ASSESSMENT OF BACTERIAL CONTAMINATION OF GROUNDWATER: A CASE OF CHUNGA DUMPSITE OF LUSAKA, ZAMBIA

By
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A dissertation submitted in fulfilment of the requirements for the degree of
Master of Science in Applied Microbiology

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DECLARATION AND COPYRIGHT

I declare that, this thesis was written and submitted in accordance with the rules and regulations governing the award of Master of Science in Applied Microbiology at the University of Zambia, and that, **ASSESSMENT OF BACTERIAL CONTAMINATION OF GROUNDWATER: A CASE OF CHUNGA DUMPSITE OF LUSAKA, ZAMBIA** is my own work and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

Signed……………………………….. Date…………………………

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This thesis for Mwansa Chilambwe has been approved as partial fulfilment of the requirements for the award of the Master of Science in Applied Microbiology by the University of Zambia.

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ABSTRACT

The community near dumpsites/landfills depend on groundwater from shallow wells and boreholes for drinking. However, leachates from dumpsites/landfills could be a source of microbial and toxic chemical contamination to the nearby soil and groundwater. Contamination of groundwater will in turn affect the nearby community by causing diseases. Solid wastes come from various sources including; domestic residences, offices, institutions, commercial buildings, restaurants, agriculture, construction, and hospitals. This study assessed whether the bacterial contaminations from the Chunga Dumpsite solid wastes, were able to infiltrate the soil and contaminate the nearby groundwater. Nine (9) soil samples were collected from the Chunga dumpsite, and nine (9) water samples from boreholes near the Chunga Dumpsite. Physical, chemical and microbiological parameters of the collected samples were analyzed. It was found that most of the parameters conformed to WHO and ZABS standards except for nitrate levels of most of the water samples which exceeded the WHO and ZABS standards. Enumeration of bacteria in the water samples was done, to determine the quality of borehole water. Bacteria were isolated from the soil and water samples using culture methods for identification, then the isolated bacteria were confirmed using PCR methods (16S rRNA gene sequencing). The following bacteria were isolated from borehole water; *Citrobacter freundi, Kluyvera georgiana, Acinetobacter indicus, Escherishia coli, Proteus hauseri, Pseudomonas sp, Aeromonas caviae, Klebsiella pneumonia and Atlantibacter hermannii*, while the following bacteria were isolated from the soil; *Bacillus sp, Klebsiella pneumoniae, Bacillus cereus, Morganella morganii, Acinetobacter variabilis, Pectobacterium carotovorum and Bacillus thuringiensis*. On the other hand, *Klebsiella pneumonia* and *Acinetobacter* sp. were isolated from both the borehole water and dumpsite soil samples. Consequenty, the Drug Susceptibility Test was done on the isolated bacteria to determine which antibiotic the bacteria were susceptible to. The findings indicated that the isolated bacteria were all susceptible to Gentamicin, Chloramphenicol and Ciprofloxacin and resistant to Penicillin and Cefoxitin Therefore, from the findings, it was concluded that the borehole water near the Chunga dumpsite is not safe to drink and that there is need for authorities to improve the waste disposal and management system ., as well as constant monitoring of the groundwater quality, which will help authority to institute appropriate action to control the groundwater contamination, and eventually prevent unnecessary disease outbreaks in the surrounding communities.

Key Terms:

Landfill/dumpsite; Groundwater; Borehole; Leachate; Contamination; Infiltrate;

Physical, Chemical and Microbiological parameters; Susceptibility.

DEDICATION

This work is dedicated to my beloved husband and my loving family .

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LIST OF ABBREVIATIONS

CBS : Centre for Biological Sequence
CFU : Colony Forming Unit
DNA : Deoxyribonucleic Acid
DSMZ : Deutsche Sammlung Von Mikroorganismen und Zellkulturen
EDTA : Ethylenediaminetetraacetic acid
EPA : Environmental Protection Agency
g : gram
GPS : Global Positioning System
LCC : Lusaka City Council
MDH : Management and Development for Health
Mgcl2 : Magnesium chloride
MH : Mueller-Hinton
NCBI : National Center for Biotechnology Information
PCR : Polymerase Chain Reaction
rRNA : Ribosomal ribonucleic acid
SSU : Small Subunit
TAE : Tris-acetate-EDTA
TCBS : Thiosulfate-citrate-bile salts-sucrose
THMS : Trihalomethanes
US : United States
WHO : World Health Organisation
WWDR: World Water Development Report
XLD : Xylose Lysine Deoxycholate
ZABS : Zambia Bureau of Standards

CHAPTER 1

1 INTRODUCTION

Groundwater is water that is beneath the earth’s surface in soil pore spaces and in the fractures of rock formations. Groundwater is recharged from the surface; it may be discharged from the surface naturally at springs and seeps, and can form oases and wetlands. Groundwater is often withdrawn for agricultural, municipal and industrial use by constructing and operating municipal wells.

According to the WWDR 2022, groundwater, which accounts for approximately 99% of all liquid freshwater on Earth, is distributed over the entire globe and half of the volume of the groundwater is withdrawn for domestic use by the global population. Yet, despite its importance, groundwater is often mismanaged. Due to inability of governments to meet the ever-increasing water demand, most people in the rural and urban areas resort to groundwater sources such as well, as an alternative water resource. Thus, humans can abstract groundwater through a boreholes or wells which are drilled into the aquifer for industrial, agricultural and domestic use. However, groundwater resources are commonly vulnerable to pollution, which may degrade their quality (Lobina and Mercy, 2015).

There are a number of sources that contribute to groundwater pollution. The most common source of groundwater contamination is the water soluble solid waste from dumpsites or landfills (Daniel, Live and Harald, 2020). Dumpsites are made up of numerous organic and inorganic materials which are able to promote the growth of microorganisms that may be harmful to the public and surrounding environment, such as soil and groundwater (Shaikh, 2012). As rainwater infiltrates through trash and garbage in a dumpsite, it accumulates a variety of chemical and biological substances (Maqbool et al 2010). The leachate, may be highly mineralized and grossly contaminated by bacteria (Michaela et al, 2018) . Eventually the leachate may reach the water table, where it flows in the direction of groundwater flow or toward a pumping well or borehole. Bacterial contamination of water may lead to water-borne diseases such as typhoid fever, cholera and dysentery (World Health Organization., 2023). Waterborne diseases are caused by pathogenic microorganisms which are directly transmitted when contaminated fresh water is consumed (Potter, 2006).

The largest dumpsite in Lusaka, Zambia, is the Chunga Dumpsite which is located in Chunga residential area. It is the official garbage disposal site for the Lusaka City Council (LCC). It was constructed in the year 2004 and was designed to service Lusaka for 25 years (Muller et al., 2017). Initially, it was meant to run as a well-engineered landfill, but due to lack of funding, the condition have deteriorated, and it has turned into a dumpsite.

According to Igboama et al (2022), groundwater pollution drops as one moves away from the landfill sites. Therefore, boreholes and wells that are near landfills or dumpsites are susceptible to solid waste contam/ination. High density residential areas in Lusaka depend on groundwater from shallow wells and boreholes for other household use. Lusaka city has numerous wells and boreholes, of which some are located near the Chunga Dumpsite, with a chance of its groundwater being contaminated.

1.1 Statement of the Problem

The quality of groundwater is threatened by a number of factors. Among these, is the presence of landfills/dumpsites. All over the world, landfills have long been, and still are, a destination to both domestic and industrial wastes (Eggen et al., 2010). Improper landfill management causes deterioration in environmental qualities (Cossu, 2013; Peng, 2017). Many communities depend on groundwater, obtained from boreholes and wells, for their daily consumption. However, some of these boreholes and wells are situated near dumpsites, whose leachate can infiltrate into the ground and contaminate the nearby groundwater. If groundwater is contaminated, it becomes a vehicle for infection. One of the causes of groundwater bacterial contamination is dumpsite solid wastes, which is a global problem that has a significant impact on human health (Igboama et al, 2022).

In view of the above, the households near the Chunga Dumpsite are at high risk of waterborne diseases due to the likelihood of groundwater contamination by solid waste and leachate from the dumpsite, due to proximity of homes.

Studies have confirmed that water from borehole/well near landfills or dumpsites have high bacterial and chemical contamination (Olusolade et al., 2019). Aina et al (2012) also carried out a bacteriological analysis of borehole water from different towns near landfills, and discovered that groundwater near the landfills was contaminated with bacteria.

However, there has been no study in Zambia, to assess the bacterial contamination of ground water due to the solid wastes from the Chunga Dumpsite. Therefore, this study attempted to assess whether the bacterial contaminations from the Chunga Dumpsite solid wastes, are able to infiltrate the soil and contaminate the nearby groundwater.

1.2 Significance of the Study

Water can be a vehicle of spreading diseases. Groundwater obtained from boreholes is generally considered to be safe for drinking and other uses (Nathaniel et al, 2020). However some of these boreholes are situated near landfills or dumpsites whose leachates can infiltrate into the ground and contaminate the nearby groundwater. This may cause water borne diseases to the community using the contaminated water.

In this regard, it was imperative that this study be carried out in order to determine whether the bacterial contaminations from the Chunga Dumpsite solid wastes were able to infiltrate the soil and contaminate the groundwater.

This study established that there is a link between dumpsites and groundwater bacterial contamination.

1.3 Objectives of the study

1.3.1 General objective

To assess the bacterial contamination of groundwater near the Chunga dumpsite.

1.3.2 Specific Research Objectives

1. To assess the physico-chemical parameters of borehole groundwater near the Chunga Dumpsite.
2. To assesss the quality of borehole water near the Chunga Dumpsite.
3. To isolate and identify bacteria at the Chunga Dumpsite soil and from borehole water near the dumpsite.

4. To determine drug susceptibility of the isolated bacteria from the Chunga Dumpsite and near by borehole water.

1.3.3 Research Questions

1. What are the physico-chemical parameters of the borehole groundwater near the Chunga Dumpsite?
2. How is the quality of the borehole water near the Chunga Dumpsite?
3. What bacterial species can be isolated from the soil at the Chunga Dumpsite and from borehole water near the dumpsite?
4. Are the isolated bacteria from the dumpsite and the borehole water near the dumpsite, susceptible to the commonly used antibacterial agents?

CHAPTER 2

2 LITERATURE REVIEW

2.1 Previous research on bacterial groundwater contamination of boreholes and wells near landfills/dumpsites.

Global perspective

A study was conducted in India by Irshad et al (2012) in which the effect of landfill leachate on groundwater was evaluated in relation to the physico-chemical and bacteriological characteristics. It was found that most of the physico-chemical parametres of groundwater in most of the sites near the landfill area were not in accordance with the normal standards of portable groundwater. Most of the water samples contained significant amount of organic matter that provides nutrition for growth and multiplication of microorganisms, (Irishad et al, 2012). Further, according to Vijaya and Mittal (2009), solid waste that is disposed off by landfills poses a threat on underground water. They stated that a large number of municipal solid waste landfills and their many hazardous materials which they contain, pose a serious threat to both the surrounding environment and human populations. One of the hazardous release from landfills is methane gas, which is one of the most potent greenhouse gases and a huge contributor to climate change. Along with methane, landfills also produce carbon dioxide and water vapor, and trace amounts of oxygen, nitrogen, hydrogen, and non-methane organic compounds. These gases can also contribute to climate change and create smog if left uncontrolled. These emissions from landfills pose a threat to the health of those who live and work around it, as it brings hazards such as odor, smoke, noise, bugs, and water supply contamination. (Kayla, 2021).Once the waste is deposited at the landfill, pollution can arise from the percolation of leachate to the porous ground surface. Contamination of groundwater by such leachate renders it and the associated aquifer unreliable for domestic water supply and other use.

