterial isolates after plasmid curing analyzed with 0.8% Agarose gel electrophoresis stained with ethidium bromide, L is 1kb plus ladder (100bp-10,000bp) DNA ladder (Molecular marker). Samples: Isolate 1, Isolate 2, isolate 3 and Isolate 4 are negative for Plasmid DNA.

Isolate 1*: Klebsiella spp*
Isolate 2*: Pseudomonas aeruginosa*
Isolate 3: *Escherichia coli*
Isolate 4*: Bacillus subtilis*

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## Antibiotics Susceptibility Standard

| Antibiotics Key | Disc code | Resistant (R) < or = (mm) | Intermediate (I) (mm) | Sensitive (S) > or = (mm) |
|---|---|---|---|---|
| Imipenem 10µg | IMI | 19 | 20 - 22 | 23 |
| Cefixime 5µg | CFM | 15 | 16 – 18 | 19 |
| Levofloxacin 5µg | LEV | 13 | 14 - 16 | 17 |
| Gentamycin 10µg | GM | 12 | 13 – 14 | 15 |
| Azithromycin 15µg | ATH | 13 | 14 – 17 | 18 |
| Ceftriaxone 30µg | CRO | 19 | 20 -22 | 23 |
| Ceftazidime 10µg | CAZ | 14 | 15 – 17 | 18 |
| Erythromycin 15µg | E | 13 | 14 – 22 | 23 |

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