OCCURRENCE AND ECOLOGICAL RISK ASSESSMENT OF THE SELECTED RESIDUAL ACTIVE PHARMACEUTICAL INGREDIENTS IN THE KINAWATAKA CHANNEL – UGANDA

BY

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DECLARATION

I, Aggrey Ndyabahika, declare that the dissertation I am submitting for the Master of Science in Conservation and Natural Resources Management at Kyambogo University is my original work and has not been submitted for any academic degree at any other university.

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APPROVAL

This dissertation by Aggrey Ndyabahika entitled “Occurrence and ecological risk assessment of the selected residual active pharmaceutical ingredients in the Kinawataka Channel-Uganda has been submitted with our approval as Supervisors.

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DEDICATION

To my loved ones: My spouse, Zawedde Vivian, my parents, and my siblings, deserve immense appreciation for their unwavering care and inspiration from the time I decided to take up this path. I am incredibly grateful to my sons, Inez Matthew and Ethan Israel, who serve as my greatest source of motivation, bringing boundless happiness into my life and providing me with the determination to persevere.

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ABBREVIATION AND ACRONYMS

ACN	Acetonitrile
AF	Assessment Factor
AIDS	Acquired Immune Deficiency Syndrome
AMR	Antimicrobial Resistance
API	Active Pharmaceutical Ingredient
AOPs	Advanced Oxidation Processes
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
CFR	Code of Federal Regulation
DGAL	Department of Government Analytical Limited
DNA	Deoxyribonucleic Acid
DOC	Dissolved Organic Oxygen
EMA	European Medicines Agency
ERA	Environmental Risk Assessment
EU	European Union
Fe^{2+} or Fe^{3+}	Iron (II) or Iron (III)
GIS	Geographic Information System
GPS	Global Positioning System
HDPE	High Density Polyethylene
H_2O_2	Hydrogen Peroxide
HIV	Human Immunodeficiency Virus
LC-MS	Liquid Chromatography – Mass Spectrophotometer
LOD	Limit of Detection

LOQ	Limit of Quantification
MEC	Measured Environmental Concentrations
mg/L	Milligrams per Litre
MS/MS	Tandem Mass Spectrophotometer
Na_2EDTA -	Sodium Editate
NCDs	Non Communicable Diseases
ng/L	Nanogram per Litre
NMS	National Medical Stores
NSAIDs	Non Steroid Anti-Inflammatory Drugs
NWSC	National Water Sewerage Cooperation
pH	Acidity/Alkalinity
PMF	Pharmaceutical Manufacturing Facility
PNEC	Predicted No Effect Concentrations
RSD	Relative Standard Deviation
RS	Remote Sensing
RT	Retention Time
SMEC	Snowy Mountains Engineering Corporation
SPE	Solid Phase Extraction
SPEC	Specification
SSD	Species Sensitivity Distribution
$T_{1/2}$	Half Life
TB	Tuberculosis
TiO_2	Titanium dioxide
TSS	Total Suspended Solids

UGX	Uganda Shillings
UNICEF	United Nations Children's Fund
UNIDO	United Nations Industrial Development Organization
WFD	Water Framework Directive
WHO	World Health Organization
WQ	Water Quality
WWTPs	Waste Water Treatment Plants
µg/L	Microgram per Litre

ABSTRACT

Kinawataka channel, its streams and wetland is an important ecosystem that plays a vital role as a drainage system in the Eastern part of Kampala City, the capital of Uganda. The pollution and floods from the surrounding environment are taken through a series of natural treatment which improves the quality of water entering in Lake Victoria Basin. The presence of 7 residual Active Pharmaceutical Ingredients (APIs), comprising of 5 antibiotics, 1 non-steroidal anti-inflammatory (NSAID) and 1 analgesic and antipyretic drugs, was analyzed in thirty-three (33) water specimens obtained from the Kinawataka channel, 2 streams, and a wetland to investigate the potential ecological risk posed by these residual APIs to aquatic life in this ecosystem. Random grab samples collected into the sampling bottles previously well cleaned and soaked in distilled water over night, were refrigerated in an ice box (preferably 2-8 ºC) and then transported to the Directorate of Government Analytical Laboratories (DGAL) for analysis. Solid phase extraction was used for sample extraction and residual APIs analysis was done with the help of high-performance liquid chromatography connected to a mass spectrometer and their Risk Quotients (RQ) calculated. Only six (6) residual APIs, Amoxicillin (0.82-607.86), Erythromycin (0.0-3431.87), Ciprofloxacin (0.0-79.17), Sulfamethoxazole (0.0-987.16), Oxytetracycline (0.0-5.34) and Paracetamol (0.09-1070.20) were detected at quantifiable concentrations (ng/l). The highest concentrations of residual APIs were erythromycin (0.0-3431.87), Sulfamethoxazole (0.0-987.16) and paracetamol (0.09-1070.20). The most contaminated sampling compartments were Jamaica and Kawooya streams, the main recipients of Pharmaceutical Manufacturing Facility (PMF) waste water. This study also revealed that the detected residual APIs posed high, moderate and low ecological risks, where Erythromycin and Sulfamethoxazole (RQ=1.92, RQ=1.12 respectively) showed a high risk suggesting a huge threat to aquatic organisms in the Kinawataka channel, Amoxicillin (RQ=0.54) also posed a moderate risk whereas Ciprofloxacin, Oxytetracycline, and paracetamol (RQ=0.02, RQ=0.01, RQ=0.06 respectively) pose a low risk. Overall, residual APIs especially Erythromycin and Sulfamethoxazole were detected in high concentrations hence posing significant ecological risks in the Kinawataka channel. This being the first study to report the residual APIs pollution in the Kinawataka channel, policy makers should priotize implementing targeted measures that reduce pollutant levels especially in the streams by adhering to the polluter pays principle, enforcing regulations on industries, imposing fines on environmental pollution and promoting sustainable practices to prevent future contamination.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Active Pharmaceutical Ingredients (API) are known as organic and inorganic materials or substances or chemicals that are used in the manufacture of prescription medicines that help to diagnose, cure, or inhibit diseases and improve organ activity. These include; non-prescription medicines, prescription medicines and veterinary drugs which are designed to give specific health impacts and deliver essential benefits to the body (Sardella, 2021). On average, the consumption of medicines is estimated at about 15,000 mg and 150,000 mg per capita per annum in low-income and developed countries respectively (Mohapatra *et al*., 2014). Currently, there are over 1500 different APIs that exhibit different pharmacological, chemical and toxicological characteristics which are present in over 2000 pharmaceutical drugs (Kamba *et al*., 2017).

The occurrence of residual APIs in aquatic ecosystems, soils, and biota is abundantly proven and is attributed to pharmaceutical manufacturing effluents, improper disposal, and human/animal excretion (Hughes *et al*., 2013). These residual APIs persist in the environment because of their slow degradation nature since they resist chemical degradation that takes place in the alimentary canal and this presents a distinctive risk when they get to the environment (Björnberg and Elenström, 2016). However, the degradation of APIs in aquatic ecosystems hinge on the water quality properties. It is, therefore, significant in such cases to determine the water quality parameters as some of them such as turbidity, pH, and dissolved oxygen can affect the degradation, solubility, and bioavailability of certain APIs (Ohoro *et al*., 2022).

In African countries, the lack of data on residual APIs in the environment is still a challenge, with only a few documented reports. However much the studies done in Africa including Uganda, found these residual APIs to be in nanograms (ng) and micrograms (µg) in pharmaceutical effluent, freshwater resources, and water for drinking, their persistence raises the risk of bioaccumulation in aquatic organism and bio magnification across the food chain (Wee *et al*., 2020; Waleng & Nomngongo, 2022). For example, on lake Victoria, concentrations of 1-5600 and 10-66 ng/l for sulfamethoxazole and erythromycin respectively were reported by (Nantaba *et al*., 2020). Furthermore, concentrations of 53.8-56.6 and 0.087-272.2 µg/l for sulfamethoxazole and amoxicillin were reported in Kenya and Nigeria respectively (Waleng & Nomngongo, 2022).

The most affected freshwater lake in Uganda is Lake Victoria which is predominantly vulnerable as the major receiver of industrial and household effluent (Dalahmeh *et al*., 2020; Nantaba *et al*., 2020). This undesirably compromises the quality of water on the lake that is used by most food processing industries for their operation. For example, despite the residual API occurrences and incidences on Lake Victoria being significant most of Uganda's food processing industries utilize this water in their manufacturing processes (Angiro *et al*., 2020). Due to this, the cost of water treatment in order to remove the API has greatly (Dalahmeh *et al*., 2020).

Like the Nakivubo channel, the Kinawataka channel carries a lot of urban run-off and waste load from the eastern part of Kampala city. Like any other drainage channel, it drains its flow into the Murchison bay on Lake Victoria hence contributing to the pollution of the lake (Wanasolo et al., 2018). Recent studies

have detected the residual APIs like Sulfamethoxazole, Erythromycin, Paracetamol, Oxytetracycline on Lake Victoria especially in the receiving waters (Dalahmeh *et al*., 2020; Nantaba *et al*., 2020).

Kinawataka channel is surrounded by the Pharmaceutical Manufacturing Facilities (PMF), livestock farming activities and households whose sanitation is poorly managed (Ndugga, 2021). This makes it susceptible to residual API pollution which will then directly affect the ecological health of the receiving waters, in this case Lake Victoria making it important to study the residual API load in this channel. Since the channel drains its water in Lake Victoria, the presence of residual APIs poses an ecological risk to aquatic organisms and humans. To understand this risk, it's important not only to measure the concentrations of these residual APIs but also to determine how they impact ecosystem health which can be achieved through an ecological risk assessment (ERA) by determining the risk quotient (RQ) of each residual API (European Medicines Agency, 2018).

The other implication is that these residual APIs may enter into drinking water or be taken up by aquatic organisms (Wee *et al*., 2020). This means they can easily be passed to humans causing notable health risks such as antibiotic resistance (Pereira *et al*., 2020). For instance, there's some antibiotic-resistant bacteria, E. coli that were found in the intestines of fish on Lake Victoria due to antibiotic pollution attributed to anthropogenic and industrial activities around lake Victoria (Kikomeko, 2016).

For many years the irresponsible use of human medicines was heavily blamed for incidences of AMR, however, drug pollution has been found to have a worrying

contribution (Lübbert *et al*., 2017). In present times, releases of APIs throughout the manufacturing of pharmaceutical drugs has been under-evaluated yet these releases are direct point sources to the environment addressing pollution from pharmaceutical manufacturing plants (PMF) is a priority area of intervention in low-income countries like Uganda (Becker, 2010; Kookana *et al*., 2014).

1.2 Statement of the Problem

Even though it's amongst the most polluted freshwater lakes globally, Lake Victoria is extensively used by several East African countries for various purposes such as fishing, transport, water for irrigation and industry. Despite this wide spread reliance, the ineffective water quality monitoring systems in Africa particularly Uganda characterized by lack of real time monitoring technologies and advanced analytical instruments could allow contaminants such as residual APIs to contaminate water sources without detection. However, the majority (about 75%) of API studies in aquatic ecosystems have focused on Europe and other developed countries, leaving insufficient data in low income countries like Uganda. This data deficiency raises concerns as residual APIs can potentially enter drinking water and aquatic foods posing health risks like antimicrobial resistance (AMR) to humans and endocrine disruptions to the aquatic organisms compromising the regional and global fight against pharmaceutical pollution. Given the government's support for industrialization and the rapid growth of the pharmaceutical industry in Uganda increases the likelihood of residual APIs being released to the aquatic ecosystem like Kinawataka Channel. Although Kinawataka channel is one of Kampala's drainage Channels, there remains limited understanding regarding the occurrence, distribution and ecological risk of residual APIs. Therefore, there is an urgent need to investigate the prevalence

and ecological risk of residual APIs in the Kinawataka Channel to provide information on its influence on API prevalence and impact in Lake Victoria. The findings will aid regulatory authorities in developing relevant policies for managing the potential environmental impacts of pharmaceutical waste discharge and ensuring the safety of water resources for both human consumption and ecosystem's wellbeing.

1.3 Objectives of the study

1.3.1 Main Objective

To establish the occurrence, distribution and ecological risk of selected API released to the Kinawataka channel, its streams and wetland.

1.3.2 Specific Objectives

i) To determine the water quality parameters along the Kinawataka channel, its streams, and the wetland.

ii) To determine variations in the concentrations of the selected residual APIs in the Kinawataka channel, its streams, and the wetland.

iii) To calculate the screening-level ecological risk of the selected residual APIs in the Kinawataka Channel, its streams and the wetland.

1.4 Research Hypothesis

i) There is no significant difference in the water quality parameters at different sampling points along the Kinawataka channel, its streams and wetland.

ii) There is no significant difference in the type and concentration of selected APIs along the Kinawataka channel, streams and wetland.

iii) The selected residual APIs detected in the Kinawataka Channel, its streams and wetland have no significant ecological effects and risks on aquatic life of the recipient fresh waters.

1.5 Significance of the Study

Studies in Uganda about API in the ecosystems have prioritized the western part of Kampala that includes the Nakivubo channel and Lubigi (Dalahmeh *et al*., 2020; Nantaba *et al*., 2020; Kayiwa *et al*., 2022). The eastern part of Kampala where the Kinawataka channel and its streams are located has been neglected, despite it receiving waste from highly urban and industrialized area with several pharmaceutical manufacturing facilities (PMF) and health centers.

This study therefore seeks to establish the contribution of Kinawataka Channel and its streams to the residual APIs prevalence in Lake. The data presented in this study can be used by environmental protection and the research fraternity to either mitigation or give valuable information conceivably giving course to any future studies.

1.6 Scope of the Study

The study was limited to Kinawataka drainage channel which crisscrosses the wetland before draining into Lake Victoria. It included two streams that originate from areas suspected to be polluted with residual APIs and drain wastewater to the Kinawataka channel. The determination of concentrations of the 7 selected residual APIs which included; sulfamethoxazole, ciprofloxacin, erythromycin, amoxicillin, oxytetracyline, ibuprofen and paracetamol too was considered. The study also included the determination of risk quotients (RQ) of these residual APIs to evaluate the ecological risk assessment. The analysis also covered total

phosphate, turbidity, total nitrogen, total suspended solids (TSS), biochemical oxygen demand (BOD), pH and chemical oxygen demand (COD), the water quality parameters that commonly associate with APIs in water systems.

1.7 Justification of the Study

Kinawataka channel is surrounded by the Pharmaceutical Manufacturing Facilities (PMF), livestock farming activities and households whose sanitation is poorly managed. This makes it susceptible to residual API pollution which will then directly affect the ecological health of the receiving waters, in this case Lake Victoria making it important to study the residual API load in this channel

1.8 Conceptual Frame work

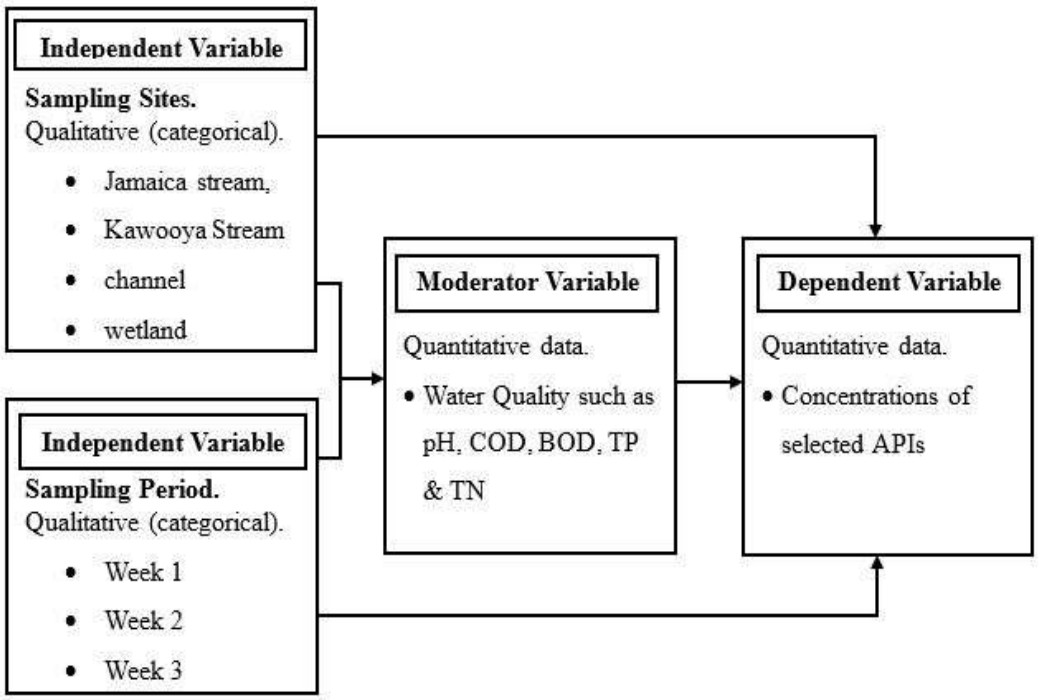

Figure 1. 1 The framework shows how the independent variables influenced the dependent variable. Water quality was determined by pH, COD, BOD, nutrients which in turn influenced the relationship between the sampling compartments, sampling weeks and concentrations of APIs. The water quality also was influenced by the variations in the sampling compartments and sampling weeks which influenced the persistence of the residual APIs in aquatic environments.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of APIs in the environment

The effects of residual API pollution problems were not well understood for a very long time after being first documented in the 1970s (Kummerer, 2001). The consequences of residual APIs are most common in advanced economies where massive volumes of prescription medicines are ingested by individuals and used in food production. The repercussions include the direct toxicity in fish, immediate toxicities of APIs on biota, and drug resistant strains in humans. However, fears have grown in recent generations (Kookana *et al*., 2014). The current reviews have been productive as a result of advancements in detection technology of residual APIs in the ecosystem. These systems have become highly robust and allowed researchers to detect residual APIs in water even at low contents (ng/L) levels (Maycock & Watts, 2012).

2.2 Pharmaceutical drug use in Uganda

The increased human dependence on pharmaceutical drugs is mainly attributed to changes in people's lifestyles including sleep routines, degree of regular exercise, stress-reduction techniques, and water intake routines (WHO, 2018). These adjustments in habits have elevated the Uganda's health issues that is monopolized by infectious diseases, which contribute almost half of death rates. Infections such as diarrhea, malaria, epidemics, TB, chronic diseases, mental illnesses and neonatal conditions that lead to illness and death, have all influenced the country's disease burden. This has increased the volumes of drugs on market as well as the increased consumption of pharmaceutical drugs has been mainly triggered by the rapid increase in population with the increasing various diseases

(WHO, 2018). Ministry of Health, National Medical Stores and their development partners like the Global Fund, which assist the government of Uganda in providing, supplying and distributing of prescription medicines, have all referred to such medications as important medicines, which address a Uganda's top health-related issues. This has prompted the government of Uganda to increase the budget allocation of essential medicines over years. For example, a total of UGX 94.6 billion was used to purchase essential medicines for years 2008/2009 (UNIDO, 2010) compared to about UGX 1.1 trillion for the 2022/2023 financial year (Budget Speech - Financial Year 2022/2023, 2022).

