

*ECOTOXICOLOGICAL CHARACTERIZATION OF MULTI-CLASS POLLUTANTS AND THEIR EFFECT ON MICROBIAL ASSEMBLAGES
IN THE WINAM GULF OF LAKE VICTORIA, KENYA*

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**A thesis submitted in partial fulfilment for the requirements for the award of the
Degree of Doctor of Philosophy in Microbiology of Masinde Muliro University of
Science and Technology**

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DECLARATION

This thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for degree or any other award.

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APPROVAL

The undersigned certify that we have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a research Thesis entitled “*Ecotoxicological characterization of multi-class pollutants and their effect on microbial assemblages in the winam gulf of lake victoria, kenya*”

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DEDICATION

This work is dedicated to my daughters Elianah Neema and Elliorah Rashona

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ABSTRACT

Lake Victoria in East Africa is an important source of fish for domestic, regional and global market. Recently the lake has suffered massive pollution from industrial and wastewater discharge. Microplastics, pharmaceutical, drugs, heavy metals, agrochemical and personal care products are ubiquitous in Winam Gulf section of the lake located in Kisumu County in Kenya. The pollutants are known to alter microbial assemblages in aquatic ecosystems with calamitous consequences to human health. Some of the pollutants are reportedly associated with human cancers and induce antibiotic resistance. There is paucity of data on the effects of pollutants on microbial profiles of this important but heavily polluted aquatic ecosystem. The objectives of this study were to identify heavy metals and pharmaceutical residues in the aquatic ecosystems of Winam Gulf Kisumu, assess the impacts on microbial communities, look into patterns of antibiotic resistance in bacterial populations, and explore genes and pathways for possible pollutant bioremediation. The presence of pharmaceutical residues was determined by the solid-phase extraction method, high-performance liquid chromatography (mass spectrophotometry), and the HACH method spectrophotometer was used in the identification of heavy metals in the samples. Total genomic DNA pooled from all samples was extracted and analyzed by whole-genome shotgun metagenomics sequencing. The analysis of antibiotic resistance genes and pathways were done using the shotgun metagenomics method. Phenotypic antibiotic resistance was carried out by exposing bacteria to discs containing common antibiotics. KEGG functional annotation was used to identify the genes and pathways capable bioremediation of pollutants. This study revealed the presence of pharmaceuticals such as Sulfamethoxazole, Caffeine, Trimethoprim, Tetracycline, Amoxillin, Artemether and Neverapine in the sediment and water samples. Further, the study reports the presence of Mercury, Nickel, lead, Aluminum, Copper, Chromium and Cadmium pollutants. Metagenomics analysis revealed three major kingdoms; Bacteria, Archaea and Eukaryotes belonging to 3 phyla, 13 classes, 14 families, 9 order, 14 genera and 10 species. *Proteobacteria*, *Betaproteobacteria*, *Comamonadaceae*, *Burkholdariales* and *Arcobacter* were the dominated phyla, class, family, order, genera and species respectively. KEGG analysis highest number of genes involved in metabolism. In addition, carbohydrate active enzymes were the most abundant and included genes in glycoside hydrolases. The study reported 9 ARGs from the 37 high-risk AMR gene families previously reported by the WHO. *Proteobacteria* (53%) had the highest relative abundance of antibiotic resistance, followed by *Bacteriodes* (4%), *Verrumicrobia* (2%), (*Plantomycetes Chlorflexi*, *Firmicutes*) (2%), and other unclassified bacteria at (39%). Classification of genes including genes that target protection, replacement, change, and antibiotic efflux were listed in order of dominance. The dominant genes were antibiotic resistant efflux. KEGG pathway analysis on antibiotic resistance to beta lactamase and vancomycin were observed. Phenotypic resistance to vancomycin, tetracycline, and sulfamethoxazole, erythromycin, trimethoprim tetracycline and penicillin was observed. Through the KEGG functional annotation the following biodegradation pathways and genes were reported; Xylene, drug and enzyme metabolism, Atrazine, Steroid and Nutrotolene. The study draws attention to the possible health concerns to human and biodiversity associated with the presence of heavy metal pollution, antibiotic-resistant genes, and pharmaceutical residues in Lake Victoria. Furthermore, the results indicate that native microbial communities may be useful for ecosystem bioremediation.

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ABBREVIATIONS AND ACRONYMS

AIDs	Acquired immunodeficiency syndrome
ANOVA`	Analysis of variance
AMR	Antimicrobial Resistance
APIS	Active pharmaceutical ingredients
(ARGs)	Antibiotic resistant genes
ARO	Antibiotic Resistance Gene Ontology
BOD	Biological oxygen demand
CNS	Central nervous stimulus
DO	Dissolved oxygen
DL-PCB	dioxin-like polychlorinated biphenyls
DNA	Deoxyribonucleic Acid
EU	European Union
EE2	Ethinyl Estradiol
FAAS	Flame Atomic Absorption
HIV	human immunodeficiency virus
L-B	Luria Bertani
MDMA	Methylene dioxy methamphetamine
PHBA	Para-hydroxybenzoic acid
PAH	Polycyclic aromatic hydrocarbons
PBDF	Polychlorinated dibenzofurans

PCR	Polymerase chain reaction
PCB	Polychlorinated biphenyls
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
THC	Tetrahydrocannabinol
TB	Tuberculosis
UV	Ultraviolet
WHO	World Health Organization
RIAT	Ramogi Institute Advanced Technology
KEGG	Kyoto Encyclopedia of genes and genomes
eggNOG	Evolutionary genealogy of genes Non-supervised Orthology
CAZY	Carbohydrate enzymes
MGRAST	Metagenomics Rapid Annotation using Sub system technology
KIWASCO	Kisumu Water and Sewerage Company
WARMA	Water Resource Management
JKUAT	Jomo Kenyatta University of Agriculture and Technology
TDS	Total Dissolved Solids
EC	Electric conductivity
COD	Chemical Oxygen Demand
RGI	Resistance Gene Identifier

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Freshwater habitats are a hotbed of biodiversity, yet they are in danger from various environmental factors (Salgado *et al.* 2022). One such factor is pollution. Pollution related disturbances in freshwater ecosystems are driven largely by anthropogenic activities, which alter natural biogeophysical processes through increased eutrophication, acidification and toxic pollutants (Sterner and Robinson 2018). For large water bodies, their integrity is degraded by changes in riparian vegetation and watershed land use, together with downstream sedimentation (Larned *et al.* 2020). The productivity, population dynamics, and species composition of aquatic ecosystems are affected by the combined impacts of human activities (Okoyen *et al.* 2020).

Many years of research on environmental toxins in water, initially concentrated on legacy contaminants such as heavy metals and persistent inorganic pollutants (Yuan *et al.* 2023; Zhang *et al.* 2022). Natural and newly developed synthetic compounds, upon their introduction into the environment, possess the potential to adversely impact the environment and human health (Barnes *et al.* 2018). Multi class pollutant such as microplastics, nanomaterials, pharmaceuticals personal care products, endocrine disrupting compounds, steroids flame retardants, illegal drugs and hormones that are frequently found in the environment but are not explicitly defined in the environmental regulation are referred to as emerging contaminants (Kumar *et al.*, 2022).

Heavy metals and pharmaceutical residue pollution of aquatic environments is a significant global concern because of their harm to the ecology and health of aquatic life (Okerefor *et al.* 2020; Rathi, Kumar, and Vo 2021a). Heavy metals such as mercury, lead, cadmium and arsenic are released into aquatic ecosystem through industrial processes, mining activities and

improper disposal of wastes (Sonone *et al.* 2020a). These metals are toxic to aquatic organisms at low concentrations and can bioaccumulate in the food chain, leading to serious ecological consequences (Jeong *et al.* 2023). Mercury contamination in water bodies has been shown to bioaccumulate as ethyl mercury in fish, posing risk to both aquatic organisms and human who consume the fish (Jinadasa *et al.* 2021).

Pharmaceutical residues enter aquatic ecosystems primarily through wastewater discharges from sewage treatment plants, hospitals, storm water from urban dumpsites, and pharmaceutical industries (Ngqwala and Muchesa 2020). The residues include various active pharmaceutical ingredients, such as antibiotics, analgesics, hormones, anticancer agents, anticoagulants, antidepressants, antidiabetics, and antipsychotics (Alessandro *et al.*, 2022). On entry into water bodies, these compounds can impact the natural physiological processes of aquatic organisms and contribute to the emergence of antibiotic resistance bacteria (Le *et al.* 2022).

Heavy metals such as lead, mercury, cadmium, chromium, and arsenic cause various biochemical and physiological changes in living things (Bhat, Hassan, and Majid 2019). For instance, heavy metals alter the activities of enzymes and proteins, degrade lipids, and harm DNA (Rai *et al.* 2021). The investigation of aquatic life distribution and biochemical profiles caused by point and non-point pollution is considered a key step in early detection of environmental changes (Jamla *et al.* 2021). In natural ecosystems, microorganisms respond to chemical stresses by changing their niche occupational systems (Abirami *et al.* 2021). Utilizing microorganisms as bio-indicators, their genetic profiles can be used to detect and track their responses to stressors across time and space (Aylagas *et al.* 2021). In fact, this considered a key step in the early detection of environmental changes is the investigation of

the distribution of aquatic life and the biochemical profiles caused by point and non-point pollution (Jamla et al. 2021). Results from biomonitoring can be used as confirmatory tests for the impacts of persistent organic pollutants, dangerous heavy metals, and environmental factors (Saravanan et al. 2022). Analyzing the distribution of aquatic life and biochemical profiles resulting from of point and non-point pollution is crucial for the early detection of environmental changes (Xie et al. 2022).

The wide spread presence of pharmaceutical residues and heavy metals in aquatic ecosystem has raised concerns about their long-term impacts on biodiversity, ecosystem functioning and human health (Impellitteri et al. 2023). These pollutants are known to cause reproductive abnormalities, development disorders and weakened immune systems in aquatic organisms such as fish, amphibians and microbes (Landrigan et al. 2020). Moreover, they can also lead to the degradation of habitat and the loss of sensitive species of plants and animals, thereby disrupting the balance of the ecosystem (Ahmed et al. 2022).

Various studies have investigated the occurrence and levels of pharmaceuticals and heavy metals in water bodies to assess their potential ecological and human health impacts (Anand et al. 2022; Han et al. 2020; Rathi, Kumar, and Vo 2021b). Advancements in ecotoxicological models and biomonitoring techniques in the field of environmental science have been driven by studies on contaminant exposure and their ecological consequences (Rosner et al. 2024). Numerous studies have been conducted on the pollution by pharmaceutical residues and heavy metals in aquatic ecosystems (Narayanan et al. 2022; Saravanan et al. 2022; Vardhan, Kumar, and Panda 2019). These studies have found that pharmaceutical residues and heavy metals can negatively impact aquatic organisms and ecosystem health, leading to potential ecological consequences (Dhiman & Pant, 2021; Garg *et al.*, 2022, Rosner *et al.*,

2021). Some studies have reported evidence of harm caused by pollution in Lake Victoria (Nassali, Yongji, and Fangninou 2020). For example, one study found high levels of heavy metals such as lead, cadmium, and mercury in the water, which can have negative effects on the health of aquatic organisms and humans who consume fish from the lake (Tolkou, Toubanaki, and Kyzas 2023) . Additionally, another study noted an increase in algal blooms in the lake, which can deplete oxygen levels and harm fish populations (Watson et al. 2016).

Agrochemicals, personal care products, illicit drugs like cocaine, and pharmaceutical residues are on the rise in the biota, sediments, and water compartments of aquatic environments at levels that could potentially harm ecosystems (Bottoni and Caroli 2018). Several of these multiclass pollutants such as heavy metals, pesticides, pharmaceuticals, and industrial chemicals .have physiochemical properties that make them difficult to remove during the water treatment process, allowing them to persist in aquatic ecosystems (Mandarić et al. 2018). These compounds and their metabolites are continuously released, making it difficult for natural breakdown processes like biodegradation to take place (Soon et al. 2019). Aquatic species, such as fish and algae, have the ability to rapidly bioaccumulate multiclass pollutants (Okoye et al. 2022). These pollutants, including antibiotics, contain chemical compounds that were designed with a specific biological mode of action targeting a particular group of species (Nannou et al. 2020; Okoyen et al. 2020). Their presence in aquatic ecosystems in diverse doses unintentionally raise eco-toxicological risks to aquatic flora and fauna (Chaturvedi et al. 2021). Continuous exposure to different pharmaceuticals in waste and natural waters poses a health risks to people (Gudda et al. 2020) . For instance, patients with HIV/AIDS who are not receiving treatment may develop resistance due to low amounts of antiretrovirals ingested through the water and food systems (Kock et al. 2023). Concerns have been raised over TB resistance in HIV patients and other antibiotics due to the

consumption of low concentrations of pharmacological combinations through the water and food chains (Ngqwala and Muchesa 2020) . Several classes of pharmaceuticals, their combinations and environmental interactions, may have detrimental effects on public health as well as aquatic habitats (Khan et al. 2022) .

Globally, antibiotic resistance is a serious problem that affects not only human health but also the fragile aquatic ecosystem (Carvalho, Forestier, and Mathias 2019). Since resistant bacteria and antibiotic residues can enter water bodies through a variety of channels, including wastewater discharge, agricultural runoff, and direct antibiotic use in aquaculture, the World Health Organization stresses the critical need to investigate and address antibiotic resistance in these environments (Jian et al. 2021; Kakkar et al. 2020; Pepi and Focardi 2021). Antibiotics in aquatic ecosystems, exert selective pressure on bacteria, favouring the survival and proliferation of antibiotic-resistant strains (Liu *et al.*, 2023). This can lead to the spread of resistance genes within the bacterial community, posing a potential threat to human and animal health (Uddin et al. 2021).

Consequently WHO emphasizes the need for comprehensive surveillance and monitoring programs to assess the levels of antibiotics and antibiotic resistance in aquatic environments (Liguori et al. 2022). Further, prudent use of antibiotics in medical and agricultural settings is recommended to minimize the release of antibiotics into water systems and reduce the dissemination of antibiotic-resistant bacteria (Dhaka et al. 2023; Ezeuko et al. 2021). Continuous antibiotic resistance surveillance in aquatic ecosystems enhances and safeguards public health and preserve the effectiveness of commonly used antibiotics for future generations (Serwecińska 2020). It is therefore crucial for researchers, policy makers, and other stakeholders to collaborate, implement and come up with innovative strategies that

mitigate the risk associated with antibiotic use, poor disposal and promote sustainable/responsible practices in both human and animal sector (Adebisi 2023).

Bioremediation technology, the use/ application microbes like bacteria to remove environmental pollutants from soil and water include membrane-based technologies, photochemical oxidation, ion exchangers, adsorbents, and coagulants (Khan et al. 2022; Ramezani et al. 2021). Microbial remediation using biofilms, membrane bioreactor, biosequestration and biological engineering among others are widely used to break down complex aquatic pollutants (Tekere, Jacob-Lopes, and Zepka 2019; Zhu and Wang 2020). In Polar Regions, microbial – based remediation is widely used to treat ocean water and eliminate toxins from agro-industrial waste (Sharma *et al.*, 2021). In Kenya, bioremediation studies on xenobiotic pesticides by bacteria species isolated from flower farm soils near Lake Naivasha confirmed that the bacteria species successfully and rapidly decomposed xenobiotics (Anode and Onguso 2021). This study provided an insight on how to develop a cost-effective bioremediation approach to pollutant elimination in aquatic ecosystems (Sanganyado and Gwenzi 2019).

There is scant data on how pollution affects aquatic microbial communities (Meng, Li, and Yao 2022). Besides, understanding aquatic microbial diversity has proven a challenge due to the intricacy of microbial interactions with species and chemical contaminants (Li *et al.*, 2023). Studies have shown that chemical and heavy metal contamination in freshwater alters the composition of microbial communities and reduces their diversity (Shuaib et al. 2021a). Furthermore, there appears to be a link between antimicrobial resistance and genetically determined resistance to contaminants such as heavy metals, agrochemicals, personal care products, and pharmaceutical residues (Ahmed et al. 2022). Exposure to various multi-class

pollutants in the water system leads to the development of antibiotic-resistant bacterial populations (Van *et al.*, 2019) . The resistance of tolerant microbes to antibiotics poses a significant challenge and traits once considered characteristic of susceptible microorganisms can now confer multiple drug resistance (Mancuso *et al.* 2021). This phenomenon in the microbial community may have considerable adverse effects on public health (Qin *et al.* 2022).

The continued presence of multiclass pollutants provide evidence for potential negative impacts on aquatic life and human health (Kumar *et al.*, 2022). Predicting and reducing future disease risks require an understanding of how these contaminants affect the development of emerging diseases in humans as well as other species (Babuji *et al.* 2023). The application of novel methods can lead to a better understanding of the ways in which genetic factors contribute to the development of diseases and how specific populations may be more vulnerable to pollution effects (Shen *et al.* 2019). The aquatic environment contains persistent carcinogenic pollutants that are caused by human activities (Sharma *et al.* 2023). High concentrations of pollutants and chemicals can be commonly detected in various environments that have been negatively impacted by human activities, such as industrial sites, agricultural areas, urban settings, landfills, and mining sites (Khandelwal *et al.*, 2022). Studies have shown that cancer is a significant contributor to animal mortality in polluted aquatic ecosystems (Baines *et al.* 2021). Pollutants such as organochlorides have been linked to cancer in Californian sea lions (Rothenberg *et al.* 2023). Many fish tumours and cancer-related deaths have been report in aquatic species in contaminated aquatic habitats (Sepp *et al.* 2019). Most human cancers are caused by environmental contaminants (Shetty *et al.* 2023). Humans come into contact with these pollutants on a daily basis through water and the food chain (Stiefel and Stintzing 2023). According to numerous studies, chemicals and heavy metals in water are a major factor responsible for the rise in cancer cases worldwide (Briffa,

Sinagra, and Blundell 2020; Malandrino et al. 2020; Scutarașu and Trincă 2023). For instance, bladder cancer rates significantly increased where arsenic pollution was present in aquatic ecosystems (Jenwitheesuk, Peansukwech, and Jenwitheesuk 2020). Lymphoma and leukemia malignancies are caused by dioxin, which is included in personal care products (Cazzolla Gatti 2021). Research conducted in Iowa and Nebraska , USA, found a correlation between higher meat consumption and levels of nitrates in community-supplied drinking water with an increased risk of developing stomach, esophagus, bladder, colon, rectum, pancreas, and kidney cancers (Langston et al. 2022). Exposure to high doses of perfluorooctanoic and polyfluoroalkyl chemicals has been shown linked to acidic renal and testicular tissue (Ojo, Peng, and Ng 2021). Unlike terrestrial organisms, aquatic organisms are exposed to various contaminants, providing an opportunity to explore the relationship between pollution and cancer at the cellular level (Fenton et al. 2021; Turner et al. 2020) . Exposure to multiple classes of pollutants can also damage the genomic integrity of cells, and potentially causing mutations that contribute to the development of cancer (Li 2023). *et al.*,The role of DNA damage is significant in the process of cancer development (Moubarz et al. 2023). Studies have connected the development of neoplasia in more than 330 aquatic species to exposure to a range of pollutants that impact DNA (Mirzaee et al. 2021). In China, studies have demonstrated a correlation between the amount of water pollution and the prevalence of liver and esophageal cancer (Zhang *et al.*, 2022).

Globally, antibiotic resistance is a serious problem that affects not only human health but also the fragile aquatic ecosystem (Carvalho et al. 2019). Since resistant bacteria and antibiotic residues can enter water bodies through a variety of channels, including wastewater discharge, agricultural runoff, and direct antibiotic use in aquaculture, the World Health Organization stresses the critical need to investigate and address antibiotic resistance in these environments (Jian et al. 2021; Kakkar et al. 2020; Pepi and Focardi 2021). Once established

in aquatic ecosystems, antibiotics exert selective pressure on bacteria, favouring the survival and proliferation of antibiotic-resistant strains (Liu *et al.*, 2023). This can lead to the spread of resistance genes within the bacterial community, posing a potential threat to human and animal health when these resistance bacteria are transmitted back to human through contaminated water or food (Uddin *et al.* 2021).

Consequently WHO emphasizes the need for comprehensive surveillance and monitoring programs to assess the levels of antibiotics and antibiotic resistance in aquatic environments (Liguori *et al.* 2022). Further, prudent use of antibiotics in medical and agricultural settings is recommended to minimize the release of antibiotics into water systems and reduce the dissemination of antibiotic-resistant bacteria (Dhaka *et al.* 2023; Ezeuko *et al.* 2021). Continuous antibiotic resistance surveillance in aquatic ecosystems enhances and safeguards public health and preserve the effectiveness of commonly used antibiotics for future generations (Serwecińska 2020). It is therefore crucial for researchers, policy makers, and other stakeholders to collaborate, implement and come up with innovative strategies that mitigate the risk associated with antibiotic use, poor disposal and promote sustainable/responsible practices in both human and animal sector (Adebisi 2023).

The use/of application microbes like bacteria to remove environmental pollutants from soil and water is a known as bioremediation technology. Current bioremediation methods include membrane-based technologies, photochemical oxidation, ion exchangers, adsorbents, and coagulants (Khan *et al.* 2022; Ramezani *et al.* 2021). Bioremediation involves the use of bacteria biological processes to reduce residual pollutants from contaminated water, including pharmaceuticals , personal care products, drugs of abuse, heavy metals, and agrochemicals (Iwar, Utsev, and Hassan 2021) . Microbial remediation using biofilms,

membrane bioreactor, biosequestration and biological engineering among others are widely used to break down complex aquatic pollutants and wastes (Tekere et al. 2019; Zhu and Wang 2020). In Polar Regions, microbial – based remediation is widely used successfully to treat ocean water and eliminate toxins from agro-industrial waste (Sharma *et al.*, 2021) . In Kenya, bioremediation studies on xenobiotic pesticides by bacteria species isolated from flower farm soils near Lake Naivasha confirmed that the bacteria species successfully and rapidly decomposed xenobiotics (Anode and Onguso 2021). The study sought to develop a cost-effective bioremediation approach aimed at addressing the issue of pollutant elimination in aquatic ecosystems. (Sanganyado and Gwenzi 2019).

Microbiologists are now confronted with the challenge of accurately characterizing the microbial community indicators that signal multi-class pollution in aquatic environments and then employ them for ecosystem health (Sundararaman et al. 2022). Shotgun metagenomics analysis is a powerful tool and technique used to study microbial communities in polluted aquatic ecosystem (Kumar *et al.*, 2021). It involves sequencing the DNA of all organisms present in a sample, providing comprehensive snapshot of the microbial diversity and functional potential within the ecosystem (Armengaud 2023). In polluted aquatic ecosystems such as contaminated rivers, lakes or marine environment affected by industrial or agricultural runoffs, the microbial assemblages play a crucial role in degradation and transformation of pollutants (Bourhane et al. 2022). Shotgun metagenomics analyses allows us to examine the genetic potential of these microbial communities, shedding light on the abilities to metabolize specific pollutants and their overall ecological function ability (Jhariya et al. 2022). By sequencing the whole genome, shotgun metagenomics analysis offers a thorough and objective investigation of the microbial communities in contaminated habitats (Akter et al. 2023). By identifying the specific microbial taxa, pathways and genes involved in degradation of pollutants, researchers can gain insight into the ecosystems resilience and

potential for bioremediation (Gangola et al. 2022). Shotgun metagenomics can detect rare or low abundance microbial species that may play significant ecological roles but might be missed in targeted approaches (Jia et al. 2022). The ability to capture the entire microbial community enhances understanding of the ecosystem dynamics in polluted environment. (Amon et al. 2022).