Kanownik and Przydatek (2019). carried out a study in Poland, to analyse changes in the physicochemical elements in groundwater in the vicinity of a small municipal solid waste landfill site. The findings of the study indicated that there was a negative impact of the municipal solid waste landfill on the state of the water environment in the immediate vicinity. The results showed that there was increased values of inorganic elements such as Cadmim (Cd) and total organic carbon (TOC). These turned out to be the determinants of the negative impact of leachate on the groundwater quality around the landfill. Consequently, it was concluded that the applied correlation relationship between physicochemical elements between leachate and groundwater can be an important tool to identify the threat of ground water pollution in the area of landfill sites.

Similarly, a study was done in the State of Mexico. The study aimed to determine the influence of leachate on the physicochemical quality and hydrogeochemical processes which determine the chemical composition of groundwater in an area near a municipal sanitary landfill site. The findings of the study showed some evaluated parameters, such as nitrate and phosphate) were outside the norm. This was attributed to the proximity of groundwater to the landfill (Dávalos, 2021).

Africa perspective

Several studies have been done in Africa on groundwater contamination by landfills. A study was coducted in Nigeria on the impact of leachate from landfill sites on water and soil quality. It was discovered that borehole water near landfills had high bacterial and chemical contamination (Olusolade et al., 2019).

In South Africa, Nevondo et al (2019) conducted a research with the aim of determining the total mercury (THg) concentrations in leachate and sediment samples collected from 4 selected landfill sites (3 sites in Gauteng Province – Soshanguve, Hatherly, Onderstepoort and 1 site in Limpopo Province – Thohoyandou). The findings from this study suggested that there was a likelihood of groundwater pollution by mercury from landfill leachate seepage, particularly for landfills that are not lined with a geomembrane.

Magda and Gaber (2015). evaluated the environmental impacts associated with solid waste landfilling, leachate and groundwater quality near the landfills in Egypt. The results of physico-chemical analysis of leachate confirmed that its characteristics were highly variable with severe contamination of organics, salts and heavy metals. It was also found that groundwater in the vicinity of the landfills had certain parameters that exceeded the WHO and EPA limits. These parameters included conductivity, total dissolved solids, chlorides, sulfates, Manganese and Iron. The results suggested the need for continuous monitoring of the groundwater and leachate treatment processes.

Zambian perspective

Similar studies have been conducted in Zambia, but non of the studies focused on groundwater bacterial contamination by solid wastes from the Chunga Dumpsite.

Nyirenda and Mwamba (2022) carried out research to investigate the physical chemical characterization of the leachate from Chunga landfill with respect to its impact on pollution of groundwater of the surrounding area. It was concluded that the operation of the Chunga landfill had compromised the groundwater quality in and around the landfill area, as evidenced by the presence of inorganic material that was detected in the leachate and groundwater. The study found that the major pollutants in the groundwater were cadmium, lead, nitrates and chlorides, generated from the leachate produced from the landfill. Nevertheless, this study did not look at the bacteriological contamination of the borehole around the Chunga Dumpsite

Another similar study was done by Siwila and Choolwe (2021) in Kitwe, Zambia, who investigated the effect of home-owned dumpsites, to the quality of groundwater. The findings of the study revealed that for boreholes within 15 metres proximity to home-owned dumpsites, the level of fecal contamination increased as the distance from the boreholes to the dumpsites decreased. However, this study did not go into details to analyse the diversity of bacteria that had contaminated the groundwater.

Nakonga et al, (2017), also conducted a cross-sectional study where microbiological assessment of borehole water in Libala South, Lusaka, was done. Borehole water samples were analysed for microbial contamination. The study revealed that 31% and 48.15% of the boreholes were contaminated with *Escherichia coli* and coliforms respectively. It was concluded that half the boreholes in Libala South was contaminated with harmful bacteria such as *E.Coli*, and posed a public health risk to the residents who use the water for domestic purposes. However, this study did not go further to determine the cause of the groundwater contamination.

2.2 Pathogenic bacteria

According to the microbiology society of England, bacteria are microscopic, single-cell organisms that live almost everywhere. They live in every climate and location on earth. Some are airborne while others live in water or soil. Bacteria live on and inside plants, animals, and people. They actually perform many vital functions for organisms and in the environment. The vast majority of bacteria are harmless to people and some strains are even beneficial. When considering all the strains of bacteria that exist, relatively few are capable of making people sick.

Pathogenic bacteria are bacteria that can cause disease, by producing virulence factors, which enable them to overcome the host's defense mechanisms, invade the tissue, and gain access to deeper locations to spread the infection. Some pathogens invade only the surface epithelium, skin or mucous membrane, but many travel more deeply, spreading through the tissues and disseminating by the lymphatic and blood streams. Pathogenic bacteria contribute to other globally important diseases, such as pneumonia, which can be caused by bacteria species belonging to the genera such as *Streptococcus, Pneumococcus and Pseudomonas*, and foodborne illnesses, which can be caused by bacteria species belonging to the genera such as *Shigella, Campylobacter, and Salmonella* (Thomas, 2002). Pathogenic bacteria also cause infections such as typhoid fever, diphtheria, hepatitis, and cholera.

Gram staining an bacterial culture, and other tests like genetic analysis can be used to identify pathogenic bacterial strains and help determine the appropriate course of treatment (Matthew, 2011).

2.3 Kirby-Bauer disk diffusion susceptibility test

The purpose of the Kirby-Bauer disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic bacteria to various antimicrobial compounds. The pathogenic organism is grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism (Jan, 2009). The Kirby-Bauer test for antibiotic susceptibility (also called the disc diffusion test) is a standard that has been used for years. First developed in the 1950s, it was refined by W. Kirby and A. Bauer, then standardized by the World Health Organization in 1961. This test is used to determine the resistance or sensitivity of bacteria to specific antibiotics, which can then be used by the clinician for treatment of patients with bacterial infections, (Anon, 2021).

The antibiotic diffuses from the disc into the agar in decreasing amounts the further it is away from the disc. If the organism is killed or inhibited by the concentration of the antibiotic, there will be no growth in the immediate area around the disc: This is called the zone of inhibition, as shown in Figure 2.1.

Disk diffusion is the most widely used AST method in microbiology laboratories because of its low cost and ease of performance and applicability of numerous bacterial species and antibiotics (Balouiri, 2016). On the other hand, the disadvantages are that some bacteria grow poorly or not grow at all on the media, and the minimum inhibitory concentration (MIC) cannot be determined (Barbara, 2014).

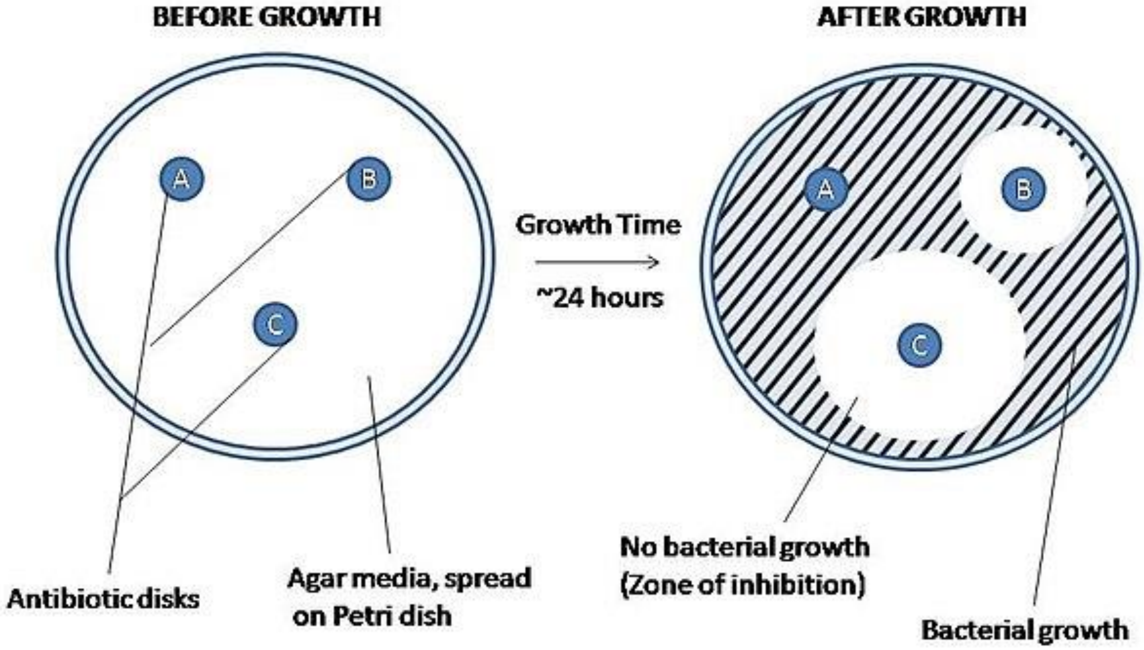


Figure 2.1 Disk diffusion test. (Wikipedia, Retrieved January 15, 2023).

The zone sizes are measured with a ruler in millimetres, and are looked up on a standardized chart to give a result of sensitive, resistant, or intermediate (Anon, 2021).

2.4 16S rRNA Sequencing

The 16S rRNA sequencing refers to sequencing the 16S rRNA gene that codes for the small subunit (SSU) of the ribosome found in prokaryotes such as Bacteria and Archaea. There are several factors that make the 16S rRNA gene the perfect target to complete your taxonomy or phylogeny studies. Because of the 16S rRNA gene codes for the SSU of the prokaryotic ribosome, researchers can rely on the fact that their target gene will be present in every cell. The 16S rRNAgene contains both highly conserved regions as well as hypervariable regions. The presence of the highly conserved regions allow researchers to design primer pairs that will accurately and reliably amplify the 16s rRNA gene hypervariable region of their choice. The presence of the hypervariable regions affords researchers the ability to differentiate between closely related genera or species detected in their samples. The overall size of the 16S rRNA gene is relatively short. ~1500bp (Vishakha et al., 2019).

One of the most attractive potential uses of 16S rRNA gene sequence informatics is to provide genus and species identification for isolates that do not fit any recognized biochemical profiles, for strains generating only a “low likelihood” or “acceptable” identification according to commercial systems, or for taxa that are rarely associated with human infectious diseases. The cumulative results from a limited number of studies to date suggest that 16S rRNA gene sequencing provides genus identification in most cases (>90%) but less so with regard to species (65 to 83%), with from one to 14% of the isolates remaining unidentified after testing (Drancourt et al., 2000; Mignard, 2006; Woo, 2003).

Although 16S rRNA gene sequencing is highly useful with regards to bacterial classification, it has low phylogenetic power at the species level and poor discriminatory power for some genera. DNA relatedness studies are necessary to provide absolute resolution to these taxonomic problems (Bosshard, 2006; Mignard, 2006).

The 16S rRNA gene sequence data cannot distinguish between recently diverged species (Petti 2007).

The usefulness of 16S rRNA gene sequencing as a tool in microbial identification is dependent upon two key elements, deposition of complete unambiguous nucleotide sequences into public or private databases and applying the correct “label” to each sequence (Micheal et al, 2007)

CHAPTER 3

3 MATERIALS AND METHODS

3.1 Study area and groundwater sampling boreholes/wells

This study was conducted at the Chunga Dumpsite and the surrounding areas, as shown in figure 2. The Chunga Dumpsite is lagest dumpsite in Zambia, and it was constructed in the year 2004, funded by the Danish Government. It is positioned at 15°00'S and 28°02'E, and located approximately 7 km from the Central Business District of Lusaka and approximately 800 metres west of the Great North Road. The site is situated on a plot of the Government Farms, and shares boundary with Chingwere Cemetery to the Southwest. According to Chishiba (2002), the Chunga Waste Disposal Site is currently the only operational municipal waste disposal site in Lusaka with authorisation from the Environmental Council of Zambia.