Kampala city, the Ugandan capital has a population of about 1.8 million generates all kinds of waste in which some of them are waste that are contaminated with drugs products which can find their way into the streams, wetlands and other sources (KCCA Strategic Plan, 2020). The fact that most pharmaceutical industries are also allocated around Kampala and their effluents can directly impact the aquatic ecosystems. Consequently, significant volume of medicines supplied by the government and what people purchase from the local pharmacies, end up in the environment which compromises the environment's health and it's important to know how much is used and how they get to the environment to come up with a complete study about their occurrences. Below is an imaginary representation of drug supply, prescription and disposal chain.

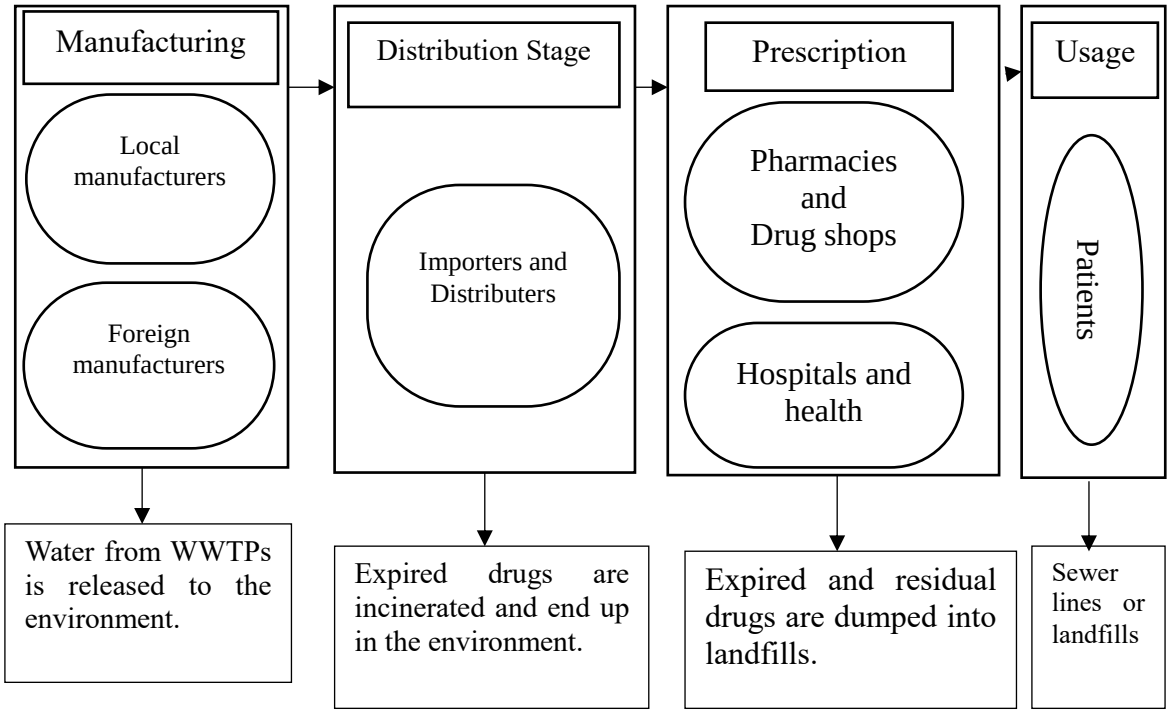

Figure 2. 1 A schematic representation of drug supply, prescription and disposal chain of medicines (UNIDO, 2010)

It is not a coincidence that most the prescribed medicines are the ones that have a high ecological foot print and persistence (Dalahmeh *et al*., 2020; Nantaba *et al.,* 2020). It's understood that past studies in Uganda about APIs in the environment have revealed that there are common residual APIs that have been found to be persistent in the environment which include sulfamethoxazole, erythromycin, ciprofloxacin, paracetamol, *(*Dalahmeh *et al*., 2020; Nantaba *et al*., 2020; Kayiwa *et al*., 2022). In 2000s, the occurrences of residual APIs in the environment were minimum mainly from non-point pollution of residual APIs from hospitals, homes and expired drugs from pharmacies and drug shops since Uganda relied on the importation of pharmaceutical drugs. But ever since, the pharmaceutical sector has evolved over the last 20 years.

Currently, Uganda has a total of 21 pharmaceutical industries which could probably have increased the levels of point source pollution and cases of environmental occurrences of residual APIs (Lubowa *et al*., 2021). The recent milestone that might help in mitigating the residual APIs to the environment is the approaches and efforts by both the government and its health partners to promote multi-sectoral approaches to prevent and control communicable, non-infectious and other nutrition-related disorders. This will not only reduce the volumes of medicine consumed per capita but also reduce their load on the environment (World Health Organisation, 2018).

2.3 Sources of residual APIs in the environment

2.3.1 The Pharmaceutical wastewater treatment plant

In a pharmaceutical Manufacturing facility (PMF), water is used as a raw material in the manufacturing process, cleaning and analysis. However, at the end of all these processes, it has gained other foreign substances or deteriorated in quality and has to undergo a series of treatment before it's discharge off (Gadipelly *et al*., 2014). The eradication of pollutants taken up during industrial operations is the primary duty of waste water treatment plants (WWTPs) and partial elimination of residual APIs during the treatment procedures makes them end up in different ecosystems causing damage to the ecosystem (Kanama *et al*., 2018). Therefore, the presence of residual APIs in aquatic ecosystems such surface water has brought the performance of pharmaceutical WWTPs in removing the residual APIs into question. Primary treatment, secondary treatment and tertiary treatment steps are what distinguish traditional from sophisticated wastewater treatment.

The first stage of treatment typically consists of screening and clarification processes. In order to remove garbage and debris and segregate aqueous from solid waste, this step comprises two practices: first, filtration and sedimentation, then clarifying (Michael *et al*., 2013). The second stage often uses trickling filters, membrane bioreactors, or traditional activated sludge (Yi *et al*., 2017). Organics and nutrients are removed by biological decomposition, which can happen in a variety of aerobic, anaerobic, and anoxic environments (Michael *et al*., 2013). In order to improve effluent quality, sophisticated WWTPs have implemented tertiary treatment, which uses chemical treatment, disinfection methods (chlorination), water purification techniques (ozonation) and sterilization methods (UV light irradiation) (Barbosa *et al*., 2016). Additionally, advanced oxidation processes (AOPs), reverse osmosis, filtration (Ultra and nano), as well as activated carbon adsorption are always used in addition to conventional steps.

2.3.2 Pharmaceutical occurrence in final wastewater effluents

Research shows that wide range of concentrations are found in pharmaceutical waste water in countries like Nigeria, Uganda, Scotland, India, and Canada *(*Frascaroli *et al*., 2021; Gadipelly *et al*., 2014; Lan *et al*., 2019) . The primary causes of the residual APIs include spills from washing of manufacturing units, unregulated dumping of faulty medicines, and cleaning of production lines (Parra-Saldivar et al., 2021).

However, the PMF raw wastewater studied by Kayiwa and others in 2022, API concentrations reported were ranging from 0.14 and 240.83 mg/L for sulfamethoxazole, losartan, clarithromycin, diclofenac and carbamazepine. Metronidazole was found to have amounts of 8.04 ± 0.56 µg/l and 2.24 ± 0.57

µg/l in effluent from PMF in an assessment in Nigeria *(*Lan *et al*., 2019). Likewise, in least developed countries (Africa and Asia), the PMFs might not be able to use sophisticated treatment processes, which could lead to releases of inadequately treated wastewater to aquatic ecosystems causing detrimental impacts on ecosystems that receive effluent (aus der Beek *et al*., 2016).

2.3.3 Hospitals, healthcare facilities and homes

Medical centers have increased in number due to the growing population, which has left less room for waste disposal landfills. All medical care systems generate waste, which when improperly handled can end up in the surroundings and cause environmental problems, rendering clinical waste control a worldwide crisis (Ali *et al*., 2017). Because there is no legislative structure in Uganda defining how medical centers and residences should discard of their residual medicines, there is a great chance that residual medicines could enter the ecosystem (Muhwezi *et al*., 2014).

The inappropriate dumping of redundant or outdated medications into household or clinical trash cans that end up in landfills is therefore without doubt likely to be another significant way that leftover drugs can leach into and persist in the environmental waters (Murdoch 2015; Ramakrishnan *et al*., 2015). This is regarded as a main cause of antimicrobial resistance (AMR) (Musoke *et al*., 2021). Due to administration of more doses than necessary, there is a significant amount of unused prescriptions in homes of residences. This suggests the necessity for recovery systems for discarded drugs to be set up (Murdoch 2015). Nevertheless, even where recovery mechanisms are in place, the most prevalent ways of getting rid of unused medicines include throwing them into toilet or

household trash cans or sinks (which frequently ends up in landfills or municipal sewage system) (Ayele & Mamu, 2018; Murdoch 2015).

2.4 Occurrences of residual APIs in environmental Waters

2.4.1 Surfaces waters

In contrast to the developed world, least developed countries have no strict regulations regarding unused drugs and how they should be handled. Exploring the prevalence and amounts of residual APIs has been a current global interest among environmentalists and the medical world (Agunbiade & Moodley, 2016). Numerous analyzed research papers have demonstrated that the main sources of residual APIs entering environmental waters are municipal wastewater effluent disposal (treated or untreated), improper PMF waste disposal, and the landfilling of excess prescriptions by patients and healthcare providers (Björnberg & Elenström, 2016; Ayele & Mamu, 2018; Parra-Saldivar *et al*., 2021). In surface water, sulfamethoxazole was one of the most often detected compound, according to monitoring data from roughly 47 different nations, a comprehensive global assessment conducted by (ausderBeek *et al*., 2016). The advancement of microbial antibiotic resistance, feminization of marine organisms on interaction with hormones, and fish endocrine disruption are just a few of the serious tensions and recognized risks of exposure to these residual APIs to ecosystems, and other life forms (Lonappan *et al*., 2016; Grenni *et al*., 2018; Duarte *et al*., 2022).

2.4.2 Drinking Waters

When residual APIs are discharged into aquatic environment, their amounts in waterbodies is elevated, and this water is frequently processed to produce tap water as a result. Unfortunately, present forms of water treatment are ineffective

for getting rid of residual APIs, which could contaminate tap water (Wee *et al*., 2020). Because of pragmatic issues like exorbitant prices, absence of clear analytical systems and laboratory facilities to trace a huge spectrum of residual APIs and their metabolites result in the lack of surveillance programs to regularly evaluate for residual APIs in drinking water (Maycock & Watts, 2012). Nevertheless, some other international studies have found relatively trace amounts of residual APIs in processed drinking water, tap water in numerous nations in developed economies and Asia, as well as the United States (Maycock & Watts, 2012; Boleda *et al*., 2014; Wee & Aris, 2017; Wee *et al*., 2020).

There is a risk that severely polluted food and water will be consumed in nations without adequate wastewater and drinking water treatment systems, which is very worrying. There are still concerns about the bioaccumulation of such substances, which could result in relevant effects such as antimicrobial resistance (AMR) and endocrine effect consequences. Although (Niemi, 2020) claimed that residual APIs levels within drinking water might not be significant enough to cause consequences for humans.

2.5 Emerging Impacts of residual APIs

2.5.1 Humans

People may be affected by the effects of residual APIs if they are subjected to them at levels above their predicted no-effect levels. The incidences of residual APIs in drinking water in European and Asian nations, such as the United States, Canada, Malaysia among others question the status of drinking water in nations where efforts have not been undertaken to detect them (Maycock & Watts, 2012; Boleda *et al*., 2014; Wee & Aris, 2017). The levels of residual APIs in water for

drinking being at detectable levels presently do not guarantee that these levels have an adverse impact on human wellbeing (Wee *et al*., 2020; Wee & Aris, 2017). However, antibiotics present in surface water may lead to the formation of bacteria with a higher risk of developing resistance thereby reducing the efficacy of antibiotics in medicines once consumed by humans. (Oğuz & Mihçiokur, 2014; Parolini, 2020).

Nonetheless, the lack of proof of repercussions does not prove that there are no consequences because the likelihood of uncertain and recurring adverse reactions from continued consumption of drinking water contaminated with residual API cannot be ruled out (Wee *et al*., 2020).

The residual APIs might also bridge with untargeted receptors, which could have worrying negative consequences on untargeted receptors. Some residual APIs, particularly estrogenic APIs, have a significant bioaccumulation propensity that may negatively impact hormonal regulation. Additionally, small doses of some medicines present in water resources, such as antibiotics may cause the spread of antimicrobial resistance, leading to over hospitalization (Lam, 2014).

2.5.2 Ecosystem

It has been discovered that certain residual APIs even at very small concentrations, affect aquatic species. For example, the feminization of fish in waters polluted with ethinylestradiol, a common contraceptive pill (Hamilton *et al*., 2022). Ecological risk assessments reveal that some residual APIs are detected in aquatic ecosystem at levels sufficient enough to endanger aquatic species (Parolini, 2020). Residual APIs have effects on aquatic species as they are frequently not degradable even at low doses and are made to withstand

deterioration (Oğuz & Mihçiokur, 2014). Additionally, because residual APIs are consistently released into surface waters, aquatic species are continuously exposed to the toxins all through. (Lan *et al*., 2019; Parra-Saldivar *et al*., 2021; Waleng & Nomngongo, 2022).

For example, Paracetamol at levels exceeding their predicted no effect levels, has an impact on marine algae, invertebrate, and fish species, affecting liver function and inducing oxidative stress in fish (Verlicchi *et al*., 2012; González *et al*., 2020). Furthermore, Ibuprofen's mode of action as a cyclooxygenase inhibitor has been reported to affect aquatic organisms, potentially disrupting physiological processes and causing toxicity (Fent *et al*., 2014). Amoxicillin's ability to inhibit bacterial cell wall synthesis can harm beneficial aquatic microorganisms

Studies have also reported sulfamethoxazole affecting the photosynthesis process in algae and the reproductive capabilities of aquatic invertebrates (Liu *et al*., 2020).

Additionally, they may accumulate on floating debris like micro- and nano-plastic, that could serve as a vehicle for the continued spread of these substances across ecosystems (Bakir *et al*., 2014). Many medicines, such as Ciprofloxacin, amoxicillin, ciprofloxacin, and tetracycline, can bind to micro plastic, it would easily spread such drugs across aquatic habitats (Vieira *et al*., 2021). Furthermore, solid-bound contaminants may leak from the initial channel and cause ecological consequences if polluted media is consumed which causes their accumulation over time in tissues of aquatic species with which they have high affinity (Brandts *et al*., 2018). Fish living in effluent-impacted rivers had been found to have

bioaccumulation in their neural tissue, which raises the prospect of bio magnification (i.e., further distribution through food webs) (Schultz *et al*., 2010).

2.5.3 Residual APIs and the increase of Antimicrobial Resistance (AMR)

Antimicrobial resistance (AMR) represents one of the most pressing global health challenges of our time, characterized by the ability of bacteria, viruses, fungi, and parasites to evolve and become resistant to antimicrobial drugs that once effectively treated infections and has threatened life-saving medications and posed risks to surgical procedures (Osabuohien et al., 2021). Pharmaceutical environmental pollution plays a critical role in AMR development and spread. Pharmaceutical pollution originates from multiple interconnected sources, including household excretion (30-90% of consumed antibiotics are excreted unchanged), hospital and healthcare facility discharge, pharmaceutical manufacturing effluents, and veterinary/agricultural practices (Ajekiigbe et al., 2025; Kotwani et al., 2021).

A global ecological analysis has demonstrated a significant positive correlation ($p = 0.36$, $p = 0.011$) between ciprofloxacin concentrations in rivers and fluoroquinolone resistance prevalence in *E. coli* across 48 countries, providing direct evidence of the environment-resistance nexus (Kenyon, 2022). In Africa, a study in Blantyre, Malawi, reported pharmaceutical pollution of urban rivers with 38 different antibiotics, 49 pharmaceuticals all exceeding Predicted No Effect Concentration (PNEC) thresholds ten times (Cocker et al., 2025). However, many African countries lack regulatory frameworks for pharmaceutical residues, with less than one-third having National Action Plans for AMR (Mshana et al., 2021; Ripanda et al., 2022).

The persistent nature of many pharmaceuticals lead to bioaccumulation and biomagnification through food chains, causing adverse effects including growth inhibition, behavioral disorders, and developmental abnormalities in amphibians, fish, and mammals (Muchesa & Nosiphiwe, 2020; Ugoeze et al., 2024). The health implications of AMR are deep as antibiotic-resistant infections require more expensive treatments, longer hospitalization, and often result in treatment failures. Consequently, the World Bank estimates that AMR could push 28.3 million people into extreme poverty by 2050, while the current projections suggest AMR could cause 10 million deaths annually by 2050 if inadequately addressed, surpassing cancer as a leading cause of mortality (Ugoeze et al., 2024).

2.6 Priority APIs

2.6.1 Analgesics/NSAIDs

Paracetamol and Ibuprofen, 2 drugs from the analgesics/NSAIDs family that are frequently utilized relieve pain and swelling Due to their substantial usage, it is thus anticipated that paracetamol and ibuprofen will reach wastewater in significant amounts (Lonappan et al., 2016; Parolini, 2020).

2.6.1.1 Paracetamol

Paracetamol (acetaminophen), one of the widely used analgesics and antipyretics (González *et al*., 2020). Its ubiquitous use has led to frequent detection in surface waters, wastewater, and occasionally in groundwater. Paracetamol enters the environment through human excretions due to its high consumption and pharmaceutical waste since it can't be completely removed from WWTPs (Vieno *et al*., 2017). Paracetamol may pose deleterious effect on aquatic organisms, particularly at higher concentrations and has been found to affect growth,

reproduction, and survival of invertebrates, fish and algae but all in all paracetamol has a short life in water (Escher *et al*., 2014).

Specifically, Paracetamol at levels exceeding their predicted no effect levels, has an impact on marine algae, invertebrate, and fish species, affecting liver function and inducing oxidative stress in fish (Verlicchi *et al*., 2012; González *et al*., 2020). Policymakers have established and enforced regulations for the disposal and use of Paracetamol to protect water quality and aquatic ecosystems and adopted methods for removing Paracetamol such as biological treatments (constructed wetlands), as well as chemical treatments (ozonation and photo catalysis) (Kümmerer *et al*., 2018).

2.6.1.2 Ibuprofen

A common non-steroid anti-inflammatory, Ibuprofen widely used for pain relief and anti-inflammatory purposes making it one of the medicines associated with environmental pollution. It's often found in all environment water with major pathways being WWTP effluents, human excretion and disposal (Fent *et al*., 2014; Vieno *et al*., 2017). Ibuprofen may be deleterious to aquatic organisms, particularly at higher concentrations. It can impact the fish, algae, and invertebrates at different stages of development and reproduction. Studies have shown that ibuprofen can disrupt endocrine functions in aquatic species, leading to long-term ecological consequences (Bebianno and Gonzalez-Rey, 2014). Ibuprofen's mode of action as a cyclooxygenase inhibitor can affect aquatic organisms, potentially disrupting physiological processes and causing toxicity (Fent *et al*., 2014).