1.2 Statement of the Problem

The sources of pollutants entering Lake Victoria include industrial discharge, agricultural runoff, sewage treatment plants, and urban storm water runoff. These sources introduce a variety of contaminants such as heavy metals, pesticides, fertilizers, and pharmaceuticals into the lake. However, little is known about the specific profiles of these pollutants and their impacts on water quality and ecosystem health. Moreover, there is need to determine the bacterial communities present in the lake and their genetic composition in relation to antibiotic resistance and bioremediation of pollutants. Through shotgun metagenomics analysis, this study sought to fill the gaps in knowledge about the health of the ecosystem and provide insights for the development of effective pollution management and mitigation strategies.

1.3 Justification of the Study

Lake Victoria, as a crucial freshwater ecosystem, supports numerous ecological processes and sustains the livelihood of millions of people (Nyamweya et al. 2023). The presence of heavy metals and pharmaceutical residues in this fresh water ecosystem possess a significant risk to human health and environment (Sonone et al. 2020a). These pollutants have the ability to bioaccumulates in food chains, resulting in adverse effects in aquatic organisms. They have the potential to impact the human population that depends on the lake a source of livelihood. Gaining an understating of the presence and potential impacts of multiclass

pollutants in Lake Victoria is crucial for ensuring its long-term health and sustainability. The analysis of microbial assemblages through shotgun metagenomics is a novel approach that provides valuable insight into the diversity and functional potential of the microorganisms' present in polluted aquatic ecosystems. This approach makes it possible to identify specific microbial genes responsible for processes such as antibiotic resistance and bioremediation of pollutants. By studying these genes, we can gain a better understanding of the microbial response to pollution and explore potential strategies for mitigating these harmful effects. These findings highlight the urgent need for continued monitoring and research to address pollution in Lake Victoria and protect its ecosystem.

1.4 General Objective

To assess the ecotoxicological profiles of multi-class pollutants in aquatic ecosystem of Winam Gulf of Lake Victoria, Kenya

1.4.1 Specific Objectives

1. To isolate and identify multi-class pharmaceutical residues and heavy metals in the aquatic ecosystems of Winam Gulf Kisumu.
2. To assess the influence of identified pollutants, on the microbial assemblages of the aquatic ecosystem of Winam Gulf.
3. To evaluate the genotypic and phenotypic antibiotic resistance patterns among the bacterial assemblages of the aquatic ecosystems of Winam Gulf.
4. To determine the genes and pathways applicable for bioremediation of multi-class pollutants in the aquatic ecosystems of Winam Gulf.

1.5 Research Questions

1. Are there presence of multi-class pharmaceutical residues and heavy metals in the aquatic ecosystems of Winam Gulf?
2. What is the influence of the identified pollutants on the microbial assemblages in the aquatic ecosystems of Winam Gulf?
3. Are there differences in the genotypic and phenotypic antibiotic resistance patterns among bacterial assemblages in the aquatic ecosystems of the Winam Gulf?
4. What specific genes and pathways are identified for the bioremediation of multi-class pollutants in the aquatic ecosystem of the Winam Gulf?

1.6 Significance of the Study

The findings from this study revealed the presence of contaminants such as heavy metals and pharmaceuticals, which are harmful to the ecosystem and human health. Antibiotic resistant genes have been found in environmental bacteria due to pollution, highlighting the complexity of antibiotic resistance transmission in the aquatic ecosystem. Understanding these mechanisms is crucial for developing strategies to combat antibiotic resistance. The study also identified genes and gene families involved in bioremediation processes, providing insights into the effectiveness of natural microbial communities in removing pollutants. Responsible wastewater management and ongoing monitoring efforts are essential to protect the environment and human populations.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter offers a detailed examination of the current knowledge and research undertaken on the pollution of the aquatic ecosystem in Lake Victoria's Winam Gulf and its related ecosystems. The influence of chemical and biological pollution on microbial diversity and its role in ecosystem function disturbances is known as microbial ecotoxicology (Naz et al. 2022). Water pollution is an escalating issue globally, presenting substantial risks to human health, aquatic life, and prompting emerging challenges that demand worldwide attention (Wolfram et al. 2021) . Water pollution occurs when detrimental substances, such as chemicals, toxins, and contaminants, are introduced into bodies of water, including rivers,

lakes, and oceans (Morin-Crini et al. 2022). These pollutants stem from a variety of sources, such as industrial processes, agricultural runoff, improper disposal of waste, and urban development (Sarker et al. 2021). Globally scene, emerging issues related to water pollution are becoming increasingly apparent (Gomiero et al. 2019). One notable trend is the presence of microplastics in water bodies, which are tiny particles resulting from the breakdown of plastic waste (Issac and Kandasubramanian 2021). Microplastics not only harm marine life but also can enter food chains and potentially impact human health (Lehel and Murphy 2021). Another emerging issue is the presence of pharmaceutical residues in water sources, which can have adverse effects on both human and aquatic life (Lee *et al.*, 2017).

2.2 Pharmaceutical Residues and Heavy Metals in Aquatic Ecosystems

The World Health Organization reports that about 100,000 metric tons of pharmaceuticals are consumed worldwide each year (González *et al.*, 2021). Chemicals and active pharmaceutical ingredients are being used and released into the environment, but the potential risks related to their disposal have not been adequately addressed (Nguyen *et al.*, 2023). The escalating threat posed by pharmaceuticals, particularly antibiotics, is a growing global concern (Bianchini, Müller, and Pelletier 2020). Numerous prominent cases of antibiotic contamination leading to the proliferation of bacteria harboring antibiotic-resistant genes have been observed (Dey *et al.*, 2023). Between 30% and 90% of the active ingredients in medicines taken orally are excreted in urine (Nyaga, Nyagah, and Njagi 2020). Around 600 different APIs have been found in the environment across the world, and there have been instances of high concentrations that are hazardous to ecosystems (O’Flynn et al. 2021). Wastes, drinking water, and natural waters have all been found to contain pharmaceuticals and their metabolites (Zoumpouli et al. 2020). The use of antibiotics is a significant factor in the rise of current health challenges, including antimicrobial resistance (AMR), which poses a

serious threat to individuals (Ahmed et al. 2022) . It is anticipated that 2050, AMR will be a burden due to illness, lost lives, healthcare costs, and decreased productivity (Dadgostar 2019). According to recent data, AMR will likely lead to a 15-fold rise in morbidity in Europe by 2050 (Venkataraman et al. 2023) . Despite the potential risks posed by pharmaceuticals, they are still being released into the environment without regulation. Adding to the problem is a lack of awareness among the public regarding the impact of active pharmaceutical ingredients (APIs) on the environment (Dhananjayan et al. 2020) . The regulation of chemical pollutants such as pesticides, biocides or industrial chemicals, the emission of pharmaceuticals into the environment to ensure adequate information on the environmental impacts of pharmaceuticals (van *et al.*, 2019).

Pollution in Lake Victoria has been worsened by human and land use activities on the lakeshores and inland catchment areas within the lake basin (D. O. Onyango et al. 2021) . Basic industries, small-scale mining, waste from waste water treatment plants, municipal landfills, and other untreated domestic wastes are major sources of pollution in Lake Victoria (Nyilitya et al. 2021). The impact of various pollutants being introduced into the environment on aquatic ecosystems is not thoroughly understood (Kümmerer et al. 2019). These highly soluble multi-residue contaminants' persistence, prevalence, and toxicity in aquatic ecosystems pose a serious threat to receiving water bodies due to their fast spread and accumulation (Chen et al. 2020). Few baseline studies have evaluated the impact of these multiple-residue contaminants in water bodies on human health (Shore et al. 2023). Wastes from municipal landfills and wastewater treatment facilities are commonly released into water bodies, however the ability of these treatment facilities to completely remove all multi-residue contaminants is not well understood (Anand et al. 2022) . The abundance and diversity of microbial communities and their roles in the ecosystems may change as a result

of exposure to several residues of pollutants in the environment (Yu et al. 2021). Reduced microbial diversity results from the interactions of the microbial community with various residual contaminants, which alter the microbial community structure (Yang *et al.*, 2021) . Eutrophication has been linked to the extinction of the Cichlid *Haplochromis* and the slow disappearance of other species in Lake Victoria, including phytoplankton and zooplanktons (Okechi 2022). The ongoing discharge of pharmaceutical residues and other contaminants into aquatic ecosystems has contributed to the emergence of multidrug-resistant bacterial strains, which present a threat to human and global health (Imran, Das, and Naik 2019). Several contaminants' environmental toxicological degradation has had detrimental effects on ecological health, plant health, and human health (Allouzi et al. 2021). The use of polluted food and water has been related to the development of numerous ailments, including cancer, immune system damage, reproductive problems, and respiratory illnesses (Segovia-Mendoza et al. 2020). The increasing number of cancer cases is not clearly linked to the presence of multi-residue pollution in Lake Victoria (Abdallah et al. 2021).

Around the world, 80 percent of municipal wastewater and runoffs are discharged into the environment without being treated (Montoya-Bautista, Mohamed, and Li 2022). These wastewater and runoffs contain millions of tonnes of hazardous waste every year, including toxic sludge, solvents, personal care products, and drugs of abuse, agrochemicals, and heavy metals (Datta et al. 2022). By altering the food web, water pollution puts aquatic ecosystems, productive activities, and human health at risk (Sonone et al. 2020a) . According to the World Health Organization, environmental pollution kills 12.6 million people annually, or close to one-fourth of all fatalities worldwide (Selvaraj et al. 2022). The toxicity of a pollutant for ecosystem and human health is based on concentration, nature of chemical; ability to persist and bio accumulate (Ali *et al.*, 2019). Pollutants including industrial waste, poor air quality, litter, and other sorts of water contamination can be seen easily (Siddiqua, Hahladakis, and

Al-Attiya 2022) . If released into the environment in unacceptable concentrations, highly hazardous chemicals, such as heavy metals, have the potential to cause cancer, genetic damage, miscarriage, birth defects, injuries, and death. They are toxic and reactive (Nivetha et al. 2022) .

2.2.1. Pharmaceutical Pollutants

The presence of drugs and their metabolites in the environment and in tissues of organisms in ecosystems referred to as pharmaceuticals pollution (Świacka et al. 2022) . Is a growing threat due to improper disposal. The main sources of pharmaceutical waste include expired medications and contamination, which render the medication unfit for use on humans or animals (Nyaga et al. 2020). Drug dumping in open landfills and wastewater systems is unsafe method of disposing of pharmaceutical waste (Alnahas et al. 2020). As a results antibiotics and their metabolites have been found in food chains, water systems, and aquatic organisms (Okoye et al. 2022) . There is urgent need to enforce safe drug disposal practices because of the negative consequences produced by pharmaceutical residual pollution (Dar, Maqbool, and Rasool 2019) .

2. 2.2. Pharmaceuticals in the Environment

Pharmaceuticals are categorized according to their origin, pharmacological qualities, and therapeutic effects, as well as their modes of action, chemical makeup, and method of administration (Nguyen & Lai, 2020) . Pharmaceuticals can be divided into several classes, such as analgesics, antimalarials, antibiotics, antiseptics, and antiretrovirals (Mandal and Ghanta 2022). Scientists have utilized biopharmaceutical classification systems to categorize orally administered medications into subgroups based on factors such as solubility, permeability, absorption, and solubilization of active ingredients in the intestines (Jacobsen et al. 2023) . Various classification techniques have their own drawbacks for instance, chemical structure-based categorization may group together substances with various modes of action (Zhou, Nutt, and Davies 2022) .

2.2.3. Analgesics as Pollutants

Analgesics, also referred to as painkillers, are medications used to ease pain. They are freely accessible without a prescription, widely utilized, and available over the counter (Chaves et al. 2022) . Many studies have reported the presence of analgesics in wastewater and surface water (Ramirez-Morales et al. 2021). The biodegradation of these compounds is a concern due to the lack of understanding surrounding their frequency, toxicity, and the processes involved in their microbial degradability (Moghaddam et al. 2023) .

2.2.4. Antimalarials on Aquatic Environment

Research on water and agricultural samples have revealed antimalarial contaminants, which is worry for the environment (Ashesh et al. 2022) . Antimalarial water pollution has increased due to improper dumping and insufficient wastewater treatment, which has had an impact on antimalarial drug resistance (Saim& Mokran, 2022) .

2.2.5. Antibiotics Pollutants

Research conducted in the past have shown that 95% of the chemicals employed in antibiotics are excreted from the body unmodified, speeding the development of antibiotic resistance by bacteria in the environment (Irfan, Almotiri, and AlZeyadi 2022) . Several researchers have documented the presence of antibiotics in aquatic environments like waste waters, surface waters, and ground waters, including ciprofloxacin, erythromycin, sulfamethoxazole, and amoxicillin (Kovalakova et al. 2020) .

2.2.6. Antiviral and Anti-retroviral Drugs Pollutants

The use of antiviral and antiretroviral medications is more prevalent in developing countries with higher HIV/AIDS rates (Feyissa et al. 2022). However, their presence in the environment poses a concern due to their potential to alter ecosystems and promote the development of drug-resistant viruses (Andreo-Vidal et al. 2021) . These antiretroviral drugs have been identified as a "highly dangerous therapeutic class" due to their toxicity in water (Dikeocha et al. 2022) .

2.2.7 Antipsychotic Drugs Pollutants

Aquatic habitats suffer because of the psychoactive drug concentration, which recorded. Antipsychotic medications bioaccumulate in the food chain, causing behavioral alterations and altering population dynamics at lower quantities, according to reports on studies (Kampepidou 2021) . Antipsychotic medication pollution and its effects on the environment have received little attention (Argaluza et al. 2021).

2.2.8 Contraceptives in the Environment

Millions of women rely on oral contraceptives to prevent pregnancies daily (Festin 2020) . However, these contraceptives, including EE2, have been discovered to exert detrimental impacts on aquatic ecosystems (Almeida et al. 2020). EE2, a hormonal contraceptive, retains its biological activity even at minimal concentrations and displays resistance to degradation, resulting in environmental pollution (Chen et al. 2020) . Current wastewater treatment methods are ineffective in breaking down EE2, leading to its accumulation in aquatic ecosystems (Abibu et al. 2021) . This accumulation has been shown to negatively impact aquatic creatures, such as fish, and interfere with their reproductive systems (Sharifinia et al. 2020). Additionally, there have been reports of contraceptives causing the development of an intersex condition in male aquatic animals, where they produce eggs in their testicles (Langston et al. 2022).

2.2.9. Depressants and Stimulants Pollutants

In the aquatic environment, the presence of central nervous system (CNS) depressants results in a deceleration of both brain and bodily functions (Moreira et al. 2022). This includes substances such as alcohol, anxiety-reducing tranquilizers (e.g., Prozac, Valium, Thorazine, and Xanax), barbiturates (e.g., beta-hydroxybutyrate or GHB), Rohypnol, and various drugs like Paxil and Zoloft, among others (Chavda, Madhwani, and Chaurasia 2021) . Notably,

cocaine and its metabolites, being among the most frequently consumed illicit narcotics, contribute significantly to this scenario (Zarei et al. 2020).

Despite the elimination of cocaine metabolites like benzoylecgonine intact in urine, only a minimal 1% of cocaine's hazardous compounds find their way into the environment, as conventional wastewater treatment plants struggle to effectively remove them (Christophoridis et al. 2021). Research indicates the ubiquitous presence of cocaine and its metabolites in both natural and sewage waters (Sulej-Suchomska et al. 2020) . Furthermore, studies have demonstrated that cocaine induces alterations in gene expression within aquatic habitats due to its ecotoxicological effects (Wang *et al.*, 2021).

Despite being the largest and most important freshwater ecosystems in the world, limited research has been conducted on the extent of, distribution and effect of pharmaceutical residues and heavy metals in the Winam Gulf. Therefore, further research is needed to fill this research gap and obtain a comprehensive understanding of the occurrence, sources, fate and effects of pharmaceutical residues and heavy metals in Winam Gulf of Lake Victoria.

2.2.10 Hallucinogens Pollutants

The presence of hallucinogenic substances in aquatic ecosystems poses a threat to water quality and ecosystem health (Chen et al. 2020) . Studies have detected elevated levels of MDMA in both wastewater and natural water sources, raising concerns about the potential negative impacts on aquatic organisms and human well-being (Haalck et al. 2021). Evidence suggests that hallucinogens such as MDMA may lead to adverse effects on aquatic ecosystems, including zebrafish motility impairment, highlighting the need for further investigation into the consequences of these substances on water pollution (Khan et al. 2022).

2.2.11 Heavy Metals

Because they are non-degradable, heavy metals cannot be converted into substances that are less hazardous (Zango *et al.*, 2020) . Many systems, including landfill runoff, volcanic eruptions, soil and rock weathering, waste waters, and human activities like metal processing and mining, are sources of heavy metals in aquatic habitats (Akhtar *et al.* 2021). Nickel, mercury, cadmium, copper, lead, and arsenic are the commonest heavy metal contaminants (Hölzle *et al.* 2022). Aquatic ecosystems, including flora and fauna, have the capacity to accumulate heavy metal residue contamination from habitats (Sonone *et al.* 2020a) . Health problems emerge from heavy metal contamination of aquatic ecosystems, which penetrates the human food chain (Isangedighi and David 2019). Research findings indicate that the inclusion of organic binders enhances the bioavailability of Cadmium (Cd) in fish and mussels, facilitating the diffusion of the hydrophilic liquid component (Sonone *et al.* 2020a). This phenomenon underscores the significance of organic binders in promoting the spread of pollutants and their impact on aquatic pollution (Macías *et al.* 2023) . Fish, phytoplankton, and zooplankton development are hampered by the presence of heavy metals in aquatic ecosystems (Amoatey and Baawain 2019). Pollutants made of metallic compounds interfere with mollusc development, reproduction, and byssus formation by lowering oxygen levels (Chong 2022). Fish and crustaceans have shown changes in histology processes like fatty acid degradation in the liver and gill necrosis (Liu *et al.*, 2023). Heavy metals may disrupt the central nervous system, which can result in blood component damage, mental illnesses, and damage to important organs like the liver, lungs, and kidney (Engwa *et al.* 2019). Long-term heavy metal deposition in the body can cause physical, muscular, and neurological deterioration (Obasi and Akudinobi 2020). Besides long-term buildup of heavy metal compounds in the human body may result in mutations, damage to nucleic acids, and hormone mimicry, disruption of the reproductive and endocrine systems and ultimately

cancer (Ungureanu and Mustatea 2022). There is need to understand of the sources, pathways and fate of heavy metals in lake ecosystem. While studies have identified certain potential sources such as industrial activities and agricultural run offs, it is necessary to studies identify all potential sources and understand how these metals are transported and accumulate in aquatic environment.

2.3 Microbial Profiles

2.3.1 Microbial Assemblages

Pollutants have significant impacts on aquatic ecosystems (Rathi et al. 2021a). Research has demonstrated the presence of pharmaceutical residues and heavy metal pollutants in aquatic ecosystems, which can have adverse effects on aquatic microbial communities (Pinto, Simões, and Gomes 2022). These effects include alterations in community structure, reduced microbial diversity, changes in microbial functions, and disruptions to ecosystem processes (Oh, Choi, and Cha 2019). Lakes, rivers, and oceans are habitats to a diverse range of microorganisms that interact with each other and their surroundings (Neale et al. 2023). The microbial composition plays a vital role in maintaining the equilibrium of aquatic ecosystems (Kumar et al., 2023).

Microbes in aquatic ecosystems are essential for the breakdown and recycling of organic matter (Suominen et al. 2021). They decompose deceased organisms and waste materials, releasing crucial nutrients back into the water (Husain et al. 2022). Nutrient cycling process is crucial for sustaining the overall productivity of the ecosystems. Certain microbial species help in regulating water clarity by managing the algae population (Prasad et al. 2021). Algal blooms can occur when nutrient levels in water are high, leading to reduced oxygen levels and harming other organisms (Wurtsbaugh, Paerl, and Dodds 2019). Bacteria and protists

consume excess algae, preventing harmful blooms and maintaining water quality (Lv et al. 2022) .

Microbes also plays significant role in cycling important elements like carbon, nitrogen and phosphorus (Wang *et al.*, 2022). They mediate the conversion of the organic forms of these elements into inorganic forms and vice versa. This cycling process is important for the availability and distribution of nutrients within the ecosystem, supporting the growth of various microorganisms (Hartmann and Six 2023). Some species of microbes act as natural biocontrol agents, regulating the population of disease causing organisms (Weng et al. 2022). They suppress the growth of harmful bacteria and fungi preventing the spread of diseases among aquatic organisms. Maintaining a diverse microbial community enhances the overall health and resilience of the ecosystems (Hamza and Zinjarde 2023) .

Microbes contribute to the formation of habitats in aquatic ecosystems. Biofilms composed of various microorganisms form on surface such as rocks, plants and submerged debris (Caruso 2020). These biofilm provides attachment surfaces for other organisms like invertebrates and alga, creating a complex and diverse ecosystem (Petersen, Kellermann, and Schupp 2020). Microbial profiles serve as indicators to environmental changes and disturbances in aquatic ecosystems. Shift in microbial structure and community (Weiman et al. 2021).

2.3.2 Pharmaceutical Residues and Heavy Metals on Microbial Profiles

Pharmaceutical residues are emerging pollutants that can enter aquatic environments through various sources such as wastewater discharge, incomplete removal through water treatment processes, and agricultural practices (Sundararaman et al. 2022). These compounds, including antibiotics, hormones, and painkillers, can have direct effects on microbial communities by interacting with microbial cells, affecting their growth, metabolism, and gene expression (Castronovo et al. 2021). Additionally, some pharmaceuticals can act as antimicrobial agents,

inhibiting the growth of susceptible microbial species while potentially promoting the proliferation of resistant strains (Varela et al. 2021). Indirect effects of pharmaceuticals on aquatic ecosystems can arise from changes in water chemistry, including nutrient availability and toxin accumulation, which can impact microbial populations, alter water quality, and introduce invasive species (Narayanan et al. 2022) .

On the other hand, heavy metals such as lead, mercury, cadmium, and copper are commonly found in industrial effluent, agricultural runoffs, and urban wastewater (Ahmed et al. 2022). These pollutants can disrupt metabolic activities of microorganisms, inhibit enzyme function, and impair cell membrane integrity (Sheng et al. 2023). Some microbial species are more sensitive to heavy metal toxicity, while others exhibit tolerance or even metal resistance mechanisms (Pal et al. 2022). In some cases, heavy metals may alter the composition and abundance of microbial populations, leading to a shift in microbial community structure (Shuaib et al. 2021b). This shift can have cascading effects on ecosystem functions, including nutrient cycling, decomposition, and overall ecosystem stability (Sveen, Hannula, and Bahram 2024) .

2.3.3 Mechanisms of Pollutant and Microbial Interaction in Aquatic Ecosystem

Pharmaceuticals and microbial communities have a complex interaction where microbes can adsorb pharmaceuticals onto their surfaces and transform them through biodegradation and biotransformation processes (Fernandes et al. 2021). Biotransformation involves modifying the chemical structure of pollutants, while bioremediation refers to breaking down pollutants into simpler, less toxic compounds (Sharma, 2020). Some microbes can detoxify pharmaceuticals by enzymatically converting them into less harmful substances, reducing their toxicity in the environment (Paraschiv et al. 2022). Pharmaceuticals can also influence microbial metabolism by altering enzyme activity and gene expression patterns, leading to

changes in metabolic pathways and overall microbial community structure (Kumar *et al.*, 2023) .