Initially, the Chunga Dumpsite was constructed to operate as an engineered landfill, however, due to inefficient management, it has lost its status as a well - engineered landfill. Further, due to lack of funding, the condition of dumpsite has deteriorated over the years. This has led to leachate drainages being blocked, leading to uncontrolled leachate generation, and leakages,. This has high potential of contaminating surface and groundwater resources. One of the leading problems caused by Municipal Solid Waste landfill/dumpsite is leachate (Bhalla et al., 2013), whose major environmental impacts are contamination of groundwater, (Kjeldsen and Christophersen, 2001; Kjeldsen et al., 2002; Słomczyńska and Słomczyński, 2004). Clean up and remediation of these water resources is very difficult and costly.

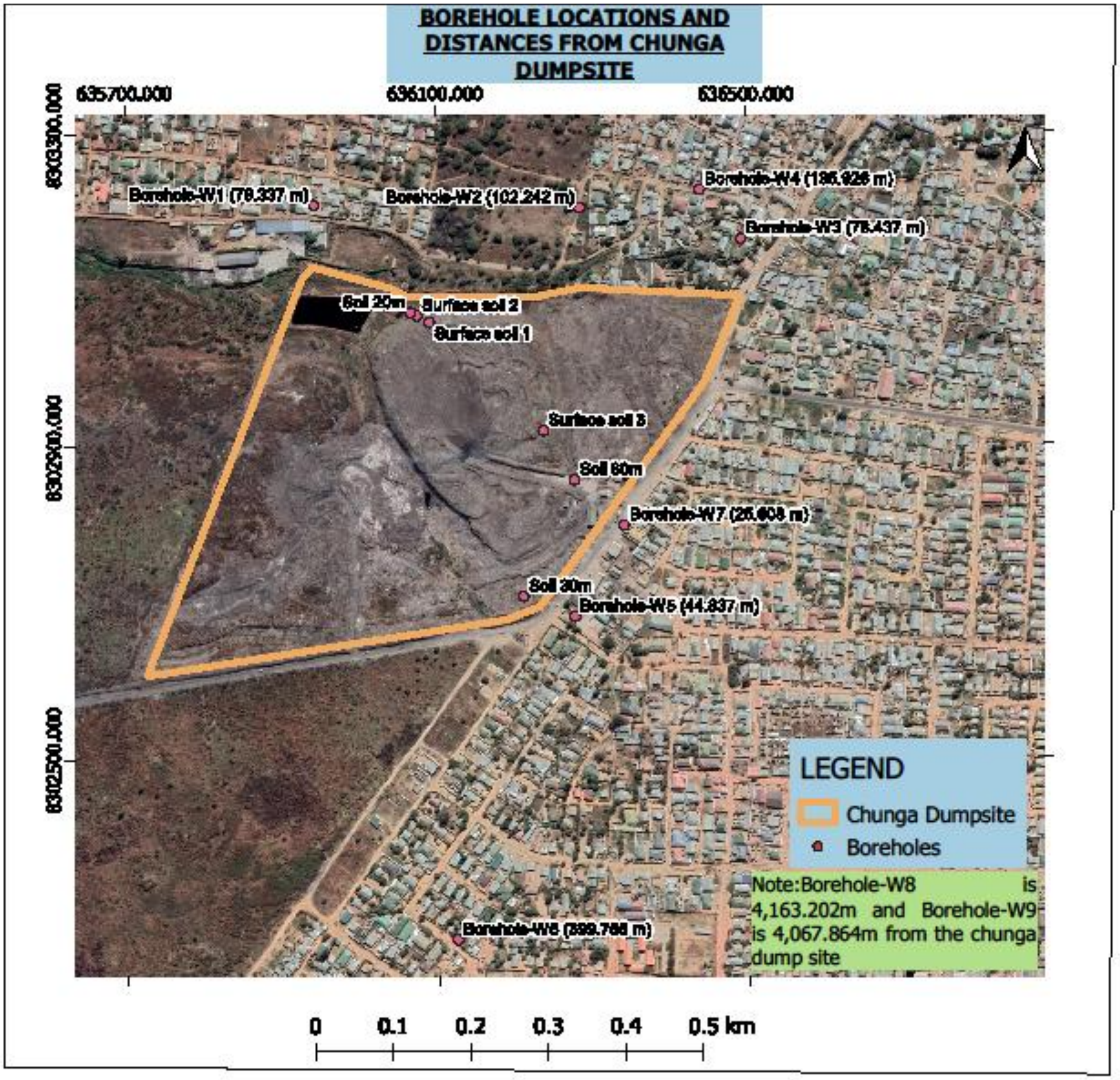
3.1.1 Sampling sites

Table 3.1 The coordinate points were soil and water samples were collected

Surface soil 1	Surface soil 2	Surface soil 3	Soil 20m	Soil 30m	Borehole - W1	Borehole - W2	Borehole - W3	Borehole - W4	Borehole - W5	Borehole - W6	Borehole - W7	Borehole - W8	Borehole - W9
0636091	0636075	0636237	0636067	0636124	0635943	0636285	0636493	0636440	0636277	0636211	0636341	0632930	0632962
8303058	8303067	8302919	8303071	8302268	8303209	8303204	8303164	8303227	8302681	8302707	8302798	8299568	8299623

Table 3.2 showing the distance of boreholes from the Chunga dumpsite

Borehole	Distance from the Chunga Dumpsite (in metres)
W1- Chazanga water and sewerage company	79.337
W2 -Chazanga water and sewerage company	102.242
W3 - Private	78.437
W4- Private	195.426
W5- Private	44.837
W6- Private	399.755
W7- Private	26.403
W8-control- Private	4,163.202
W9-control- Private	4,067.864



Note:Borehole-W8 is 4,163.202m and Borehole-W9 is 4,067.864m from the chunga dump site

Figure 3.1 Location of the Chunga Dumpsite and sampling boreholes; W1-W9 [Map source: National Remote Sensing Centre

3.2 Study design

This was a cross-sectional study design and was conducted at the Chunga dumpsite and surrounding areas.

Soil samples were randomly collected at 3 different depths for analysis. The following media were used for isolating bacteria from the soil samples; nutirent agar, TCBS, XLD and Macconkey agar. Water samples were aseptically collected from the study area and control samples further away from the study area. GPS was used to measure the distance from the dumpsite to the boreholes and mapped accordingly. The following parameters were analysed on the water samples: pH, Total dissolved solids, Sulphates, Ammonia, Nitrite, Nitrate, Chloride, Phospahates, color, turbidity and ordor. Bacteria from the water samples were isolated using the following media; nutrient agar, Macconkey agar, XLD agar and TCBS agar.

Bacteria were identified by PCR and DNA Sequencing of 16S rRNA Gene. The antibiotic susceptibility test of the isolated pure bacterial cultures, were determined following Kirby-Bauer disc diffusion antibiotic susceptibility test.

3.3 Sample collection and transportation

The samples were collected in September 2022. Cluster random sampling was used to collect soil samples from the Chunga Dumpsite. The dumpsite was roughly divided into 4 parts, and in each quadrant, a set of 4 soil samples were randomly aseptically collected. To collect the soil samples on the surface of the dumpsite, a spatula was sterilised using 70% alcohol and was used to scoop soil on the dumpsite. To collect the soil samples at 10 and 20 centimetres (cm), a hoe was sterilised with 70% alcohol, and was used to dig the through the soil, and a sterilised ruler was used to measure the length, and soil samples were collected on the sides of the dug hole.

4 sets of dumpsite soil samples were collected at 3 different depths, that is; 4 soil samples were collected at the surface, 4 samples at 10 cm and 4 samples at 20 cm.The 4 soil samples at each depth, were mixed thouroughly and 25g was obtained from the soil mixtures at each depth and put in a polyethene plastic for analysis.

Further, Seven (7) samples of 1000 mls borehole water were aseptically collected from the boreholes near the Chunga Dumpsite, the distances are shown in table 3,11, and two (2) borehole water samples were collected away from the Chunga Dumpsite, form George compound, to act as control. Similarly, the borehole water samples were collected from residents near the Chunga dumpsite, and transported in cooler boxes containing ice packs, to the University of Zambia School of Veterinary Medicine laboratory for analysis. Water samples could not be collected from the monitoring wells, because it was not possible because they were non-functional due to the deteriorated condition of the dumpsite.

The distance from the water sample collection sites and the Chunga Dumpsite was measured using a global positioning system (GPS), also the soil sample collection points were also mapped using the GPS,and the measurements were sent to the National Remote Sensing Centre to be mapped on the Chunga Dumpsite map, as shown in figure 2 .

3.4 Sample preparation

3.4.1 Serial dilutions of the soil samples

Aseptic Laboratory Techniques were used in the handling and treatment of the samples as outlined by Sanders (2012) and Sagar (2019).

Sterile test tubes containing, each 9 ml of sterile normal saline were labelled 10, 10^{-1},10^{-2}, and 10^{-3}. Soil samples weighing 1g, was added to the test tube labelled 100 and mixed well. Using a sterile pipette, 1 ml of the sample in test tube labelled 10 was drawn into the pipette and added to the test tube labelled 10^{-1} to make the total volume of 10 ml. This provided an initial dilution of 10-1. The dilution was thoroughly mixed by emptying and filling the pipette several times. The pipette tip was discarded, and a new pipette tip was attached to the pipette. Using a new pipette, 1 ml of mixture was taken from the 10-1 dilution, using a new pipette, and was added into the test tube labelled 10^{-2} to make a dilution factor of 10^{-2}. The same process was then repeated for the remaining tubes, to make a dilution of 10^{-3}. The serial dilutions were done for all the 4 soil samples.

3.4.2 Standard plate count of water samples

The 'standard plate count' was used to determine the quality of borehole water near the Chunga dumpsite.

Serial dilutions were done on all the water samples, at 10, 10^{-1} and 10^{-2}. All the test tubes containing the dilutions, were well labelled. From each water dilution, 0.1 ml was inoculated on nutrient agar plates, using Spread method. Then the inoculated plates were inverted and incubate at 37°C for 24 hrs. Thereafter, the bacterial colonies were counted and the results were recorded.

The number of bacteria in the borehole water samples were calculated using the formula below;

$$\text{Number of bacteria/mL} = \frac{\text{number of colonies x dilution factor}}{0.1\text{ mL}}$$

3.4.3 Analysis of physical and chemical parametres of the water samples

The following physical and chemical parameters were analysed on the 9 water samples, in the laboratory; pH, Total dissolved solids, Sulphates, Ammonia, Nitrite, Nitrate, Chloride, Phospahates, color, turbidity and ordor, (Lenore et al., 1999).

3.5 Isolation of Bacteria from soil and water samples

The following media were used for isolating bacteria; nutirent agar, TCBS, XLD and MacConkey agar. The media were prepared according to the manufacturer's instructions. Isolation of bacteria from soil samples.

Nutrient agar is a general purpose, nutrient medium used for the cultivation of microbes and supporting growth of a wide range of non-fastidious organisms. It can grow a variety of types of bacteria, and contains many nutrients needed for the bacterial growth (Sagar 2021). TCBS agar is used for the selective isolation of *Vibrio cholerae* bacteria and other enteropathologic *Vibrio.*

On the other hand, XLD agar is a selective differential medium for the isolation of Gram-negative enteric bacteria. It is especially suitable for the isolation of *Shigella* and *Salmonella* species (Sagar 2021), while MacConkey agar MacConkey agar is used for the isolation of gram-negative enteric bacteria and the differentiation of lactose fermenting from lactose non fermenting gram-negative bacteria.