2.6.2 Antibiotics

The top residual APIs chosen included antibiotics such as Amoxicillin, Erythromycin, Ciprofloxacin and Sulfamethoxazole which are used to treat bacterial infections. In WWTPs that operate secondary biological degradation without additional treatment, antibiotics typically exhibit poor clearance while treating wastewater (i.e., tertiary disinfection or clarification) (Li *et al*., 2021; Tran *et al*., 2016). Antibiotics lead to significant eco toxicological issues, typically in regard to the possibility of promoting the development of bacterial strains resistant to particular APIs in microorganisms which aid AMR in microbial flora in groundwater. Antibiotics present in groundwater may impede bacterial growth or lead to the formation of bacteria with a higher risk of developing resistance (Oğuz & Mihçiokur, 2014; Giebułtowicz *et al*., 2020; Ngigi et al., 2020; Parolini, 2020).

2.6.2.1 Amoxicillin

Amoxicillin, a β-lactam antibiotic, helps to treat infections including otitis media, upper respiratory tract infections, pneumonia, and urinary tract infections (Tran *et al*., 2016). Amoxicillin enters the environment through different ways including human excretions due to its high consumption, pharmaceutical waste since it can't be completely removed from WWTPs and improper disposal of medications, however, Amoxicillin is very unstable due its β-lactum ring which get hydrolyzed easily (Kümmerer *et al*., 2018). Amoxicillin's ability to inhibit bacterial cell wall synthesis can harm beneficial aquatic microorganisms, leading to imbalances in microbial communities hence spreading of resistance bacteria strands (Kümmerer *et al*., 2018).

Furthermore, Amoxicillin and its degradation products can pose ecological risks. It also affects non-target microbial communities in soil and water, potentially leading to increased antibiotic resistance. Literature reveals that amoxicillin can change the structure and function of microbes, impacting nutrient cycling and ecosystem health (Wang *et al*., 2020). Biological treatments, such as membrane bioreactors, and chemical treatments like chlorination and ozonation, have been explored for better amoxicillin removal with varying efficiency (Verlicchi *et al*., 2015).

2.6.2.2 Erythromycin

Erythromycin, belonging to a class of macrolide antibiotics, used to treat respiratory, sexually transmitted and skin infections (Grenni *et al*., 2018). Erythromycin's main path to the environment is human excretion and WWTP effluents and its frequent detection in wastewater and surface waters is attributed to extensive use and inadequate elimination during treatment. Erythromycin can be degraded by light, however, its persistence in low light medium and bioactivity pose risks to aquatic environments (Gulkowska *et al*., 2014).

Erythromycin can also significantly impact aquatic ecosystems by inhibiting the growth of beneficial bacteria in aquatic environments, leading to disruptions in microbial community structures. Additionally, evidence shows that erythromycin is detrimental to reproduction and growth of aquatic organisms including the invertebrates, even at low concentrations (Teng *et al*., 2018). AOPs technics, such as UV/H_2O_2 and Fenton processes, as well as biological treatments like activated sludge, exhibit significant success in removing erythromycin from wastewater (Luo *et al*., 2014).

2.6.2.3 Ciprofloxacin

Ciprofloxacin belongs to the fluoroquinolone class of antibiotics, mostly employed in the treatment of wide spectrum of infections including respiratory, urinary tract, and skin infections (Martínez *et al*., 2014). Ciprofloxacin is introduced into the environment via human and animal excretion, pharmaceutical manufacturing effluents, and improper disposal which contributes to its persistence in aquatic environments due to its stability and poor biodegradability as a result of its strong fluorine groups which strongly get attracted to the sediments (Kümmerer *et al*., 2018). Since it's used to treat a wide spectrum of infections, it stops the activity of bacterial DNA gyrase and topoisomerase IV, which can cause affect in non-target aquatic organisms and enhancing the multiplication of antibiotic-resistant bacteria (Martínez *et al*., 2014).

Ciprofloxacin can also exhibit toxic properties on aquatic organisms including invertebrates, disrupt microbial communities in water bodies, potentially leading to an increase in the extent resistant bacteria (Li *et al*., 2018). Ciprofloxacin occurrence is particularly concerning because of its potential to induce resistance mechanisms, thereby reducing the efficacy of antibiotics in medicines (Wang *et al*., 2020). Removal techniques such as ozonation, photocatalysis, and constructed wetlands have been studied for ciprofloxacin removal, with varying degrees of success (Kovalova *et al*., 2013).

2.6.2.4 Sulfamethoxazole

In humans, sulfamethoxazole, a sulfonamide antibiotic, is extensively used in combination with trimethoprim to treat infections including bronchitis and most urinary tract infections which are all caused by bacteria (Kumar *et al*., 2019).

Sulfamethoxazole's main environmental pathway is human and animal excretion and inappropriate disposal of medications. WWTPs often do not fully remove this compound, leading to its presence in surface waters (Gulkowska *et al*., 2014). Sulfamethoxazole's bacteriostatic action, which inhibits bacterial synthesis of dihydrofolic acid, can disrupt aquatic microbial communities, leading to antibiotic resistance and altered ecosystem functions (Michael *et al*., 2014).

Studies have also reported sulfamethoxazole affecting the photosynthesis process in algae and the reproductive capabilities of aquatic invertebrates (Liu *et al*., 2020). Sulfamethoxazole's high environmental resistance to degradation and persistence raises important ecological concerns. Evidence indicates that it exhibits toxic properties on aquatic organisms at environmentally relevant concentrations, impacting bacterial communities and potentially leading to an increase in the extent resistant strains (Zhou *et al*., 2019). The methods of wastewater treatment that are abit advanced including use of activated carbon, membrane filtration and advanced oxidation processes (AOPs) have shown effectiveness in eliminating sulfamethoxazole from wastewater (Rodriguez-Mozaz *et al*., 2015).

2.6.2.5 Oxytetracycline

Oxytetracycline is a tetracycline antibiotic used mainly in veterinary medicines to treat a range of bacterial infections. It is frequently detected in agricultural runoffs and water bodies near livestock operations due to its extensive use in animal husbandry (Nantaba et al., 2020). Oxytetracycline is introduced into the environment through agricultural runoff, aquaculture, and livestock excretion. It is known for its persistence in water and soil (Sarmah *et al*., 2018).

Oxytetracycline strongly adsorbs to the sediments which enhances environmental persistence, and impact microbial communities, potentially increasing the spread of antibiotic resistance. It has been shown to inhibit bacterial growth and disrupt microbial community structures in soil and water, which can affect nutrient cycling and ecosystem functions (Zhou *et al*., 2017).

Oxytetracycline can also be detrimental to the reproduction and growth of aquatic organisms including the invertebrates, at environmentally relevant concentrations hence inducing resistance genes in environmental bacteria which are particularly concerning (Liu *et al*., 2020). Strategies including construction of wetlands and advanced oxidation processes are exhibiting success in removing oxytetracycline from wastewater. Techniques such as adsorption using bio char, photo catalysis, and advanced biological treatments have also shown effectiveness in removing oxytetracycline from water (Barbosa *et al*., 2016).

2.7 Ecological risk assessment

Because these APIs relate with receptors and metabolic route in humans, with some preserved in non-target animals, there is growing fear that exposure to them might harm ecosystem health (Gunnarsson *et al*., 2019) by posing a risk of ecotoxicity to numerous organisms. These risks include early-stage toxicity in fish, inhibition of algal growth, decreased Daphnia magna reproduction which impacts the entire food chain, as the affected organisms are all important components of it and contributing to the extent of genes that are resistant to antibiotic (Ashfaq *et al*., 2017). It is therefore central to know how much of these residual APIs do exist and establish the risk of their ecotoxicology potential to be

able to understand the likelihood of negative effects occurring to the aquatic organisms (Whomsley *et al*., 2019).

When a drug is already in use, the risk assessment compares the Predicted No Effect Concentrations (PNECs) representing three trophic levels of fish, Daphnia, and algae with the Measured Environmental Concentrations (MEC) indicating actual concentration of the substance in the aquatic ecosystem (Whomsley et al., 2019). The highest detected environmental concentrations are taken as measured environmental concentrations (MEC) to represent a devastating situation (Nantaba *et al*., 2020). To estimate the PNEC, data of at least three aquatic species must be provided, representing the first three levels of the food chain: primary producers (algae), first-level consumers (Daphnia magna), and second-level consumers (e.g. fish) (Whomsley *et al*., 2019). The PNEC is usually generated using assessment factor (AF) technique (Duarte *et al*., 2022). The AF technique is the most commonly used method because it is based on the idea that protecting the weakest link in the food chain can help to preserve the entire ecosystem. It also has the advantage of requiring the least amount of data (S. Zhou *et al*., 2019).

2.8 API and drug product degradation in the environment

Residual APIs behavior and organic reduction via breakdown after being released into the environment, remains unclear. The two primary mechanisms for API breakdown in groundwater are thought to be photolysis and biological degradation (Lonappan *et al*., 2016; J. Wilkinson *et al*., 2017). Although APIs can undergo an extensive breakdown by microbes in surface water, this process may be constrained by the bioactivities and bioavailability of the drug component (Z. Li & Mclachlan, 2019).

When substances enter ecosystems, a lot happens including biodegradation, environmental reactions such as hydrolysis and photolysis occur and they are sometimes influenced by extraneous aspects like the seasons, Water quality (WQ) and surface water hydrology (J. Wilkinson *et al*., 2017). Light irradiance that helps to breakdown these residual APIs can also be affected by water clarity and turbidity, among other parameters, and photo degradation rates are impacted by the way residual APIs are distributed and transported within the water (Batchu *et al*., 2014). For instance, certain APIs degraded in freshwater quite quickly under real sunshine, with the NSAID diclofenac having a half-life ($t_{1/2}$) of 39 minutes and chlorination breaks down paracetamol into products N-acetyl-p-benzoquinone imine and 1,4-benzoquinone (Niemi, 2020).

Although screening analysis can be used to identify metabolites after degradation, the residual APIs may undergo chemical or biological breakdown and change into new molecules which make their quantification difficult due to the absence of standards for their detection, the prevalence at trace levels, and the need for sophisticated analysis such as nuclear magnetic resonance, isotope analysis and high resolution mass spectrometry (HRMS) to verify and identify drug metabolites (Evgenidou *et al*., 2015; Sabater-Liesa *et al*., 2021).

2.9 Potential of wetland to remove APIs

Wetlands' ecological and economic benefits are now more widely acknowledged, although they were formerly largely disregarded. Wetlands are ecosystems with wonderfully changeable physical and chemical properties that provide a favorable habitat to get rid of a range of environmental toxins. Municipal wastewater is treated all around the world using the natural and artificial wetlands'

capacity to purify water (Conkle *et al*., 2012). Wetlands used to treat by removing and digesting nutrients and reducing biological oxygen demand (Anderson *et al*., 2015), but in more recent years, wetlands have started using phytoremediation to eliminate organic contaminants (Nguyen *et al*., 2019).

For low-income countries where the expenses of constructing and operating advanced wastewater treatment facilities are not viable, it is vital to research mitigation measures that might effectively remove these residual APIs before they reach the fresh waterways (Kookana *et al*., 2014; Lan *et al*., 2019; Angiro *et al*., 2020). This is why wetlands are frequently used to reduce nutrients and organic pollutants in wastewater before it is drained into the open waters and this makes them a lower priced and more practical way to address the concern of residual APIs in wastewater (Anderson *et al*., 2015; Nguyen *et al*., 2019).

The two mechanisms that are probably more in charge of pollutant removal in wetlands, degradation and sorption, require the study to comprehend the unique water quality factors of wetlands that impact them (Conkle *et al*., 2012; Wu *et al*., 2012). Therefore, it is crucial to comprehend how frequently existing residual APIs degrade in marine ecology and identify the factors that facilitate or prevent such deterioration.

2.10 Advances in analytical and detection technology

Accurate and sensitive technologies must be used to explore the incidences and fate of these trace level pollutants (Batt *et al*., 2008). The advancements in the sensitivity and accuracy technology of analytical instruments are primarily responsible for the rise in published data of residual APIs at trace levels in different ecological portions such as water for drinking, treated effluents and

surface water (Maycock & Watts, 2012). Sophisticated technologies like liquid chromatography and tandem mass spectrometry (LC-MS/MS) are frequently used in the identification of residual API in water and wastewater because they can identify target substances to the ng/l scale (Gago-Ferrero *et al*., 2015).

While detection techniques such as HPLC, LC-MS/MS, and GC-MS have advanced API monitoring, each method has unique limitations. For instance, LC-MS/MS provides high sensitivity and selectivity at ng/L levels, but it is costly and requires skilled personnel, limiting its application in low-income countries (Maycock & Watts, 2012). Conversely, HPLC is more accessible but has lower resolution for complex mixtures. Some studies also highlight the absence of standardized protocols across laboratories, which leads to difficulties in comparing results globally (Gago-Ferrero *et al*., 2015). A critical gap remains in developing affordable, field-deployable technologies for API detection in regions with limited resources.

The choice of technique is influenced by the physicochemical properties of the target substances, for instance to evaluate target molecules that are more polar and extensively dissolved in water but overall, LC-MS/MS analysis is preferable (Sargent (Ed.), 2015). New researches are focusing on the detecting and analyzing residual APIs and metabolites because the new hybrid high resolution mass spectrometers (HRMS) that can combine non-targeted testing in deep scan configuration with targeted MS/MS investigation have been developed (Pugajeva *et al*., 2017). The sole purpose of LC-MS/MS is to identify and quantify these substances. The outputs and findings can then be combined with existing

techniques for determining the dangers to humans and ecosystems to evaluate the risks involved with their existence.

2.11 Relationship between Water quality and concentrations of APIs

Because the quantities and withdrawal of these pollutants in ecological media rely solely on water quality (WQ) parameters and it's crucial to identify them in such studies (Kayiwa *et al*., 2022). These variables include pH, COD, BOD, turbidity, and dissolved oxygen as well as nutrients (Ohoro *et al*., 2022). It is problematic to conclude on the impact of WQ on the quantification of residual APIs because other factors such as change in climate, nature of water, cause of contamination, and resilience may have a significant impact too. However, it was observed that an increase in turbidity, gives higher concentrations of residual APIs, and neutral pH gives higher residual APIs concentrations (Ohoro *et al*., 2022).

Even though TDS, DO, total carbon and temperature are thought to be the main factors that influence the total concentrations of residual APIs (Ferguson *et al*., 2013), their concentrations can also rise with the increase in DOC content establishing it as a transporter of residual APIs (Zhao *et al*., 2015). As the amount of suspended sediment in water rises, a growing number of residual APIs get adsorbed to TSS which facilitates their removal hence decreasing concentrations of residual APIs (Lara-Martín *et al*., 2014; J. Zhou & Broodbank, 2014). Even though there has been numerous international researches about residual APIs, very few publications have clarified on how water quality affects their levels. Therefore, its crucial to investigate further the linkage between water quality and API levels.

2.12 Summary Literature and Research gaps.

The literature shows that APIs of various types have been used and detected in the environment since the 1970s, but the level of pollution has only increased in recent years (Kummerer, 2001). This rise is largely linked to the growing disease burden and lifestyle changes that have driven higher consumption of medicines, which in turn has stimulated increased pharmaceutical production (WHO, 2018). The main sources of residual APIs include hospitals, healthcare centers, pharmaceutical manufacturing plants, and improper disposal of unused medicines (Ali *et al*., 2017). These pollutants are primarily detected in surface waters, soils, and drinking water, highlighting the widespread environmental presence of APIs (Maycock & Watts, 2012). However, there is a gap in understanding how water quality parameters influence the behavior and fate of these compounds in aquatic ecosystems.

Once in the environment, APIs can have significant adverse effects. Literature reports include the development of antimicrobial resistance (AMR) in humans and feminization of fish caused by ethinylestradiol, as well as impacts on liver function, oxidative stress, and disruption of physiological processes in aquatic organisms (Hamilton *et al*., 2022). Ecological risks can be categorized as high, moderate, or low, providing an assessment of ecosystem health. These findings emphasize the serious potential consequences of residual APIs on both human and ecological health (Whomsley et al., 2019). Nonetheless, gaps remain in assessing the long-term impacts of chronic, low-dose exposure to these compounds and understanding the combined ecological risks of multiple APIs.

While detection technologies such as LC-MS/MS and high-resolution mass spectrometry have advanced the study of residual APIs, each technique has limitations. Some require specialized personnel and sophisticated infrastructure, which are often lacking in low-income countries, affecting consistent monitoring and evaluation (Maycock & Watts, 2012). The choice of technology depends on its suitability for specific compounds and environmental matrices. However, there are limitations to the accessibility and applicability of these detection methods in optimizing their application in resource-limited countries to integrate them water quality monitoring of residual APIs.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

3.1.1 Kinawataka Channel

Kinawataka channel which is about 8.3 Km long was designated by the KCCA as a primary drainage channels that drains the areas of Kireka, Ntinda, Mbuya I, Banda, Mbuya II, Mutungo, Butabika, Kirinya, Luzira all in Nakawa Division in Kampala district as reported by (SMEC, 2017). It crisscrosses the wetland before it proceeds into Lake Victoria, Uganda's most important source of fresh water.

3.1.2 The streams

Two major streams supply the Kinawataka channel, the first stream (Jamaica stream) which is about 2.6 Km and originates from the Ntinda area, in northeast Kampala and crosses Ntinda Industrial Area which has many industries including one PMF which crosses Jinja Road Kyambogo side.

The Second stream (Kawooya stream) which is about 2.7 Km and starts from upper Kireka, flows in the valley between Kireka and Banda, and comes out around Norbrook Pharmaceuticals where it joins the major Kinawaka channel which flows through Banda slum, Kinawataka, Mbuya, Butabika and Port Bell on Lake Victoria. The streams have been named letters A and B

3.1.3 The wetland

Part of the wetland of interest is before Lake Victoria on the side of Port Bell in Luzira area in Nakawa Division, Kampala and it's about 0.016 sq.km. The dominant vegetation in this wetland is a seasonal swamp. This wetland is

important because it is used for the natural filtration of waste as they purify wastewater with hazardous chemicals before it's discharged into the lake.

In general, the Kinawataka catchment area were the Kinawataka channel, Jamaica and Kawooya streams and the wetland were characterized by anthropogenic activities such as informal settlements, industrial setting most common ones being pharmaceutical and paint, car washing bays, solid waste dumping, crop and livestock farming, local merchandise and drug shops.