On the other hand, heavy metals pollutants can also have significant impacts on microbial communities (Zhao et al. 2020). Microbes may possess specific genes or genetic adaptations that enable them to survive and thrive in the presence of heavy metals (Bhat et al. 2019). Heavy metals can inhibit key enzymes, disrupt electron transport chains, and impair energy production in microbes (Elnabi *et al.*, 2023). Long-term exposure to heavy metals can lead to adaptations in microbial populations, potentially resulting in the development of resistant strains or altered metabolic capabilities (Duan et al. 2021). Elevated concentrations of heavy metals in the aquatic environment can inhibit microbial growth, disrupt metabolic pathways, and alter the overall functioning of microbial communities (Caracciolo & Terenzi, 2021). Some metal-tolerance microorganisms may thrive in these conditions, leading to a shift in community structure towards more resistant species (Ojuederie and Babalola 2017) .

2.3.4 Ecological Implication of Pollutants in Aquatic Ecosystems

Studies have shown that pollutants in the Lake Victoria ecosystem have significant effect on delicate balance of the ecosystem (Omwoma et al. 2019). In the Winam Gulf, studies have revealed that pollutants from industrial wastes, agricultural runoff, and urban wastewater have led to water quality degradation (Githaiga et al. 2021). Excessive nutrients from fertilizers have led to eutrophication, resulting in algal bloom (Aziz et al. 2021). Multi-class pollution has caused biodiversity loss in Lake Victoria, the second-largest freshwater lake (Anagnostopoulpou et al. 2022). There have been cases of biodiversity loss in the Winam Gulf ecosystem due to death and abnormalities in reproductive processes that have been attributed to pollution (Nyakeya et al. 2022). In recent days, the accessibility of pharmaceuticals in the environment is progressively a worldwide concern (Okechi 2022). The significant wellspring of these contaminations in water assets is drugs for human use or

veterinary medications (Sivaranjanee et al. 2022). Intermediates, active metabolites and raw materials present in water from pharmaceutical industry waste because of incomplete sewage treatment systems (Bavumiragira, Ge, and Yin 2022). The conventional effluent treatment methods are not sufficient to remove API (active pharmaceutical ingredients) from this water effectively (Taoufik et al. 2021). There is necessitate for continuous monitoring of the pharmaceutical compounds in aquatic ecosystem to save the environment and living form of lives from health hazards (Rathi, Kumar, and Vo 2021c).

2.3.5 Shotgun Metagenomics Analysis of Microbial Profiles

The microbial communities in the aquatic ecosystems of Winam Gulf are heavily influenced by the presence of pollutants, which can lead to structural changes in the microbial population (Aura, Odoli, and Musa 2023). The extensive biodiversity of these microbial communities can be revealed through DNA sequencing, allowing for a deeper understanding of their composition and function (Wani et al. 2021). However, a higher proportion of environmental microbes are difficult to culture in the laboratory, making it challenging to fully comprehend the role of these community members in the ecosystem (Sheng et al. 2023).

One potent method that has been developed to study microbial biodiversity and community function is shotgun metagenomics DNA sequencing (Fadiji and Babalola 2020). This technique provides an in-depth analysis of the genetic content of microbial communities, allowing researchers to identify metabolic pathways, gene functions, and potential interactions within the community (Mishra et al. 2021). By examining the entire genetic content, researchers can gain valuable insights into how microbial communities respond to pollution and their potential for pollution degradation in polluted aquatic ecosystems (Bhat et al. 2019) .

In polluted ecosystems like Winam Gulf, understanding the microbial response to pollution is crucial for monitoring environmental health and developing targeted interventions (Nandy and Kapley 2024). The use of shotgun metagenomics analysis can help researchers discover novel microorganisms with unique adaptations and capabilities that may be relevant to polluted environments (Kirubakaran et al. 2020). By comparing DNA sequences to existing databases, researchers can identify known organisms and uncover new species or genetic variants that can provide valuable information for environmental monitoring and biotechnological applications (Chac and Thinh 2023). This information can also lead to the discovery of novel enzymes, pathways, or mechanisms that can be harnessed for the remediation of polluted aquatic ecosystems (Lema, Gemed, and Woldeamay 2023) .

2.4 Genotypic and Phenotypic Drug Resistance Patterns

The rising presence of antibiotics resistance genes in aquatic environments is a growing concern, posing a risk to both human and animal health (Hossain et al. 2022). Research indicates that surface water and urban water systems harbor various antimicrobial resistance genes that can be transferred horizontally (Kusi et al. 2022). The discharge of pharmaceutical residues, industrial effluent, and other pollutants into water bodies contributes to the proliferation of antibiotic resistance genes, exacerbated by heavy metals and other pollutants (Anand et al. 2022). These genes can reduce the effectiveness of antibiotics in treating infections and impact human health indirectly through bioaccumulation in the food chain (Zhao et al. 2020). Shotgun metagenomics analysis provides a comprehensive view of the microbial community and can track changes in gene abundance and diversity over time in aquatic ecosystems (Behera et al. 2021).

Genotypic antibiotic resistance patterns involve the genetic characteristics of bacteria that allow them to resist the effects of antibiotics, such as specific genes or mutations (Baquero,

Martínez, and Cantón 2008). These genetic changes can be transmitted through bacterial DNA replication. Phenotypic antibiotic resistance patterns, on the other hand, refer to the observable resistance of bacteria to antibiotics in a laboratory setting, determined by conducting tests like disc diffusion or minimum inhibitory concentration tests (Mukami 2021).

In environmental bacteria, both genotypic and phenotypic antibiotic resistance patterns can vary due to exposure to antibiotics, presence of antibiotic-resistant genes in the bacterial population, and selective pressure from the environment (Larsson and Flach 2022). Environmental bacteria can acquire antibiotic resistance genes through horizontal gene transfer or mutations in their genetic material (Jian et al. 2021).

2.5 Key Aspects to Consider In Bioremediation

Various microorganisms possess specific genes that code for enzymes capable for breaking down different types of pollutants (Zango Usman et al. 2020). Certain bacteria possess genes for production of enzymes like dehalogenases, which aid in degradation of halogenated compounds such as chlorinated solvents (Oyewusi, Wahab, and Huyop 2020). Pollutant degradation often involves multiple enzymatic reactions, forming metabolic pathways. These pathways produce systematic instruction for breakdown of pollutants (Yang *et al.*, 2021). Specific enzymes encoded by the respective genes facilitate each step. Understanding these pathways is crucial for developing effective bioremediation strategies (Singh *et al.*, 2019).

Aquatic ecosystems harbor diverse microbial communities with varied genetic potential for pollutant degradation (Sun et al. 2021). This genetic diversity increases the likelihood of encountering microorganisms with necessary genes for degrading specific pollutants (Rout et al. 2022). Specific genes and pathways in bioremediation of pollutant in aquatic ecosystem can vary depending on the nature of the pollutants and the microbial communities present

(Weiman et al. 2021). Research and ongoing studies continues to uncover new insight and potential bioremediation strategies (Gaur et al. 2022).

One research gap in the field of pharmaceutical pollution is the need for comprehensive monitoring and assessment of pharmaceutical residues in different environmental compartments across various geographical locations and ecosystems. Another research gap concerns the fate and behavior of pharmaceutical residues in the environment. While some studies have identified microorganisms capable of degrading pollutants in water bodies, there is a lack of understanding of the genetic mechanisms governing their bioremediation potential. Further research is needed to unravel the genes and pathways involved in pollutant degradation, as well as to explore gene expression patterns and regulatory networks controlling the biodegradation process in aquatic ecosystems. Identifying these genetic factors could lead to more targeted and efficient bioremediation strategies.

CHAPTER THREE

MATERIAL AND METHODS

3.1 Introduction

This chapter provides detailed information on the methods and materials used in the study. It outlines multiple research methods, with specific section dedicated to study area, study sites, research design, sample collection and processing, pollutants in aquatic ecosystem, analysis of microbial assemblages, phenotypic and genotypic antimicrobial resistance, identification of genes and pathways of bioremediation and data analysis.

3.2 The Study Area

The Winam Gulf is a prominent geographical feature extending from the northeastern part of Lake Victoria into the western Kenya. Previously referred to as Kavirondo Gulf, Nyanza Gulf, or Lake Nyanza Gulf, it is a shallow inlet that is linked to the main lake through the Rusinga Channel, which is 4.8 kilometers wide. The gulf is partially concealed from the main body of Lake Victoria by several islands such as the Maboko, Rusinga, and Ndere Islands.

The gulf has an average width of 25 kilometers and stretches for a distance of 40 miles from Kisumu to the channel. Notable bays within the gulf include Naya Bay, Nyakach Bay, Osodo Bay, Kendu Bay, Homa Bay, Ruri Bay, Mirunda Bay, Asembo Bay, and Olambwe Bay. The Winam Gulf has a population of 397,957 according to the Kenya Population and Housing Census (KPHC, 2019). Kisumu city, situated on the northeastern shore of the Winam Gulf, is the third-largest city in Kenya. The gulf is home to small-scale agricultural markets, fishing activities, and small-scale industries including tourism, food processing, oil refining, plastics, furniture, and cement production. The area also features various commercial establishments, educational institutions and healthcare and research facilities that accommodate a large population of people.

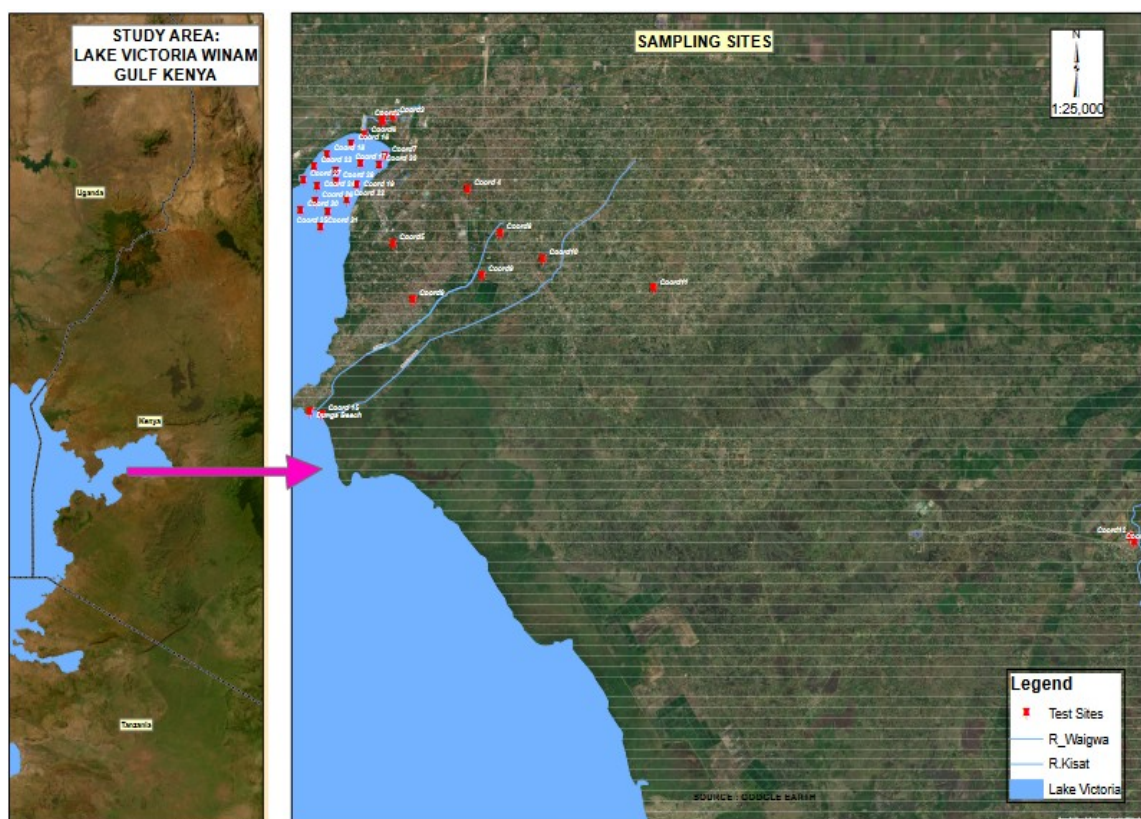


Figure 1. Location of Winam Gulf in Lake Victoria Kenya and GPS location of the sampling sites (Appendix 1) .

3.3 Sampling Sites

Sampling was carried out at the Inlet Rivers (Kisat, Wigwa, Nyamasaria, Nyando), Kisumu waste water treatment plant's (KWWTP) effluent discharge into the lake, Kisumu industrial effluent, Fish landing beaches, and storm water entrance points to the lake , Kisumu Water and Sewerage Company (KIWASCO) treatment facility and inside the and Lake

3.4 Research Design

This study on the eco-toxicological profile of multi-class pollutants and their effects on microbial communities in the Winam Gulf employed an experimental research design. Important sources of pollution identified by reconnaissance visits, and sampling locations chosen using a purposive sampling strategy that took into account factors including pollutant entrance point. To find different kinds of pollution, samples of water and sediment were

taken from these locations and examined in a laboratory. To arrive at significant findings, the collected data were subjected to statistical and metagenomics analysis.

3.5 Sample Collection

Purposive sampling was used to ensure that a diverse range of organic and inorganic pollutants of interest could be obtained from the sediment and water samples collected from the fifteen different sites in Winam Gulf (Figure 1) and the table of the GPS locations of the sampling sites are provided in appendix 1. Purposively choosing particular sites enabled the study focus on areas where contaminants were believed or known to enter into the lake, allowing a more in-depth analysis of the region's environmental pollution. Three water samples replicates were collected and placed in sterile plastic bottles (500ml) that were then sealed and transported to the laboratory in cooler boxes (4°C) within 12 hours and stored at -80°C for laboratory analysis (Abia et al., 2015). The samples of sediment and water were collected from 31 sampling points during both the dry and wet seasons. At each sampling point, three samples of water and three samples of sediment were obtained, resulting in a cross-sectional study design where data was collected from multiple sampling points at a single point in time. A total of 130 water and sediment samples were collected from different sampling sites during two seasons.

3.6. Pollutants in Aquatic Ecosystems

A 100ml water sample and a 50g sediment sample were collected from each sampling site for pollutant analysis.

3.6.1 Heavy Metals Analyses in the Water and Sediment Samples

The analysis of heavy metals in water and sediment samples was conducted using the Hach method technique with a spectrophotometer for accurate measurement. Any organic debris was removed from the sediment samples, then dried, ground to a fine powder, and dissolved in distilled water. Filtration of water samples was done to remove any suspended solids. A set of calibration standards containing precise concentrations of heavy metals, including lead,

mercury, nickel, copper, cadmium, zinc, and aluminum, was prepared for analysis (Ghale Askari et al., 2022). The standards covered the expected range in the samples according to the appropriate reagent of the Hach standard preparation. The digestion process for each sample of heavy metal involved weighing 0.5 gram of the sample and adding concentrated nitric acid and hydrochloric acid to a digestion vessel.

Sample digestion was performed to break down the sample matrix and release the metal for analysis. The mixture was heated to dry, and then hydrochloric acid was added until fully dissolved. The solution was transferred to a volumetric flask and diluted with distilled water to a final volume of 1 liter. The digested samples were analyzed using a spectrophotometer that was calibrated to the absorption maximum of each heavy metal being tested. Standard solutions with known concentrations were prepared to create a calibration curve by measuring their absorbance. After digestion, the samples were analyzed for their absorbance to determine the concentration of heavy metals using the calibration curve. The calibration and selection of the optimal wavelength followed the Hach method (Maidan et al., 2023). The absorbance of each sample was measured and recorded, with quality control measures in place such as analyzing blank samples and conducting test replicates to assess precision and accuracy.

3.6.2 Analysis of Pharmaceuticals Residues

3.6.2.1 Solid-phase Extraction (SPE) by Cartridge

The SPE cartridges were conditioned with methanol followed by water according to the manufacturer's instructions. This step was important for proper wetting of the sorbent and removal of any impurities in the cartridges

The mixture of water and sediment samples was filtered using a suitable filtration apparatus to remove particulate matter. The pH of the filtered sample was adjusted to enhance the extraction efficiency of the target analytes.

The prepared sample was loaded onto the pre-conditioned SPE cartridge at a slow and steady flow rate using a vacuum manifold or an SPE system. It was ensured that the sample volume did not exceed the capacity of the cartridge. The sample was allowed to fully pass through the cartridge, retaining the analytes on the sorbent bed while washing away interfering compounds.

3.6.2.2 HPLC-MS/MS Analysis.

The samples, quality control (QC) and pharmaceutical standards were analyzed using Agilent's series 1100 HPLC (Santa Clara, California) tandem a waters' Micromass, Quattro Ultima mass spectrometer (Milford, Massachusetts). Column used is Sunshell C18, 2. 6um, 4.6mmi, 50mm samples.

3.7 Analysis of Microbial Assemblages

3.7.1. Physico-chemical Analyses of the Samples

Salinity, total dissolved solids, pH, temperature conductivity, total dissolved solids, and dissolved oxygen were all measured using procedures according to (Nahhal et al., 2021). Prior to taking water and sediments samples, the temperature of the water was measured in-situ with a thermometer, and the values recorded. PH was measured using the PH meter which was calibrated for accuracy using standard buffers with pH values of 4.0, 7.0, and 10.0 prior to determining the pH levels of the samples. Following calibration, 4 ml of each water sample were collected, and pH measurements made at each of the 89 water sample. To avoid contamination, the pH probe was cleaned using distilled water in between each measurement. The conductivity of the water samples was determined using a conductivity meter. The meter was calibrated using a standard solution before electrical conductivity testing in the lab, and the outcomes were recorded. Salinity was measured using a refractometer by first determining the refractive index of water sample placed on the device's prism. The refractive index was then translated to salinity using a conversion table (Le Menn & Naïr, 2022). Turbidity was measured by Salinity was measured with a refractometer by determining the

refractive index of a water sample placed on the device's prism. This refractive index value was then converted to the corresponding salinity level using a conversion table.

Total dissolved solids were measured by heating 5ml of water samples to evaporation in a pre-weighed container. The volume of the original sample was divided by the weight of the solids produced after drying, and the result recorded in milligrams per liter to calculate the TDS (Total Dissolved Solids) concentration in a sample. Dissolved oxygen levels were measured using a dissolved oxygen meter. The probe was immersed directly into the water samples, and the instrument measured the partial pressure of oxygen dissolved in the solution. The readings were recorded as dissolved oxygen concentration.

3.7.2 Shotgun metagenomics sample processing

A total of 90 samples of water and 40 samples of sediment from various sampling sites were combined to create a homogenous sample. The sample was then processed by sieving through grade 1 Whatman™ filter papers to eliminate large particles and debris. 10 mL of the homogeneous sample was centrifuged and the supernatant was decanted, with this process being repeated for the entire homogenous sample. The sediment cell debris was vortexed and transferred to 2ml Eppendorf tubes, followed by centrifugation at 5000×g for 10 minutes to obtain pellets for DNA extraction in preparation for metagenomics sequencing (Xue et al., 2023).

3.7.3. Extraction of Genomic DNA and Shotgun Metagenomics Sequencing.

The DNA extraction was conducted using a protocol that combines enzymatic and chemical methods according to (Stewart et al., 2020). The sample was placed in 2 ml Eppendorf tubes to which 2% of 0.7 ml of extraction CTAB buffer (20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2 % CTAB) was added. 0.4 % Beta-mercaptoethanol was then added and incubated at 65 °C for 45 min while gently mixing by inversion after every 15 min. 0.6 mL

chloroform-isoamyl in a ratio (24:1) was added to the mixture and gently mixed for 1 min followed by centrifugation for 10 min at 16000×g and procedure repeated twice. 0.7 ml of cold isopropanol (-20 °C) was added to the mixture and gently mixed by inversion. The solution was then centrifuged at 16000×g for 10 min to form the DNA pellets. The DNA pellets was washed twice with 1 ml of 70 % ethanol to eliminate salt residues and left to dry overnight with the tubes inverted over a filter paper at room temperature. DNA Pellet was then was eluted in 100 ml TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) and stored at -20 °C for shotgun metagenomics. Agarose gel electrophoresis was used to check the quality and integrity of the DNA sample while Qubit 2.0 Fluorimeter (ThermoFisher Scientific) was used for quantitation of DNA concentration. The sequencing of the DNA sample was performed at Novogene ((UK) Company Ltd) which involved genomic DNA fragmentation, end repair and A-Tailing, adapter ligation and PCR amplification.

3.8. Phenotypic and Genotypic Antimicrobial Resistance Analysis

Genotypic analysis was conducted using shotgun metagenomics, while phenotypic analysis was conducted by culturing water and sediment samples and disc diffusion method as described below.

3.8.1. Isolation of bacteria from water and sediment samples

A serial dilution technique was employed on the pooled water samples to reduce the bacterial concentration. Subsequently, these samples were inoculated into nutrient broth and incubated at 37°C for 24 hours. The grown cultures were then inoculated on MacConkey and nutrient agar plates for colony differentiation, and then subcultured to isolate pure colonies. Further identification of the bacteria was carried out using the Gram staining technique.

3.8.2 Evaluation of Antibiotic Resistance to Selected Drugs

The pure cultures were inoculated onto Muller-Hinton agar (Merck, Germany) for the antibiotic disc diffusion technique (Kouadri, 2018). The commonly used drugs from the

following classes were used for the drug diffusion test: Ampicillin (10 g), Penicillin (10 g), Imepenem (10 g), Vancomycin (30 g), Ceflazidime (30 g), Gentamycin (120 g), Tazobactam (110 g), Erythromycin (15 g), Tetracycline (30 g), Cefoxitin (30 g), Ceftriaxone (30 g), Sulfamethoxazole (25g). The growth and inhibition zones of each antibiotic were measured and assessed in accordance with established protocol (Smith et al., 2023).

3.8.3. Shotgun Metagenomics Analysis for Antibiotic Resistance

The drug resistance genes in the bacteria were analyzed by conducting shotgun metagenomic (Abdel Salam et al., 2021) using IDBA-UDv1.1.1(Iterative De Bruijn graph Assembler for RNA-Seq Data version 1.1.1) and aligned to the assembled contigs with the Burrows-Wheeler transform (BWT) and Ferragina-Manzini (FM) index. Gene prediction was then carried out using Prodigal v2.6.

3.9 Data Analysis

3.9.1. Analysis of Data on Heavy Metals

The data was analyzed using graphpad prism software version 10. All the results were tested for normality using Shapiro-wilk test and expressed as a mean $\pm$ SD using descriptive statistics. Two-way ANOVA was used to test the significance difference of heavy metals in water and sediments. Post-hoc analysis was done using Dunnett's multiple comparisons test to check the significance differences across the sediments and water against WHO standards. The test data was statistically significant at $p \leq 0.05$.

3.9.2. Analysis of data on pharmaceutical residues

The HPLC-Ms used in the analysis of the pharmaceuticals was installed with a library of compound in its system and therefore the identification and data analysis were automated

3.9.3. Analysis of Metagenomics Data

Taxonomical abundance was determined by comparing metagenomics reads to a database of taxonomically informative gene families (MicroNR database). Gene prediction was done by Meta Gene Mark based on the scaftigs length and gene catalogue for each sample was

obtained through CD-HIT by keeping clustering threshold at 95 %. Species annotation was done using DIAMOND software (V0.9.9.110) for alignment of Unigenes sequences with those of bacteria, fungi, archaea, and viruses extracted from NCBI's NR database. Functional annotation was inferred based on its similarity to the sequence in the databases (KEGG, egg NOG, and CAZy) while functional category hits distribution was annotated using MG-RAST Subsystems classification.