3.5.1 Media preparation

3.5.1.1 Nutrient and MacConkey agar

About 49.53 grams of dehydrated medium was suspended in 1000 ml of distilled water. This mixture was heat to boiling to dissolve the medium completely, and was then sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. After which it was cooled to 45°C -50°C, was well mixed before pouring into sterile Petri plates.

3.5.1.2 XLD

Preparation of Selenite Broth

Ingredients	**Grams / Litre**
Part A –	
Tryptone	5
Lactose	4
Sodium phosphate	10
Part B -	
Sodium hydrogen selenite	4 gm

About 4.0 grams of Part B was suspended in 1000 ml distilled water, then 19.0 grams of Part A was added to the mixture. The broth was mixed well and warmed to dissolve the medium completely, and then 9 ml of the broth was distributedyuh in sterile test tubes. The the tubes were put in a boiling water bath for 10 minutes to sterilize the broth (HiMedia, 2018).

3.5.1.3 TCBS agar

Preparation of alkaline Peptone water

About 5g of alkaline peptone powder was added to 250 ml of distilled water and mixed well. The mixture was distributed by putting 9 ml in different test tubes and the test tubes were sterilized by autoclaving at 121°C for 15 minutes. After autoclaving, the mixture in the test tubes were let to cool down at room temperature.

3.5.2 Isolation of bacteria from soil samples

From each of the 10^{-3} dilution soil sample mixture, 0.1 ml was transferred onto the surface of the dried sterilized agar plates of nutrient, MacConkey agar, XLD agar and TCBS agar, using aseptic methods as outlined by Sanders (2012) and Sagar (2019) using a sterile loop, and streaked as described in Laboratory Manual for General Microbiology by Matthew (2011). The streaked plates were covered and incubated at 37°C for 24 hours.

3.5.3 Isolation of bacteria from water samples

3.5.3.1 Inoculation of water samples on Nutrient and Macconkey agar

To inoculate the water samples on other nutrient media, a loop was sterilised by heating it to red hot on a flame, and when cooled, it was used to scoop the water sample and streaked on a plate with solid nutrient agar and MacConkey agar, as described by Matthew (2011). This process was repeated for all the water samples. The streaked plates were covered, inverted and incubated at 37 °C for 24 hours.

3.5.3.2 Inoculation of the water samples on XLD agar

The water samples were centrifuged at 10000 *xg* for 5 minutes, and 1 ml of the centrifuged water samples were inoculated using a pipette dropper, in the cooled test tubes containing the selenite broth, and the tubes were incubated at 37°C for 24 hours.

After 24 hours, a loop of the broth was inoculated on solid XLD agar and streaked as described by Matthew (2011). The streaked plates were covered and incubated at 37°C for 24 hours.

3.5.3.3 Inoculation of the water samples on TCBS agar

One (1) ml of the centrifuged water samples were inoculated in the cooled test tubes containing the alkaline peptone water, and the tubes were incubated at 37°C for 24 hours.

After 24 hours, a loop of the alkaline peptone mixture was inoculated on solid TCBS agar, using a pipette dropper and streaked as as described by Matthew (2011). The streaked plates were covered and incubated at 37°C for 24 hours.

3.5.4 Isolation of pure bacterial colonies from the cultured soil and water samples.

A sterile inoculating loop was used to pick isolated colonies from the incubated nutirent, TCBS, XLD and Macconkey agar plates, with soil and water samples isolates, and subcultured by streaking on a fresh similar agar plate to isolate pure colonies, as described by Matthew (2011). That is to say that if a colony was picked from nutrient agar, it was subcultured on nutrient agar to obtain pure colonies.The streaked plates were covered and incubated at 37°C for 24 hours.

3.6 Identification of bacteria by PCR and DNA Sequencing of 16S rRNA Gene

3.6.1 DNA extraction from the isolated pure colonies

Using a sterile loop, a loop full of the pure colonies were put in Eppendorf tubes, each containing 0.1 ml of sterile distilled water and mixed well. 19 colonies were from water samples and 14 from soil samples. The isolated pure colonies were The Tubes were labelled according to the pure colony that it contains, and then were heat in a heat block at 95°C boiled for 15 minutes. After heating, the tubes were centrifuged for five minutes at 10,000 *xg* for 2 minutes to get the supernatant to be used for PCR. The quality of the DNA was assessed using a nanodrop method focorresponded to a DNA concentration of 50µg/ml

3.6.2 PCR DNA amplification

A mastermix was prepared, with a total volume of 25 µl, containing 2µl bacterial DNA template, 5 µl of 10x ExTaq buffer, 3.75 µl dnTPs (10mM), 0.5 µl forward primer, 0.5 µl reverse primer, 0.125 µl Taq DNA polymerase, 1 µl Cofactor - ($Mgcl_{2)}$ (25mM) and 12.125 µl of distilled water. Since there were 33 DNA templates to be amplified, all the reagents in the mastermix were multiplied by 35 just in case of spillage and evaporation. The mastermix was gently mixed by vortexing and briefly centrifuged to collect all components at the bottom of the tube.

The PCR tubes were labelled and 23 µl from the mastermix was transferred to each of the 33 labelled PCR tubes. About 2 µl template DNA was then transferred each to its PCR tube. The 16S rNA highly conserved regions were used , with the following primers, the forward primers were AKGTGTAGCGGTGAAATGCGTAG while the reverse primers were TGGTGTGACGGGCGGTGTGTACAAGG. These primers were sourced from Inqaba Biotec (South Africa).

The tubes were then loaded in the PCR machine for amplification, with the following cycle conditions: an initial denaturation step at 94°C for 2 min; 35 cycles of 94°C for 1 minutes, 55 °C for 30 seconds, and 72 °C for 1.5 minutes, and a final extension step at 72 °C for 5 min. After amplication, the PCR product was evaluated by agarose gel electrophoresis.

3.6.3 Gel electrophoresis

The agarose-TAE solution was prepared by mixing 15g of 1.5% agarose gel and 100ml 1x TAE buffer. The solution was then heated in the microwave to dissolve the agarose, and was poured into a casting tray that, once the gel solution has cooled down and solidified, a gel slab with a row of wells at the top was created.

The solid gel was placed into a chamber filled with TAE buffer in such a way that the chamber wells were closest to the negative electrode of the chamber. The first and last gel chamber wells were markers, then second well was loaded with distilled water to act as a negative control.The other gel chamber wells were loaded with the mixture of 0.5 µl of DNA sample and 1µl of 6x loading dye.

The negative and positive leads were connected to the chamber and to a power supply where the voltage was set. The power was turned on to set up the electric field and the negatively charged DNA samples started to migrate through the gel and away from the negative electrode towards the positive.

Once the blue dye in the DNA samples had migrated through the gel, the power supply was turned off and the gel was removed and analysed using a UV transilluminator. The bands were cut and put in tubes for purification.

3.6.4 DNA gel purification

The gel with PCR products was subjected to cleaning using the clean-up recovery kit. Briefly, 10 µl of membrane binding solution was added to the PCR product, incubated at room temperature for 1 minute, and then transferred to a spin column for centrifugation at 13000 *xg* for 30 seconds. Next, the flow through was discarded, and 600 µl Membrane wash buffer was added and centrifuged at 13000 *xg* for 30 seconds. As in the previous step, the flow through was discarded, and this step was repeated one more time. Finally, 50 µl of nuclease-free water was added directly to the column, incubated at room temperature for 2 minutes, and centrifuged at 23,000 *xg* for 2 minute. The minicolumn was discarded and the eluted DNA was stored at - 4 ºC.

3.6.5 Sequencing reaction

The Big Dye^{TM} Terminator V3.1sequencing kit (Thermo Fisher scientific) was used to sequence the purified PCR product. Two master mixes were prepared by; 1) mixing 1 µl big dye, 3.75 µl sequencing buffer, 0.33 µl forward primer, and 12.92 µl distilled water, and, 2) mixing the same reagents but instead of forward primer, 0.33 of reverse primer was used for the second master mix. The volumes of each master mix were multiplied by 35 and 33 tubes were labelled according to the DNA sample and indicated reverse, and the other 33 tubes were labelled according to DNA samples and labelled forward. 18 µl of reverse master mix was added to each of the 33 tubes labelled reverse, and 18 µl of the forward master mix was added to each of the 33 tubes labelled forward. The 2 µl of DNA samples were added to the tubes, and placed in the PCR machine.

The thermal cycler conditions were set under the following conditions: initial denaturation at 95º C for 1 minutes to break the hydrogen bonds between the two strands of the DNA double helix to create single-stranded DNA templates, followed by 25 cycles of denaturation at 96ºC for 10 seconds, annealing at 50ºC for 5 seconds to allow the primers to bind to the single-stranded DNA templates to provide a starting point for the polymerase enzyme, and extension at 60ºC for 1.15 minutes for the polymerase enzyme to extend the primers by adding nucleotides to the 3' end of the DNA template, creating a new double-stranded DNA molecule.

3.6.6 Ethanol Precipitation of the DNA Amplicons

Excess buffers and dNTPs were removed from the cycle sequencing products using the ethanol precipitation method.

The mixture was prepared by mixing 20 µl sample DNA, 2µl of 3mNa Acetate, 2 µl of 125 mM EDTA and 54 µl of 70% ethanol in a tube. The mixture was incubated at room temperature for 15 minutes and then centrifuged at 15,000rpm for 15 minutes. The supernatant was discarded and 70 µl of 100% ethanol was added to the tube and centrifuged at 10,000rpm for 10 minutes. The ethanol was then discarded gently using a pipette. The tube was covered in aluminium foil and then dried up in the safety cabinet for 2 minutes. 15 µl of Formamide was added and votexed for 2 minutes.

The tubes were then placed in the PCR machine and thermal cycled for 2 minutes at 95ºC. The tubes were then placed in the sequencer for sequencing.

3.6.7 Data analysis

Nucleotide sequences were assembled using GENETYX software version 4.0 (GENETYX cooperation Tokyo). The sequences were subjected to the BLAST tool on the National Centre for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/BLAST), which was used for comparing and analysing the nucleotide sequences. The blaCTX-M nucleotide sequences were then aligned with other nucleotide sequences obtained from GenBank using Clustal X2 incorporated in Genetyx version 4.0(GENETYX cooperation Tokyo).

3.7 Drug Susceptibility Testing

The antibiotic susceptibility test of the isolated pure bacterial cultures, were determined following Kirby-Bauer disc diffusion antibiotic susceptibility test as described by Jan (2009).

The following were the antibiotics used; Gentamycin Gen, (10) Ampicillin (AMP10), Azithromycin (AZM10), Levofloxacin (LEV5), Chloramphenicol (C30), Streptomycin (S10), Nalidixic Acid(NA30), Penicillin (P10U), Cefoxitin (Fox30), Ciprofloxacin (Cip5), Enfloxacin (EX5), Ceftriaxone (CRO30), Doxycycline (DO30), Vancomycin (Va5) and Cefadroxil (CFR30). The antibiotics used were selected based on the kind of bacteria that were isolated, which were all gram negative enteric bacteria. Therefore, the selected antibiotics were the available ones that were suitable for enteric bacteria.