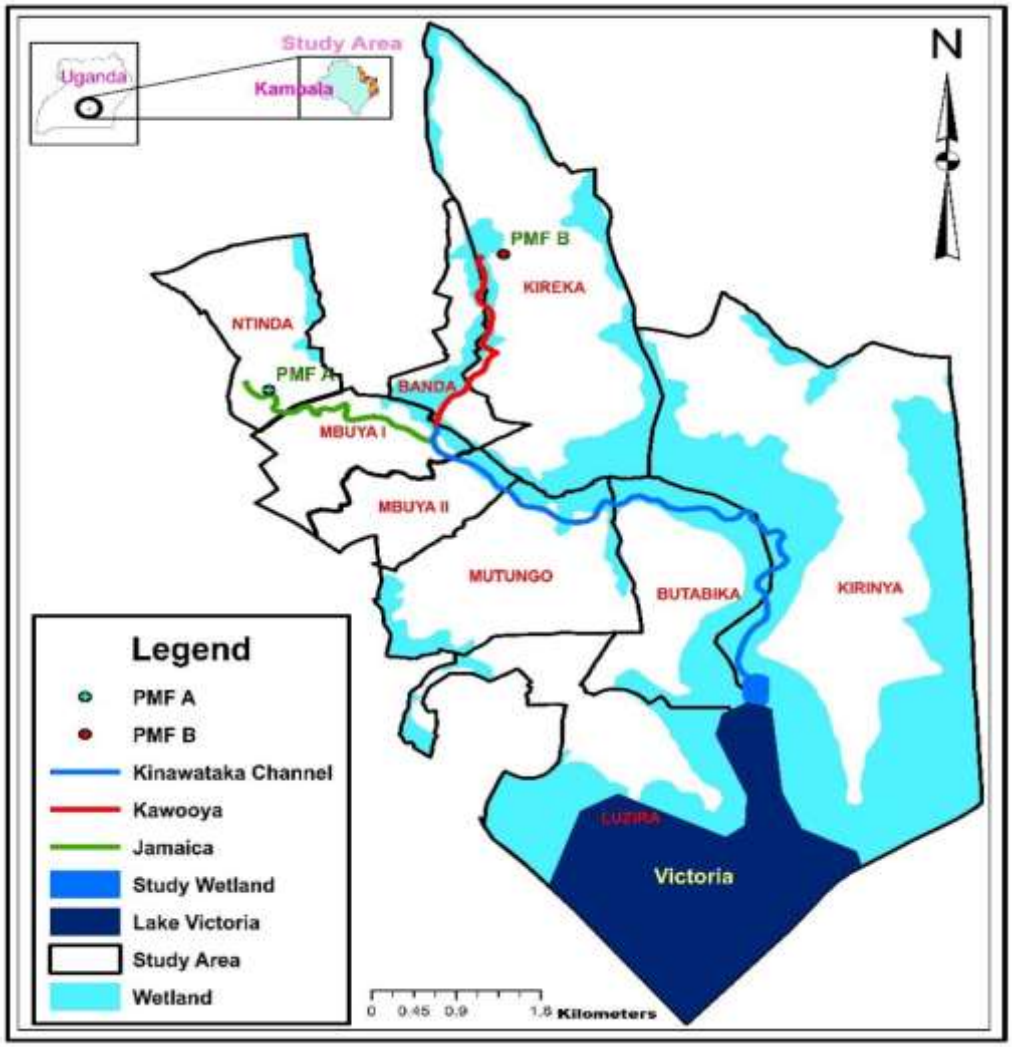

Figure 3. 1 A map showing the study Area including Kinawataka channel and the major streams that feed it.

3.2 Research Design

The study was a completely cross-sectional design on the sampling and analysis of waste water and estimated the water quality parameters and concentrations of the 7 selected residual APIs and the samples were all collected at random.

The quantification of the selected residual APIs was achieved by two strong analytical techniques that included solid phase extraction to clean and concentrate samples and instrumental analysis by the LC-MS/MS system.

The distilled water blanks were analyzed with the samples which served as a control to measure the background contamination present in the laboratory environment and reagents used in the analysis. Seven calibration standards at concentrations of 1, 5, 10, 25, 50, 75, and 100 µg/l and a respective QC sample (at 100 µg/l) which made it possible to quantify the residual APIs and ascertain the performance of the method.

An experimental research design was preferred because of its higher levels of control, hence making the results highly acceptable and allowing firmer and more specific conclusions to be made and provides for replication of the experiments.

3.3 Selection of target APIs

The residual APIs investigated in this study were selected based on literature evidence of their high consumption, frequent detection in aquatic systems, and potential ecological risks. Several of these APIs are also included on the EU Water Framework Directive (WFD) Surface Water 2nd Watch List (Decision 2020/1161/EU, 2020). Previous studies have repeatedly reported their presence in drinking and surface waters, such as research on the Nakivubo wetlands and

Lake Victoria, Kampala (Dalahmeh *et al*., 2020), Lake Victoria, Uganda (Nantaba *et al*., 2020), wastewaters and surface waters in Scotland (Niemi, 2020), effluents and run-offs from pharmaceutical manufacturing plants and wastewater treatment plants in Kampala (Kayiwa *et al*., 2022), pharmaceutical residues in wastewater (Pugajeva *et al*., 2017), and active pharmaceutical ingredients in Malaysian drinking water (Wee *et al*., 2020). Most of the medicines analyzed are classified by the World Health Organization as essential medicines (WHO, 2021) and represent different therapeutic classes such as macrolide, sulfonamide, fluoroquinolone, and tetracycline, non-steroidal anti-inflammatory drug (NSAID) and analgesic and antipyretic. The selection criteria for the target APIs therefore considered their ecological risk profile, high consumption in Kampala, likelihood of entering aquatic environments, and their frequent detection in freshwater systems. Details of the residual APIs, including therapeutic class, molecular formula, molecular weight, environmental pathways, persistence, and ecological characteristics, are presented in Appendix 5.

3.4 Collection of samples

Surface water samples were taken right from downstream to upstream to avoid disturbance caused by sampling upstream first. The common formula $(ts/u)^2$ for estimation of minimum number of sample for a mobile matrix such as water was used with relevant assumptions (Baird *et al*., 1990).

Where t = student's statistic for at 95% confidence interval with appropriate degrees of freedom. S = overall standard deviation and u = level of uncertainty. Using the recommended ratio of overall standard deviation/level of uncertainty

(2.5), the minimum number of samples that was required to give a good statistical power is 26.

But to give the study more statistical power, the actual number of samples sampled was 33. A total of (6) samples was taken from each of Jamaica stream and Kawooya stream before they join the main channel, samples (15) were taken from the Kinawataka channel before the wetland and then the other samples (6) were taken from the wetland (at the end of the wetland as it enters Lake Victoria). Random grab samples were collected from sites described above (i.e. Kinawataka channel, its streams and wetland). The Samples were picked weekly for three consecutive weeks between 09th and 23rd June 2023 from 7-10 AM where the activities in this area were minimal hence little disturbance on the flow of the waste water.

For every set of sampling bottles used, were soaked overnight and washed with distilled water the following morning and rinsed three (3) times. The samples for water quality parameters analysis were collected in the umber HDPE bottles (1L) where stream water was collected directly into respective sample bottles. While at the sampling points, bottles were rinsed with the sample three times before taking the actual sample and labelled accordingly, the labelling included location, stage, date, and time of collection.

Samples were refrigerated in an ice box (preferably 2-8 ºC) and then transported to the Directorate of Government Analytical Laboratories (DGAL) for analysis, analysis was done within 24 hours of collection. The GPS coordinates were also picked from all the sampling points for a better description of the study area and a map with detailed sampling location is provided in appendix 1.

3.5 Determination of water quality parameters

Several parameters defining the water quality were estimated using the methods published by the American Public Health Association, American Water Works Association and Water Environment Federation for the examination of water and wastewater. Table 3.2 details different parameters that were estimated with respective method used.

Table 3. 1 Summary of Water Quality parameters estimated and the respective method utilized for their estimation according to the American Public Health Association, American Water Works Association and Water Environment Federation for the examination of water and wastewater.

WQ Parameter	Method and equipment used
Total Suspended Solids (TSS)	Gravimetric method
Biochemical Oxygen Demand (BOD)	Azide modification of the Winkler method
Chemical Oxygen Demand (COD)	COD reactor
Total Nitrogen	Alkaline Persulphate digestion method
Phosphate	Persulphate method
pH	pH meter

3.6 Determination of the concentration of selected residual APIs

3.6.1 Preparation of Samples and Solid Phase Extraction (SPE)

The samples were extracted following the (Batt *et al*., 2008) protocol as follows; The Samples were first filtered using filter funnels and whatman filter paper (5.0 µm) in order to remove the suspended particles and then filtered through a vacuum filter of about 0.45 µm in order to clean them by removing the particulate matter and protection of SPE Cartridges used in extraction. To every 300 ml of

the filtered samples, 1.2 ml of di sodium editate (0.5% w/v), to help in chelation and ascorbic acid (0.0025%w/v) was also added to reduce or eliminate any residues of chlorine existing in the samples. Oasis HLB cartridges containing 0.5g of sorbent were used for extraction. Acetonitrile (6 mL) was passed through the SPE cartridges to facilitate conditioning followed by distilled water (6 mL). Samples were pushed through the cartridges at a rate of 3-5 mL/min by use of a vacuum manifold.

Using about 6 M of Formic acid (2%), cartridges were washed and allowed to dry in vacuum. Elution of analytes (acidic and neutral) was achieved with 4 mL of acetonitrile into glass tube while different glass tube was used to elute basic analytes which was achieved with Acetonitrile containing 5% ammonium hydroxide and later dried using a nitrogen evaporator at 40 °C.

The acidic elutions were reconstituted using acetonitrile (20%), while the basic one was reconstituted using methanol (20%). The sample solutions reconstituted were filled into clean liquid chromatography (LC) vials for analysis. The quantification of the selected residual APIs was achieved by a strong analytical LC-MS/MS system.

3.6.2 Instrumentation for the determination of residual APIs

To determine the concentrations of the selected residual APIs, the analysis of the extracted samples was conducted using liquid Chromatography-Tandem mass spectrometer (LC-MS/MS) system according to (Furlong *et al*., 2014). The detection unit composed of a mass spectrometer (MS/MS) and this was due to its high specificity and sensitivity since the residual APIs are typically present at

levels of micrograms or nanograms per liter. Sample analysis was done in duplicate.

At the DGAL laboratory the analysis was assigned to equipment LC 1260 internally identified with No. DGAL/TOX/LCM/OO1/HQS. The column used was a C-18 of ZORBAX type, 2.1×100 mm, 1.8 μm and the column was kept at 40 °C throughout the analysis.

Mobile phases A & B were used, where A was composed of 0.1% formic Acid adjusted to PH 5.5 using ammonium hydroxide (NH_4OH) and B was purely acetonitrile. The elution was majorly gradient where mobile A & B were varied according to the table below. The flowrate of the system was maintained at 0.3 ml/min while injecting 1.0 μl of the sample.

Table 3. 2 Summary of gradient elution profile indicating run time and the corresponding percentage composition of mobile phase A and mobile phase B during the Analysis.

Run time	**% mobile phase A**	**% mobile phase B**
0	100	0
15	0	100
20	0	100
21.5	100	0

The mass spectrometer used was an Agilent G6420A QQQ operating in positive (ESI+) and negative (ESI-) electrospray ionization mode. The instrument's mass range was set between 125 and 800 amu while scanning for 300 milliseconds. A voltage of 3,500 volts was applied, while the nebulizer gas pressure was

maintained at approximately 2.5 bars. The drying gas flow rate was 9 liters per minute while its temperature was set to 350°C.

The above settings for the mass spectrometer were tailored for each set of analyte were carefully adjusted to achieve ion transition and the ions with the greatest signal were used for quantifying respective compounds, while the transition with the next greatest signal was used to confirm the compound. Sample analysis was conducted using multiple reaction monitoring (MRM) mode whereas, ChemStation, version LTS 01.11 software was used to quantify of target compounds.

3.7 Ecological risk assessment of the selected residual APIs

The European medicines Agency (EMA) has always guided on how to assess the environmental risk posed by medicinal products for human use which was determined as a risk quotient (RQ) (Whomsley et al., 2019). The RQ is in simple terms the ratio of Measured Environment Concentration (MEC) to the Predicted No Effect Concentration, expressed mathematically as RQ = MEC/PNEC. The maximum measured environment concentrations were used instead to depict a worst case scenario. Predicted No Effect Concentrations (PNECs) represent the concentration of residual APIs below which an undesirable effects are expected not occur and was derived from the acute toxicity data available on the Norman Ecotoxicoligical Database (Zhou *et al*., 2019).

The PNECs was determined by the AF (assessment factor) method which is also the most commonly used method due to its lowest data requirements and PNEC was derived as a ratio of the smallest acute toxicity value either median effective concentration (EC50) or lethal concentration (LC50) reported in the Norman

toxicity Database literature representing three trophic levels of fish, algae and Daphnia magna to the assessment factor (AF) of 1000 which is used to cater for uncertainties associated with raw toxicity data and all this was computed using Microsoft Excel (Version 16). However, this stage of risk assessment does not provide a complete ecological risk assessment but provides baseline data for further such investigations.

The risk ranking criteria utilized to categorize the risk associated with the residual APIs i.e. RQ less than 1 (minimal risk), RQ between 0.1 and 1 (moderate risk) and RQ greater than 1 (high risk) was highly appreciated by (Zhou et al., 2019).. The risk caused by all the residual APIs detected was determined using the concentration addition (CA) model ($RQ_T = \sum_{l=1}^{n} RQi$) which helped to obtain a broad analysis of the risk associated with Kinawataka Channel and its streams, where; RQ_T = Total risk quotient, n = total residual APIs detected and RQ_i = Risk quotients of individual residual APIs.

3.8 Quality control and Quality Assurance

In the lab, all sample bottles for water analysis were soaked overnight and cleaned thoroughly using tap water and then rinsed three times using distilled water before use, and separate pipettes and filters were used to avoid cross-contamination. Samples were handled in a clean space and benches were cleaned before and after analysis. The samples were analyzed with distilled water blanks which served as a negative control to assess the background levels of contaminants present in the laboratory environment and reagents used in the analysis and the equipment was also calibrated to ensure accuracy. Seven calibration standards at concentrations of 1.00, 5.00, 10.00, 25.00, 50.00, 75.00, and 100.00 µg/l were analyzed where

the Linearity (R^2 > 0.99) for all the residual APIs was achieved and a representative calibration Curve is presented below in figure 4.6.

Method validation parameters, LOD and LOQ were obtained from the formula 3.3σ / S and 10σ / S respectively where σ is the standard deviation of the response of 10 replicate blank injections, S is the slope of individual residual API from the calibration curves (Harron, 2013; Wenzl et al., 2016). (Table 4.1).

Every set of calibration standards was analyzed with a respective QC sample of known concentration (100 µg/l) and its recovery calculated where accuracy of the method was calculated according to (Conference et al., 2005; Gad, 2007). (Table 4.1). This was achieved by the formula

$$\% \text{ Recovery} = \left(\frac{\text{Measured Concentration}}{\text{Known Concentration}}\right) * 100$$

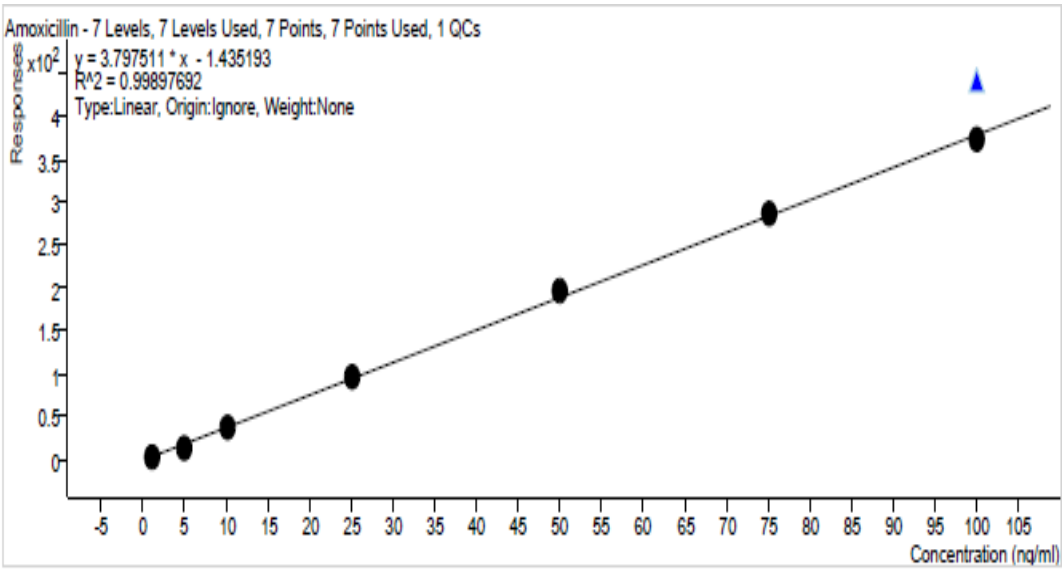

Figure 3. 2 A representative calibration curve (Amoxicillin) at seven (7) different concentration levels of 1.00, 5.00, 10.00, 25.00, 50.00, 75.00, and 100.00 µg/l and a QC sample of a known concentration (100.00 µg/l).

Table 3. 3 The LOD and LOQ values for method validation which were calculated from 10 replicate blank responses and the % recovery obtained as a ratio of the response from 3 replicates of respective QC samples of known concentrations (10 µg/l) to the expected concentrations.

Residual API	**LOD (ng/l)**	**LOQ (ng/l)**	**% Recovery**
Amoxicillin	0.2987	0.9050	100.48
Sulfamethoxazole	0.0240	0.0727	99.55
Ciprofloxacin	0.0033	0.0101	101.72
Erythromycin	0.8412	2.5489	101.51
Oxtetracycline	0.0425	0.1289	98.38
Paracetamol	0.0097	0.0295	99.84

3.9 Statistical analysis

Microsoft Excel (Version 16) and IBM SPSS Statistics version 20 were used for statistical data analysis. Analysis of variance (ANOVA) taking the confidence level as 95% was used to evaluate the variation in API concentrations and water quality parameters between sampling points and sampling periods. Microsoft Excel (Version 16) was also used to calculate the risk quotient (RQ). The histograms were used to analyze the variations of the water quality and residuals APIs in different sampling compartments while statistical tables were used to present the summaries of the data.

One-sample Kolmogorov-Smirnov (One-sample K test) for normality was used to test for the normality of the data and one-way ANOVA was used to identify particular differences in the means once the data was found to be normally while Tukey multiple comparison of means (Tukey's Post hoc tests) was done to locate particular differences between the groups. When the data was found not be normally distributed, Kruskal-wallis test was used to determine the whether there exist differences between the means and Post hoc Dunn's test was also used to determine the exact differences between the groups. One-way ANOVA and Kruskal-wallis test was used for comparing group means the dependent and independent variables.

To measure the association of water quality (WQ) parameters and API concentrations, spearman's correlation coefficients, correlation matrix and linear regression were used. Data on WQ of the wastewater of the Kinawataka channel, its streams and wetland was compared with CFR effluent guidelines and standards and the national environment standards for discharge of effluent into

water or land. Data on the concentrations of API was compared to the existing literature to give a new understanding of the study, conceivably giving course to any future studies.

CHAPTER FOUR

RESULTS

4.1 Water quality parameters along the Kinawataka channel, its streams and wetland

Waste water in the four sampling compartments i.e. Channel, Jamaica, Kawooya streams and Wetland were analyzed for water quality parameters and then compared with the NEMA specifications. The WQ parameter studied were pH, TSS, COD, BOD, Phosphate and Nitrates. The summary of WQ parameters, including their means ± standard deviations (SD), ranges, and the corresponding National Environment Management Authority (NEMA) specifications, is provided in detail in Appendix 2.

4.1.1 pH

pH was in the range of 7.08 - 7.99 in all the sampling compartments i.e. channel, Jamaica stream, Kawooya stream and wetland respectively and were all within the specifications of NEMA of (5.0 -8.5). The Channel (7.42 ± 0.26) and Wetland (7.43 ± 0.33) had relatively high mean pH. Kawooya stream (7.26 ± 0.23) and Jamaica stream (7.39 ± 0.19) had relatively low mean pH compared to the channel and wetland (Figure 4.1).