3.9.4. Annotation of Antibiotic Resistance Genes

Unigenes were aligned with the CARD database (<https://card.mcmaster.ca/>) using the Antibiotic Resistance Gene Identifier (RGI) software provided by the CARD database with a default E value of less than $1e-30$. The RGI bioinformatics tool was utilized for the identification of Antibiotic Resistance Genes (ARGs) in bacterial genomes. The relative abundance of each ARO was calculated based on the RGI alignment results and Unigenes abundance information. Subsequently, an abundance histogram, abundance clustering heat map, abundance distribution circle map, ARO database, and difference analysis between groups was generated. Antibiotic resistance genes were identified in the analysis (Unigenes annotated as ARO) and the specific species to which antibiotic resistance mechanisms were attributed were determined through species analysis. Some AROs with lengthy names were abbreviated using the first three words followed by underscores.

3.9.5. Identification of Genes and Pathways Depicting Bioremediation of the pollutants

Through shotgun metagenomics analysis functional annotation utilizing KEGG was employed to identify genes and pathways implicated in bioremediation of pollutants in microbial communities present. Using KEGG the study identified and analyzed the specific genes and pathways responsible for bioremediation process within these microbial communities.

CHAPTER FOUR

RESULTS

4.0 Introduction

This chapter provides detailed information on the findings of this study. It outlines the concentration of heavy metals in water and sediments, concentration of pharmaceutical residues in water and sediments, physicochemical parameters, taxonomic classification of microbial communities, functional annotation of bacterial communities present in water and sediment samples, phenotypic and genotypic antibiotic resistance and Kegg pathways depicting bioremediation.

4.1. 1 Concentration of Heavy Metals in Water and Sediments in the Winam Gulf

Kisumu

The following metals were detected in the water and sediments from different sampling locations; Nickel, Lead, Copper, Cadmium, Aluminum, and Mercury. Table 1 & 2 shows the differences in concentrations (mean and standard deviation) of all heavy metal characteristics analyzed in sediments, water and the acceptable WHO standards for water. Nickel concentrations were lowest in Kiwasco water and highest in the River Kisat. River Kisat water had the lowest concentration of cadmium; whereas the lake water had the highest. The lake water had the lowest lead levels, whereas Kiwasco water had the highest. River Kisat water had the lowest copper concentration, whereas Kiwasco water had the highest. The zinc content was the highest at Kiwasco, but lowest in in Nyando River. The Kisat River had the lowest aluminum concentration, while the Nyando River had the highest. The investigation also revealed the concentration of certain heavy metals at several Winam Gulf locations. The sediments from Kiwasco had the most nickel, whereas those from lakes had the least. The lake had the least amount of cadmium in its sediments, while the sediments from the river Nyando had the most. Between Kiwasco and Nyando, lead concentrations were greater and

lower, respectively. Copper concentrations were higher in lake sediments and lower in Nyando River sediments.

4.1.3 Heavy Metals in Water and Sediments

Table 1. Mean and Standard Values of Heavy Metals in Water in Winam Gulf and WHO Standards (Mg/l)

	N	Ni	Pb	Cu	Cd	Zn	Al	Hg
The lake	13	0.238±0.005	0.033±0.002	0.002±0.02	0.001±0.067	0.025±0.126	0.026±0.017	0.00032±0.001
	4			0				
Kiwasco	13	0.304±0.077	0.016±0.004	0.282±0.00	0.0247±0.026	0.823±0.007	0.007±0.024	0.00051±0.001
	4			5				
R Nyando	13	0.204±0.033	0.007±0.102	0.121±0.00	0.097±0.001	0.049±0.013	0.004±0.042	0.00047±0.001
	4			1				
R. Kisat	13	0.275±0.029	0.135±0.019	0.019±0.00	0.024±0.002	0.073±0.009	0.015±0.002	0.00049±0.001
	4			2				
WHO STDS		0.07mg/l	0.01mg/l	2mg/l	0.003mg/l	5mg/l	0.09mg/l	0.006mg/l

Table 2. Mean and Standard Values of Heavy Metals in Sediments (mg/l)

	N	Ni	Pb	Cu	Cd	Zn	Al	Hg
Kiwasco	134	0.052±0.034	0.044±0.21 7	0.124±0.008	0.033±0.04 9	0.072±0.012	0.020±0.00 4	0.00021±0.001
R. Nyando	134	0.226±0.269	0.032±0.00 8	0.142±0.019	0.031±0.02 7	0.055±0.011	0.015±0.00 3	0.0001±0.001
R. Kisat	134	0.416±0.016	0.010±0.02 9	0.003±0.040	0.004±0.00 7	0.041±0.021	0.015±0.00 2	0.00046±0.001
Lake	134	0.181±0.184	0.003±0.09 1	0.171±0.642	0.050±0.00 5	0.053±0.006	0.022±0.04 7	0.00047±0.001
WHO STDS		0.007mg/l	0.001mg/l	2mg/l	0.03mg/l	5mg/l	0.09mg/l	0.007mg/l

There was a significant difference between heavy metal concentration in water samples from different sampling points (F= (p<0.05). The results indicate that copper, zinc, and aluminum were within acceptable WHO standards while nickel, lead, and cadmium were above the WHO acceptable standards

4.1.1.2. Data Analysis for Heavy Metals by Graph Prism Pad

Results of the analysis of heavy metals in sediment showed significant levels of heavy metals in sediments ($F_{(4, 401)}=19.63$, $p<0.001$). The analysis indicated also that there was significant difference between the rivers ($F_{(4, 401)} = 19.63$, $P<0.0001$. Sediments and WHO standards as shown in (table 3). There were significant levels of heavy metals in sediment samples were significant as shown in table 4. The post hoc analysis showed that there was a significant difference between WHO standards levels and heavy metals levels in the river sediments (Table 4).

Table 3 post-hoc analysis of WHO standards and sediments.

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
WHO Standard vs. Kiwasco s	0.9247	0.6825 to 1.167	Yes	****	<0.0001
WHO Standard vs. Nyando S	0.9582	0.7156 to 1.201	Yes	****	<0.0001
WHO Standard vs. Kisat S	0.9569	0.7146 to 1.199	Yes	****	<0.0001
WHO Standard vs. Lake s	0.9569	0.7146 to 1.199	Yes	****	<0.0001

ANOVA of the heavy metals present in waters samples showed a significant difference among the groups $F_{(4, 408)} = 23.80$ $P<0.0001$

Table 4. Post-hoc comparison of WHO standards and heavy metals in water

Dunnett's	multiple	Mean	95.00% CI of	Below	Summar	Adjusted P
-----------	----------	------	--------------	-------	--------	------------

comparisons test	Diff.	diff.	threshold?	y	Value
WHO Standard vs. Kiwasco					
W	0.9719	0.7447 to 1.199	Yes	****	<0.0001
WHO Standard vs. Nyando W	0.9749	0.7476 to 1.202	Yes	****	<0.0001
WHO Standard vs. Kisat W	1.005	0.7776 to 1.232	Yes	****	<0.0001
WHO Standard vs. Lake W	0.9528	0.7256 to 1.180	Yes	****	<0.0001

4.1.2 Concentration of Pharmaceutical Residues in Water and Sediments

Representative classes of pharmaceutical residues detected in the water samples included, artemether (antimalarial), caffeine (stimulants), nevirapine (antiretroviral) sulfamethazine, sulfamethaxole, trimethoprim, tetracycline and amoxil (antibiotics) (figures 2 & 3).

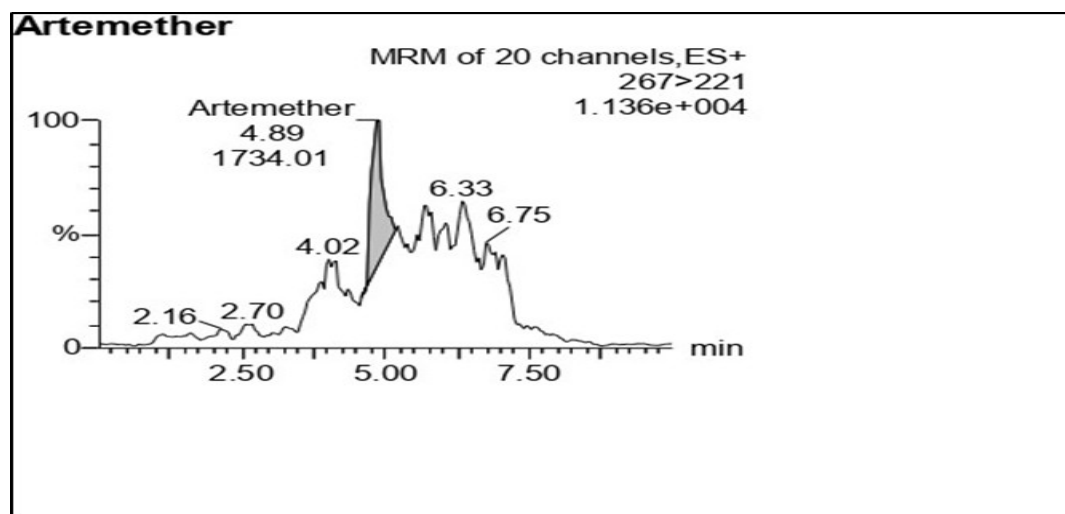


Figure 2. Identified Pharmaceutical residues grey peaks represent the pharmaceutical compounds present. The peaks represent the detection of artemether residues, which belong to the class of antimalarial, found in water and sediment samples.

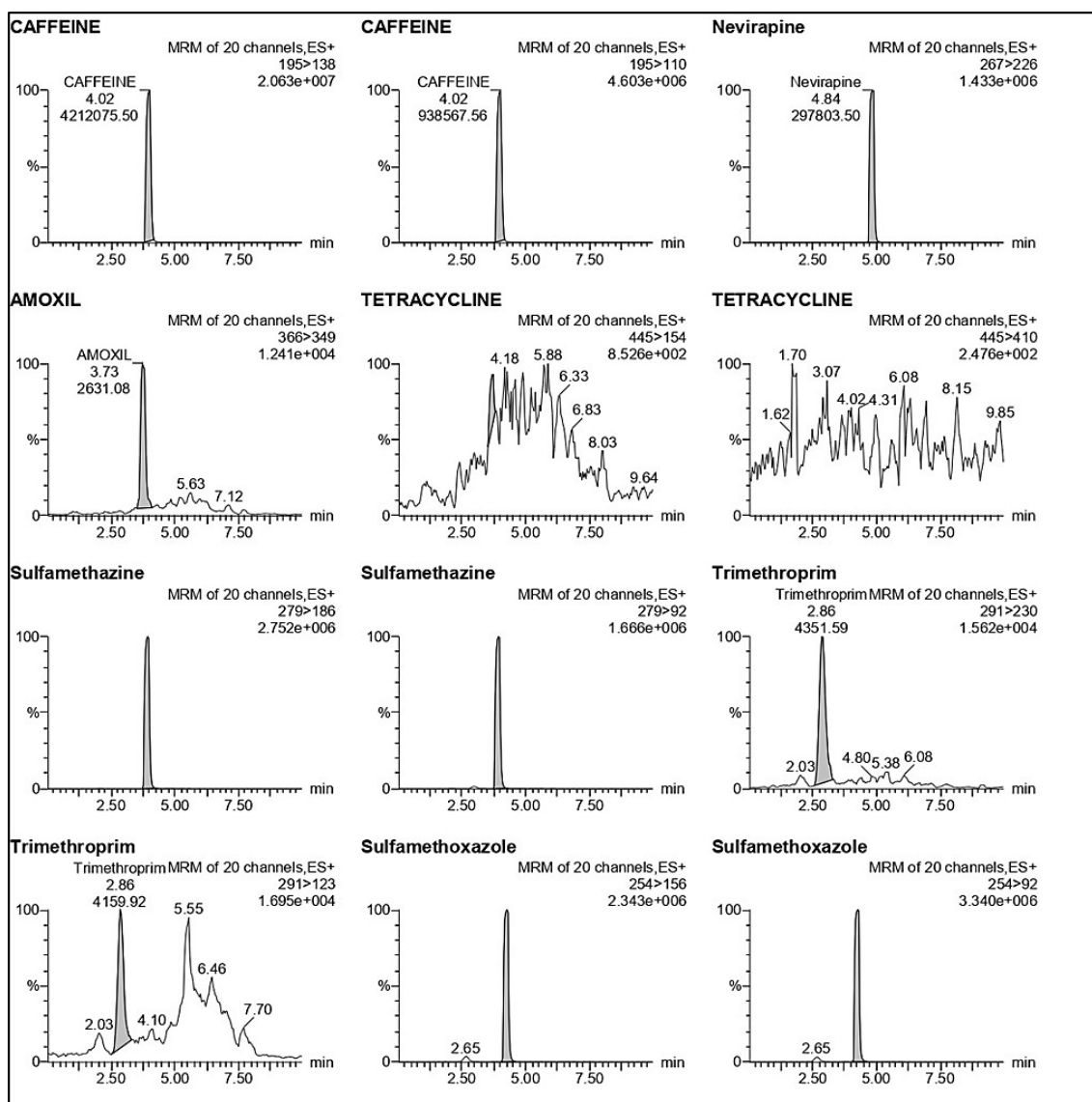


Figure 3. Identified Pharmaceutical residues grey peaks represent the pharmaceutical compounds present. The peaks represent the detection of pharmaceutical residues present in the water and sediment samples; caffeine residues, which belong to the class of stimulants, Nevirapine residues, which belong to the class of Antiretrovirals, trimethoprim, sulfamethaxazole, sulfamethaxine tetracycline, Amoxil all these belongs to the class of antibiotics.

4.2.1. Physicochemical Parameters

Table 5 shows mean and standard values of physico-chemical characteristics of water sample from study sites. There were significant differences between physicochemical parameters in various water samples from different sites ($F = (P < 0.01)$). The values of pH, salinity, TDS, EC and COD from water samples from different sites were within acceptable WHO standards whereas turbidity was above the WHO acceptable standards (Table 5).

Table 5. Physicochemical Parameters of Water Samples and WHO Standards

Site	N	pH	Salinity	Turbidity	TDS	EC	COD
		ppt	NTU		S/m		mg/O ₂ l
Kiwasco	89	8.50±0.23	0.11±0.04	6.93±2.02	184.42±2.74	368.45±11.60	520.60±39.40
Nyando	89	7.06±0.05	0.10±0.01	7.29±1.29	182.87±7.02	365.21±3.27	381.50±4.92
Kisat water	89	7.04±0.09	0.04±0.02	6.67±1.28	299.20±1.36	604.00±4.17	272.33±52.54
Lake water	89	7.07±0.38	0.02±0.01	7.12±0.17	41.9±1.46	84.43±0.67	321.00±6.76
Nyamasaria	89	7.66±0.07	0.21±0.04	11.06±1.54	266.53±28.6	535.84±11.40	397.25±6.64
6							
WHO		6.5-8.5	>0.5ppt	>5NTU	>500	>1000S/m	<5mg/O₂l
STDS							

4.2 .2. Taxonomic Classification of Microbial Communities

Analysis using the Krona chart revealed that bacteria were the most dominant group at 95% followed by Eukaryotes and Archaea at 0.03% and 0.01% respectively in the Winam Gulf. The unclassified microbes accounted for 0.3% while the unknown microorganism were at 4% of the community (Figure 4). The relative abundance of annotated taxa of bacteria phyla revealed *Proteobacteria* was highly abundance at 75%, *Bacteriodes* enriched at 15% and *Verrucomicrobia* was least abundance at 2% (Figure 5)

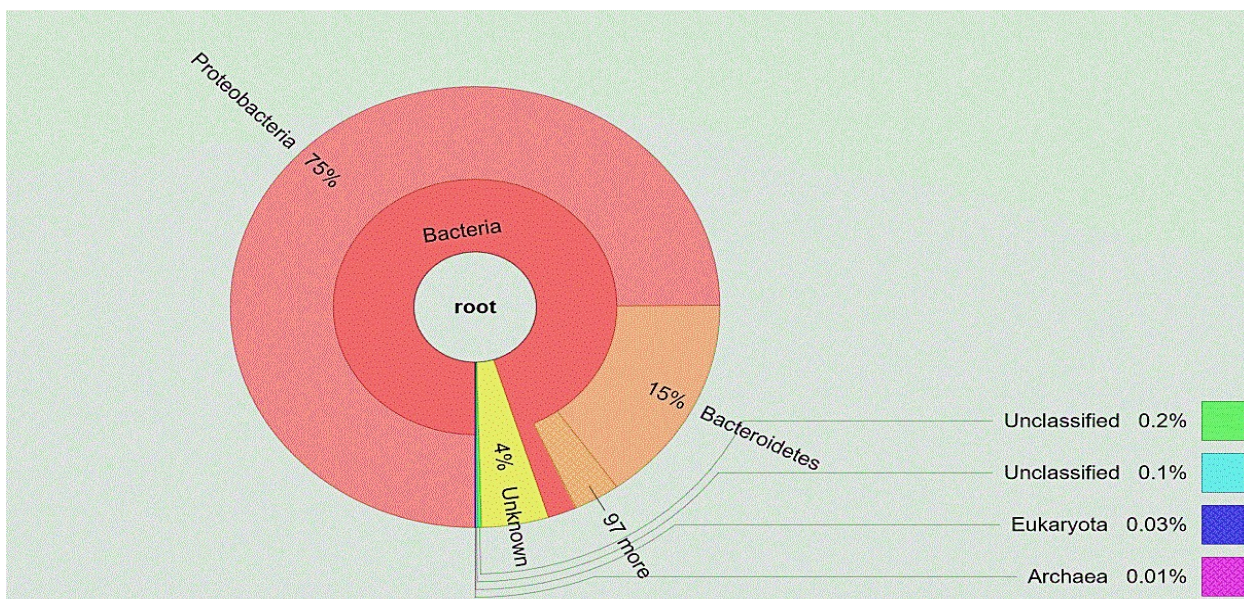


Figure 5. Relative abundance of bacteria in water and sediment samples

Betaproteobacteria (36.7%) was the most abundant class and *Cytophagia* (0.3%) was the least abundance (Figure 6A). On the other hand, indicates that *Comamonadaceae* (16.4%), was found to be the most abundance family and the least abundance was *Sphingomonadaceae* (1%) (Figure 6B).

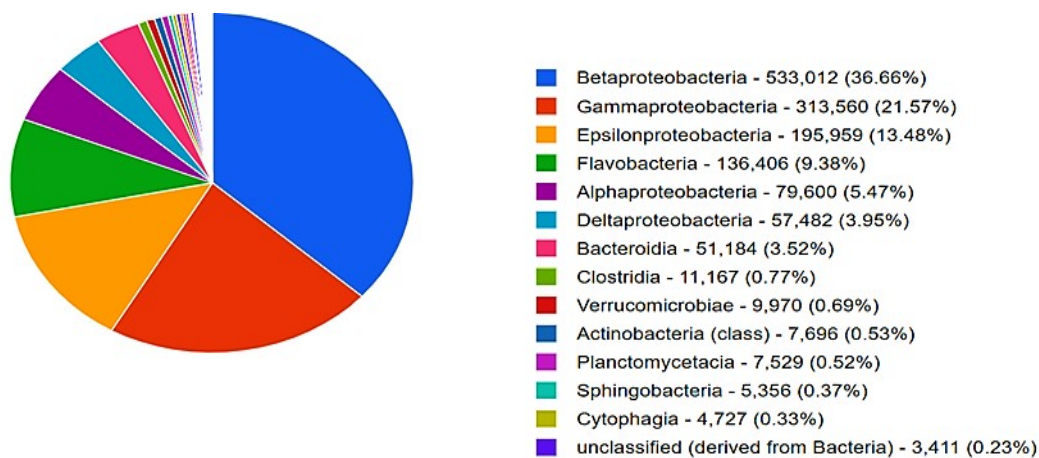


Figure 6. (A) Frequencies of bacteria taxa class of the bacteria in the sample

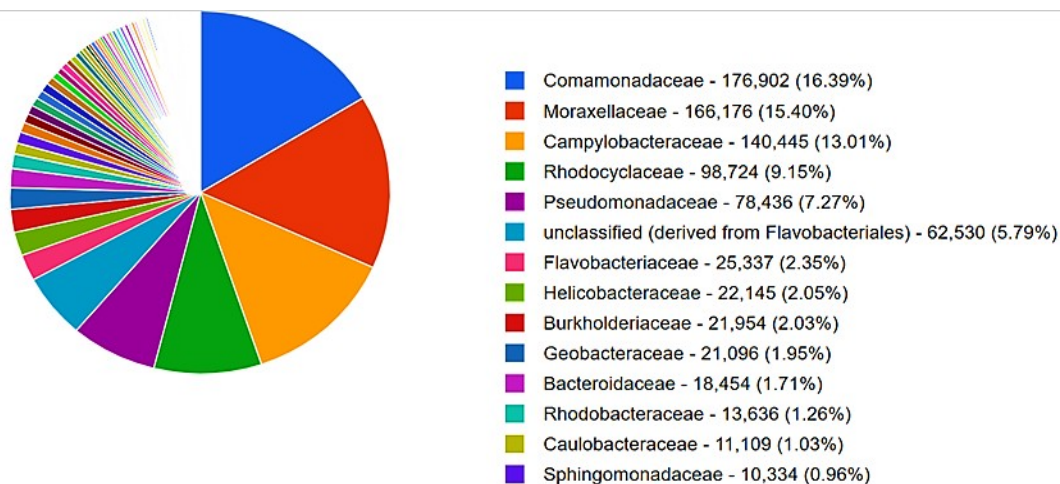


Figure 7. (B) Frequencies of bacteria taxa family of the bacteria in the sample

At the order level, *Burkholderiales* (24.7 %) was the most relatively abundant order followed by *Pseudomonadales* (19.2%), *Campylobacterales* (13.8%) and *Rhodocyclales* (7.7%), *Bacteroidales*, (4%), *Sphingomonadales* (1.7%), *Desulfuromonadales* (1.1%), *Rhodobacterales* (1.30%) and the least abundance was *Thiotrichales* (1. %) ((Figure 7).

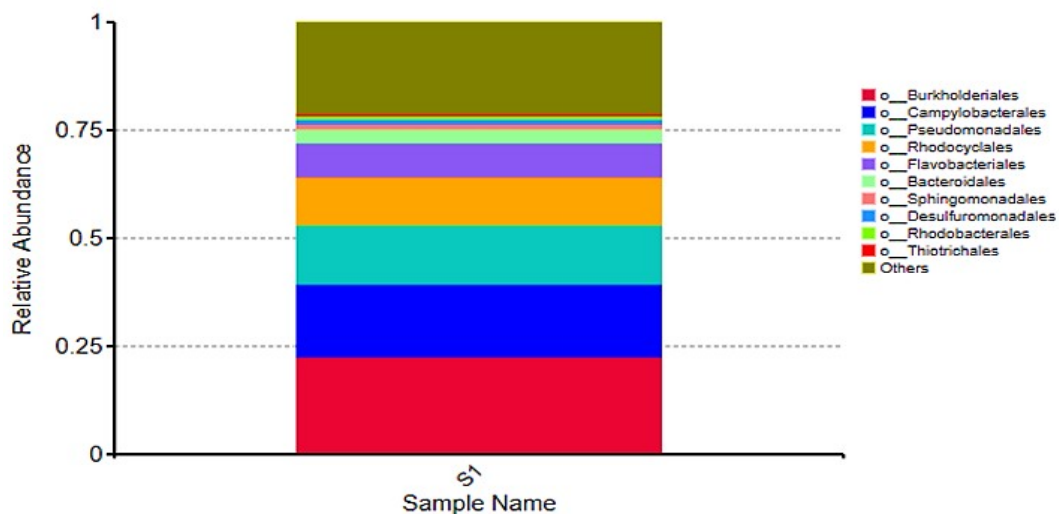


Figure 8. Relative abundance of bacteria taxa at order level. X-axis represents the sample name l and Y-axis represents the relative abundance.