The Mueller-Hinton (MH) Agar plate was inoculated with a suspension of the pathogen to be tested prior to the placing a 6-mm filter paper disk, impregnated with a known concentration of an antimicrobial compound, on the MH agar plate. Immediately water is absorbed into the disk from the agar, the antimicrobial compound began to diffuse into the surrounding agar. The rate of diffusion through the agar is not as rapid as the rate of extraction of the antimicrobial out of the disk, therefore the concentration of antimicrobial is highest closest to the disk and a logarithmic reduction in concentration occurs as the distance from the disk increases (Jorgensen, 2007). The rate of diffusion of the antimicrobial through the agar is dependent on the diffusion and solubility properties of the drug in MH agar (Bauer, 1966), and the molecular weight of the antimicrobial compound. Larger molecules will diffuse at a slower rate than lower molecular weight compounds. These factors, in combination, result in each antimicrobial having a unique breakpoint zone size indicating susceptibility to that antimicrobial compound.

The Zones of inhibition, or the clear areas around the antibiotic disc, were measured by calculating the radius of the clear zone with a meter rule in milliliters. The value or length of the inhibited zones by the antibiotics, determines how susceptible or resistant a particular bacterium is to the applied antibiotic.

CHAPTER 4

4 RESULTS

Table 1. below, summerises the physico-chemical parametres of borehole water samples collected near the Chunga Dumpsite, compared to ZABS standards for drinking water. The pH values ranged between 6.6 - 7.27 which was within the limit by ZABS.

Table 4.1 Analysis of Physical and Chemical parameters of the water samples

	boreholes water sample									**ZABS standards**
Parameters	**W1**	**W2**	**W3**	**W4**	**W5**	**W6**	**W7**	**W8**	**W9**	
pH	6.67	6.70	6.83	7.11	6.56	6.49	6.6	7.27	6.79	6.5-8.0
Sulphates (mg/l)	59.30	65.10	53.50	108.80	91.80	47.90	28.70	77.00	136.50	400
Total dissolved Solids (mg/l)	1,100	901	530	587	417	354	381	446	951	1,000
Ammonia (as NH_{4-} Nmg/l)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.20	< 0.70	1.5
Nitrites (as NO_{2-} N mg/l)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.800	1
Nitrates (as NO_{3-} N mg/l)	2.30	6.50	15.90	18.50	28.500	22.50	32.70	15.10	84.40	10
Chloride (mg/l)	275.0	205.0	60.0	90.0	55.0	45.0	43.0	40.0	132.0	250
Phosphates (mg/l)	< 0.01	< 0.01	< 0.01	< 0.01	1.2	0.4	0.3	< 0.01	0.1	5.0
Color	-	-	-	-	-	-	-	-	-	-
Turbidity	-	-	-	-	-	-	-	-	-	-
Odor	-	-	-	-	-	-	-	-	-	-

Standard plate count

A bacterial count was done on the borehole water samples and the results are shown in table 2. The result indicated that the bacterial count for all the boreholes were within WHO and ZABS limit.

The number of bacteria were calculated using the formula below;

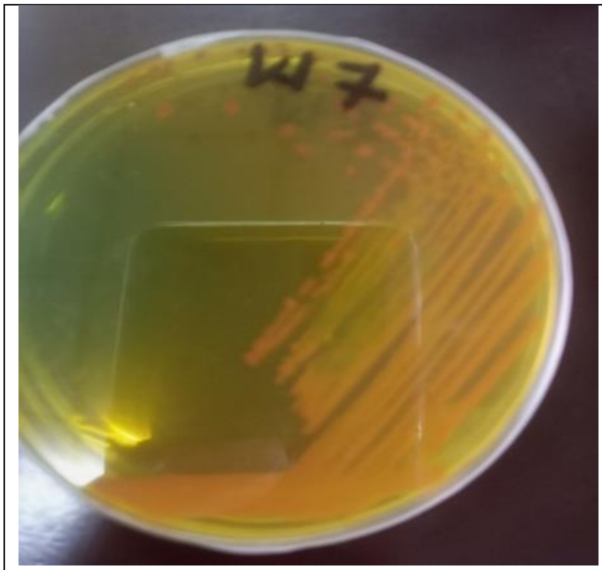
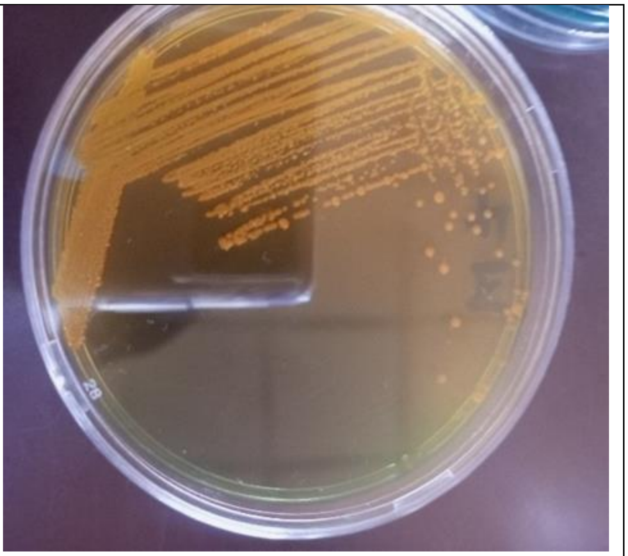
$$\text{Number of bacteria/mL of water} = \frac{\text{number of colonies x dilution factor}}{0.1\text{mL}}$$

Table 4.2 showing bacterial colony forming unit and total number of bactreial count per ml

	Number of bacterial CFU	**Number of bacteria**
Borehole 1	0	0
Borehole 2	1	100
Borehole 3	30	3000
Borehole 4	7	700
Borehole 5	3	300
Borehole 6	6	600
Borehole 7	42	4200
Borehole 8	7	700
Borehole 9	4	400
Acceptable standard		≤500 CFU/ mL

Isolated bacteria from borehole and dumpsite soil

Bacteria were isolated from borehole water and dumpsite soil, on different media, which included nutrient, Macconkey, TCBS, and XLD agar. Bacterial colonies presented different shapes and colors on the different media they were cultured on. Figures 4.1 and 4.2 in appendix, show some of the bacterial colonies on Macconkey and TCBS agar.

 **Figure 4.1 bacterial colonies on Thiosulfate-citrate-bile salts-sucrose (TCBS) agar**	

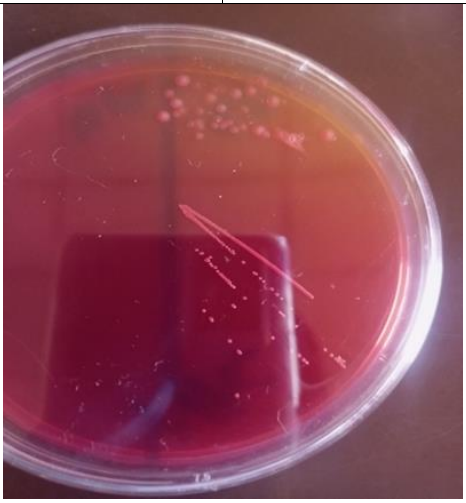


Figure 4.2. Bacterial colonies on MacConkey agar

Eight (8) bacteria were isolated from the borehole water near the Chunga Dumpsite, the distances from the dumpsite are shown in table 3.2, and one (1) bacteria was isolated from the control sample away from the dumpsite. dentified using 16S rRNA sequencing. Seven (7) bacteria were isolated from the Chunga Dumpsite and were also identified using 16S sequencing. The summary of the results are shown below, in tables 4.3 and 4.4, respectively. Out of the isolated bacteria, two (2) bacteria were common in both the borehole water samples and the dumpsite soil samples, as summerised in figure 4.3. Below.

Table 4.3 16S results for borehole water samples

Water sample site	**organism code**	**Identified microorganisms**	**% identification**
Borehole-W2	**12**	*Citrobacter freundi* strain UMH 16S chromosome, complete genome	98.07%
Borehole-W3	**4**	*Kluyvera georgiana* strain 392 chromosome	98.49%
	30	*Acinetobacter indicus* strain TQ23 chromosome, complete genome.	99.80%
Borehole-W4	**5**	*Escherishia coli strain PJ-T13 chromosome, complete genome*	99.45%
	10	*Proteus hauseri* strain 15H5D-4 chromosome, complete genome	99.18%
Borehole-W6	**11**	*Pseudomonas* sp strain R21 16s ribosomal RNA gene, partial sequence	98.59%
Borehole-W7	**2**	*Aeromonas caviae* GSH 8M-1 DNA, complete genome	99.18%
	28	*Klebsiella pneumonia* isolated KP 9201 genome assembly, chromosome	98.92%
Borehole-W8	**9**	*Atlantibacter hermannii* strain IADC ACS 22, 16s ribosomal RNA gene partial sequence	98.03%

Table 4.4 16S results for Chunga Dumpsite soil samples

Soil sample site	**organism code**	**Microorganisms isolated**	**% identification**
Surface	**25**	*Bacillus* sp L J-13 16s ribosomal RNA gene, partial sequence	99.11%
10 centimetres	**14**	*Klebsiella pneumoniae strain MS 14393 chromosome, complete genome*	99.44%
	27	*Bacillus cereus strain* P2lc 16s ribosomal RNA gene, partial sequence	98.96%
20 centimetres	**17**	*Morganella morganii* strain NCTC 235 genome assembly, chromosome	97.77%
	26	*Acinetobacter variabilis* strain FDAARGOS_1487 chromosome, complete genome	95.48%
Leachate	**13**	*Pectobacterium carotovorum* subsp. Carotovorum PCCSI chromosome, complete genome	100%
	24	*Bacillus thuringiensis* strain CGAP GP BS-096, 16s ribosomal RNA gene, partial sequence.	98.81%

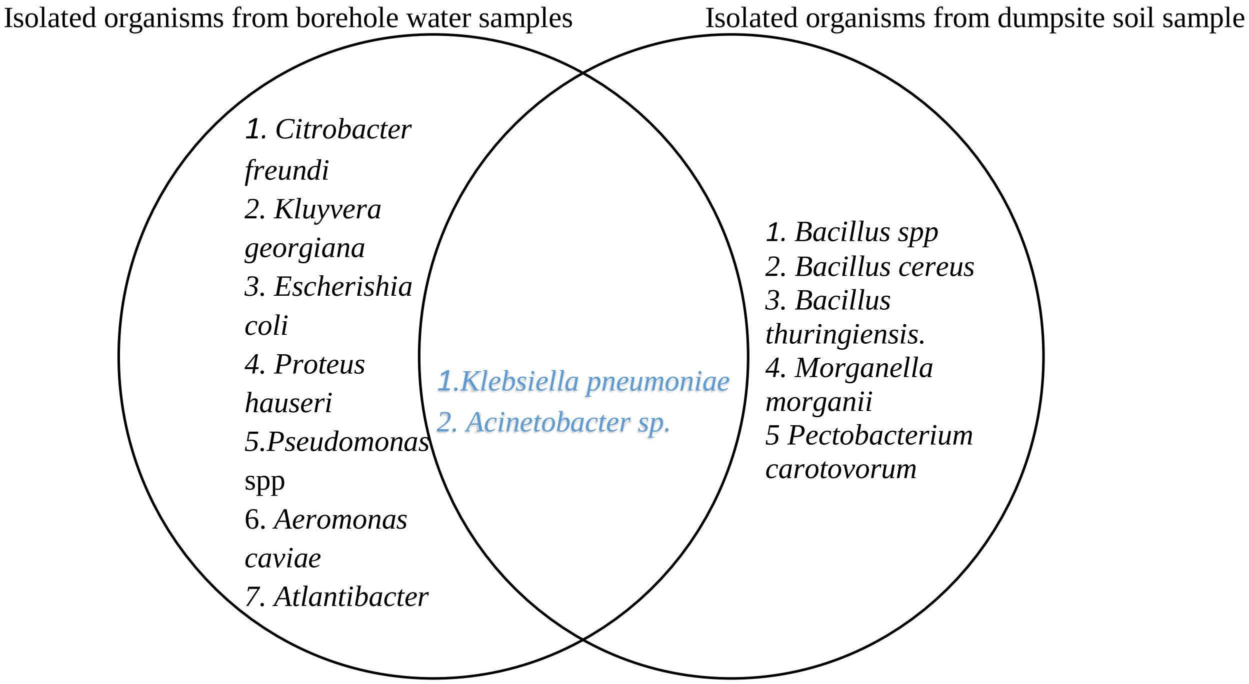


Figure 4.3 diagram showing bacteria isolated from water and soil, and bacteria isolated from both water and soil.