One-way Anova was conducted for the pH values and it was found out no significant differences existed in pH values in the sampling compartments (P value = 0.523) which suggests that the sources contributing the acidic and basic polluting substances remained low in the sampling compartments. Similarly, no significant difference existed in the pH values between sampling weeks (P value = 0.051).

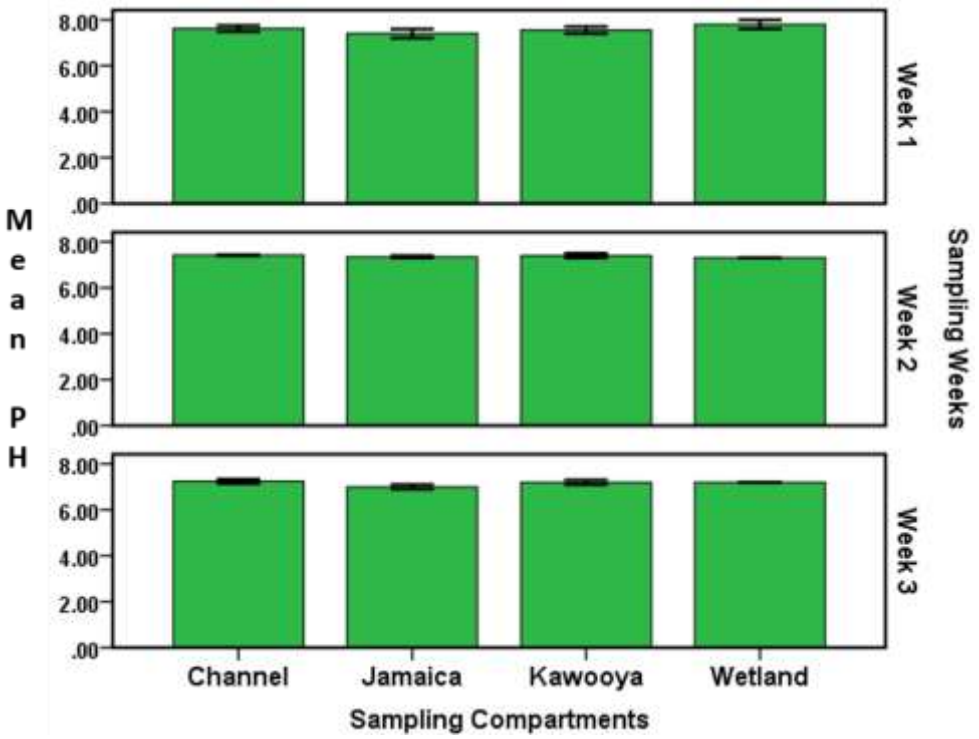

Figure 4. 1 Comparison of pH across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of pH with standard error bars presented to indicate variability in of the test groups.

4.1.2 Total Suspended Solids (TSS)

The Channel and Wetland which were in the ranges of 37.00 – 389.00 mg/L and 20.00 - 269.54 mg/L respectively were the only sampling areas with values of the individual sampling points complying with NEMA specifications (50 mg/L).

The rest of the sampling areas i.e. Jamaica stream, 58.00 – 412.00 mg/L and Kawooya stream, 133.29 – 312.00 mg/L were entirely out of NEMA specifications. All the means of TSS in the sampling compartments i.e. Channel (221.80 ± 88.11 mg/L), Jamaica stream (166.17 ± 45.25 mg/L), Kawooya stream (241.67 ± 68.82 mg/L) and Wetland (105.83 ± 50.82 mg/L) were all out of NEMA

specifications with Kawooya stream having the highest levels of TSS. The mean TSS in Kawooya stream > Channel > Jamaica stream > Wetland. (Figure 4.2)

The TSS data obtained was normally distributed, and the statistical comparisons to estimate the variations of concentrations of TSS in the sampling compartments and during sampling periods were established. There was no significant difference in the concentrations of TSS in all the sampling compartments (P value = 0.063) and between the sampling weeks (P value = 0.759) which implies that the sources of pollution contributing TSS to the study remained high throughout.

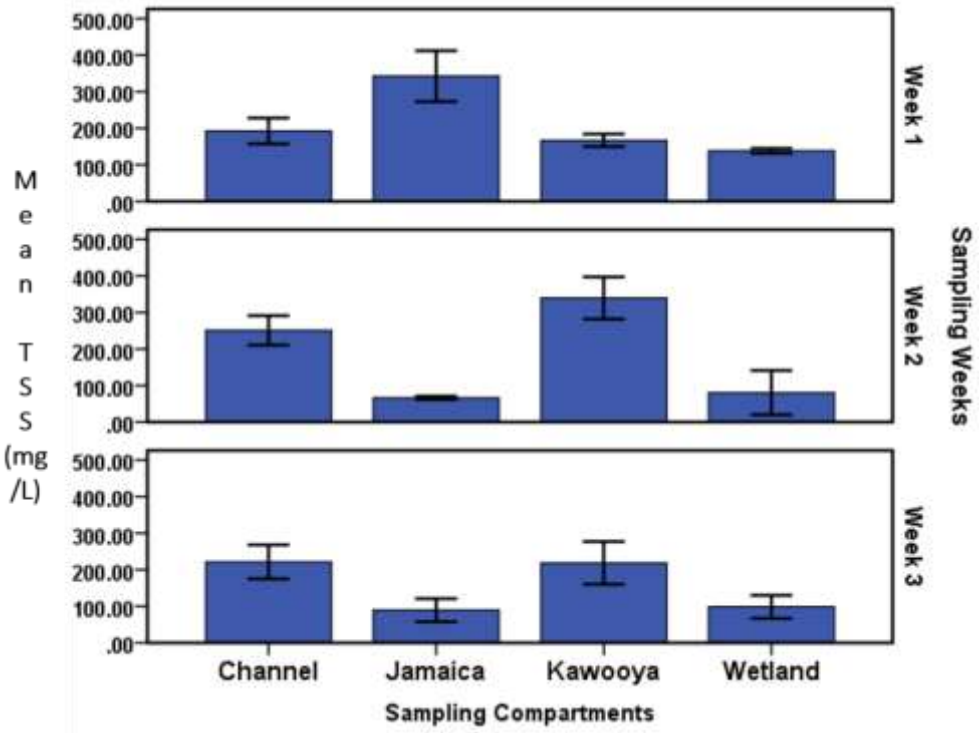

Figure 4. 2 Comparison of total suspended solids (TSS) across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of TSS with standard error bars presented to indicate variability in of the test groups.

4.1.3 Chemical Oxygen Demand (COD)

The COD in the channel, Jamaica stream and wetland were in the ranges of 7.48 – 334.70 mg/L meaning some individual sampling points had values with in NEMA specifications (70 mg/L). But Channel (131.24 ± 72.39 mg/L) and Jamaica stream (98.43 ± 129.67 mg/L) had mean values that were largely out of specifications whereas the Wetland in the range of (5.83 – 99.07 mg/L) had some sampling points with values above the NEMA specifications but was the only sampling area that had mean COD (37.83 ± 32.92mg/L) complying with NEMA specifications. The COD for individual sampling points in Kawooya stream with range (133.29 – 312.10 mg/L) were entirely and largely out NEMA specifications and the mean (173.47 ± 68.82) was also out of specifications (Figure 4.3).

The COD values were normally distributed but there existed a significant difference in the COD concentrations observed in the sampling compartments on conducting One-Way Anova (P value = 0.038). The Post hoc test revealed that the difference in COD concentrations existed between Kawooya and Jamaica streams and wetland suggesting that the pollution with organic substances in Kawooya stream was much higher than that of the wetland. However, it also suggests that pollution of organic substances in Kawooya stream, Jamaica stream and channel remained high and similar. On the contrary, there was no significant difference in COD concentrations observed between the sampling weeks, meaning there were similar levels of pollutions between the sampling weeks.

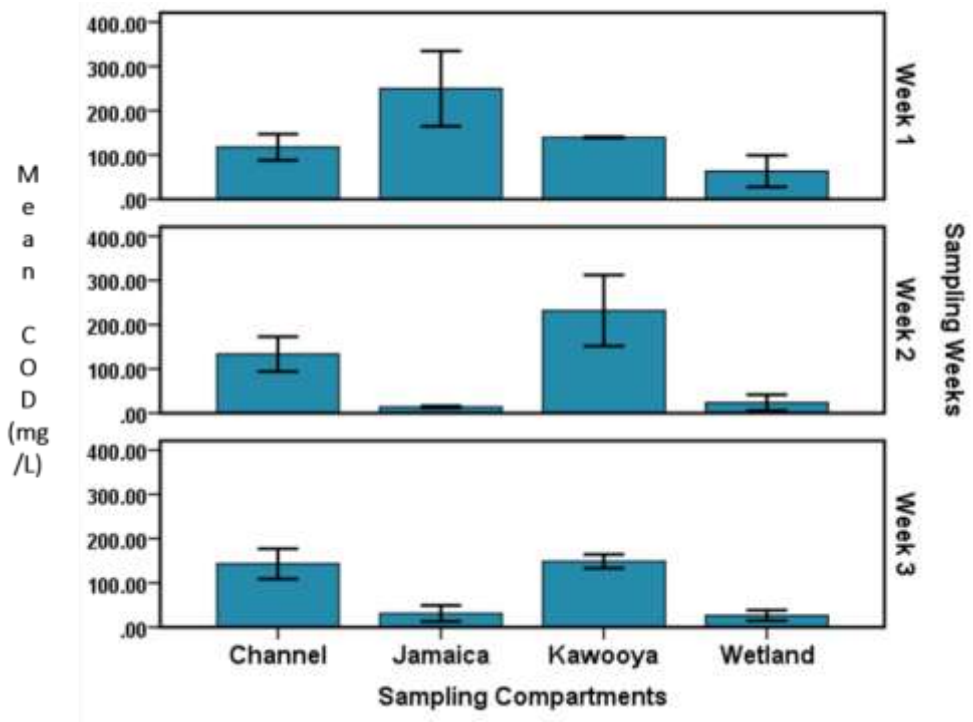

Figure 4. 3 Comparison of chemical oxygen demand (COD) across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of COD with standard error bars presented to indicate variability in of the test groups.

4.1.4 Biological Oxygen Demand (BOD)

The BOD in the Channel, Jamaica stream and wetland were in the ranges of 2.59–159.26 mg/L where a few individual sampling points had values with in NEMA specifications (50 mg/L). The means of the channel (61.75 ± 25.37 mg/L) and Jamaica stream (65.36.43 ± 47.16 mg/L) were just above the specifications whereas the wetland with BOD range of (2.59 – 47.67 mg/L) had all sampling points with values with in the NEMA specifications and was the only sampling area that had mean BOD (31.00 ± 17.52 mg/L) complying with NEMA specifications. The BOD for individual sampling points in Kawooya stream with

range of (54.58 – 142.48 mg/L) was entirely out of NEMA specifications and the mean (73.20 ± 34.12) was also slightly above the specifications. The COD was generally higher than BOD in all the sampling compartments (Figure 4.4).

The BOD data was normally distributed and presented no significant differences in both the sampling compartments (P value = 0.109) and between sampling weeks (P value = 0.679) which means that generally there was a continued high pollution with organic pollutants to the Kinawataka catchment area throughout the sampling period.

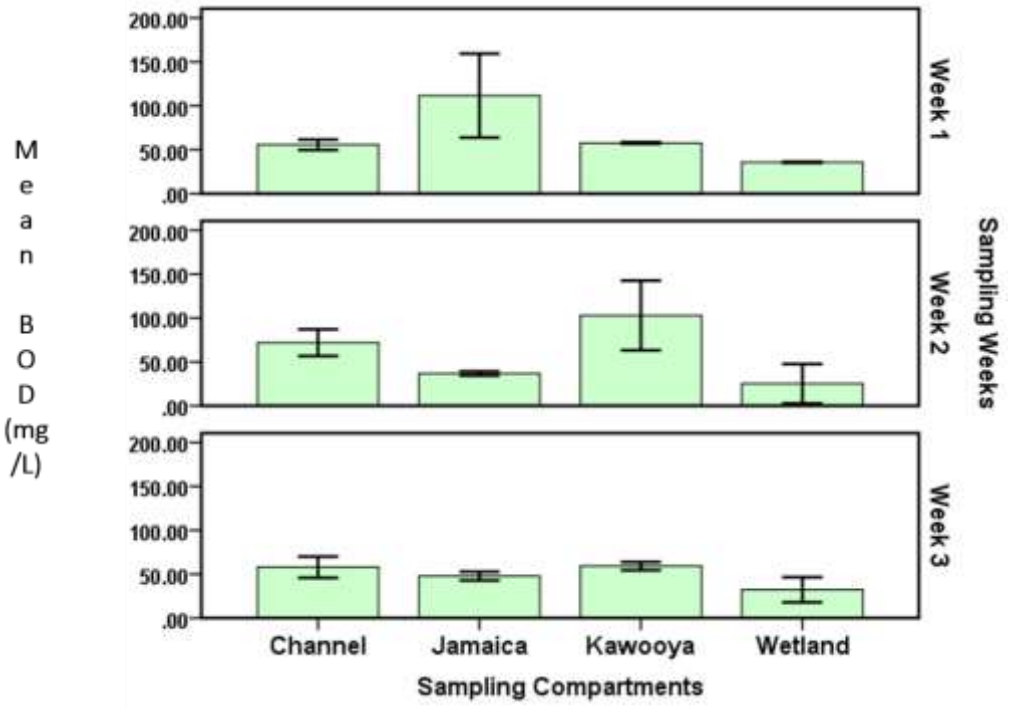

Figure 4. 4 Comparison of biological oxygen demand (BOD) across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of BOD with standard error bars presented to indicate variability in of the test groups.

4.1.5 Total Nitrates

Total Nitrates were ranging from 0.18 - 0.51 mg/L in all the sampling compartments i.e. channel, Jamaica stream, Kawooya stream and wetland respectively were all within the specifications of NEMA of 10 mg/L. The means of the Channel (0.33 ± 0.07 mg/L), Jamaica stream (0.30 ± 0.03 mg/L), Kawooya stream (0.29 ± 0.08 mg/L) and wetland (0.24 ± .04 mg/L) which were all within the specifications of NEMA where the Channel > Jamaica stream > Kawooya stream > Wetland (Figure 4.5).

The data of total nitrates was normally distributed and on conducting a One-Way Anova, there was no significant difference in the total nitrates levels in both the sampling compartments (P value = 0.056) and between the sampling weeks (P value = 0.570), meaning the sources of pollution sources with nitrates load were low throughout this study.

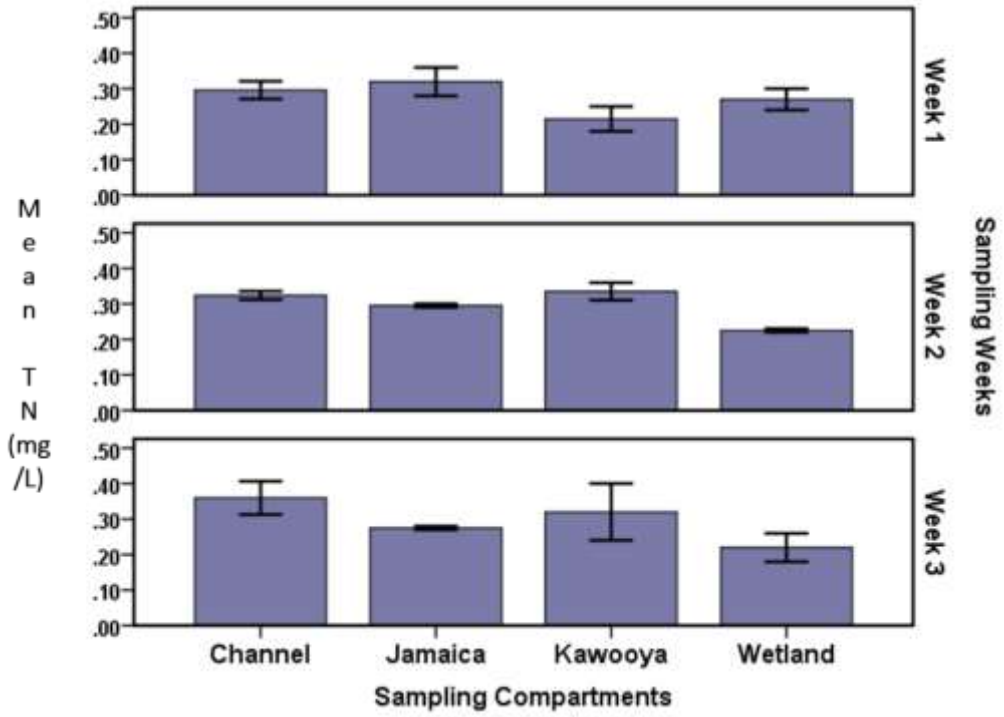

Figure 4. 5 Comparison of total nitrate (TN) across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of TN with standard error bars presented to indicate variability in of the test groups.

4.1.6 Total phosphates

The total phosphate in the Channel, Jamaica stream, Kawooya stream and Wetland had ranges of 0.12-43.60 mg/L meaning individual sampling points had values within and out of NEMA specifications (5 mg/L). All the means 13.50 ± 10.46 mg/L, 12.66 ± 16.51 mg/L, 20.91 ± 12.13 mg/L, 5.26 ± 5.53 mg/L of the above sampling areas respectively were out of NEMA specifications too, with the mean of the Wetland slightly above the specifications (Figure 4.6).

Statistically, there was no significant difference in the total phosphates concentrations in the sampling compartments (P value =0.155) and between

sampling weeks (P value = 0.824), implying that the level of pollution in these areas with waste high in levels of phosphates was constant and consistent through sampling period.

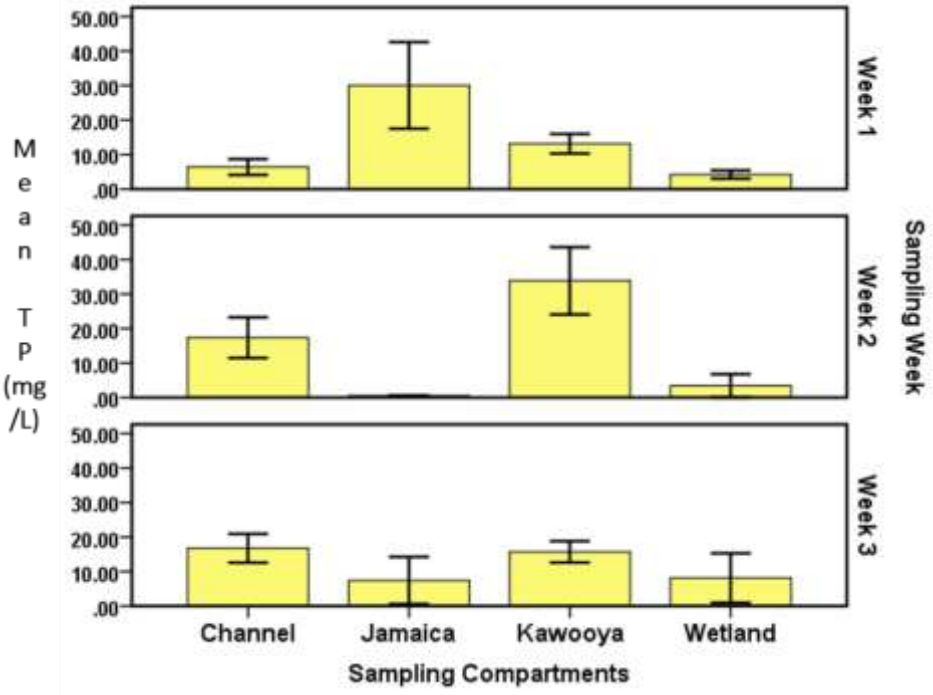

Figure 4. 6 Comparison of total phosphate (TP) across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of TP with standard error bars presented to indicate variability in of the test groups.