At the genus level, *Acinetobacter* (18.9%) was the most abundant genus, followed by *Arcobacter* (14.1%), unclassified derived from *Flavobacteriales* (*Flavobacterium*) (7.3%), *Dechloromonas* (6.6%), *Pseudomonas* (5.5%), *Geobacter* (2.5%), *Thauera* (2.3%), *Acidovorax* (2.2%), *Sulfuricurvum* (2.2%), *Bacteroides* (2.2%), *Polaromonas* (1.5%), *Burkholderia* (1.1%), *Desulfovibrio* (1%.) and the least abundance was *Verrucomicrobium* (0.8%) (Figure 8).

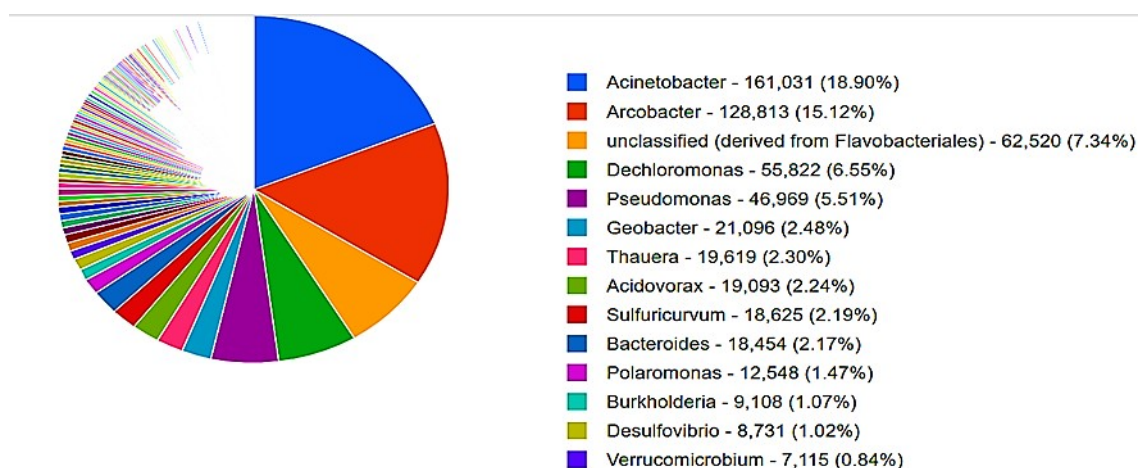


Figure 9. Relative abundance of bacteria taxa at genus level; taxonomic category based on shotgun metagenomics datasets showing the percentages of the bacteria in the sample from Winam Gulf Kisumu

At the species level, *Arcobacter spp* (6%) was the most relative abundant species, followed by *Burkholderiales bacterium* (6%), *Azonexus hydrophilus* (2%), *Flavobacterium sasangense* (2%), *Dechloromonas agitata* (2%), *Arcobacter butzleri* (1%), *Comamonas aquatic* (1%) *Flavobacterium cucumis* (1%), *Cloacibacterium normanemse* (1%) and the least abundance was *Giesbergeria annulus* (1%) ((Figure 9).

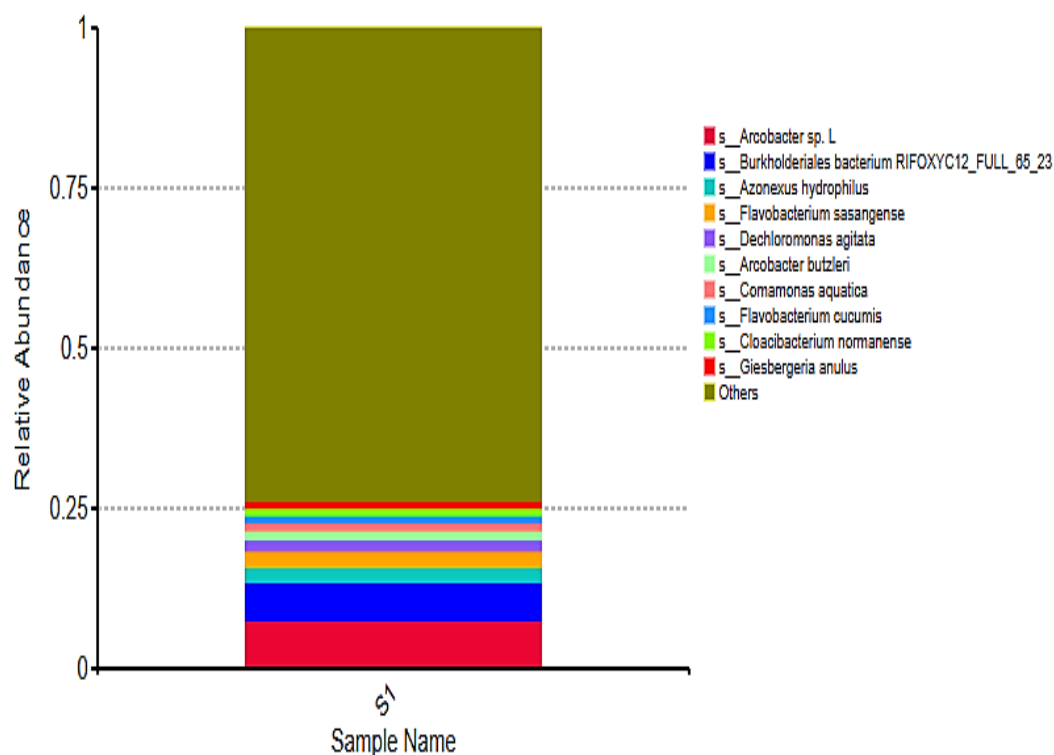


Figure 10. Relative abundance of bacteria taxa at species level; taxonomic category based on shotgun metagenomics datasets showing the percentages of bacteria in the sample from Winam Gulf Kisumu.

4.2.2.1 Functional Annotation: Carbohydrate Metabolism

Glycoside hydrolases displayed highest number of matched genes (≥ 4000), followed by Glycosyl transferases with 3800 number of matched genes (Figure 10). Carbohydrate-binding modules matched 1500 genes while Carbohydrate esterase matched approximately 600 genes. Auxiliary activities and polysaccharide lyases matched genes less than 500.

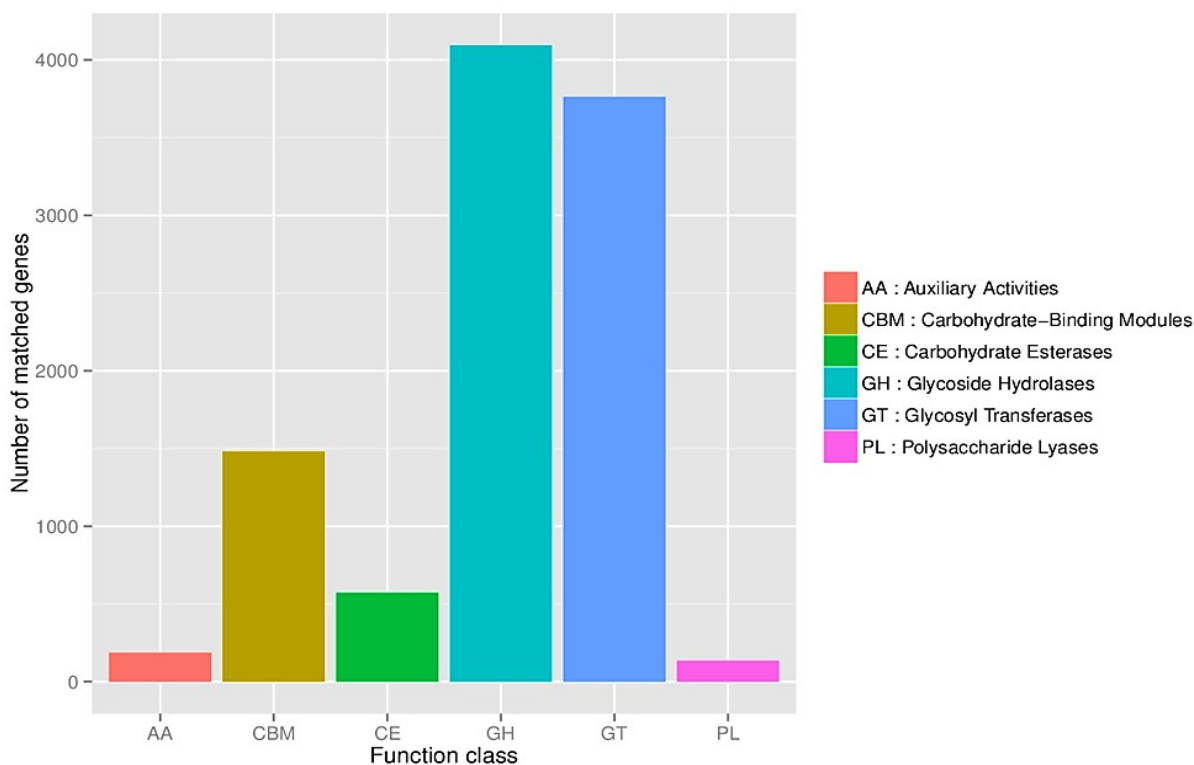


Figure 11. Predicted functional groups of operating taxonomic units based on CAZY enzymes based on shotgun metagenomics data set for fresh water and sediments. The Glycoside Hydrolases and Glycosyl Transferases were among the highest relative abundance genes.

4.2.2.2 MG-RAST Functional Annotation

Results of the Metagenome Rapid Annotation using Subsystem Technology revealed that the most abundant subsystem was clustering-based, which accounted for 14.1% of the total, followed by carbohydrates at 10.8% and amino acids at 10.02%. Other notable subsystems included miscellaneous (7.5%), protein metabolism (7.3%), and co-factors vitamins being the least abundance at (5.86%) (Figure 11).

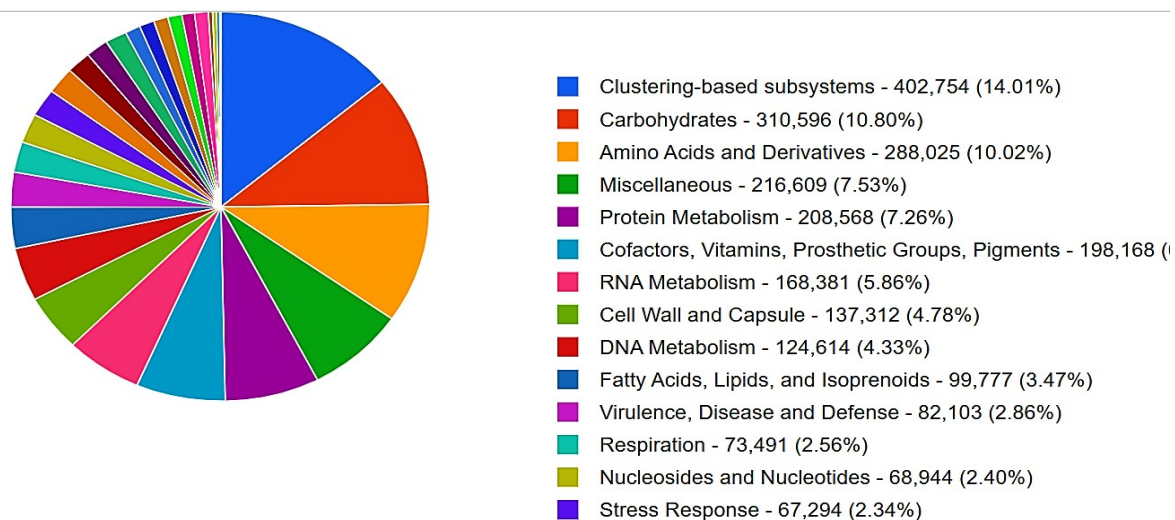


Figure 12. Relative abundance of MG-RAST Functional category hits distribution annotated Subsystem's classification based on shotgun metagenomics data set for fresh water and sediments.

4.2.2.3 EGGNOG Functional Classification

Analysis of the Orthologous Groups (OGs) of proteins revealed that the genes for unknown functions had highest relative abundance with more than 70,000 matched genes, followed by Amino acid derivatives and metabolism genes, replication, recombination and repair, energy production, conversion while among the least was RNA processing and modification, chromatin structure and dynamics and cytoskeleton (Figure 12).

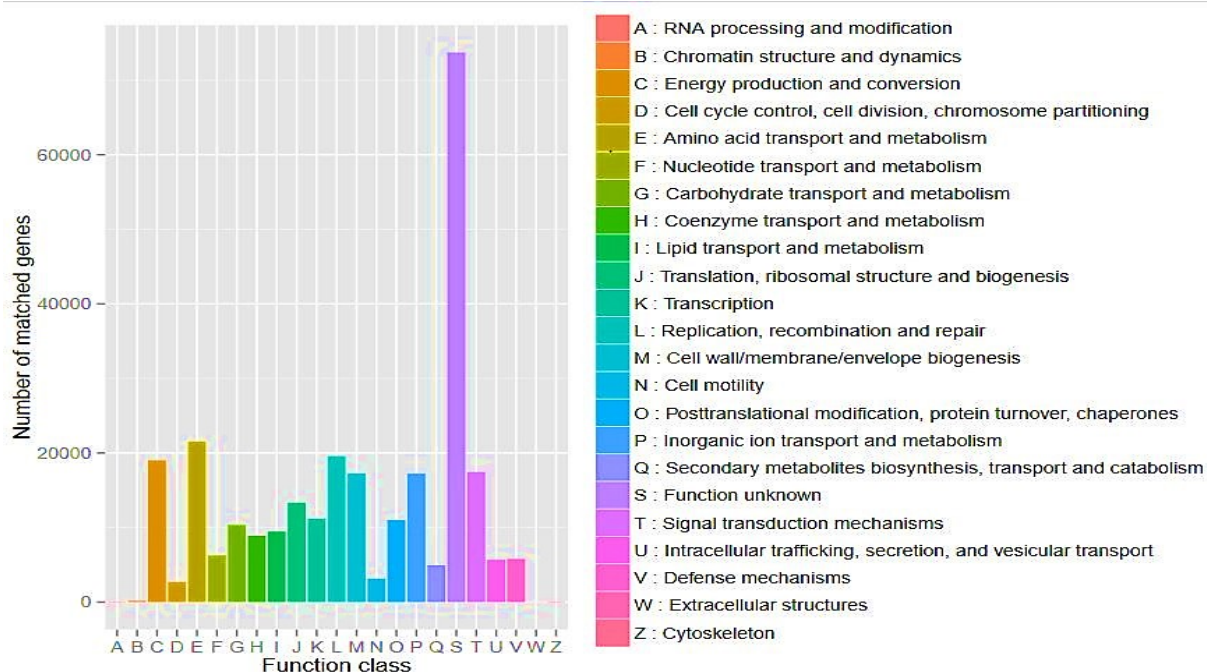


Figure 13. Relative abundance of eggNOG, Orthologous Groups (OGs) of proteins at different taxonomic levels; functional categories based on shotgun metagenomics database for fresh water and sediments.

4.2.2.4 Functional Annotation KEGG (Kyoto Encyclopedia of Genes and Genomes)

Figure 13 show that 594,646 genes involved in metabolism activities (57.8 %) had the highest abundance followed by 191,290 genes involved in genetic information processes at (18.6%), 156,580 genes involved environmental information processes (15.22 %), 68,939 genes for cellular process (7%), 12,969 genes involved in human diseases 1.26 % and the least abundant were genes for organismal systems (4,137 genes) at 0.40 %.

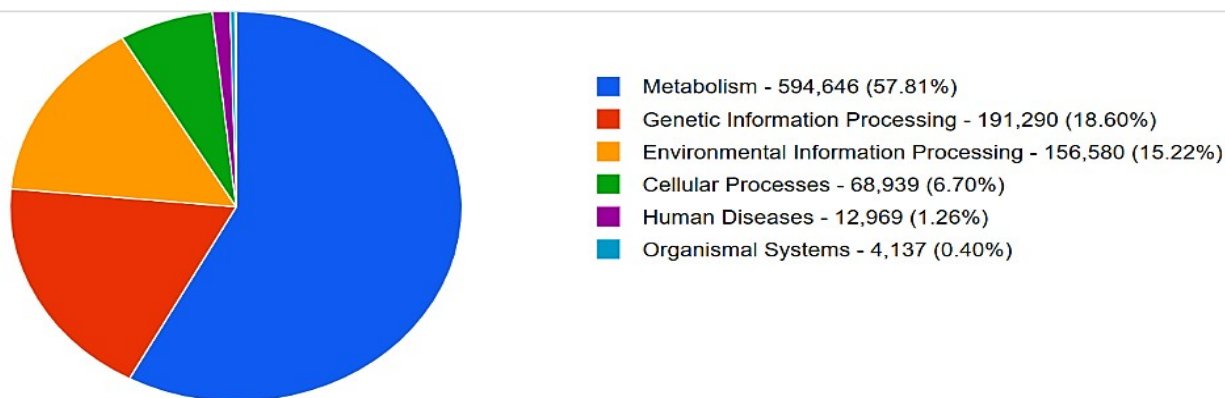


Figure 14. Relative abundance of KEGG level functional categories based on shotgun metagenomics dataset for fresh waters and sediments showing the percentages and genes.

4.3 Phenotypic and Genotypic Antibiotic Resistance

4.3.1 Genotypic Antibiotic resistance gene profiles

A total of 21 antibiotic resistant genes were detected in the sediments and water samples from Winam Gulf of Lake Kenya (Figure 14). The genes Sul 1, sul2, APH6-1d, msrE, APH3-1b dominated.

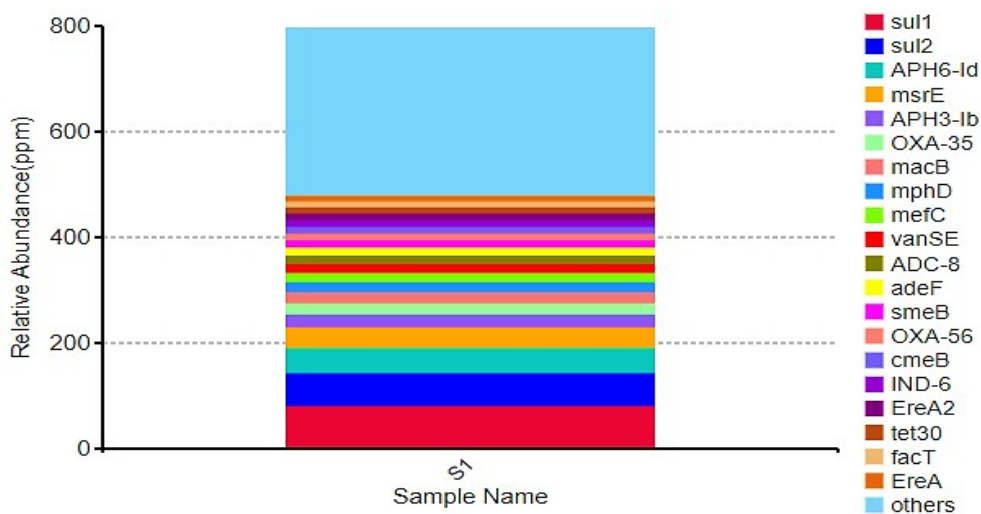


Figure 15. Relative abundance of antibiotic resistance gene

The phyla *Proteobacteria*(75%), *Bacteriodes* (15%), *Verrucomicrobia* (2%) and others, 8%. *Planctomycetes*, *Fermicutes*, and *Chloroflexi* were less than 1%. The relative abundance of antibiotic resistance genes present in each phylum were; *Proteobacteria* 53%, *Bacteriodes* 4% other 39%, *Verrumicrobia* 2%, *Planctomycetes*, *Firmicutes* and *Chloroflexi* remaining 2% (Figure 15).

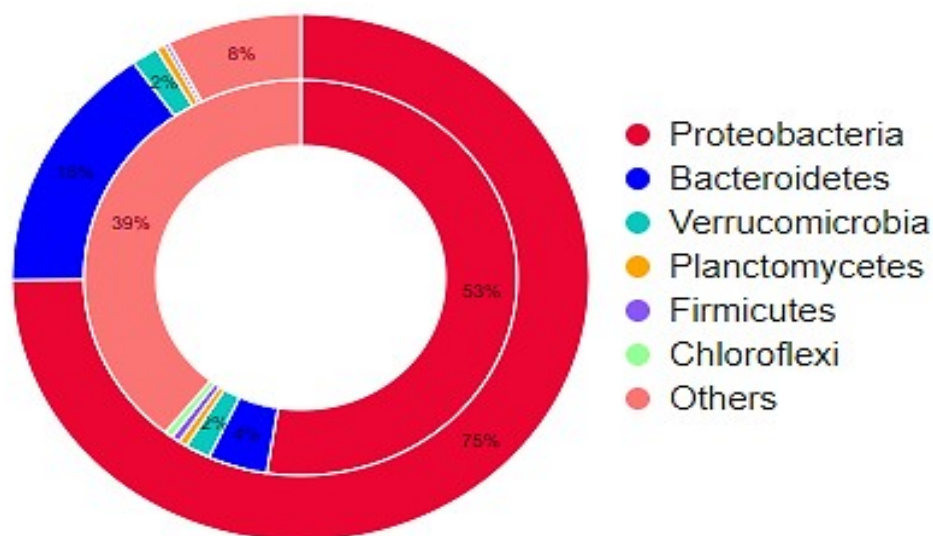


Figure 16. Donut chart (inner circle represents the proportion of different phyla hosting the ARGs and the outer ring is proportion of genes in different phyla of the same group in the Winam Gulf Kisumu

4.3.2 Mode of Action of Antibiotic Resistance Genes

The following modes of action were demonstrated by the antibiotic resistance genes: antibiotic efflux, antibiotic inactivation, antibiotic target change, antibiotic target replacement, and antibiotic target protection. The *Proteobacteria*, *Bactrioides*, *Verrucomicrobia* showed antibiotic efflux. Antibiotic inactivation was present in *Proteobacteria*, *Bactrioides* and *Verrucomicrobia* *Planctomycetes*. Antibiotic target changes in *Proteobacteria*, *Bactrioides*, *Verrucomicrobia* *Planctomycetes*, and *Chloflexi*. Antibiotic target replacement, and antibiotic target protection was seen in all the phyla present (Figure 16).