Drug susceptibility Test

The isolated bacteria were subjected to various antibiotics to determine susceptibility and/or resistance and the results are shown in figure 4.4.

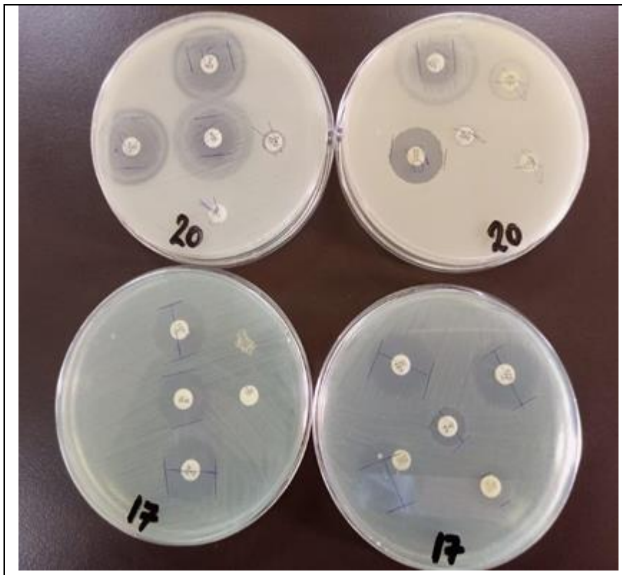
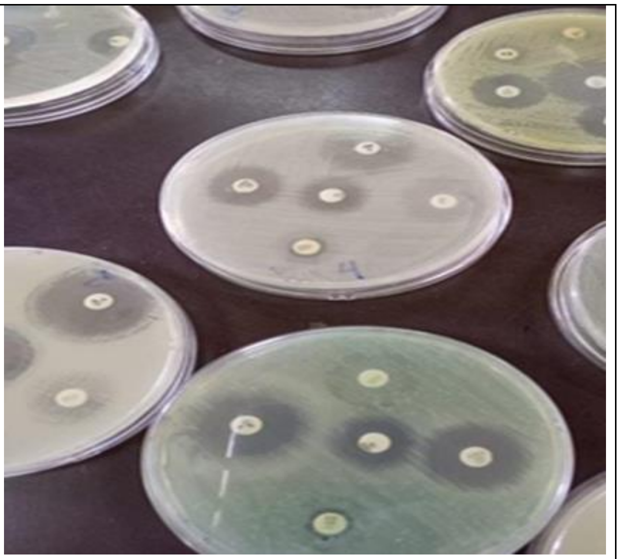
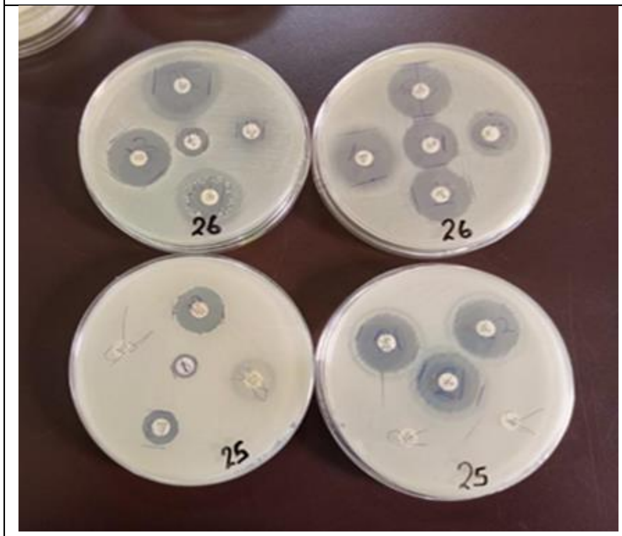


Figure 4.4 Petri-dishes showing cultured bacteria with Zones of Inhibition by antibiotics.





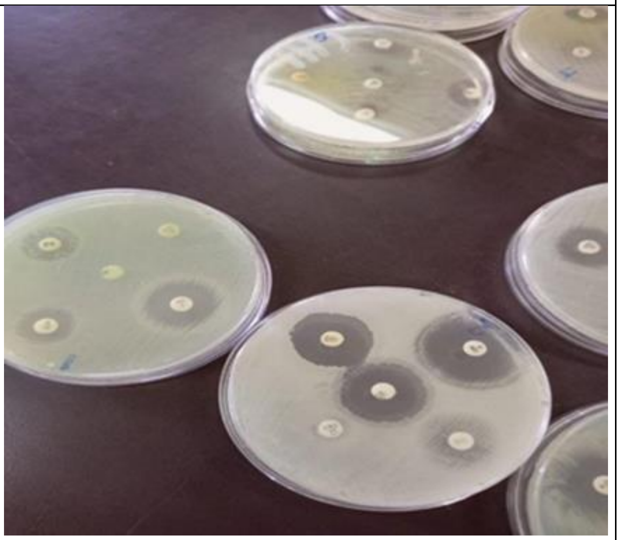


Figure 4.4 Petri-dishes showing cultured bacteria with Zones of Inhibition by antibiotics.

Table 4.5. shows the values in millimetres of the measured zones of inhibition of the isolated bacteria, and figures 4.5 below, shows the summerized percentage Antimicrobial Resistance profile for bacteria in form of a chart.

Table 4.5 Showing measured zones of inhibition of the isolated bacteria

Aeromonas caviae					
Antibiotics	**Resistant**	**Intermediate**	**Susceptible**	**Inhibited zones in (mm)**	
Gentamycin Gen(10)	≤ 12	13-14	≥15	23	Susceptible
Chloramphenicol (C30)	≤ 12	13-17	≥18	13	Intermediate
Streptomycin (S10)	≤ 11	12-14	≥15	18	Susceptible
Nalidixic Acid(NA30)	≤ 13	14-18	≥19	18	Intermediate
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Cefoxitin (Fox30)	≤ 14	15-22	≥23	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	30	Susceptible
Enfloxacin (EX5)	≤ 16	17-20	≥21	24	Susceptible
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	6	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	12	Intermediate
Kluyvera georgiana					
Gentamycin Gen(10)	≤ 12	13-14	≥15	14	Intermediate
Chloramphenicol (C30)	≤ 12	13-17	≥18	22	Susceptible
Streptomycin (S10)	≤ 11	12-14	≥15	6	Resistant
Nalidixic Acid(NA30)	≤ 13	14-18	≥19	8	Resistant
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Cefoxitin (Fox30)	≤ 14	15-22	≥23	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	26	Susceptible
Enfloxacin (EX5)	≤ 16	17-20	≥21	19	Intermediate
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	20	Intermediate
Doxycycline (DO30)	≤ 10	11-13	≥14	8	Resistant
Escherishia coli					
Gentamycin Gen(10)	≤ 12	13-14	≥15	18	Susceptible
Chloramphenicol (C30)	≤ 12	13-17	≥18	14	Intermediate
Streptomycin (S10)	≤ 11	12-14	≥15	13	Intermediate
Nalidixic Acid(NA30)	≤ 13	14-18	≥19	6	Resistant
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Cefoxitin (Fox30)	≤ 14	15-22	≥23	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	22	Susceptible
Enfloxacin (EX5)	≤ 16	17-20	≥21	22	Susceptible

Ceftriaxone (CRO30)	≤ 24	25-26	≥27	19	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	8	Resistant
Atlantibacter hermannii					
Gentamycin Gen(10)	≤ 12	13-14	≥15	20	Susceptible
Chloramphenicol (C30)	≤ 12	13-17	≥18	22	Susceptible
Streptomycin (S10)	≤ 11	12-14	≥15	16	Susceptible
Nalidixic Acid(NA30)	≤ 13	14-18	≥19	6	Resistant
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Cefoxitin (Fox30)	≤ 14	15-22	≥23	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	17	Intermediate
Enfloxacin (EX5)	≤ 16	17-20	≥21	16	Resistant
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	26	Intermediate
Doxycycline (DO30)	≤ 10	11-13	≥14	20	Susceptible
Proteus hauseri					
Gentamycin Gen(10)	≤ 12	13-14	≥15	24	Susceptible
Chloramphenicol (C30)	≤ 12	13-17	≥18	29	Susceptible
Ampicillin (AMP10)	≤ 13	14-16	≥17	21	Susceptible
Streptomycin (S10)	≤ 11	12-14	≥15	6	Resistant
Nalidixic Acid(NA30)	≤ 13	14-18	≥19	6	Resistant
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Cefoxitin (Fox30)	≤ 14	15-22	≥23	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	21	Susceptible
Enfloxacin (EX5)	≤ 16	17-20	≥21	21	Susceptible
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	32	Susceptible
Doxycycline (DO30)	≤ 10	11-13	≥14	22	Susceptible
Pseudomonas sp					
Ampicillin (AMP10)	≤ 13	14-16	≥17	6	Resistant
Streptomycin (S10)	≤ 11	12-14	≥15	21	Susceptible
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	38	Susceptible
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	6	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	25	Susceptible
Vancomycin (Va5)	≤ 9	10-11	≥12	6	Resistant
Cefadroxil (CFR30)	≤ 14	15-17	≥18	6	Resistant
Azithromycin (AZM10)	≤ 13	14-17	≥18	18	Susceptible
Levofloxacin (LEV5)	≤ 13	14-16	≥17	35	Susceptible
Citrobacter Freundi					
Gentamycin Gen(10)	≤ 12	13-14	≥15	15	Susceptible

Chloramphenicol (C30)	≤ 12	13-17	≥18	16	Intermediate
Streptomycin (S10)	≤ 11	12-14	≥15	10	Resistant
Nalidixic Acid(NA30)	≤ 13	14-18	≥19	18	Intermediate
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Cefoxitin (Fox30)	≤ 14	15-22	≥23	10	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	32	Susceptible
Enfloxacin (EX5)	≤ 16	17-20	≥21	24	Susceptible
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	18	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	11	Intermediate
Pectobacterium carotovorum subsp					
Ampicillin (AMP10)	≤ 13	14-16	≥17	19	Susceptible
Streptomycin (S10)	≤ 11	12-14	≥15	19	Susceptible
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	24	Susceptible
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	19	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	6	Resistant
Vancomycin (Va5)	≤ 9	10-11	≥12	6	Resistant
Cefadroxil (CFR30)	≤ 14	15-17	≥18	6	Resistant
Azithromycin (AZM10)	≤ 13	14-17	≥18	6	Resistant
Levofloxacin (LEV5)	≤ 13	14-16	≥17	21	Susceptible
Klebsiella pneumoniae					
Gentamycin Gen(10)	≤ 12	13-14	≥15	18	Susceptible
Chloramphenicol (C30)	≤ 12	13-17	≥18	19	Susceptible
Streptomycin (S10)	≤ 11	12-14	≥15	9	Resistant
Nalidixic Acid(NA30)	≤ 13	14-18	≥19	16	Intermediate
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Cefoxitin (Fox30)	≤ 14	15-22	≥23	12	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	21	Susceptible
Enfloxacin (EX5)	≤ 16	17-20	≥21	24	Susceptible
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	23	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	13	Susceptible
Morganella morganii					
Ampicillin (AMP10)	≤ 13	14-16	≥17	19	Susceptible
Streptomycin (S10)	≤ 11	12-14	≥15	16	Susceptible
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	17	Intermediate
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	15	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	11	Intermediate
Vancomycin (Va5)	≤ 9	10-11	≥12	10	Intermediate