4.3 Determination of the concentrations of the selected residual APIs.

A total of 33 samples from all the four sampling compartments i.e. Kinawataka Channel, Jamaica stream, Kawooya stream and Wetland were analyzed over a period of three weeks. Residual APIs Amoxicillin, Erythromycin, Ciprofloxacin, Sulfamethoxazole, Oxytetracycline and Paracetamol were all detected at quantifiable amounts (ng/l**)** in at least one sampling point. Only one residual API (Ibuprofen), out of the seven that were analyzed, was not detected in any of the sampling compartments.

The summary of quantified residual APIs including the number of detects (n), detection frequency (% DF), Mean concentration (± standard deviation), range and the median of residual APIs quantified in the all the sampling compartment is provided in detail in Appendix 3.

A sample of the APIs detected in the samples across the sample compartments (Jamaica stream, Kawooya stream, Channel and wetland) with each mass spectrum showing a molecular ion at a specific mass-to-charge ratio (m/z) corresponding to the respective molecular weight of each compound, thereby confirming their presence in the samples analyzed are presented in detail in Appendix 4.

4.3.1 Amoxicillin

Kawooya stream had the highest mean concentrations of amoxicillin 173.91 ± 269.28 ng/l and Jamaica stream had the lowest mean concentrations 5.53 ± 7.44 ng/l. Kinawataka Channel and Wetland had relatively low mean concentrations 11.00 ± 15.80 ng/l and 10.01 ± 21.21 ng/l respectively compared to Kawooya

stream. Week 1 had the most detected amoxicillin while other weeks (1&2) had relatively very low amoxicillin detected. (Figure 4.7)

Kruskal-wallis test was conducted for amoxicillin concentrations and it was found out that no significant differences existed in amoxicillin concentrations in the sampling compartments (P value =0.177) which suggests that the pollution of amoxicillin in the sampling compartments remained the same throughout. However, there was a significant difference in the amoxicillin concentrations between sampling weeks (P value = 0.001). Post hoc test (Turkey's Honest test) confirmed that there existed differences between the sampling weeks specifically between week 2, 3 and week 1.

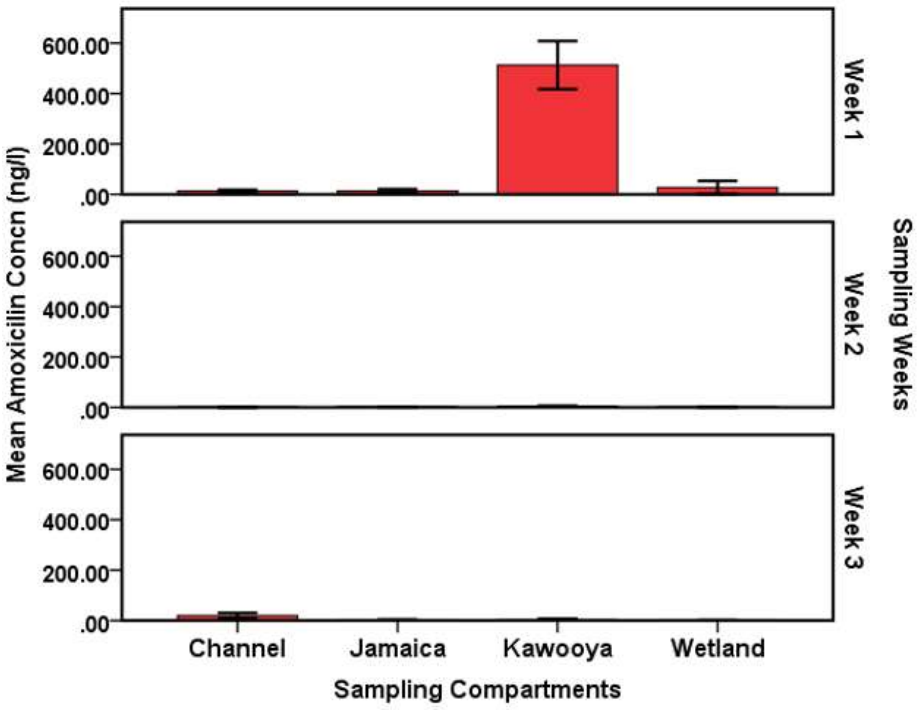

Figure 4. 7 Comparison of amoxicillin across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of amoxicillin with standard error bars presented to indicate variability in of the test groups.

4.3.2 Erythromycin.

Jamaica stream had the highest mean concentrations of erythromycin 936.55 ± 1503.31 ng/l and Wetland had the lowest mean concentrations 49.76 ± 121.90 ng/l. Kinawataka Channel and Kawooya stream had relatively high mean concentrations 441.29 ± 804 ng/l and 499.30 ± 815.38 ng/l respectively compared to Jamaica stream while Week 1 & 2 had the most detected erythromycin compared to week 3 (Figure 4.8)

The erythromycin concentrations from all the sampling compartments were found not to be normally distributed after testing for normal distribution with P value (0.001) using a non-parametric test (one-sample Kolmogorov-Smirnov test) using SPPS version 20.

Kruskal-wallis test was conducted for erythromycin concentrations and it was found out that no significant differences existed in erythromycin concentrations in the sampling compartments (P value =0.619) which suggests that the pollution of erythromycin in the sampling compartments remained the same throughout. However, there was a significant difference in the erythromycin concentrations between sampling weeks (P value = 0.001). Post hoc (Turkey's Honest) test confirmed that there existed a significant difference between week 1 and week 3 and between week 2 and week 3.

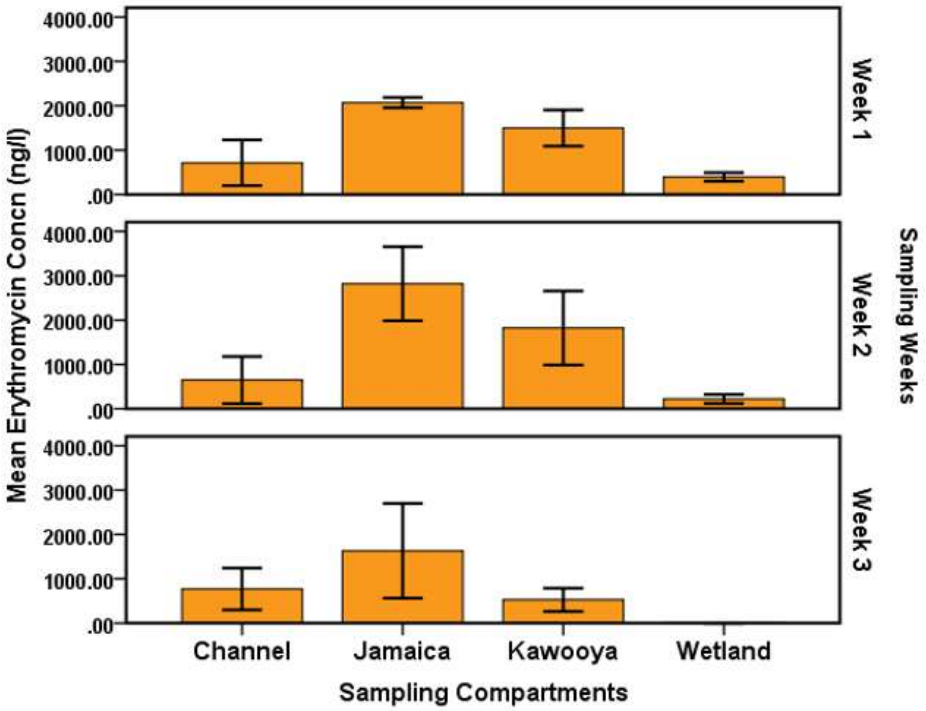

Figure 4. 8 Comparison of erythromycin across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of erythromycin with standard error bars presented to indicate variability in of the test groups.

4.3.3 Sulfamethoxazole

Furthermore, Kawooya stream had the highest mean concentrations of sulfamethoxazole 388.91 ± 363.53 ng/l, the wetland also had relatively high mean concentrations of sulfamethoxazole 192.28 ± 297.59. Jamaica stream and Kinawataka Channel had relatively low mean concentrations of Sulfamethoxazole 154.74 ± 163.33 ng/l and 189.21 ± 134.09 ng/l respectively. With Jamaica stream having the lowest mean concentrations. All sampling weeks had relatively high concentrations of sulfamethoxazole detected but week 3 & 2 had relatively higher sulfamethoxazole than week 1. (Figure 4.9)

Sulfamethoxazole is the only API whose concentrations were normally distributed with (P value 0.30) and this was confirmed after testing for normal distribution using a non-parametric test (one-sample Kolmogorov-Smirnov test).

Conducting ANOVA for sulfamethoxazole concentrations established that no significant differences existed in sulfamethoxazole concentrations in the sampling compartments (P value = 0.268) suggesting constant pollution of sulfamethoxazole in the sampling compartments. However, there existed a significant difference in the sulfamethoxazole concentrations between sampling weeks (P value = 0.009) and it was established by post hoc test (Turkey's Honest test) that there were differences between the sampling week 1 and week 3.

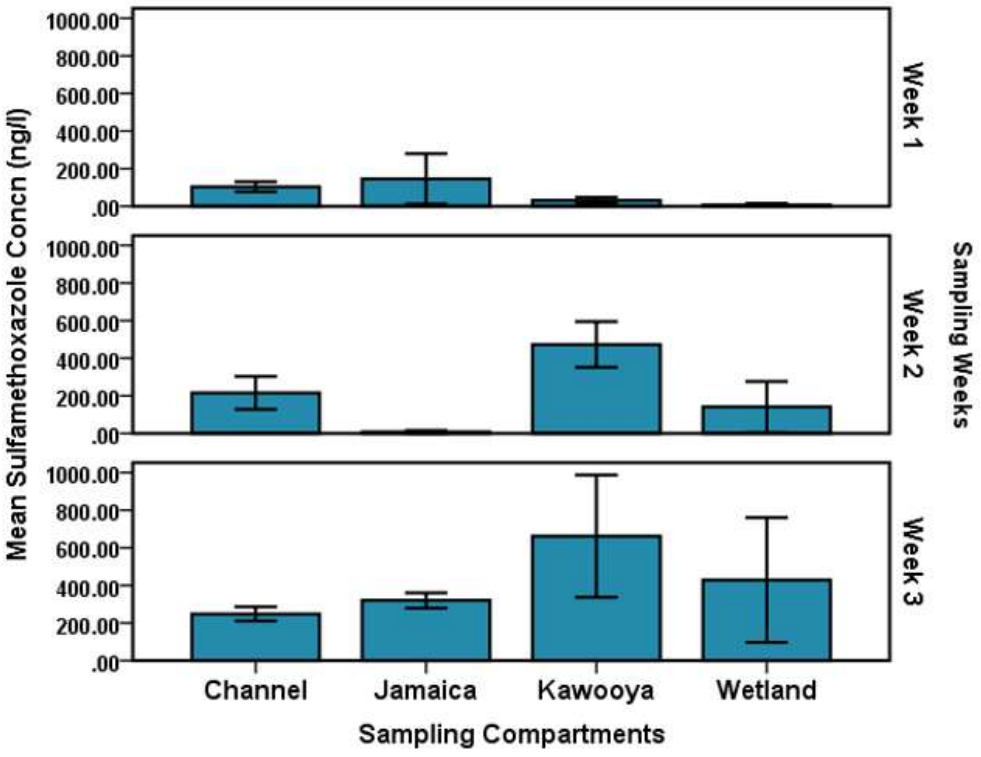

Figure 4. 9 Comparison of sulfamethoxazole across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values

of sulfamethoxazole with standard error bars presented to indicate variability in of the test groups.

4.3.4 Paracetamol

In addition, Jamaica stream had the highest mean concentrations of paracetamol 261.33 ± 376.23 ng/l. Kinawataka Channel, Kawooya stream and Wetland relatively had equal mean concentrations of Paracetamol 122.18 ± 265.50 ng/l, 138.00 ± 188.40 ng/l and 101.58 ± 150.29 ng/l respectively and the wetland had the lowest mean concentration compared to the rest. Comparing the weeks of sampling, week 1& 2 had relatively higher concentrations of paracetamol than week 3 (Figure 4.10)

A non-parametric test (one-sample Kolmogorov-Smirnov test) established that paracetamol was another API whose concentrations were not normally distributed (P value 0.001). Kruskal-wallis test established that no significant differences existed in paracetamol concentrations in the sampling compartments and between sampling weeks (P value = 0.478) and (P value = 0.839) respectively.

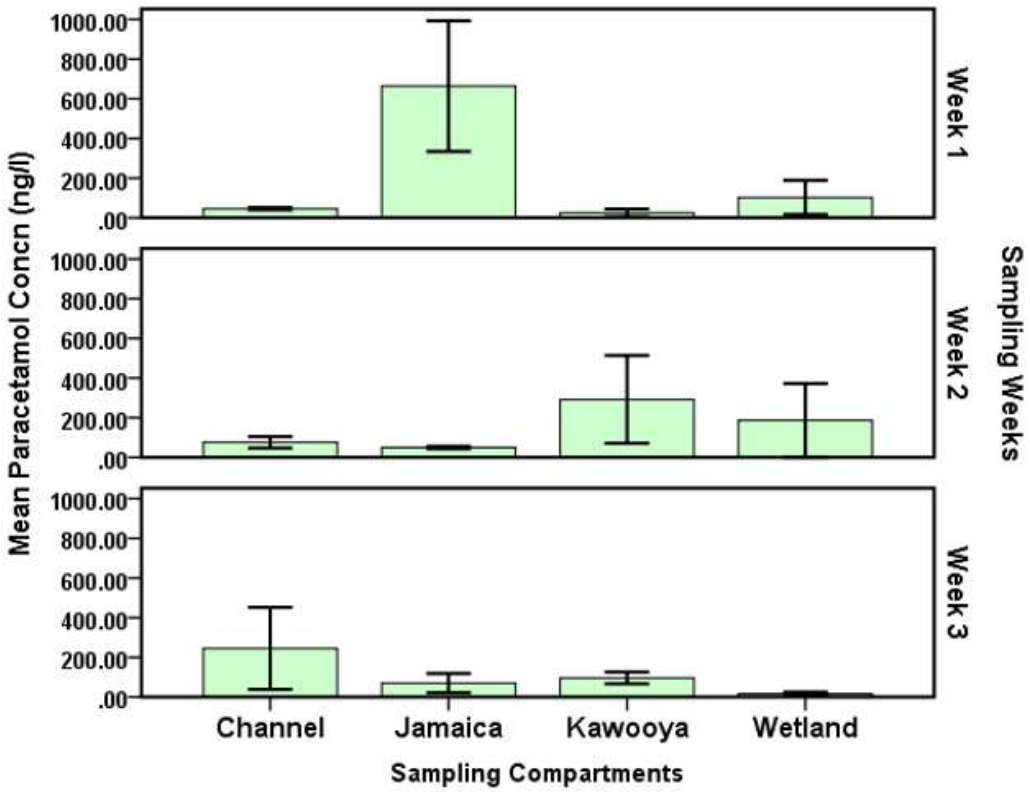

Figure 4. 10 Comparison of paracetamol across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week

sampling period i.e. week 1, week 2 and week 3. The Mean values of paracetamol with standard error bars presented to indicate variability in of the test groups.

4.3.5 Ciprofloxacin

Kinawataka Channel had the highest mean concentrations of Ciprofloxacin 13.68 ± 19.02 ng/l and Wetland had the lowest mean concentrations 8.50 ± 6.95 ng/l. Jamaica stream and Kawooya stream had relatively very low mean concentrations 12.03 ± 6.26 ng/l and 9.43 ± 1.96 ng/l respectively compared to Kinawataka Channel. However, the amount of ciprofloxacin detected in all the sampling weeks were relatively similar. (Figure 4.11)

Ciprofloxacin concentrations from all the sampling compartments were not normally distributed (P value 0.01) as confirmed by a non-parametric test (one-sample Kolmogorov-Smirnov test). A non-parametric test, kruskal-wallis test established that no statistical significant differences existed in ciprofloxacin concentrations in the sampling compartments and between sampling weeks (P value = 0.333) and (P value = 0.216) meaning that the pollution sources of ciprofloxacin in the sampling compartments and sampling weeks were constant.

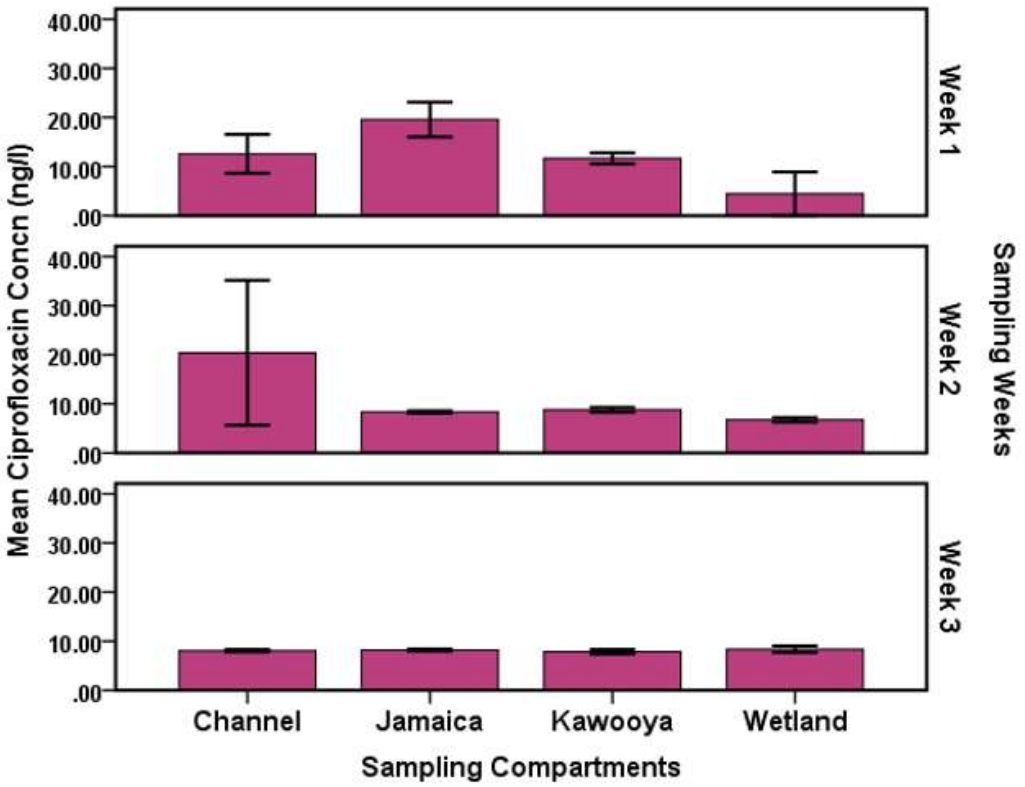

Figure 4. 11 Comparison of ciprofloxacin across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of ciprofloxacin with standard error bars presented to indicate variability in of the test groups.