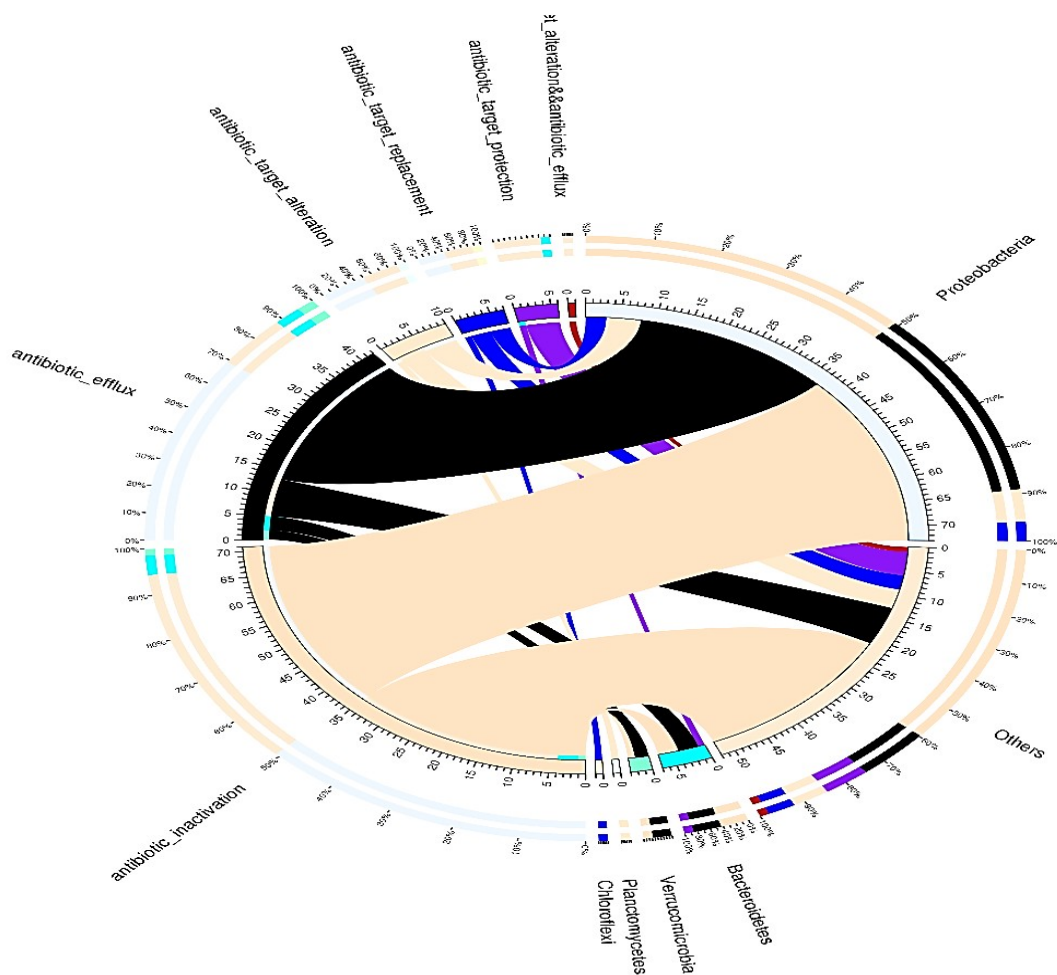


Figure 17. overview circle charts showing the antibiotic resistance genes. The right side of the circle sample information and the left side of the circle is antibiotic information. Inner, different antibiotics; the scale represents the relative abundance, and the unit is ppm. The left part represents the sum of relative abundance of different samples in ARO, the right part versa. Outer circle, the left part represents the relative percentage composition of different samples in ARO, the right part versa.

4.3.3. Phenotypic Antibiotic-Resistant Patterns

Antibiotic resistance was observed in the commonly used antibiotics in clinical set up (Figure 17). Plate 1 (gram -ve) bacteria showed resistance to Ampicillin, penicillin, Imepen and sulfamethoxazole, Plate 2 (gram +ve) bacteria exhibited resistance in Piperaccillin, Meropenem vancomycin, Plate 3 (gram-ve) resistance was observed in Imepem,

Tetracycline, ceflazidime and Erythromycin, plate 5 (gram -ve) showed resistance to piperacillin/tozabactam and trimethoprim and Plate 4 did not reveal any drug resistance.

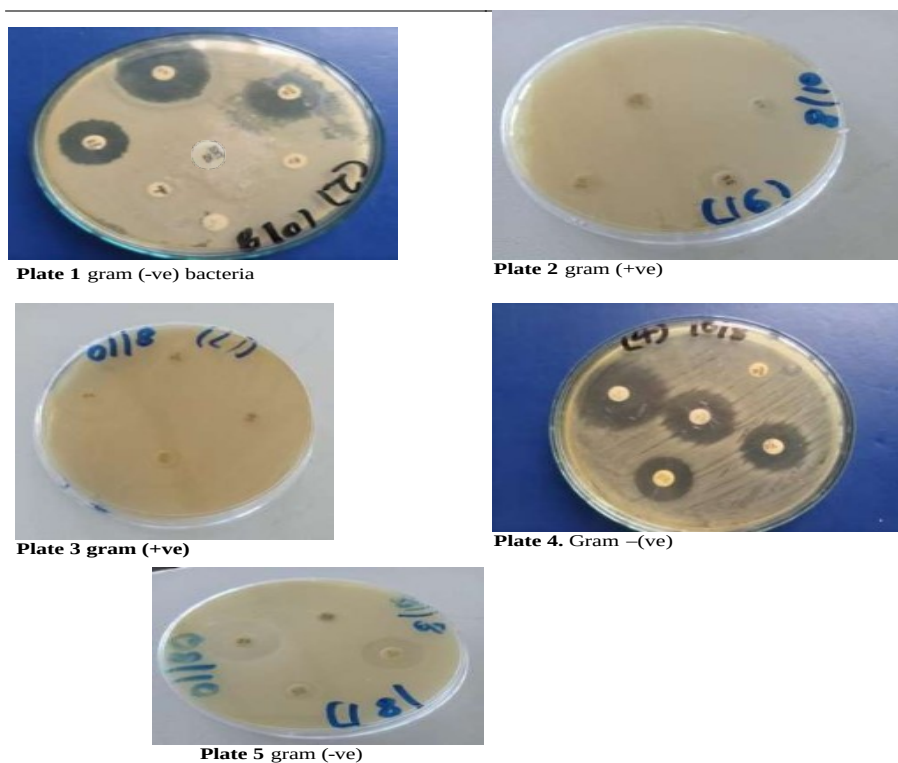


Figure 18. The antibiotic resistance patterns of gram negative and positive bacteria

CHAPTER FIVE

DISCUSSION

5.1 Introduction

This chapter outlines a comprehensive analysis of the study's findings and their implications, taking into consideration the existing literature in the field. The results are interpreted to provide a deep understanding of their significance. The practical implications of the findings explore the potential applications.

5.2 Heavy Metals in Water and Sediment Samples

The results clearly show the levels of concentration of heavy metals in the various samples. The Means of Nickel, copper cadmium and zinc were higher in lake sediments than water samples while Aluminum and lead were higher in lake water samples than sediments. This was attributed to the sediments acting as a sink effectively accumulating them over time ,also the benthic organisms plays a role in bioaccumulation of heavy metals upon there death its deposited in the sediments (Topaldemir et al. 2023). The findings on Nickel, copper cadmium and zinc higher in sediments than water samples agrees with previous studies in Mangla dam Lake (Muneer et al. 2022). Samples from Kiwasco showed that heavy metals concentration were higher in the water samples than sediments this is because in wastewater treatment plants heavy metals tend to mix uniformly in water, and tent to accumulate in sediment and settle in solid form, which are removed through sedimentation and infiltration (Pratap, Mani, and Prasad 2020). The findings of this study agrees with the findings of previous studies indicating that the levels of heavy metals in water samples from wastewater treatment plants are higher than those found in sediment samples (El-Magd et al. 2021). In River Nyando sediments and water samples showed little differences in heavy metal concentration this agrees with previous studies on river Isiukhu (Oremo et

al. 2019). Samples from Kisa showed that heavy metals concentration were higher in water samples than sediments agreeing with previous studies on rivers receiving from wastewater treatment plants (Swana et al. 2020).

Nickel, lead, and cadmium concentration exceeded the WHO acceptable standards at all study sites, findings agrees with previous studies that documented similar findings in Kisha city in Iran (SeyyediBidgoli et al. 2022). The concentration of nickel lead and cadmium concentration above WHO recommended standards points to a potential health hazard to human and aquatic biodiversity (El-Magd et al. 2021). Nickel, Cadmium and lead have been reported to directly harm aquatic organisms due to their chemical toxicity, which disrupt the functioning of vital biological process (Singh et al. 2022). In addition, the presence of these heavy metals impairs the respiratory, reproductive, and immune system of aquatic organisms leading to reduced population sizes and biodiversity (Kataoka and Kashiwada 2021). Studies have shown that the exposure to heavy metals such as Nickel, Cadmium in aquatic environment contributes to development of antibiotic resistance through expression of antibiotic resistance genes in bacteria (Cheng et al. 2019). This phenomena may lead to the up and down regulating of specific genes involved in resistance mechanism further promoting antibiotic resistance in aquatic bacteria (Lu et al. 2020).

Heavy metals pollutants in Lake Victoria originate from both natural and anthropogenic activities, and anthropogenic activities takes the highest sources of heavy metals pollution in lake (Baguma et al. 2022). Inadequate wastewater treatment and improper disposal practices in industrial wastes increase heavy metal contamination in the lake (Qayoom et al. 2022). Excessive fertilizers from agricultural activities around the lake is also another

source of heavy metals contamination in Lake Victoria (Githaiga et al. 2021). Illegal and unregulated mining operations in the Lake Victoria catchment have been associated with the release of high levels of cadmium, and lead in the lake ecosystem (Nassali et al. 2020).

5.2 Pharmaceutical Residues in Water and Sediment Samples

Results of pharmaceutically analyses revealed the presence of pharmaceutical compounds namely, sulfamethoxazole, trimethoprim, caffeine, tetracycline, Amoxil, Artemether and Neverapine in the aquatic ecosystem of Winam Gulf. These findings were comparable to previous studies with similar pharmaceutical residues in aquatic ecosystem in sub-Saharan Africa (Ripanda et al. 2021). Pharmaceutical pollutants are disposed in aquatic ecosystems through multiple sources (Narayanan et al. 2022). Improper pharmaceutical disposal, agricultural runoff, inadequate urban waste management, and ineffective wastewater treatment have been identified as the main sources of pharmaceutical residue pollution in aquatic habitats (Ngqwala and Muchesa 2020).

The pollution caused by sulfamethoxazole, trimethoprim, caffeine, tetracycline, Amoxil, Artemether and Neverapine has far-reaching detrimental effects on both aquatic microbes and human health (Samal, Mahapatra, and Ali 2022). These pollutants are commonly found in wastewaters and once they enter aquatic ecosystem their effect can be devastating, ranging from altering microbial communities to causing serious health problems such as antibiotic resistance (Kulik, Lenart-Boroń, and Wyrzykowska 2023). Aquatic microbes are utmost importance in maintaining healthy aquatic ecosystems, as they are responsible for nutrient cycling and play crucial role in the food web (Arias-Andres, Rojas-Jimenez, and Grossart 2019). However, pollutants like sulfamethazine, trimethoprim, caffeine and neverapine can negatively affect their survival and function of aquatic microbes (Bala et al.

2022). These pollutants can alter microbial community structure. Furthermore, they can cause antibiotic resistance among environmental microbes thus making it difficult to treat bacterial infections (Abebe 2020).

The antimalarial medication called Artemether is commonly used to treat malaria. When it gets into the water bodies due to inappropriate disposal or runoff from pharmaceutical companies and poor disposal of medicines (Ripanda et al. 2021). Artemether is known to have harmful effects on fish and amphibians, including death, physical and behavioral abnormalities, and reproductive problems (Abdel Salam et al. 2021). Artemether has been shown to bioaccumulate over time in species, increasing concentrations at higher trophic levels (Daniel et al. 2024). Human health may be at risk from continuous bioaccumulation to artemisinin in the environment (Gwenzi et al. 2022).

The finding of this study on pharmaceutical residues and heavy metals pollutants may have the potential to disrupt aquatic ecosystems by impacting the growth, survival, and biodiversity of aquatic organisms (Thanigaivel et al. 2023). This disruption can alter the food chain, influence the carbon cycle, and contribute to climate change (Mehmood et al. 2020) . The accumulation and bioaccumulation of heavy metals in sediments and organisms can lead to health problems for both humans and wildlife, as well as impact water quality, oxygen levels, and greenhouse gas emissions (Sonone et al. 2020b). These effects can also influence the water cycle, precipitation patterns, and extreme weather events in the region, thereby exacerbating climate change and affecting the health of Lake Victoria and surrounding ecosystems (Luhunga and Songoro 2020) .

Heavy metals and pharmaceutical pollutants can have harmful effects on fish populations and biodiversity in aquatic ecosystems (Malik et al. 2020) . These contaminants, such as lead, mercury, cadmium, antibiotics, and hormone disruptors, can accumulate in fish tissues, leading to toxicity, altered behavior, and reduced reproductive success (Samuel et al. 2023) . Additionally, they can bioaccumulate in the food chain, impacting larger predatory fish and ultimately affecting the entire ecosystem (Saidon et al. 2024). The presence of these pollutants can result in reduced biodiversity, economic burdens on fishing industries, and long-term consequences for aquatic ecosystems (Ogidi and Akpan 2022). It is crucial for regulatory agencies and stakeholders to take action to reduce the release of these contaminants and protect the health of aquatic environments and their inhabitants.

5.3 Physico-chemical Properties of Sampling Sites

Analysis of physical chemical properties of the sampling sites showed that pH ranged from 6.5-8.5 which is often considered as an ideal optimal health of fresh water ecosystems and suitable for the survival of most fresh water organisms (Kandie et al. 2020). This pH is favorable for the survival of diverse plants and animals and for natural breakdown of organic matter and nutrient cycling (Nahhal et al. 2021). This was consistent with the findings of a previous study on Lake Victoria (Bwire et al. 2020). The values of pH, salinity, TDS, EC and COD were within acceptable WHO standards whereas turbidity was above the WHO acceptable standards, consistent with previous studies (Yona et al. 2023). Elevated turbidity in freshwater samples has biological implications such as reduced light penetration, thus affecting photosynthesis in aquatic plants. This phenomenon can disrupt food chain and impact in the overall ecosystem health. Furthermore, excessive turbidity

can also reduce oxygen levels in water, potentially leading to hypoxia and negatively affecting fish and other aquatic organisms.

5.4 Shotgun Metagenomics Analysis on Microbial Assemblages present in the Water and Sediment Samples.

According to the study's findings, which were displayed in pie charts, bar graphs, and Krona charts, bacterial assemblages were the most abundant. A more in-depth analysis revealed that *Proteobacteria* was the most dominant phylum and these findings were consistent with those reported by previous studies in freshwater lakes (Boeraş et al. 2021). *Bacteriodes* was the second most dominant phylum, and the least relative abundance was the *Verrucomicrobia* at 2%; which agree with previous studies reported (Nho et al. 2018). The dominant relative abundance of *Proteobacteria* has been associated with pollution by hydrocarbons in water sediments (Oyetibo et al. 2021) The phylum *Bacteroidetes* is diverse which includes *Flexibacter*, *Cytophaga* and *Bacteroides* (Johnson et al. 2017; Niestępski et al. 2020). The *Bacteroidetes* phylum is made up of four classes: *Flavobacteria*, *Bacteriodes*, *Sphingobacteria* and *Cytophaga*, over 7,000 diverse species (Sebastião et al. 2021). *Bacteroidetes* phylum found in the Winam Gulf included the *Flavobacteria*, and *Bacterioidia*. *Flavobacteria* which is made up many aquatic species, comprise species that include opportunistic human pathogens as well as genes associated with antibiotic resistance (Sebastião et al., 2021). *Bacteroidetes* species have economic impacts on freshwater fish, which causes infections that may have negative effects on wild and farmed fish (Grande Burgos et al., 2018; Pękala-Safińska, 2018). *Bacteroidetes* are naturally found in the human gut therefore their presence in the fresh water signifies sewage pollution (Niestępski et al. 2020). According to reports, a large incidence of cancer

cases among the local population can be attributed to water contamination in the area surrounding Lake Victoria (Hatta et al. 2021). *Verrucomicrobia* are naturally found in freshwater habitats; however, studies have shown that their relative abundance decreases in the presence of pollution (Sazykina et al. 2022). This indicates that pollution has the potential to alter the microbial community structure in freshwater ecosystem (Qian et al. 2018). *Betaproteobacteria* have a broad habitat range, spanning from the natural aquatic environments to human pathogens: therefore, their presence can indicate pollution in a given ecosystem (Szekeres et al. 2018). *Epsilonproteobacteria* are known to be autotrophic and play an important role in CO₂ fixation in aquatic ecosystem and are prevalent in animal and human digestive tracts as pathogens or symbionts (Dang et al. 2019). *Gammaproteobacteria* are widely distributed and abundant in various ecosystems such as soils, fresh water lakes and rivers (Tian et al. 2021). The findings in relation to family were as *Commamonadaceae* followed by *Moraxellaceae* then *Campilobacteriaceae*, *Rhodocycliaceae*, *Pseudomonaceae*, *Flavobacteriaceae*, among others. *Campylobacteriaceae* naturally inhabit birds and warm-blooded animals thus may find their way to fresh water systems through animal waste pollution. *Commamonadaceae*, *Pseudomonaceae*, which make up a major group of human pathogens may find their way in water systems through fecal contamination (Shrestha 2019).

Burkholderiales was the most abundant order; and naturally occurs in soils and water and is known to infect both humans and animals by spreading zoonotic illnesses and respiratory disorders through contaminated water. It has also been known to possess antibiotic resistant genes and not easy to be removed from the environment (Bombaywala, Purohit, and Dafale 2021; Magana-Arachchi and Wanigatunge 2020). *Rhodocycliales*, an abundant

bacteria order in wastewater treatment plants in denitrification (Wang *et al.*, 2018). *Pseudomonas* is known to inhabit soils, water and vegetation but also pathogenic to humans (Kumar *et al.*, 2021). *Pseudomonas spp* have been proved to possess high level of intrinsic resistance to antibiotic through restricted outer membrane permeability thus pump antibiotic out of the cell and producing enzymes such as β -lactamase (Abdul Hussain and Mshachal 2022). Analysis of samples from aquatic ecosystems of the Winam Gulf Kisumu has revealed the presence of taxonomic diversity, indicating the existence of bacteria with antibiotic resistance capabilities. The unclassified group of *Burkholderiales bacterium* exhibits resistance to antibiotics and represented a significant proportion of antibiotic-resistance bacteria. This phenomenon raises concerns regarding the potential risk to public health. Studies have shown that *Acinebacter* presents moderate to highly resistant to several groups of antibiotics such as Fluoroquinolones, Aminoglycoside, tetracycline, and other classes of antibiotics (Abdi et al. 2020) . *Sulfuricurvum*, a group of bacteria that metabolises sulfur, was the least abundance within the microbial community (Saghatelian et al. 2021).

Results of functional annotation through four methods; KEGG pathways, CAZY databases, EGGNOG and MG-RAST. By employing KEGG pathways this study was able to demonstrate the relationship between metabolic and genomic network, by highlighting how encoded genes contribute to biochemical reactions (Taussat et al. 2020).

The findings of this study revealed the most abundance functional category within KEGG database were related to metabolisms, gene information, environmental formation process, human disease and organismal systems. The abundance of genes associated with

metabolism was higher, consistent with previous studies by Curran (2022). Microbes have to biosynthesize substrate and products to get energy for survival (Park and Kim 2019). Genetic information processing was second dominant genetic information systems of microbes which are responsible to gene transcription, translation, replication, folding sorting and degeneration (Zhang *et al.*, 2020). Environmental information processing genes were the third predicted by the KEGG pathway. Environmental information processing are key due to the interaction of organisms and environment for a period of time results to evolution and diversity (McFall-Ngai and Bosch 2021). The fourth functional genes were for the cellular processes of the microbes which are important in carrying specialized function like providing the body structure, nutrients uptake from food and converting them to energy (Li *et al.*, 2022). KEGG pathway and annotation shows the genes for human diseases was ranging from cancers, metabolic disease, cardiovascular disease, endocrine substance dependence, and drug resistance which may have been triggered by the presence of pollutants (Sendra *et al.* 2021). Understanding the functional capability of these microbial communities is essential for identifying potential sources of human exposure and to develop effective intervention to prevent or treat it (Yan *et al.* 2020). KEGG functional annotation can be used to identify the specific microbial genes and pathways involved in human diseases. Studies have shown certain bacteria e.g. species in aquatic ecosystem can contribute to development of gastrointestinal infections, respiratory infections and other diseases in human (Sehnal *et al.* 2021).

The CAZY pathway has been used to encode carbohydrate enzymes in microbial genome which utilize complex carbohydrates (Cabral *et al.* 2022). According to the analysis, of CAZY database, the most relatively abundance genes was GH followed by GT, CBM, CE, AA and Pl, with the latter two being least abundance. This findings are in agreement with

previous studies that observed similar patterns of gene abundance within the CAZY database (Cabral et al. 2022; Su et al. 2021). The GH enzymes that are primarily involved in metabolism of polysaccharides such as chitin, cellulose, Xylan and starch (McKee et al. 2021). GT enzymes play a crucial role in biosynthesis of carbohydrates and are particularly important for synthesizing of saccharides and facilitating glycosylation of molecules in aquatic environment (Na, Li, and Chen 2021). These roles are important in understanding molecular processes related to carbohydrate metabolism (Mohideen et al. 2021). Results of relative abundance from the CAZY analysis indicated that enzymes involved in carbohydrate metabolism were influenced by the microbial profile and community structure in specific environments. This shows that composition and characteristics of microbial communities has the potential to influence the prevalence and activity of carbohydrate-metabolizing enzymes (S. O. Onyango et al. 2021). These findings highlight the intricate relationship between microbes and the regulation of carbohydrate metabolism in different ecological context (Rowland et al. 2018). Results of MG-RAST functional annotation revealed sets of proteins that share protein-coding sequence derived from assembled contigs. This analysis provided a comprehensive understating of the functional capabilities and potential ecological roles of microorganism present in the samples. Presence of least relative abundance of the stress response enzymes signifies the microbial community structures at the Winam Gulf have adapted to the multi-class polluted ecosystem. The clustering –based subsystem that couples with functional evidence among proteins (Figueroa et al. 2018). EGGNOG (Non –supervised orthologous) functional annotation, of Amino acids sequences that predicts genes aligned with the EGGNOG through BLAST database. Twenty-six eggNOG functional categories were observed through the functional analysis. The metagenomics findings revealed significant

involvement of microbial communities in various essential biological processes including amino acid transport and metabolism, replication, recombination and repair, energy production and conversion, cell wall/membrane/envelop biogenesis, inorganic ion transport and metabolism, Signal transduction and mechanism and lastly, Translation ribosomal structure and biogenesis. These diverse functions categories highlighted the metabolic versatility and adaptability of the microbial communities present in the study environment, displaying their ability to thrive and perform a wide range of important cellular processes. The present of the signal transduction and mechanism which was among the dominant genes in the EGGNOG functional annotation. Pollution suggesting that organisms may have adapted to changing environmental conditions (Yang et al. 2019).