Cefadroxil (CFR30)	≤ 14	15-17	≥18	19	Susceptible
Azithromycin (AZM10)	≤ 13	14-17	≥18	18	Susceptible
Bacillus thuringiensis					
Ampicillin (AMP10)	≤ 13	14-16	≥17	6	Resistant
Streptomycin (S10)	≤ 11	12-14	≥15	11	Resistant
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	10	Resistant
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	6	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	10	Resistant
Vancomycin (Va5)	≤ 9	10-11	≥12	6	Resistant
Cefadroxil (CFR30)	≤ 14	15-17	≥18	6	Resistant
Azithromycin (AZM10)	≤ 13	14-17	≥18	12	Resistant
Levofloxacin (LEV5)	≤ 13	14-16	≥17	13	Resistant
Bacillus sp					
Ampicillin (AMP10)	≤ 13	14-16	≥17	6	Resistant
Streptomycin (S10)	≤ 11	12-14	≥15	21	Susceptible
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	22	Susceptible
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	6	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	19	Susceptible
Vancomycin (Va5)	≤ 9	10-11	≥12	9	Resistant
Cefadroxil (CFR30)	≤ 14	15-17	≥18	6	Resistant
Azithromycin (AZM10)	≤ 13	14-17	≥18	13	Resistant
Levofloxacin (LEV5)	≤ 13	14-16	≥17	21	Susceptible
Acinetobacter variabilis					
Ampicillin (AMP10)	≤ 13	14-16	≥17	21	Susceptible
Streptomycin (S10)	≤ 11	12-14	≥15	19	Susceptible
Penicillin (P10U)	≤ 28	-	≥29	13	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	23	Intermediate
Ceftriaxone (CRO30)	≤ 24	25-26	≥27	21	Resistant
Doxycycline (DO30)	≤ 10	11-13	≥14	23	Susceptible
Vancomycin (Va5)	≤ 9	10-11	≥12	12	Susceptible
Cefadroxil (CFR30)	≤ 14	15-17	≥18	11	Resistant
Azithromycin (AZM10)	≤ 13	14-17	≥18	10	Resistant
Levofloxacin (LEV5)	≤ 13	14-16	≥17	21	Susceptible
Bacillus cereus					
Ampicillin (AMP10)	≤ 13	14-16	≥17	27	Susceptible
Streptomycin (S10)	≤ 11	12-14	≥15	24	Susceptible

Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	39	Susceptible
Ceftriaxon (CRO30)	≤ 24	25-26	≥27	26	Intermediate
Doxycycline (DO30)	≤ 10	11-13	≥14	27	Susceptible
Vancomycin (Va5)	≤ 9	10-11	≥12	6	Resistant
Cefadroxil (CFR30)	≤ 14	15-17	≥18	6	Resistant
Azithromycin (AZM10)	≤ 13	14-17	≥18	23	Susceptible
Levofloxacin (LEV5)	≤ 13	14-16	≥17	38	Susceptible
Acinetobacter indicus					
Gentamycin Gen(10)	≤ 12	13-14	≥15	17	Susceptible
Chloramphenicol (C30)	≤ 12	13-17	≥18	21	Susceptible
Streptomycin (S10)	≤ 11	12-14	≥15	6	Resistant
Nalidixic Acid(NA30)	≤ 13	14-18	≥19	17	Intermediate
Penicillin (P10U)	≤ 28	-	≥29	6	Resistant
Cefoxitin (Fox30)	≤ 14	15-22	≥23	6	Resistant
Ciprofloxacin (Cip5)	≤ 15	16-20	≥21	22	Susceptible
Enfloxacin (EX5)	≤ 16	17-20	≥21	28	Susceptible
Ceftriaxon (CRO30)	≤ 24	25-26	≥27	6	Resistant
Doxycyclin (DO30)	≤ 10	11-13	≥14	21	Susceptible

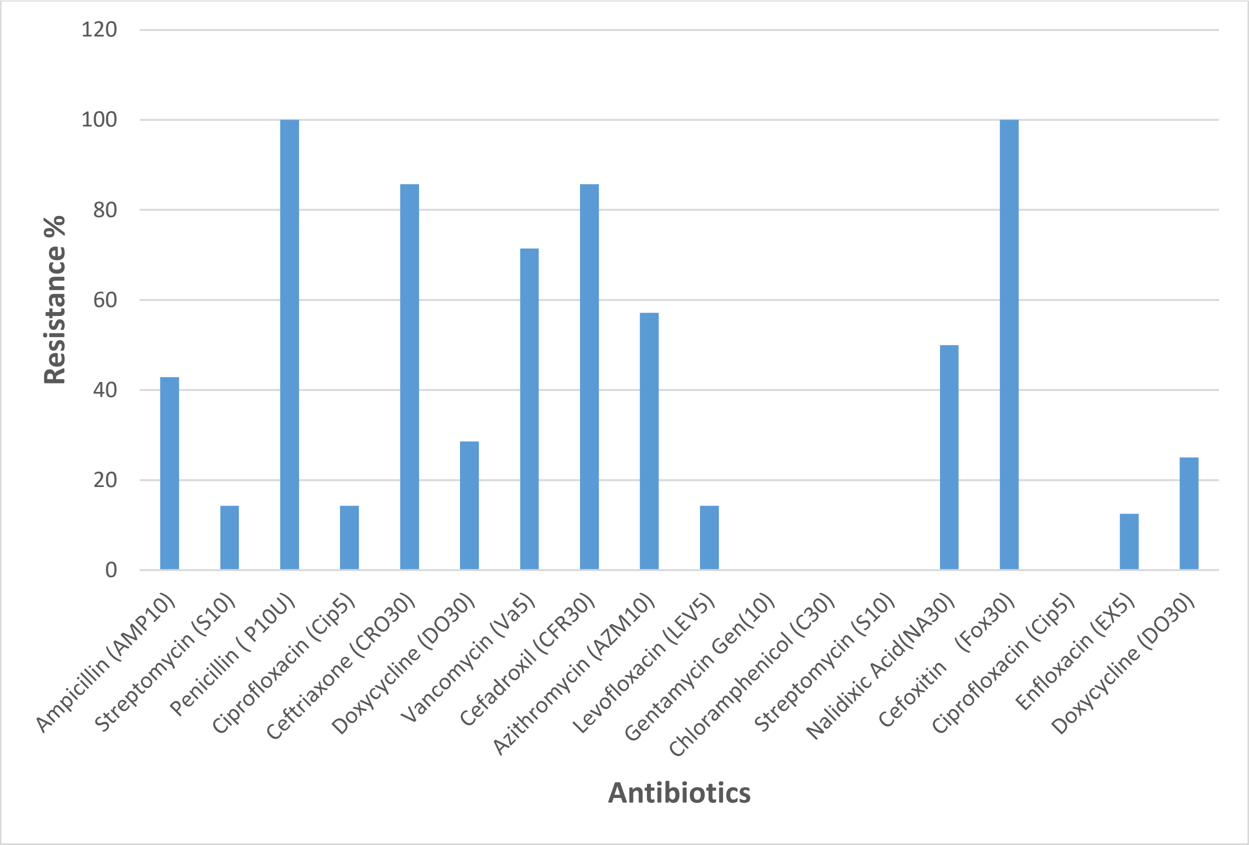


Figure 4.5 Antimicrobial percentage resistance profile for Bacteria

CHAPTER 5

5 DISCUSSION

The analysis of borehole water near the chunga dumpsite showed that there was bacterial contamination of groundwater near the Chunga dumpsite. This was because common bacteria were isolated from both the solid waste at the chunga dumpsite and the borehole groundwater near the dumpsite. In view of the above, the findings showed that bacterial contaminations from the Chunga Dumpsite solid wastes, were able to infiltrate the soil and contaminate the nearby groundwater.

During the study period, the physical and chemical parameters of nine (9) water samples were analysed and the results are shown in table 4.1. in the in results section. The following 11 parameters were analysed; pH, Sulphates (mg/l), Total dissolved Solids (mg/l), Ammonia (as NH4- Nmg/l), Nitrites (as NO2- N mg/l), Nitrates (as NO3- N mg/l), Chloride (mg/l), Phosphates (mg/l), color, turbidity and odor. Out of the analysed parameters, 8 conformed to ZABS standards except for nitrate, Chloride and dissolved solids levels of some water samples which were higher than normal. Boreholes 1 and 2 (W1 and W2) had nitrate levels within ZABS standards, while the rest of the boreholes had high Nitrate levels exceeding the ZABS standards.

The high nitrate levels could be attributed to contamination from the Chunga Dumpsite. According to Rui (2019), a land fill or dump site should be at least 200 meters away from the nearest dwelling. However, this is not the case at the Chunga Dumpsite as some houses are built as close as less than 30 meters away from the dumpsite. This might have greatly contributed to the high nitrate levels in the borehole water, indicating a possible leachate infiltration and contamination into the groundwater table.

Borehole 6 (W6) had higher levels of nitrate compared to the ZABS standards. The presence of nitrate in W6 could have led to the presence of *Pseudomonas*, which was isolated from W6. Since nitrate serve as an electron acceptor to nitrate reducing bacteria like *Pseudomonas*, its presence in W6 could have promoted the growth and sustenance of *Pseudomonas* (Sudhir et al, 2021).

Borehole 1 (W1) showed higher chloride levels than the ZABS standards. The high Chloride levels could be attributed to addition of Chlorine to disinfect the water, as the bacterial count results for borehole (W1) had no growth. Drinking water chlorination is the addition of chlorine to drinking water systems. It is the most common type of drinking water disinfection. Disinfection kills bacteria, viruses, and other microorganisms that cause disease and immediate illness. Chlorine is effective and continues to keep the water safe as it travels from the treatment plant to the consumer's tap, (The Minnesota Department of Health (MDH), 2022).

However, according to Long's EcoWater Systems. (2023), ingesting chlorine in tap water can have a number of adverse health effects. Some of these effects occur because chlorine tends to form trihalomethanes. Trihalomethanes (THMs), including chloroform, form when chlorine reacts with tiny organic particles found in water. These chemical compounds have been implicated in several adverse health outcomes. Below are some of the potentially harmful effects of chlorine water

Isolation of bacteria from soil and water samples

The results of the total bacterial colony forming unit (cfu/mL) ranged from 0-4200 bacteria/mL of borehole water. It indicated that 5 borehole water samples, out of the 9 analysed samples, had higher concentration of microorganisms than the nomal range of $\leq$500 cfu/mL of water (National Research Council (US), 1977). The high presence of bacteria in the borehole water samples could have been as the result of the presence of significant amounts of organic matter in most of the water samples, which provided nutrition for the growth and multiplication of microorganisms (Irshad et al, 2012 and Kanownik and Przydatek, 2019). The presence of bacteria in water may affect individuals with compromised immune systems (Rusin et al., 1997; Pavlov et al., 2004).

The National Primary Drinking Water Regulations established by the U.S. EPA, indicate that less CFU in drinking water may indicate a better maintenance of the treatment and distribution systems (U.S. EPA, 2009).