4.3.6 Oxytetracycline

Considerably, Kawooya stream had the highest mean concentrations of Oxytetracycline 1.61 ± 1.88 ng/l, Jamaica stream and Kinawataka Channel also had fairly equal mean concentrations of Oxytetracycline 1.46 ± 0.66 ng/l and 1.48 ± 0.97 ng/l respectively. The Wetland had the lowest mean concentrations of Oxytetracycline 1.28 ± 1.04ng/l. However, week 2 & 3 presented comparable results of oxytetracycline while week 1 presented higher concentrations. (Figure 4.12)

Oxytetracycline concentrations too, were not normally distributed (P value 0.001) as tested by a non-parametric test (one-sample Kolmogorov-Smirnov test). Kruskal-wallis test established that no significant differences existed in oxytetracycline concentrations in the sampling compartments and between sampling weeks (P value = 0.958) and (P value = 0.133) respectively.

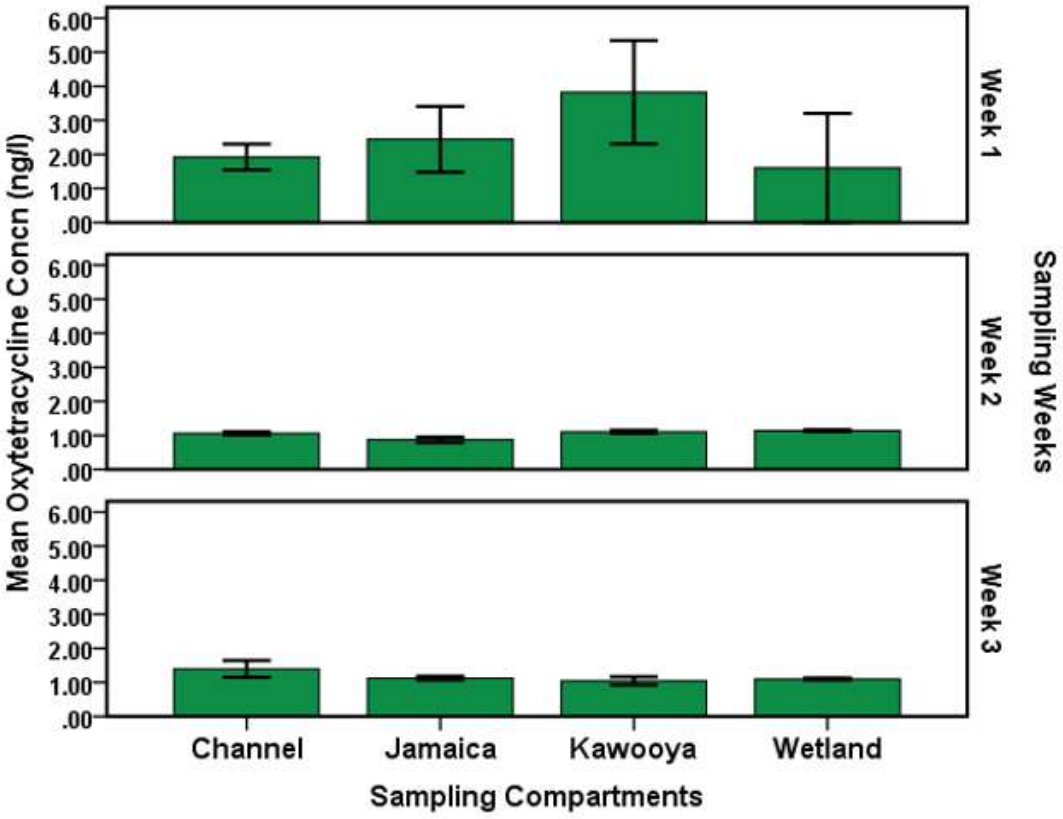

Figure 4. 12 Comparison of oxytetracycline across different sampling compartments i.e. Channel, Jamaica stream, Kawooya stream, and Wetland over a three-week sampling period i.e. week 1, week 2 and week 3. The Mean values of oxytetracycline with standard error bars presented to indicate variability in of the test groups.

4.4 Ecological risk assessment of the selected residual APIs.

The risk quotients (RQ) associated with the residual APIs quantified in Kinawataka Channel were determined considering the worst-case scenario are

summarized below (Table 4.2). The RQs of sulfamethoxazole (1.12), erythromycin (1.92) were greater than 1, a high risk and a sign that these residual APIs bring about detrimental properties to the aquatic organisms in the channel and the Lake. From table 4.2, it can also be confirmed that the two residual APIs were very much unsafe for algae which is a non-target organism in this case.

Amoxicillin (0.54) is another residual API that had RQ although less than 1, it is greater than 0.1 exhibiting a medium risk to algae which is a non-target organism in this case. Ciprofloxacin (0.02), paracetamol (0.06) and oxytetracycline (0.01) all exhibited RQs below 0.1 indicating a minimal risk. The total risk caused by all the 6 residual APIs detected was determined using the concentration addition (CA) model ($RQ_T = \sum_{I=1}^{n} RQi$) = 3.67 which helped to obtain a broad analysis of the risk associated with Kinawataka Channel and its streams.

Table 4. 1 Acute toxicity endpoints (LC 50 & EC50), Organisms level representing the aquatic food chain, Maximum environmental measured concentrations (MEC), predicted-no-effect concentrations (PNEC) and the RQ

for each of the residual APIs studied that help to measure the ecological risk they pose to the aquatic environment.

API	Organisms Levels	Acute Toxicity Data*		PNEC (ng/l)	MEC (ng/l)	RQ
		LC50 (ng/l)	EC50 (ng/l)			
Amoxicillin	Daphnia Magna	603624660				
	Algae		1124270	1124.27	607.89	0.54
	Pimephales Promelas	166467100				
Sulfamethoxazole	Daphnia Magna	99202400				
	Algae		3076080	3076.08	3431.87	1.12
	Pimephales Promelas	39318240				
Ciprofloxacin	Daphnia Magna	51513790				
	Algae		3765160	3765.16	79.17	0.02
	Pimephales Promelas	2458000				
Erythromycin	Daphnia Magna	38372870				
	Algae		515400	515.40	987.16	1.92
	Pimephales Promelas	45329160				
Oxtetracycline	Daphnia Magna	774304110				
	Algae		429440	429.44	5.34	0.01
	Pimephales Promelas	136269980				
Paracetamol	Daphnia Magna	15819560				
	Algae		43187570		992.99	0.06
	Pimephales Promelas	429353790		15819.56		

*The acute toxicity data (LC50 & EC50) was obtained from the Norman toxicity database, where each organism level had only a single data present for each residual API.

4.5 Relationship between residual API concentrations and water quality parameters

Some of the residual APIs suggested significant linear relationship with the measured water quality parameters. For every unit change in pH, concentrations of COD and total phosphates, there was an increase in the concentrations of Amoxicillin whereas there was a decrease in the concentrations of Amoxicillin for every unit change in the concentrations of TSS, BOD and total nitrates (Table 4.3).

The linear relationship of Erythromycin and water quality parameters was that for every unit change in pH, concentrations of TSS, COD and BOD there was an increase in the concentrations of Erythromycin and a decrease for every unit change in the concentrations of total phosphates, total nitrates.

Ciprofloxacin exhibited a similar relationship with a slight increase in its concentrations for every unit change in the concentrations of TSS, COD, BOD and total phosphates whereas there was a decrease for every unit change in pH and total nitrates. Paracetamol also exhibited this kind of relationship but with a change in total phosphates reducing paracetamol concentrations and a change in total nitrates increasing the paracetamol concentrations.

Table 4. 2 Regression analysis to understand the average relationship between the residual APIs and water quality parameters in the Kinawataka channel, its streams and wetland.

		Regression co-efficient					
	Regression constant	**pH**	**TSS**	**COD**	**BOD**	**Phosphate**	**Nitrate**
Amoxicillin	14.745	48.354	-0.853	1.238	-1.062	1.109	-345.021
Erythromycin	-51.454	125.493	3.904	5.139	12.969	-78.364	-297.681
Ciprofloxacin	83.525	-10.038	0.081	0.015	0.016	0.643	-25.615
Sulfamethoxazole	205.920	-236.934	-0.304	-0.426	-1.044	16.816	-526.451
Oxytetracycline	-1.080	0.347	-0.006	0.018	-0.007	-0.061	0.943
Paracetamol	15.270	-182.920	0.072	0.939	2.170	-3.141	380.305

It can be further noted that for a unit change in pH, concentrations of TSS, COD, BOD and total nitrates there was a decrease in the concentrations of sulfamethoxazole and only total phosphates indicated an increase in the concentrations. The relationship between Oxytetracycline and water quality

parameters was that for the unit change in pH, concentrations of COD and nitrates caused an increase in the Oxytetracycline concentrations whereas a unit change in the concentrations of TSS, BOD and total phosphates resulted in a decrease in concentrations.

Generally, pH and total nitrates had greater impacts on the concentrations of different APIs since it exhibited bigger coefficients whereas TSS, COD, BOD and total phosphate had minimal impact on the concentrations of the residual APIs since it exhibited smaller coefficient.

The spearman's coefficients presented the degree of relationship between the residual APIs and the WQ parameters. All residual APIs at least had a relationship, the spearman's coefficients were used to estimate this relationship by magnitude and direction (positive or negative). (Table 4.4).

Amoxicillin showed a positive but rather small correlation with all the water quality parameters which was rendered an insignificant correlation meaning that the variations in the concentrations of Amoxicillin can't be best explained by the water quality parameters.

Erythromycin showed insignificant, weak but positive correlation with TSS, COD, BOD and negative correlation with total phosphates and total nitrates which were insignificant too, therefore the variations in concentrations of Erythromycin can't be best explained by the water quality parameters. On the contrary, it showed strong positive correlation with pH (0.492) which was still insignificant.

Ciprofloxacin showed a weak and positive correlation with pH, TSS, BOD, total phosphates and negative correlation with total nitrate which was rendered insignificant whereas it was found to have a positive and significant correlation.

For Sulfamethoxazole, apart from total nitrates and total phosphates which had a positive but insignificant correlation, the rest of the water quality parameters (TSS, COD, BOD) showed a positive and significant correlation except for pH which had a negative significant correlation. Oxytetracyline had a positive and insignificant correlation with all the water quality parameters. Paracetamol had strong, positive but insignificant correlation for all the water quality parameters.

Table 4. 3 Correlation analysis to understand the degree (magnitude/direction) of relationship between the residual APIs and water quality parameters in the Kinawataka channel, its streams and wetland.

	Spearman's correlation Co-efficient					
Residual APIs	**pH**	**TSS**	**COD**	**BOD**	**Total Phosphate**	**Total Nitrate**
Amoxicillin	0.022	0.252	0.298	0.208	0.185	0.054
Erythromycin	0.492	0.247	0.214	0.187	-0.020	-0.121
Ciprofloxacin	0.067	0.177	0.349*	0.227	0.233	-0.085
Sulfamethoxazole	-0.353*	0.376*	0.385*	0.461	0.598	0.087
Oxytetracycline	0.078	0.093	0.302	0.155	0.087	0.050
Paracetamol	0.013	0.526	0.585	0.520	0.590	0.305

*The correlation is significant at 0.05.

CHAPTER FIVE

DISCUSSION

5.1 Variations in water quality parameters along the Kinawataka channel, its streams and wetland

Waste water in the four sampling compartments i.e. Channel, Jamaica stream, Kawooya stream and Wetland were analyzed for water quality parameters and then compared with the NEMA specifications. The WQ parameters studied were pH, TSS, COD, BOD, Phosphate and Nitrates.

5.1.1 pH

The observed pH values (7.26-7.43) were within the specifications set by NEMA (5.0 - 8.5) indicating that pH levels in all the sampling compartments in this study were within the acceptable environment limits (National Environment Management Authority, 2020). The pH values were found to be comparable with the pH values (7.71 ± 0.35) reported in Kinawataka channel by (Ndugga, 2021). These findings were also comparable with the results from Nakivubo channel (6.29 ± 0.27) as reported by (Kayiwa et al., 2022). pH, a key parameter in regards to determination of API concentrations in water where it was established that an increase in pH levels to neutral levels can reduce the concentrations of APIs in water (Ohoro *et al*., 2022).

The higher pH in the channel and wetland could have been caused about by the usage and discharge of soaps and detergents, paints from the surrounding slums, residential areas, garages and car washing bays which were common along the Kinawataka channel and were absent along the two streams A and B as reported by (Ndugga, 2021). The differences that existed between the sampling weeks could

be due to the pollution discharge of soaps and detergents which probably influenced the acidity/alkalinity as was reported in a study in Kinawataka channel as reported by (Wanasolo et al., 2018).

5.1.2 Total suspended solids (TSS)

Kawooya stream (241.67±68 mg/L) and Kinawataka Channel (221.80±88.11 mg/L) had the highest TSS concentrations which were out of NEMA specifications (50 mg/L). These results were way lower than what was reported in the Kinawataka channel (478.03±12 mg/L) by (Ndugga, 2021). This could have been influenced by an increase in the run offs from urban areas surrounding the Kinawataka channel overtime. Furthermore, the values of the TSS in this study were also comparable with the TSS values of another study in the Nakivubo Channel (268±4 mg/L) as reported by (Kayiwa et al., 2022).

Total suspended solids are important because as their concentrations increase, they reduce on the concentrations of residual APIs (J. Zhou & Broodbank, 2014). However, residual APIs get adsorbed to the TSS and they can easily be carried by a stream for long distances or picked up by other agents such as birds to new locations (Lara-Martín *et al*., 2014). The data analysis suggested no difference between the concentrations of TSS which implied that the sources of pollution contributing TSS to the study remained high throughout which suggested that they could be of anthropogenic origin such as agricultural runoffs as suggested by (Lara-Martín *et al*., 2014).

5.1.3 Chemical Oxygen Demand (COD)

COD measures the amount of oxygen required to decompose the organic matter including residual APIs. The high COD in Kawooya stream (173.47 ± 68.82), the

Channel (131.24 ± 72.39), and Jamaica stream (98.43 ± 129.67) which are comparable to the results reported in the Nakivubo channel (150 ± 10.0) by (Dalahmeh *et al*, 2020) are indicating the presence of organic substances in these areas that need oxygen for decomposition (Wanasolo et al., 2018). Furthermore, the sources of these organic substances could be pharmaceutical manufacturing facilities, improper disposal of waste, agricultural runoffs and waste from other industries as reported (Ndugga, 2021). However, in this study, the wetland was the only sampling compartment whose mean COD (37.83 ± 32.92 mg/L) was complying with NEMA specifications (70 mg/L) and was comparable to the COD results reported in the Kinawataka channel (30.14 ± 75.96) by (Ndugga, 2021). The low levels of COD in the wetland could be explained by the ability of the wetlands to breakdown nutrients and organic pollutants in wastewater (Anderson *et al*., 2015; Nguyen *et al*., 2019).

The difference in COD concentrations that existed between Kawooya stream and wetland suggested that pollution with organic substances in Kawooya stream was much higher than that of the wetland, however, it also suggests that pollution of organic substances in Kawooya stream, Jamaica stream and channel remained high.

5.1.4 Biological Oxygen Demand (BOD)

Similarly, to COD, the wetland was the only sampling compartment with a mean BOD (31.00 ± 17.52 mg/l) complying with NEMA specifications (50 mg/l) which were lower than the BOD values reported in Kinawataka wetland (6.08±5.453) by (Ndugga, 2021). This could have been to the due to the reduction in pollution with organic wastes. However, the findings in this study contradicted with BOD

values in different study on the Kinawataka wetland (7.15-102 mg/L) by (Wanasolo et al., 2018). In contrast, the findings in this study were contradicting with the findings in the study that was conducted in the Nakivubo ecosystem (185-190 mg/L) by (Dalahmeh et al., 2020; Kayiwa et al., 2022).

The high BOD concentrations in Jamaica stream, Kawooya Jamaica stream and channel could be accredited to industrial discharges (both pharmaceutical and non-pharmaceutical), animal waste, food-processing plants; failed septic and urban water runoffs as suggested (Wanasolo et al., 2018). This BOD data meant that generally there was an increased high pollution with organic pollutants to the Kinawataka catchment area throughout the sampling period.

5.1.5 Total Phosphates

Phosphates are essential nutrients for microorganisms and can increase the rate of degradation of APIs when available at permissible levels, however, it can lead to water quality problems linked to eutrophication and rapid algal growth (Dalahmeh *et al*., 2020).

The means of phosphate concentrations (5.26 ± 5.53 - 20.91 ± 12.13 mg/L) in this study were all out of NEMA specifications (5mg/l) (National Environment Management Authority, 2020). These results differed from phosphate concentrations (1.11 ± 2.40 mg/L) reported in the Kinawataka wetland, which could be attributed to increased nutrient-rich waste pollution in this area as reported by (Ndugga, 2021). However, the phosphate concentration of this study were comparable to the studies in Nakivubo channel (6.15±0.42 -8.32 mg/L) by (Dalahmeh et al., 2020; Kayiwa et al., 2022).

Furthermore, along Kawooya stream, it was highly characterized by agriculture, industries whose waste and runoffs could have contributed to the phosphate concentrations. The area around Kinawataka channel is also highly industrial, commercial and residential and this increases the discharge of sewage and a lot of waste water containing high phosphates load, detergents from car washing bays are also potential pollution sources contributing to elevated phosphate levels in the Kinawataka channel as reported by (Wanasolo et al., 2018).

The Wetland whose mean phosphate concentration was relatively lower compared to the other sampling compartments, however slightly above NEMA specifications, may be playing a mitigating role in reducing on the phosphate concentrations as reported by (Nguyen *et al*., 2019).

5.1.6 Total Nitrates

The mean total nitrate concentrations observed in this study (0.24–0.33 mg/L) were slightly lower than those reported in earlier studies at the Kinawataka wetland (0.5–6.2 mg/L) (Ndugga, 2021; Wanasolo et al., 2018). However, the concentrations of Nitrates in this study contradict the findings in similar studies in Nakivubo Channel where the Nitrate concentrations were in the ranges of 30±9 - 45 mg/L (Dalahmeh et al., 2020; Kayiwa et al., 2022).

However, these low concentrations of nitrates compared to other water quality parameters can be attributed to self-purification of these areas, factors like increased stream flows which increases dilution factor, absorption and conversion of NO_3 to ammonia could have reduced on these concentrations as suggested by (Ndugga, 2021). The higher nitrates levels in channel, Jamaica stream and Kawooya stream compared to other sampling compartments can be accounted for small scale

economic activities near these areas and disposal of municipal and industrial waste (Wanasolo et al., 2018).

The concentrations of total Nitrate were within the NEMA specifications (10 mg/l) (National Environment Management Authority, 2020) which is a positive indication of a balanced nutrient load from an environmental perspective. Nitrates are other important nutrients for microorganisms and their presence at permissible levels can increase the rate of degradation of residual APIs as reported by (Dalahmeh *et al*., 2020). In general, compliance with regulatory standards is essential for maintaining water quality and ecosystem health.

The Wetland sampling compartment which had the lowest mean total nitrate concentrations, may be acting as a natural filter in reducing the concentration of nitrates before they reach Lake Victoria (Dalahmeh *et al*., 2020).