The findings of this study, which involve a shotgun metagenomics analysis of microbial profiles in contaminated freshwater Lake Victoria, would contribute valuable information for mitigating the impact of climate change (Odhiambo et al. 2023). This analysis can provide insights into the diversity and abundance of microbial communities present in the lake (Ahmed et al. 2022) . Understanding these microbial communities is essential for assessing the effects of pollution and environmental stressors on the microbial ecosystem and their implications for climate change (Retter, Karwautz, and Griebler 2021)

Microbial communities play a crucial role in biogeochemical cycles and nutrient cycling in aquatic ecosystems (Shi et al. 2022) . Pollution and human activities can disrupt these processes, thereby affecting ecosystem functioning and contributing to climate change by producing greenhouse gases (Muruganandam et al. 2023). By utilizing shotgun metagenomics analysis, researchers can identify sensitive microbial species that are

impacted by stressors and monitor changes in their populations over time (Michan, Blasco, and Alhama 2021) .

5.5. Antibiotics Resistance among the Microbial Communities in the Aquatic Ecosystem of Winam Gulf Kisumu

The current study identified 21 antibiotic resistance genes consistent with the findings of previous study (S. Zhang et al. 2020). These genes practically identical in quantity to the 26 antibiotic resistance genes discovered in the aquatic ecosystem of Lake Lonar Soda in India (Bortolaia *et al.*, 2020). The study identified antibiotic resistance genes to aminoglycosides and sulfonamides, consistent with the findings from studies by Huang *et al.*, (2019b). This is consistent with research conducted in fresh water environments where Sulfonamides 1 was the major ARGs followed by Sulfonamide 2 (Kokoszka et al. 2021). Long-term antibiotic pollution in aquatic ecosystems may be responsible for the presence of 9 ARGs from the 37 WHO identified high risk AMR gene families, which has led to the evolution of resistance microbial genomes (Jian *et al.*, 2021).

Proteobacteria was the dominant phyla with antibiotic resistance genes, followed by *Bacterioidetes*, *Verrucomicrobia*, *Planctomycetes*, *Chloroflexi* and *Firmicutes* respectively (Shen *et al.*, 2022). *Proteobacteria* have a high ARGs abundance, which is related to their flexible metabolism and capacity for environmental adaptation (Zhou *et al.*, 2020). *Proteobacteria* is a diverse group of bacteria commonly found in aquatic environments (Buijs *et al.*, 2019). Studies have shown that *Proteobacteria* possess high levels of antibiotic resistance genes that enable them to survive in environments polluted with antibiotics such as lakes and rivers (Sharma *et al.*, 2023). This constant exposure to

antibiotics has placed selective pressure that has favoured the survival and proliferation of antibiotic resistance in *Proteobacteria* (Chand *et al.*, 2023). *Proteobacteria* often live in close association with other microorganisms such as algae and protozoa thus allowing inter-species exchange of antibiotic resistance genes (Ijaz, 2022). The high level of antibiotic resistance genes of *Proteobacteria* in aquatic ecosystems is a public health concern as they may contribute to the spread of antibiotic resistance in clinical and environmental set-ups (Crits-Christoph *et al.*, 2022). Examples of human and animal pathogenic *Proteobacteria* found in aquatic ecosystem have been shown to possess antibiotic resistance genes: *Vibrio cholerae*, which inhabit rivers, lakes and coastal waters, have been shown to possess genes that confers antibiotic resistance to multiple antibiotics (Adenaya *et al.*, 2023). *Pseudomonas aeruginosa*, a common opportunistic pathogen that infects humans and animals often found in aquatic environments, has been shown to possess a variety of antibiotic resistance genes (Diggle & Whiteley, 2020). *Proteobacteria* found in aquatic ecosystems including *Escherichia coli*, *Salmonella enterica* and *Klebsiella pneumoniae*, pathogenic to humans, and animals possess genes that confer resistance to multiple antibiotics (Bombaywala *et al.*, 2021).

Due to its several antibiotic resistance mechanisms, *Bacteriodes*, a common gut flora, has been reported to have multi-drug ARGs (McInnes *et al.*, 2020). *Bacteriodes* possess antibiotic resistance genes in aquatic ecosystems due to their great capacity to evolve and develop new ways to survive in different environments (Smith *et al.*, 2023). The findings of antibiotic resistance in this phylum provides insight in understanding the mechanisms of antibiotic resistance. *Bacteriodes* have the ability to quickly produce and spread antibiotic resistance genes in response to changing environments which allows them to continue to

thrive despite the presence of antibiotics (Sharma *et al.*, 2023). Some *Bacterioidetes* species that cause diseases in human and animal are *Bacterioidetes vulgatus*, *Bacterioidetes thetaiotaomicron*, and *Clostridium difficile* are some of the most noxious human pathogens with demonstrable antibiotic resistance to multiple antibiotics thus, a public health concerns (Lindell *et al.*, 2022). *Verrumicrobia*, a gram-negative bacterium, has been shown to have ARGs in water sediments (Yitayew *et al.*, 2022). A few species of this phylum have been isolated from fresh seawater, soils, and human feces (Meng *et al.*, 2022). Both aquatic and terrestrial *Planctomycetes* can be found, with *Chloroflexi* inhabiting deep freshwater lakes and *Firmicutes* occupying a variety of environments, including commensals on skin surfaces (Gupta *et al.*, 2021). The Winam Gulf revealed the least antibiotic resistance genes in *Planctomycetes*, *Chloroflexi*, and *Firmicutes*, which is consistent with studies by Fu *et al.*, (2023) in aquatic environments. These microbes possess the least antibiotic resistance genes compared to other bacterial phyla groups because they have evolved other mechanisms to survive in their environment that do not involve antibiotic resistance (Rajasekar *et al.*, 2023). *Planctomycetes* have been shown to have the ability to metabolize complex organic compounds; such as proteins, lipids and polysaccharides which may provide them with a competitive advantage over other bacteria (Chen *et al.*, 2020). Additionally, *Chloroflexi* and *Fermicutes* have been shown to be highly adaptable to changing environmental conditions, thus contributing to the ability to survive in all habitats (Liu *et al.*, 2023).

Genes for additional, unidentified antibiotic resistance were also very abundant; this may be attributed to mutation brought about by the severe circumstances induced by multiple classes of contaminants (Feng *et al.*, 2020). The genetic diversity of free-living microbes

has led to the existence of diverse natural habitats, which have become focal points for the development and dissemination antibiotic resistance genes and pathogenic bacteria. These habitats serve as hotspots for the evolution and propagation of resistance mechanisms, contributing to the global concern of antibiotic resistance (Li *et al.*, 2022). As a defense strategy, aquatic bacteria naturally produce ARGs. These genes can be multi-class pollutant-mediated, which is prevalent in aquatic ecosystems. The Winam Gulf's main antibiotic resistance mechanism was antibiotic efflux, followed by drug inactivation and target modification, which agrees with the findings of a previous study conducted in freshwater habitat (Wang *et al.*, 2020). Antibiotic resistance genes that can develop because of chronic exposure to multiple classes of contaminants, such as pharmaceuticals, agrochemicals, and heavy metals, may also be present in the antibiotic resistance genome (Perković *et al.*, 2022). For instance a study conducted in Baltic Sea found that antibiotic resistant genes in bacterial community were acquired due to human derived pollutants (Xue *et al.* 2023) . Another study conducted in the Mediterranean Sea found that antibiotic resistance genes were present in bacterial communities of the sea, and were likely acquired through chronic exposure to pharmaceuticals, heavy metals and other pollutants (Sabatino *et al.* 2020). The efflux pump's capability to recognize a wide range of substrate expressed by multiple pathogens makes antibiotic efflux the dominant for bacterial resistance acquisition (Gaurav *et al.* 2023). Various gram-negative biofilm bacteria, especially those in aquatic biofilms have been documented to possess resistance mechanisms involving efflux pumps (Pompilio *et al.* 2021). This highlighted the significance of studying and understanding the efflux phenomenon in these bacteria to combat antibiotic resistance effectively (Reza, Sutton, and Rahman 2019; Zuo and Suo 2022). The antibiotic efflux was first identified in the tetracycline resistance of *E. coli*, as well as in the multidrug resistance

efflux (Lamut *et al.*, 2019). Bacteria that produce B-lactamases, aminoglycoside enzymes, and chloramphenicol acetyltransferases have been found to exhibit antibiotic inactivation resistance (Nadeem *et al.*, 2020). Resistance to penicillin, cephalosporins, carbapenem, amoxil, and erythromycin, among other antibiotics, has been linked to antibiotic inactivation (Aurilio *et al.*, 2022). Small modifications to the target molecules and entire bacterial cells have been used to alter antibiotic targets in bacteria, thereby preventing the antibiotics from reaching the intended target (Breijyeh *et al.*, 2020). Selection in the presence of the antibiotic and spontaneous mutation of the bacterial gene on the chromosome cause the target position to alter (Montserrat-Martinez *et al.*, 2019). Pathogens are increasingly modifying antibiotics, such as glycopeptide and polymyxin antibiotics, by chemically altering the cell wall components. Similar to antibiotics that target the ribosome but hindered by rRNA methylation (Schaenzer & Wright, 2020). The least frequent responses were antibiotic target replacement, antibiotic target protection, antibiotic target modification, and efflux, which was consistent with earlier studies on aquatic environments (Li *et al.*, 2023). Antibiotic resistance in microorganisms has long been demonstrated using phenotypic antibiotic resistance patterns (Hendriksen *et al.*, 2019). Gram-positive and gram-negative bacteria were exposed to a variety of drugs; some were sensitive, whereas others displayed antibiotic resistance patterns (Belete & Saravanan, 2020). (Hayes *et al.*, 2020). The subsequent bacteria showed signs of antibiotic resistance: In both gram-negative and gram-positive bacteria, clindamycin is a broad-spectrum antibiotic that prevents early chain elongation by interfering with protein synthesis (Hayes *et al.*, 2020). Tetracycline prevents bacterial growth by interfering with protein synthesis and damaging the cell membrane. It is broad-spectrum for both gram-negative and positive modes of action. A broad-spectrum antibiotic called

sulfamethoxazole prevents bacterial growth by blocking the manufacture of nucleic acids and proteins by inhibiting the sequential step in bacterial folic acid (Millanao *et al.*, 2021). Vancomycin resistance in enterococci is a very serious threat to humans with few treatment options being available (Guffey & Loll, 2021). Vancomycin works on gram-positive bacteria by blocking the second step of cell wall formation, which prevents bacterial development (Rashid *et al.*, 2021). Pollution is a potential source of resistance for glycopeptide-producing organisms in the presence of genes encoding homologous VanA, VanR, VanS, VanR, VanH, and VanX (Andreo-Vidal *et al.*, 2021).

A key development in our understanding of the molecular and genetic basis of vancomycin resistance is the discovery of the vancomycin-resistant pathway in the bacterial community in the Winam Gulf. This highlights the importance of studying the bacterial communities and their interactions in the spread of antibiotic resistance and thus developing new strategies to combat antibiotic resistance. Beta lactamase resistance in gram-positive bacteria primarily occurs because of alteration of penicillin binding proteins with enzymatic degradation as a minor pathway (Fisher & Mobashery, 2020). This study revealed OprD, OmpF, OmpC, OmpU and PIB was altered by hydrolytic degradation blocking them from binding to penicillin binding proteins leading to the resistance of non-dulation division efflux pump. This mechanism eliminates numerous antibiotic classes A, B, C, and D from inside of bacterial cells, which contributes to multidrug resistance. In Gram-negative bacteria, RND efflux pumps offer intrinsic antibiotic resistance with the potential for cross-resistance to several drugs. The genotypic profiles of antibiotic resistance agree with other investigations and support the phenotypic antibiotic resistance patterns that were reported in previous studies. (Pakbin *et al.*, 2021).

5.6 Shotgun Metagenomics Bioremediation Potential in the Microbial Assemblages in the Aquatic Ecosystem of Winam Gulf Kisumu.

The KEGG functional annotation pathways presented in the metagenomics data, gave an in-depth of specific enzymes and metabolic pathways that were involved in degrading the pollutants (Rout et al. 2022). Xylene, drug and enzyme metabolism, Atrazine, Steroid and nitrotoluene biodegradation were significant KEGG analytical pathways. Xylene is a cyclic hydrocarbon commonly used as a solvent in industries such as dyes, paint, medical technology. However, it possesses environmental risks and is considered unsafe for the environment. In humans and animals, xylene has negative effects on the respiratory, neurological, cardiovascular, and renal systems (Abduljalel and Al-Saadi 2022). The metabolic pathway demonstrated the presence of genes exploited in the biodegradation and bioremediation of this harmful chemical. Nitrotoluene from a manufacturing plant's waste water has been dispersed into the atmosphere and surface water (Bunke et al. 2019). Public health concerns from nitrotoluene include breathing difficulties, skin and eye irritation, coughing, a rapid heartbeat, nausea, vomiting, convulsions, and even death. By employing the local bacteria, the bioremediation processes provide a remedy by eliminating the dangerous chemical from the environment. Drugs like isoniazid that are used to treat tuberculosis and its biodegradation involves series of pathways and genes in microbial communities found in polluted aquatic ecosystem (Magwira, Aneck-Hahn, and Taylor 2019). The breakdown of isoniazid into simpler compounds that can be easily assimilated with microorganisms. KEGG can be used to identify genes and pathways involved in biodegradation of isoniazid (Thamke et al. 2022). Key genes important in biodegradation of isoniazid are *inhA*, *katG*, and *ahpC*, which encode for enzymes responsible for breakdown of antibiotics (Singh et al., 2019). Understanding the genes and

pathway capable of biodegradation of isoniazid can be used to develop strategies in managing the pollution of aquatic ecosystem and mitigating the potential environmental impact of antibiotics on microbes thus reducing antibiotic resistance (Okeke et al. 2022).

Biodegradation of Azathiopurine & 6-mercaptopurine (immunosuppressive drugs class purines and analogs involve pathways and genes are highly depended on specific microorganism present in the polluted aquatic ecosystem (Becker et al. 2023).

Azathiopurine & 6-mercaptopurine, degradation genes encoding for enzymes such as thiopurine S-methyl transferase, xanthine oxidase and aldehyde oxidase have been identified in various microbial communities (Singh and Bhardwaj 2023). These enzymes are involved in the initial steps of the biodegradation process where the compounds are converted into intermediate metabolite that can be further metabolized by downstream enzymes (Pimviriyakul et al. 2020). Pathway and genes involved in the biodegradation of by microbial communities in aquatic ecosystem are carboxylesterase pathway, which involve the hydrolysis of the prodrug irinotecan to its active form SN-38 and the β -glucuronidase pathway, which is responsible for degradation of SN-38. (Pant et al. 2022)

Other genes involved are UDP-glucuronosyltransferase gene and the cytochrome p540 gene. These genes are responsible for the conjugation of SN-38 to inactive metabolite respectively (Sharma, 2020).

Atrazine biodegradation is a complex process that involves the cooperation of multiple microbial communities the genes involved in the process are responsible for encoding enzyme that are necessary for the degradation of atrazine (Pileggi, Pileggi, and Sadowsky 2020). Genes involved in atrazine biodegradation in microbial communities are atzA, atzB, atzC, atzD atzE, atzF, atzG, and atzH. These are responsible for encoding enzymes

involved in aforementioned biodegradation pathway other genes also have been identified and are responsible for encoding enzymes involved in alternative pathway for atrazine degradation into products easily utilized by microbes as source of nutrients (Bhatt et al. 2021). These enzymes play crucial roles in breaking down different components of steroids ultimately leading to their degradation (Han et al. 2023). By comparing, the metagenomics data against the KEGG database to identify specific pathways that were associated with steroid degradation in polluted aquatic ecosystem of Winam Gulf (Thakur et al. 2020). Studying the biodegradation of steroids in polluted aquatic ecosystem is important for several reasons. Steroids, which are often, present in industrial and agricultural effluent, which may have detrimental effects on aquatic organisms and ecosystems (Plessis *et al.*, 2023).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Introduction

This chapter provides an overview of the major findings as concluded from the study and recommendation based on the results for further implementation to curb pollution in the Winam Gulf ecosystem.

6.2 Conclusions

The study sites within the Winam Gulf of Lake Victoria revealed excessive concentrations of heavy metals (nickel, cadmium, lead, and zinc) and pharmaceutical substances (caffeine, trimethoprim, and sulfamethoxazole), which exceed WHO acceptable standards and pose serious risks to aquatic biodiversity and human health.

Shotgun metagenomics analysis of microbial communities revealed a wide range of bacterial assemblages, dominated by the phyla *Proteobacteria* and *Bacteroidetes*, and abundant families and orders, such *Burkholderiales*, *Pseudomonaceae*, and

Campylobacteriaceae, which point to possible contamination and pollution of the Winam gulf.

There is clearly antibiotic resistance in the microbial communities of the Winam Gulf, which explains the significant frequency of antibiotic resistance genes found in dominant phyla, including Proteobacteria and Bacteroidetes phyla. The study also identified several antibiotic resistance mechanisms, including target alteration, efflux, and drug inactivation, which all support multidrug resistance in the bacterial community. There were also unknown antibiotic resistance genes, which demonstrates the complexity of antibiotic resistance in the aquatic environment of the Winam Gulf. For instance, the discovery of ARGs in polluted aquatic ecosystems is a threat to human health and the environment and is relevant to SDG 3 and SDG 6.

Data from metagenomics revealed KEGG functional annotation pathways that shed light on the particular enzymes and metabolic processes involved in the breakdown of several harmful substances, including steroids, nitrotoluene, atrazine, xylene, and pharmaceuticals. These pathways demonstrated the existence of genes involved in the biodegradation and bioremediation of toxic substances found in the polluted aquatic environment of the Winam Gulf. Shotgun metagenomics and KEGG functional annotation provides information for assessing the effectiveness of natural microbial communities in removing pollutants and designing targeted bioremediation strategies.

6.3 Recommendations

- Developing sustainable solutions, such pollution control strategies and partnerships for environmental stewardship, requires close collaboration with stakeholders, local communities, and government agencies.

- To effectively address pollution in aquatic environments, further research is needed into the use of native bacterial communities for bioremediation.
- Implement a comprehensive monitoring and surveillance programs to combat the growing threat and spread of antibiotic resistance in aquatic ecosystems.
- Develop sustainable pollution management solutions in aquatic habitats requires customizing tactics for the targeted degradation of certain pharmaceuticals and other hazardous compounds

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APPENDICES

Appendix 1. Coordinates in Universal Transverse Mercator (UTM)

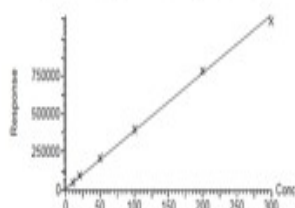
Site Name	Easting (m)	Northing (m)	Location
Coord 1	695021.44	9990768.83	On Kisat River
Coord 2	695041.95	9990805.53	On Kisat River
Coord 3	695292.15	9990885.55	On Kisat River
Coord 4	697142.34	9989179.06	Stormwater
Coord 5	695293.26	9987900.89	Stormwater
Coord 6	694588.07	9990444.19	In Lake Victoria
Coord 7	695101.12	9989950.29	In Lake Victoria
Coord 8	697948.19	9988145.57	On Waigwa River
Coord 9	697495.98	9987143.58	Kiwasco Outlet
Coord 10	699004.77	9987553.38	Stormwater
Coord 11	701749.26	9986846.43	Stormwater

Coord 12	713574.33	9980978.86	On Nyando River
Coord 13	713691.33	9980855.95	On Nyando River
Coord 14	707222.92	9966758.02	On Nyando River
Coord 15	693520.54	9983875.81	On Nyamasaria River
Coord 16	694262.00	9990245.00	In Lake Victoria
Coord 17	694490.00	9989777.00	In Lake Victoria
Coord 18	693660.00	9989999.00	In Lake Victoria
Coord 19	694399.00	9989296.00	In Lake Victoria
Coord 20	694953.00	9989745.00	In Lake Victoria
Coord 21	693878.00	9989606.00	In Lake Victoria
Coord 22	694154.00	9988933.00	In Lake Victoria
Coord 23	693338.00	9989711.00	In Lake Victoria
Coord 24	693415.00	9989258.00	In Lake Victoria
Coord 25	693677.00	9988651.00	In Lake Victoria
Coord 26	693367.00	9988909.00	In Lake Victoria
Coord 27	693072.00	9989401.00	In Lake Victoria
Coord 28	693885.00	9989355.00	In Lake Victoria
Coord 29	693367.00	9988909.00	In Lake Victoria
Coord 30	693004.00	9988677.00	In Lake Victoria
Coord 31	693499.00	9988294.00	In Lake Victoria

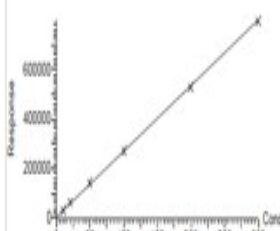
Appendix 2. The standard curves.