After analysing the borehole water near and away from the Chunga Dumpsite, 8 bacteria and 1 bacterium were isolated isolated respectively. These were; *Citrobacter freundi, Kluyvera georgiana, Acinetobacter indicus, Escherishia coli, Proteus hauseri, Pseudomonas sp, Aeromonas* *caviae, Klebsiella pneumoniae and Atlantibacter hermannii* was isolated from the control site, as shown in table 4.3. The diversity of the isolated bacteria from borehole water near the Chunga Dumpsite, agree with a similar study that was done in Nigeria by Uzoigwe and Agwa (2012), who isolated *Escherichia* spp, *Pseudomonas* spp, *Proteus* spp, among other bacteria, in borehole water near dumpsites. All the isolated bacteria from the borehole water, were Gram negative rods. Six bacteria (*Citrobacter freundi, Kluyvera georgiana, Escherishia coli, Proteus hauseri, Klebsiella pneumonia and Atlantibacter hermannii*) out of the nine isolated bacteria belonged to the family of Enterobacteriaceae, except for *Acinetobacter, Pseudomonas and Aeromonas* which belonged to different families namely; Moraxellaceae,Pseudomonadaceae and Aeromonadaceae respectively.

On the other hand, not all the pure colonies from the soil samples were analysed due to limitation in reagents and other analysis materials. Therefore, only seven bacterial species were isolated from the dumpsite soil; *Bacillus spp, Klebsiella pneumoniae, Bacillus cereus, Morganella morganii, Acinetobacter variabilis, Pectobacterium carotovorum and Bacillus thuringiensis,* as shown in tables 4.4 .

The results revealed that *Klebsiella pneumoniae and Acinetobacter spp* were isolated from both borehole water and dumpsite soil. Based on this, there is a likelihood that there could be a relationship between the bacteria at the Chunga Dumpsite and the bacteria in the borehole water near the dumpsite. This result showed that the dumpsite might have contaminated the ground water in its vicinity. However, the bacteria in the water samples could have come from other sources, because bacteria was also isolated from the control water sample (W8), which was collected away from the Chunga dumpsite.

It was also noted that more than 50% of the bacteria isolated from the borehole water belonged to the group of coliforms. These are *E.coli, Proteus, Citrobacter, Aeromonas* and *Klebsiella*. The presence of coliforms in water indicates fecal contamination. According to Charles (2009), the presence of coliforms in water has been used as a standard for assessing fecal contamination for drinking water. This means that there might be presence of pathogens in the water, which may pose an immediate health risk to anyone consuming the water. Fecal coliforms are used as indicators of fecal contamination, and presence of pathogenic bacteria because they behave in a similar way to most pathogenic bacteria, (Cisneros, 2011).

Most of the isolated bacteria from the borehole water were members of the normal intestinal flora. The Gram-negative bacilli of the genera *Escherichia, Klebsiella, Aeromonas, Citrobacter* and *Proteus* are members of the normal intestinal flora of humans and animals . These organisms are known to be responsible for major health problems worldwide (Neal, 1996).

Drug Susceptibility Testing

The isolated bacteria were all susceptible to Gentamicin, Chloramphenicol and Ciprofloxacin. These finding were in line with the findings of a study which was conducted in Nigeria by Gideon et al. (2017), where bacteria were isolated from borehole water and subjected to various antibiotics for sensitity tests. The results indicated that the majority of the isolated bacteria were sensitive to Ciprofloxacin and gentamycin, among other antibiotics. Therefore, this indicates that the first choice of antibiotics to treat infections that arise from consumption of the contaminated borehole water near the Chunga Dumpsite, are; Gentamicin, Chloramphenicol and Ciprofloxacin. Further, all the bacteria, except *Bacillus thuringiensis*, were susceptible to Levofloxacin. Similarly, all the bacteria were susceptible to Enfloxacin, except for *Atlantibacter hermannii*.

All the isolated bacteria showed resistance to Penicillin and Cefoxitin. More than 85% of the isolated bacteria showed resistance to Cefriaxone and Cefadroxil, where as above 71% showed resistance to Vancomycin and about 57% showed resistance to Azithromycin. These results agree with the findings of Borquaye et al. (2019), who evaluated isolates from an active and abandoned landfill sites, and reported that enterobacteriaceae family proved to be resistant to beta- lactam groups of antibiotics.

The most resistant bacteria to the antibiotics that were used were; *Pseudomonas* spp, *Kluyvera* spp and *Bacillus thuringiensis*. These results are backed up by the study that was done by Gideon et al. (2017) which revealed that the most resistant bacteria among the isolates from the borehole water, was *Pseudomonas*. Another study that was done in Ethiopia by Hadish et al. (2018), found that *Klebsiella* spp showed resistance to most antibiotics that were used. Several studies revealed different results on enteric bacterial resistance to antibiotics. This is mainly because, different antibiotics were used in the studies.

CHAPTER 6

6 CONCLUSION

Most of the borehole water samples had higher bacterial contamination due to the presence of higher amount of organic matter, which provided nutrients for the growth and multiplication of bacteria. The high bacterial contamination and presence of coliforms in the borehole water samples, indicates the likelihood that the contamination could have come from the Chunga Dumpsite as supported by the two common bacteria isolated from both the borehole water and the Chunga Dumpsit. However, there is a possility that the bacteria in the water samples could have come from other sources. Therefore, there is need for improved waste disposal and management system and constant monitoring of the groundwater quality. This will help authority to institute appropriate action to control the groundwater contamination, and eventually prevent unnecessary disease outbreaks in the surrounding communities.

The following are some of the recommendations to the policy makers; there is need to consider re-engineering the Chunga dumpsite so that it does not discharge leachate into the environment, and make the monitoring wells at the dumpsite functional so that the quality of borehole water in the area is checked on a regular basis to prevent disease outbreaks or health hazards, and there is need to encourage residents near the chunga dumpsite to be treating borehole water before drinking or domestic use.

CHAPTER 7

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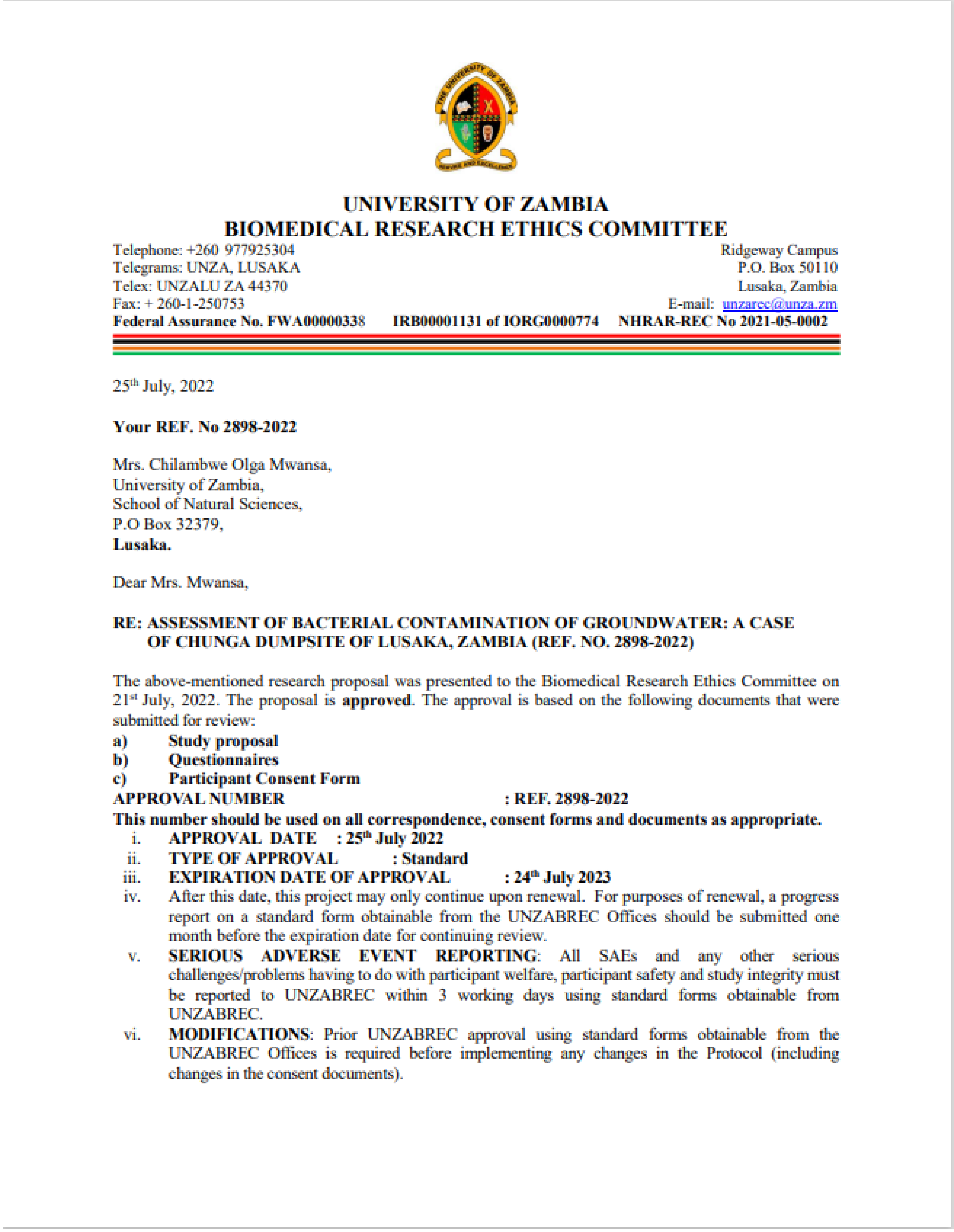
CHAPTER 8

8 APPENDICES

8.1 Ethical clearance



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Federal Assurance No. FWA00000338 IRB00001131 of IORG0000774 NHRAR-REC No 2021-05-0002

25th July, 2022

Your REF. No 2898-2022

Mrs. Chilambwe Olga Mwansa,
University of Zambia,
School of Natural Sciences,
P.O Box 32379,
Lusaka.

Dear Mrs. Mwansa,

RE: ASSESSMENT OF BACTERIAL CONTAMINATION OF GROUNDWATER: A CASE OF CHUNGA DUMPSITE OF LUSAKA, ZAMBIA (REF. NO. 2898-2022)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee on 21st July, 2022. The proposal is **approved**. The approval is based on the following documents that were submitted for review:

a) **Study proposal**
b) **Questionnaires**
c) **Participant Consent Form**

APPROVAL NUMBER : REF. 2898-2022

This number should be used on all correspondence, consent forms and documents as appropriate.

i. **APPROVAL DATE : 25th July 2022**
ii. **TYPE OF APPROVAL : Standard**
iii. **EXPIRATION DATE OF APPROVAL : 24th July 2023**
iv. After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.
v. **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.
vi. **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).

vii. **TERMINATION OF STUDY**: On termination of a study, a report has to be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.

viii. **NHRA:** You are advised to obtain final study clearance and approval to conduct research in Zambia from the National Health Research Authority (NHRA) before commencing the research project.

ix. **QUESTIONS**: Please contact the UNZABREC on Telephone No. +260977925304 or by e-mail on unzarec@unza.zm.

x. **OTHER**: Please be reminded to send in copies of your research findings/results for our records. You are also required to submit electronic copies of your publications in peer-reviewed journals that may emanate from this study. Use the online portal: unza.rhinno.net for further submissions.

Yours sincerely,

Munsaka.

Sody Mweetwa Munsaka, BSc., MSc., PhD
CHAIRPERSON
Tel: +260977925304
E-mail: s.munsaka@unza.zm

8.2 Photo of Chunga Dumpsite leachate

Figure 8.1 Leachate collecting at Chunga dumpsite due to blocked collecting pipes.