Method: C:\New Folder\MassLynx\2022_NEW_PROJECT.P1
Calibration: 24 May 2023 14:09:26

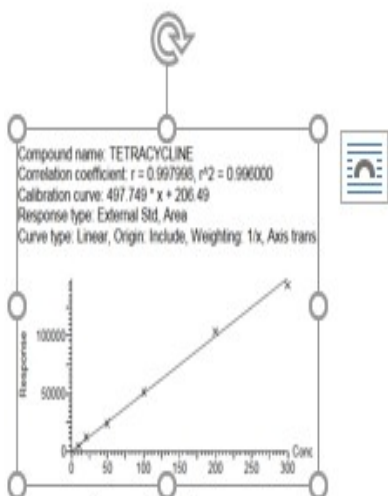
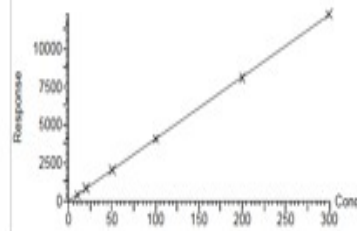
Compound name: CAFFEINE
Correlation coefficient: $r = 0.998884$, $r^2 = 0.997770$
Calibration curve: $3805.45 * x + 1072.09$
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: f



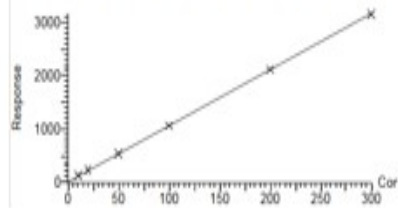
Compound name: Nivirapine
Correlation coefficient: $r = 0.999528$, $r^2 = 0.999057$
Calibration curve: $2651.53 * x + 3780.42$
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: f



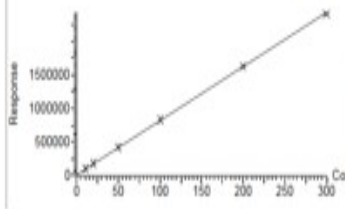
Compound name: AMOXIL
Correlation coefficient: $r = 0.999961$, $r^2 = 0.999921$
Calibration curve: $40.7208 * x + -3.70586$
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: f



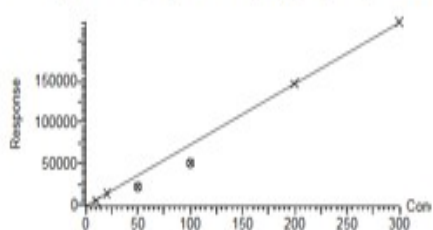
Compound name: Artemether
Correlation coefficient: $r = 0.999780$, $r^2 = 0.999561$
Calibration curve: $10.5035 * x + 12.1905$
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: f



Compound name: Sulfamethazine
Correlation coefficient: $r = 0.999657$, $r^2 = 0.999314$
Calibration curve: $8078.0 * x + 11489.8$
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: f

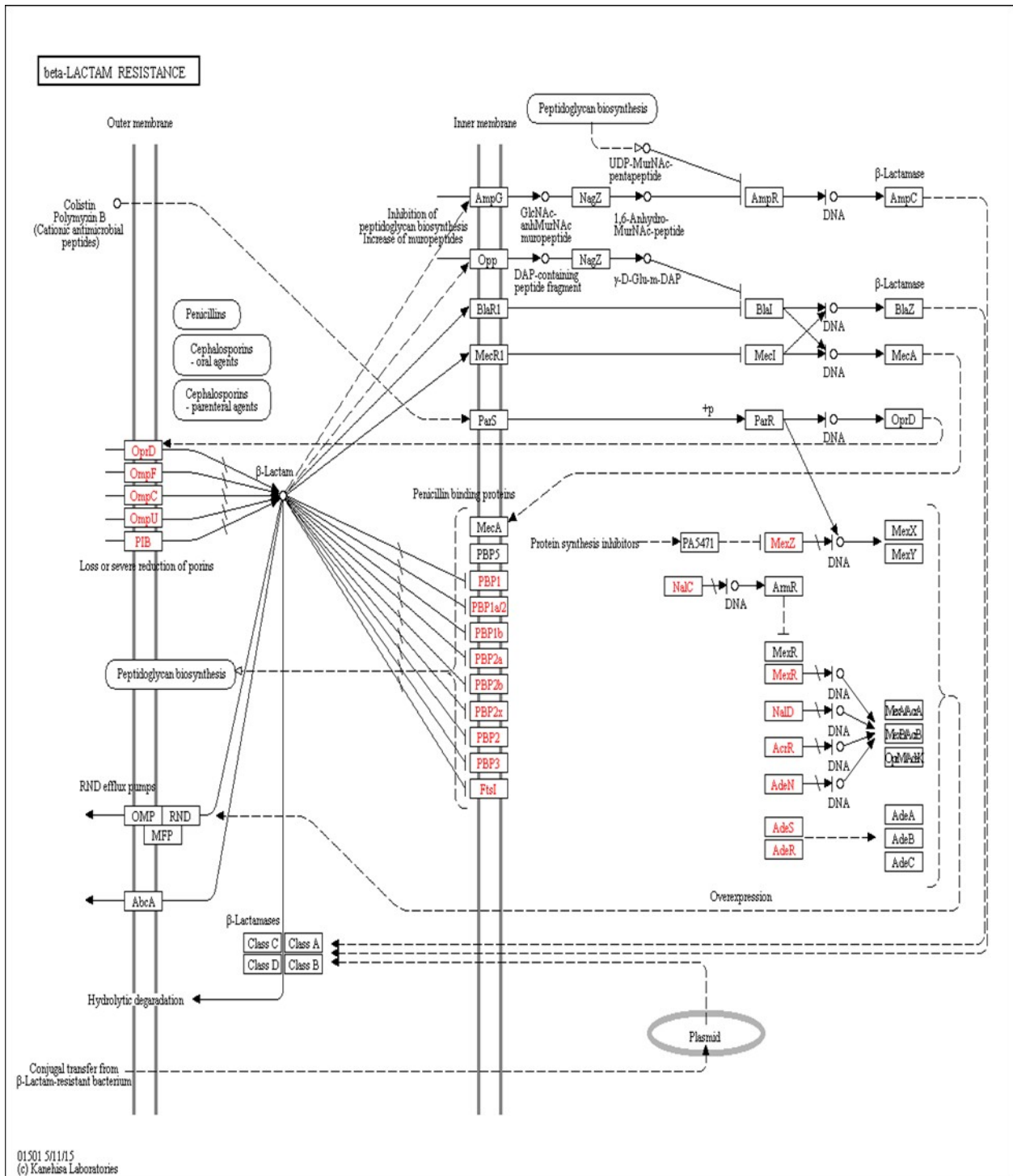


Compound name: Sulfamethoxazole
Correlation coefficient: $r = 0.999096$, $r^2 = 0.998192$
Calibration curve: $742.612 * x + -1487.88$
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: 1/x, Axis trans: f



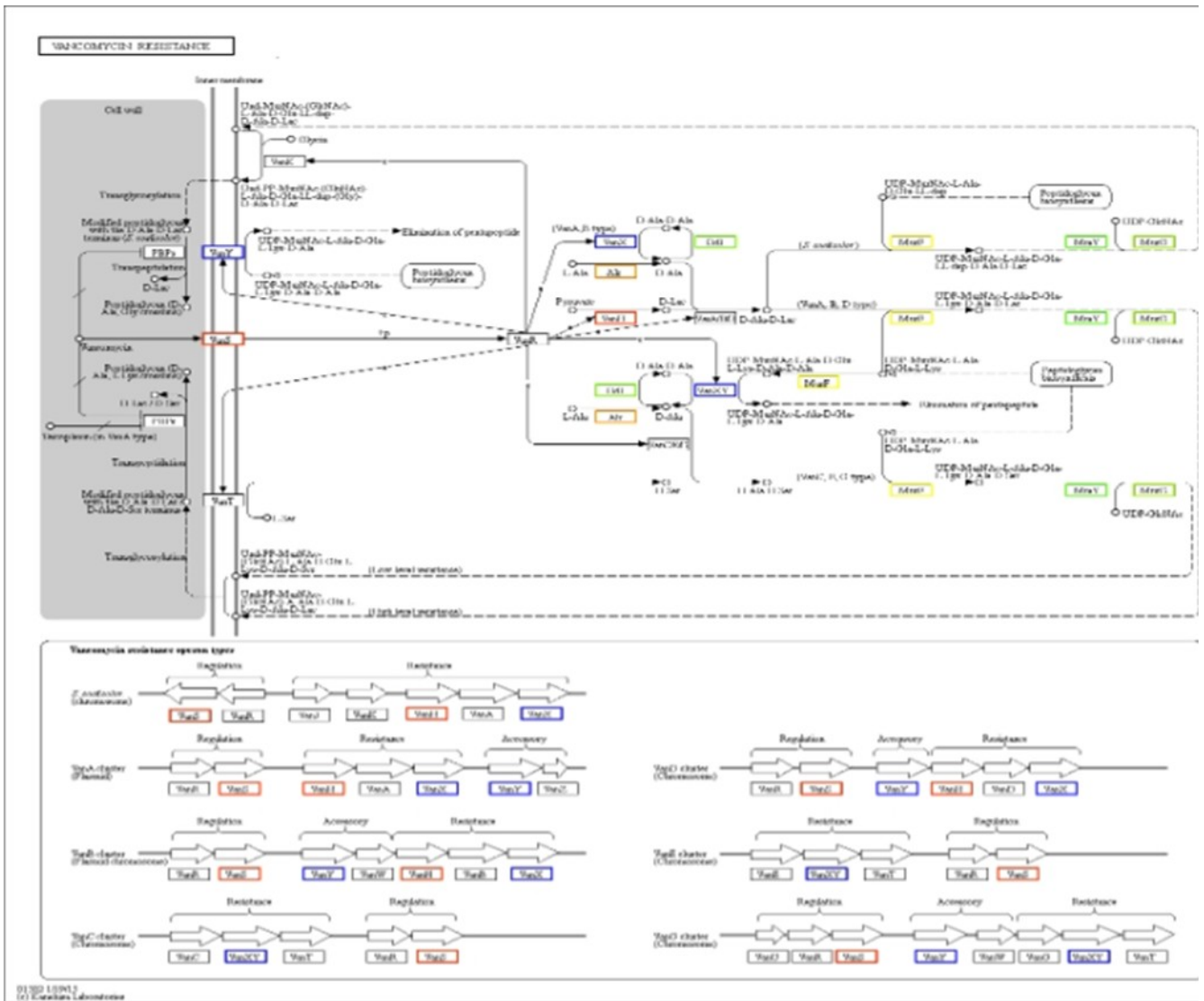
Appendix 2. The standard curves an efficiency $r^2 =$ tending to 1 meaning the standards were efficient in all compounds.

Appendix 3. Pathway showing Beta-lactamase antimicrobial resistance



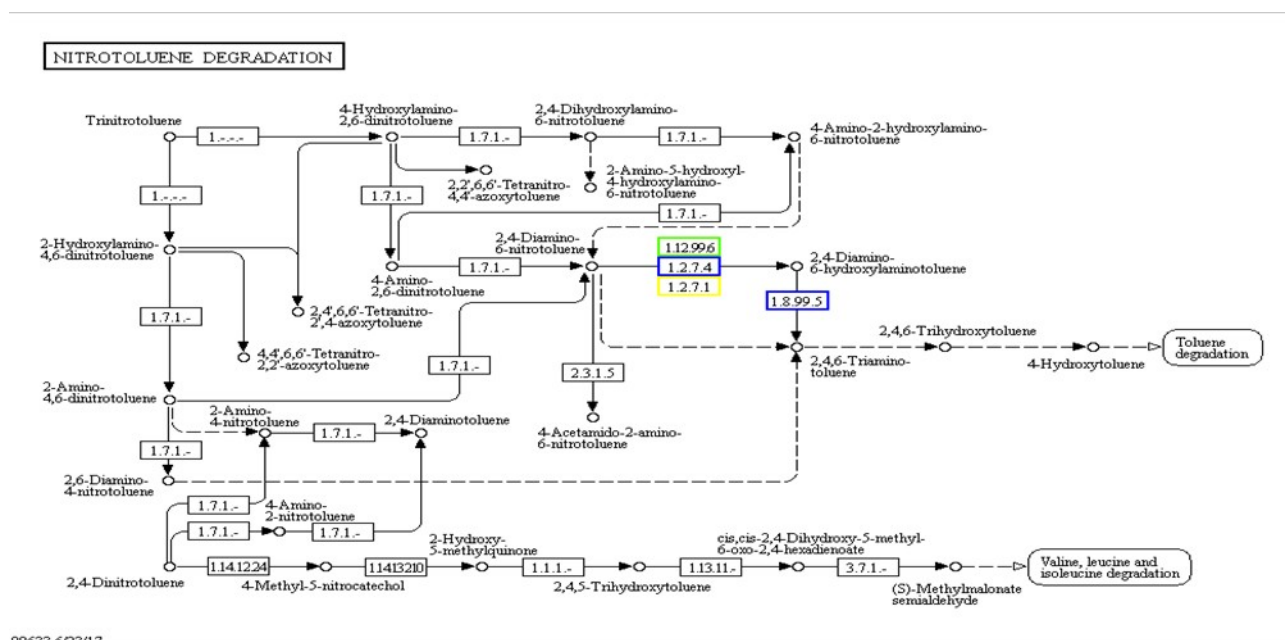
Appendix 3. Pathway showing Beta-lactamase antimicrobial resistance is caused by the reduction of production of OprD porin, ompF, OmpC, OmpU, and PIB, which are broken by hydrolytic degradation in the intermembrane space and pumped out.

Appendix 4. Pathway showing vancomycin resistance.



Appendix 4. Pathway showing VanS and VanR regulate the expression of vancomycin resistance, while VanX reprograms cell wall biosynthesis to produce peptidoglycan chain precursors. Van A and B confer acquired resistance, carried on by plasmids.

Appendix 5. Nitrotoluene degradation pathway.

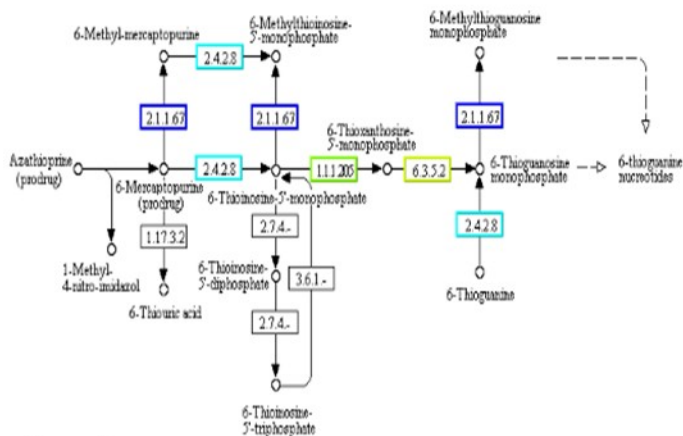


Appendix 5. Nitrotoluene degradation pathway showing 1.12.99.6 hydrogenase large subunits biodegradation by microbes. 1.8.99.5 Dissimilatory sulfite reductase alpha subunits responsible for biodegrading nitrotoluene and finally degrade toluene and amino acids namely valine leucine and isoleucine.

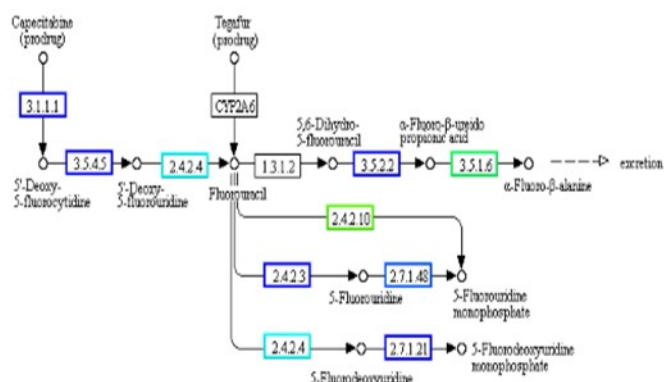
Appendix 6. Pathway showing microbial genes.

DRUG METABOLISM - OTHER ENZYMES

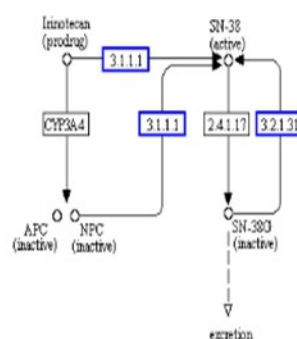
Azathioprine & 6-Mercaptopurine



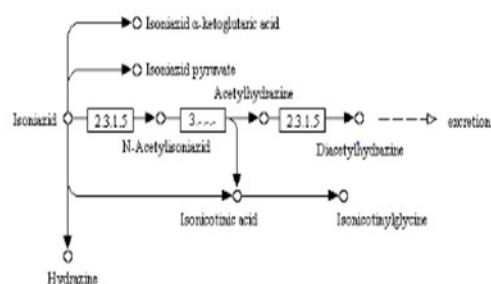
Fluorouracil



Irinotecan



Isoniazid



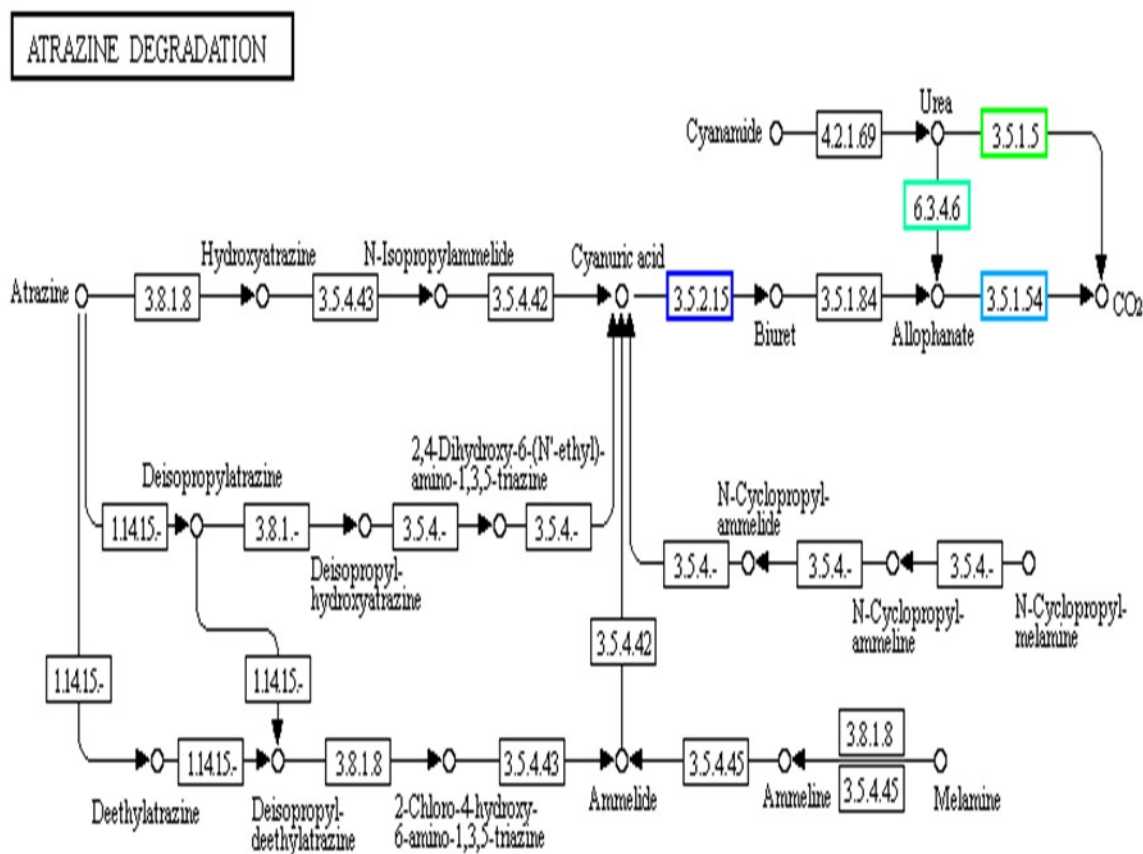
00983 9/26/16

Appendix 6. Pathway showing microbial genes such as hypoxanthine phosphoribosyl transferases, carboxylestrase KEGG orthologus genome, and thymidine kinase are responsible for drug metabolism.

[illegible]

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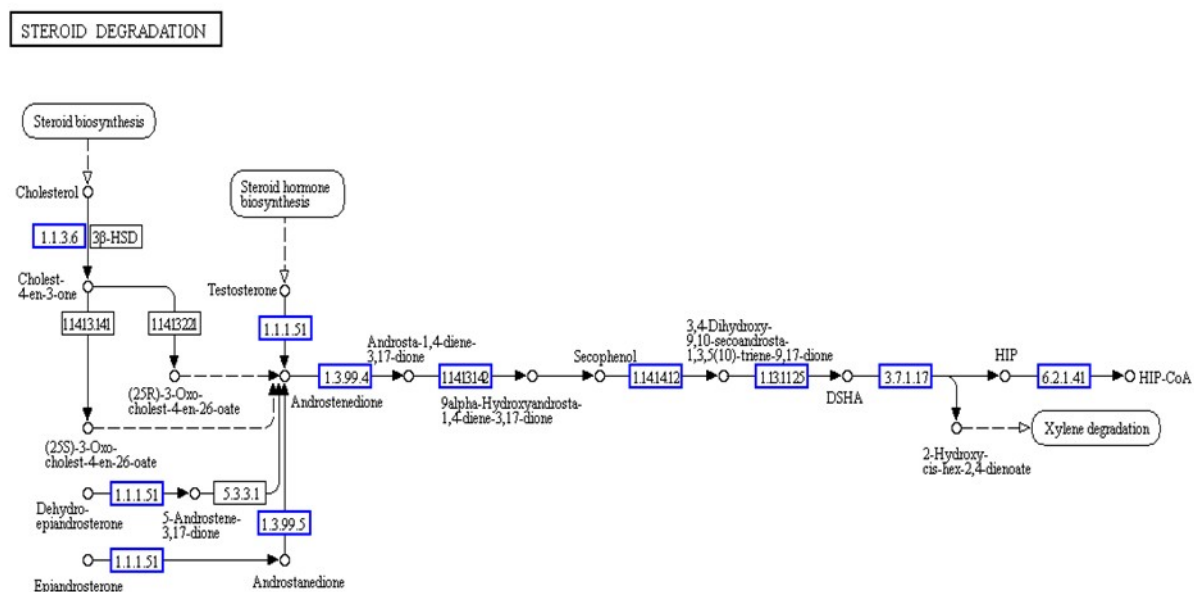
Appendix 8. Atrazine Degradation Pathway



00791 4/20/17
(c) Kanehisa Laboratories

Appendix 8. Bacteria using atrazine chlorohydrolase, cyanuric acid amidohydrolase, urease, and allophanate hydrolase break down Atrazine.

Appendix 9. Steroid degradation pathway



00984 10/17/16
(c) Kanehisa Laboratories

Appendix 9. Microbes use cholesteroxydase, beta-hydroxysteroid dehydrogenase, 3-oxosteroid 1-dehydrogenase, and CoA ligase enzymes to catalyze steroid degradation.

Appendix 10 Nacosti Permit

 REPUBLIC OF KENYA	 NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION
Ref No: 868443	Date of Issue: 02/August/2023
RESEARCH LICENSE	
	
This is to Certify that Mr. KHATIKI SANDRA of Mashaka Multira University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2017 (Rev.2014) in Kiburu on the topic: ECOTOXICOLOGICAL PROBLEMS OF MULTICLASS POLLUTANTS AND THEIR IMPACTS ON MICROBIAL ASSEMBLAGES IN THE AQUATIC ECOSYSTEM OF WINAM GULF KISUMU for the period ending : 02/August/2024	
Applicant Identification Number	License No: NACOSTI/P/23/20002
 Director General	
NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION	
Verification QR Code	
	
NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR Scanner application.	
See overleaf for conditions	

Appendix 11. Ethical Approval



MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke

P. O. Box 190,
50100,
Kakamega,
KENYA

Institutional Scientific and Ethics Review Committee (ISERC)

REF: MMU/COR: 403012 Vol 6 (01)

Date: June 2nd, 2023

To: Ms. Khatiebi Sandra

Dear Ms.

RE: ECOTOXICOLOGICAL PROFILING OF MULTI-CLASS POLLUTANTS AND THEIR IMPACTS ON MICROBIAL ASSEMBLAGES IN THE AQUATIC - ECOSYSTEM OF WINAM GULF KISUMU.

This is to inform you that the *Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMUST-ISERC)* has reviewed and approved your above research proposal. Your application approval number is MMUST/IERC/162/2023. The approval covers for the period *June 2nd, 2023 to June 2nd, 2024*.

This approval is subject to compliance with the following requirements;

- i. Only approved documents including informed consents, study instruments, MTA will be used.
- ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by **MMUST-ISERC**.
- iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **MMUST-ISERC** within 72 hours of notification
- iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to **MMUST-ISERC** within 72 hours
- v. Clearance for export of biological specimens must be obtained from relevant institutions.
- vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
- vii. Submission of an executive summary report within 90 days upon completion of the study to **MMUST-ISERC**.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <https://research-portal.nacosti.go.ke> and also obtain other clearances needed

Yours Sincerely,

Prof. Gordon Nguka (PhD)

Chairperson, Institutional Scientific and Ethics Review Committee

Copy to:

- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

Appendix 12. Genbank ID

Dear Sandra khatiebi,

This is an automatic acknowledgment that your recent submission to the SRA database has been successfully processed and will be released on the date specified.

Please reference PRJNA992979 in your publication. This BioProject accession number is provided instead of SRP and should be used in your publication as it will allow better searching in Entrez.

Accession to cite for these SRA data:
PRJNA992979
Temporary Submission ID:
SUB13654580
Release date: 2023-07-12

Your SRA records will be accessible with the following link after the indicated release date:

<https://www.ncbi.nlm.nih.gov/sra/PRJNA992979>

Send questions and update requests to sra@ncbi.nlm.nih.gov; include the

Appendix 13. Laboratory work



Appendix 14. Laboratory work