5.2 Variations in the concentrations of the selected residual APIs in the Kinawataka channel, its streams and wetland

5.2.1 Amoxicillin

This study revealed relatively lower concentrations of Amoxicillin with an exception of Kawooya stream which presented a higher average concentration (173.91 ± 269.28 ng/l). In all the sampling compartments, amoxicillin had 100% detection frequency except for the wetland where it had about 83% probably due to it being amongst the most prescribed penicillin around Kampala as reported by (Dalahmeh *et al*., 2020). In Uganda, little has been reported about amoxicillin in the environment probably due its β-lactum ring which is often unstable and gets hydrolyzed after a few hours of exposure in the environment as reported in Mexico (Elizalde-Velázquez *et al*., 2016).

However, the amoxicillin concentrations reported in this study were way much lower than those reported in a comprehensive study about amoxicillin's presence in surface waters of Nigeria and Sri Lanka (0.9 –272 μg L^{-1} and 101 μg L^{-1} respectively) due to high manufacturing of medicines and pharmaceutical unregulated waste disposal in these countries as reported by (Waleng & Nomngongo, 2022). This could be attributed to lack of comprehensive data in Uganda.

Other studies in Spanish and American waters, very low or no traces of Amoxicillin were reported and this may be due to the strict pharmaceutical products disposal policies and expensive penalties to manufacturing plants (Boleda *et al*., 2014). The differences that existed between week 2 and week 1 where there was a reduction in the pollution of amoxicillin was probably due the changing weather conditions during sampling period and also sampling away from the PMFs that were major polluters of these residual APIs.

5.2.2 Erythromycin

Erythromycin was detected in high concentrations, however, had the lowest detection frequency with most detection mainly from week one of sampling (46.67%). The highest concentration of Erythromycin (936 ± 1503.31 ng/l) was detected in Jamaica stream, coincidentally there is a pharmaceutical manufacturing facility upstream. The study on Lake Victoria in Murchison bay found erythromycin in the ranges (10.0-66.0 ng/l) which was much lower than the average concentration of the erythromycin in this study (470.70 ng/l) (Nantaba et al., 2020).

In Africa, investigations on waters in South Africa found about 1.0 ng/l of erythromycin in pharmaceutical waste water, which is smaller than what was detected in this study probably due to the stricter pharmaceutical waste disposal practices (Madikizela *et al*., 2017). Studies on pharmaceutical pollution in Africa reported about 10.6 µg/L of erythromycin in hospital water of Kumasi hospital in Ghana and up to 16.4 µg/L of erythromycin in pharmaceutical WWTP in Tunisia which is way higher than the concentrations reported in this study probably due to high pharmaceutical waste pollution reported in west and north Africa (Waleng & Nomngongo, 2022).

High concentrations of erythromycin have also been reported before, in Europe, USA and Asia in both surface water and pharmaceutical waste water by (Hughes *et al*., 2013; Deo, 2014; Ebele *et al*., 2017;). Conclusions that erythromycin can persist in environment for about a year were documented by (Kumar *et al*., 2022).

5.2.3 Ciprofloxacin

The concentrations of ciprofloxacin obtained in this study ranged from 7.40 to 79.17ng/l in all the sampling compartments, however, these values are relatively lower than most of the residual APIs detected. Ciprofloxacin was another residual API that was frequently detected (DF=90.91%) in all the sampling compartments possibly due to being highly sold in pharmacies and health centers (Nantaba et al., 2020).

From another data collected in Uganda reported that ciprofloxacin was amongst the most sold drugs in Kampala (Dalahmeh *et al*., 2020). In another study on lake Victoria, ciprofloxacin was quantified in the ranges of (2.0-41 ng/l) which more less the same as the results obtained in this study (Nantaba et al., 2020).

On contrary, ciprofloxacin was detected in much higher concentrations (27.1 µg/l) referring to pharmaceutical waste waters in South Africa, while comparing them with the concentrations from 8 pharmaceutical WWTPs in Italy, were in the ranges of 27 ng/l - 514 ng/l way higher than what was quantified in this study as reported by (Agunbiade & Moodley, 2014). Another study reported that ciprofloxacin had high concentration up to 14.3 µg/l in river water samples from Western Cape Province WWTPs (Waleng & Nomngongo, 2022). A study in Pakistan also concluded that there were traces (2.2 µg/l) of ciprofloxacin in pharmaceutical waste water (Ashfaq *et al.*, 2017). The concentrations of ciprofloxacin detected in other countries were much higher than what was detected in this study probably due to its high manufacture and consumption world-wide as reported by (Waleng & Nomngongo, 2022).

Another study reported worrying concentrations of ciprofloxacin in Turkey and other parts of Europe and America (Oğuz & Mihçiokur, 2014). Concentrations of 3.0-8.7 µg/l and 384-481 ng/l ranges of ciprofloxacin were reported in Switzerland and Canada respectively which was higher than what is reported in this study.

5.2.4 Sulfamethoxazole

Sulfamethoxazole is another residual that was detected in high concentrations and where found in the ranges of 15.38-987.16 ng/l with Kawooya stream having the highest concentrations of sulfamethoxazole probably from the PMF along this channel. Sulfamethoxazole was also frequently detected (DF) with about 87.88 % detection frequency and this could be attributed to the frequent use the trimethoprim-sulfamethoxazole drug by malaria and HIV/AIDS infected patients

in Uganda (Nantaba et al., 2020). High concentrations of sulfamethoxazole ranging from 170-800 ng/l in Nakivubo channel which was slightly similar to the concentrations reported in this study by (15.38-987.16 ng/l) (Dalahmeh *et al*., 2020) . Another study reported concentrations of sulfamethoxazole (1-5600 ng/l) in all the regions of Lake Victoria which are way higher than what was reported in this study suggesting that all the drainage channels were polluted heavily with sulfamethoxazole (Nantaba et al., 2020).

On the African Continent, sulfamethoxazole is one of the residual APIs that has been reported most and in high concentrations, for example it has been reported in Uganda, Kenya, Nigeria, South Africa and Ghana (ausderBeek *et al*., 2016). Another report reported sulfamethoxazole amongst the most detected residual APIs in America (Ferguson *et al*., 2013). These findings were confirmed by a comprehensive study which collected data from 41 countries from Asia, America, Europe and Africa and reported that sulfamethoxazole was always amongst the top five detected APIs with concentrations ranging (1-10.000 ng/l) (Hughes *et al*., 2013).

5.2.5 Oxytetracycline

All the APIs quantified in this study, Oxytetracycline was detected in very low concentrations, ranging from 0.82-5.34 ng/l with Kawooya stream having the highest concentrations of Oxytetracycline, however, it had the detection frequency (DF) of 96.97%. This frequent detection of Oxytetracycline could be due to farming that included cattle keeping and piggery that were most common along the sampling area as reported by (Nantaba et al., 2020).

In the study on Lake Victoria reported concentrations of Oxytetracycline (17.0 - 300 ng/l) which are way higher than what is reported in this study (Nantaba et al., 2020). Oxytetracycline was also reported in comprehensive study which was conducted on African and Asian waters including pharmaceutical WWTPs (Waleng & Nomngongo, 2022). Oxytetracycline was detected in higher concentration in other studies probably due to the fact that farming a round wetland is cheaper due to availability of free water for animal consumption, housekeeping and easy means of waste disposal as reported by (Nantaba et al., 2020).

5.2.6 Paracetamol

The concentrations of paracetamol obtained in this study ranged from 5.11 to 1070.20 ng/l in all the sampling compartments which makes it another highly quantified API in this study. Unlike many other APIs in this study, paracetamol was the most frequently detected API with (DF=100%), meaning every sample had traces of paracetamol. On the contrary in the studies reported the detection frequencies of 72% and 60% respectively (Ashfaq et al., 2017; Nantaba et al., 2020). The high detection frequencies and high concentrations of paracetamol can be best explained by its high production and consumption patterns where only its consumption constitutes about 6% of the total volume of drugs consumed worldwide (Patel *et al*., 2019).

Studies on Lake Victoria, reported concentrations of paracetamol (27.0 ng/l) which are lower than what is being reported in this study probably due to lack of pharmaceutical manufacturing facilities around the Nakivubo channel which are direct pollution sources as reported by (Nantaba *et al*., 2020). Comparing the

findings of this study with other similar studies in Pakistan, the reported concentrations (12.0 µg/l-64 µg/l) were way higher than what was reported in this study probably due to Pakistan being amongst the biggest producer of paracetamol in Asia (Ashfaq et al., 2017).

In the past studies, paracetamol has been recognized as the most frequently detected API globally (Barra Caracciolo *et al*., 2015). Although it is quickly catabolized by microorganisms and then consistently removed from wastewater (Baena-Nogueras *et al*., 2017). There were no significant differences in paracetamol concentrations meaning that there was constant pollution of paracetamol in the sampling compartments and sampling probably from the PMFs, poor waste disposal by drugs shops around the sampling area and many more others.

5.3 Relationship between residual API concentrations and water quality parameters

Water quality parameters exhibited different characteristics from sampling point to sampling point. It was reported that some quality parameters associate with residual APIs affecting removal hence affecting the concentration (Ohoro *et al*., 2022). Some of the residual APIs suggested significant linear relationship, whereas some exhibited weak relationship with the measured water quality parameters as reported by (Niemi, 2020). On the other hand, an increase in the concentrations of TSS have been reported to trigger a reduction in the concentrations of residual APIs especially amoxicillin, sulfamethoxazole and Oxytetracycline as reported by (Lara-Martín et al., 2014; J. Zhou & Broodbank, 2014).

Regression and correlation investigation indicated that water quality was strongly associated with concentrations of residual APIs either negatively or positively where all the 6 residual APIs were significantly associated all the 6 water quality as was reported by (Nantaba *et al*., 2020). It was revealed that some measured water quality parameters such as pH, TSS, COD and BOD trigger an increase in the concentrations of some residual APIs especially erythromycin and ciprofloxacin as reported by (Ohoro *et al*., 2022).

For instance, Sulfamethoxazole which had a positive and significant correlation with most water quality parameters suggested that the variations in Sulfamethoxazole concentrations can be well explained by these water quality parameters. Furthermore, Paracetamol had negative but significant correlation with TSS, COD, BOD suggested that these water quality parameters can explain the variations in Paracetamol concentrations. Comparing the above residual APIs with Oxytetracycline which had a positive but insignificant correlation with water quality parameters, suggested that the water quality parameters can't explain the variation in concentrations (J. Zhou & Broodbank, 2014).

5.4 The ecological risk assessment of the selected residual APIs

The RQs of sulfamethoxazole (1.12), erythromycin (1.92) were greater than 1, a high risk and a sign that these residual APIs bring about detrimental properties to the aquatic organisms in the channel and the Lake meaning that the two residual APIs were very much unsafe for algae which is a non-target organism in this case.

In some studies, in Uganda, sulfamethoxazole had high RQs (9.0) and (537) respectively in which they were higher than what has been reported in this study probably due to the detection of sulfamethoxazole in high concentrations as

reported by (Nantaba et al., 2020b; Kayiwa et al., 2022). Other studies also reported higher RQs (3.3) of erythromycin than what was reported in this study and this is due to their extensive use and detection in high concentrations (Deo, 2014; Nantaba et al., 2020).

The RQs of amoxicillin (0.54), a residual API although contributed a RQ of less than 1, it was still greater than 0.1, signifying a medium risk to the aquatic organisms in Kinawataka channel and Lake Victoria. This could probably due to being detected in slightly higher concentrations. Similarly, a slightly higher RQ of 0.87 but comparable to the RQ in this study was reported in the Nigerian aquatic environment as reported by (Waleng & Nomngongo, 2022).

Ciprofloxacin (0.02), paracetamol (0.06) and Oxytetracycline (0.01) all contributed RQs below 0.1, indicating a very low risk not likely have a significant threat to lives of organisms in the aquatic environment around Kinawataka channel. Although other studies, found ciprofloxacin to have slightly higher RQs (0.41-28.63) in lake Victoria in Uganda and lake Michigan in USA probably due to the high consumption of ciprofloxacin hence resulting in its high concentration as reported by (Nantaba et al., 2020; Oğuz & Mihçiokur, 2014).

A study on Lake Victoria, Uganda reported higher RQs of Oxytetracycline (4.48) and this was due detection of Oxytetracycline in higher concentrations as reported by (Nantaba *et al*., 2020). In the same study, the reported RQs of paracetamol (0.005) were lower than what is being reported in this study and this could be attributed to high PNEC that was used. On the contrary, a study on Lake Michigan, USA reported RQs of paracetamol (2.8) higher than what is being reported in this study probably due to high concentrations of paracetamol

detected in Lake Michigan and also the lower PNEC that was used to calculate the RQ in that study as reported by (Deo, 2014).

CHAPTER SIX

SUMMARY OF FINDINGS, CONCLUSION AND RECOMMENDATION

6.1 Summary

This study investigated the occurrence and ecological risks of residual active pharmaceutical ingredients (APIs) in the Kinawataka Channel, its streams, and wetland. The findings showed the presence of several APIs at varying concentrations, with some posing potential ecological risks, providing an important baseline for understanding pharmaceutical pollution in Uganda. The study emphasizes the need for improved wastewater management, stronger regulatory action, and collaborative efforts to protect both aquatic ecosystems

6.2 Conclusion

6.2.1 The water quality parameters along the Kinawataka channel, its streams, and wetland

Overall, the samples showed variations in pH, COD, BOD, turbidity, temperature, and nutrient concentrations, reflecting potential influences from surrounding land use and anthropogenic activities. However, no statistically significant differences were observed in water quality parameters along the Kinawataka Channel, streams, and wetland, except for COD; therefore, the null hypothesis was accepted. Notably, only pH and total nitrates met the NEMA standards, while COD, BOD, and TSS exceeded the permissible NEMA limits.

6.2.2 The concentrations of the selected residual API

Generally, the samples showed variations in the concentrations of residual APIs, reflecting possible influences from anthropogenic activities within the catchment.

However, there were no statistically significant differences in API concentrations along the Kinawataka Channel, streams, and wetland, therefore, the null hypothesis was accepted. Erythromycin, sulfamethoxazole, and paracetamols were the most detected and identified residual APIs in this study with erythromycin and sulfamethoxazole being detected in concentration over and above their predicted no effect concentrations.

6.2.3 Ecological risk assessment of the selected residual APIs

Overall, the ecological risk assessment confirmed that erythromycin, sulfamethoxazole, and amoxicillin were present as residual APIs with the potential to pose significant threats to aquatic life in the Kinawataka Channel. Therefore, I reject the null hypothesis that the residual APIs pose no ecological risks. In contrast, ciprofloxacin, paracetamol, and oxytetracycline were associated with minimal ecological risk

6.3 Recommendation

6.3.1 The water quality parameters along the Kinawataka channel, its streams, and wetland

The communities around Kinawataka area should avoid direct discharge of domestic waste into streams and wetlands and should resort to using proper waste disposal and sanitation facilities, and engage in community clean-up drives along the Kinawataka channel to reduce organic load in the Kinawataka area. Furthermore, the PMFs should conduct routine monitoring of their effluents for water quality parameters before discharge to ensure compliance with NEMA standards. Unfortunately, the study was not able to comprehensively establish the exact relationships between water quality parameters and residual APIs. The associations reported in this study were only estimated through statistical tools

such as correlation analysis, which provide only indicative rather than definitive evidence. To better explain these relationships, I therefore recommend that future studies undertake more detailed investigations into how water quality parameters influence the occurrence, persistence, and transport of residual APIs in aquatic ecosystems.

6.3.2 The concentrations of the selected residual API

The communities around Kinawataka area should implement safe disposal methods of unused or expired medicines by taking them back to the public hospital or pharmacies that prescribed them instead of flushing them into toilets or dumping into rubbish bins. Likewise, the PMFs should immediately improve in-house waste handling by separating and pre-treating pharmaceutical wastewater before releasing it to sewer systems. However, this study did not assess seasonal or long-term variations in residual APIs, which limits the ability to understand temporal variations in pharmaceutical pollution. I therefore recommend that future studies extend sampling across different seasons and time periods to capture seasonal and diurnal fluctuations of APIs in aquatic systems. In addition, future studies should focus on tracing the pathways of APIs from their primary sources, particularly wastewater treatment plants and pharmaceutical manufacturing facilities, in order to evaluate removal efficiencies and identify critical points of contamination.

6.3.3 Ecological risk assessment of the selected residual APIs

The community leaders should promote proper disposal of the unused medicines and awareness campaigns on the ecological impacts of improper drug disposal and encourage reduced misuse of antibiotics to limit environmental loading. The PMFs should initiate the installation of low-cost treatment systems like the

constructed wetlands as catchment areas for the pharmaceutical before they interact with the environment to reduce high-risk residues such as erythromycin and sulfamethoxazole from going directly to the drainage channels. This study employed a screening-level ecological risk assessment, which provided only an initial indication of the potential risks posed by residual APIs to aquatic organisms. To strengthen future ecological risk evaluations, more comprehensive approaches beyond screening-level models should be adopted. This may include laboratory or field-based toxicity exposure experiments using locally relevant aquatic species to assess both acute and chronic effects under realistic environmental conditions to understand long-term ecological risks.

6.3.4 Policy Recommendation

Generally, this study reported that residual APIs such as erythromycin, sulfamethoxazole, and amoxicillin pose ecological risks in the Kinawataka Channel, highlighting the need for urgent policy action. National Environment Management Authority should integrate pharmaceutical residues into water quality monitoring, set permissible thresholds and enforce them. National Drug Authority should only license PMFs that have installed and maintained effective wastewater treatment systems while verifying with audits verifying compliance. National Water Sewerage Corporation must upgrade treatment plants with advanced technologies and routinely test for pharmaceutical residues in both wastewater and drinking water. Ministry of Water and Environment should coordinate a multi-agency taskforce with Ministry of Health to develop a national action plan on pharmaceutical pollution. These actions would protect aquatic ecosystems, reduce risks of antimicrobial resistance, and contribute to SDG 3 (Health), SDG 6 (Clean Water), and SDG 14 (Life below Water).

6.4 Contributions of the study

This study has contributed to the understanding of residual API pollution in Uganda by providing objective evidence of residual APIs in the Kinawataka Channel, which has been identified as an important pathway through which these contaminants enter Lake Victoria, thereby adding to the limited information on pharmaceutical pollution in Ugandan aquatic ecosystems. The Probable sources of residual APIs in Kinawataka channel its streams and wetland include pharmaceutical wastewater discharges from PMFs around this area, municipal waste water effluents, and livestock farms around this channel.

6.5 Limitations of the Study

Table 6. 1 Shows the limitations/challenges of the study and how they were overcome

Limitations / Challenges	How they were overcome in this Study
Some sampling sites were difficult to access due to flooding, encroachment, and safety issues.	Selected alternative accessible points nearby and engaged local people to facilitate safe site access.
Sampling limited to a single period, so seasonal variations could not be captured.	Conducted sampling during a representative period of flow and explicitly noted this limitation in reporting.
Restricted entry in certain areas such as PMFs due to private ownership or security concerns even when I had a letter of introduction from the University.	I relied on accessible sites within the channel, streams, and wetland system.
Limited availability of comparable local studies hindered proper discussion.	Compared findings with regional and international studies to provide context.

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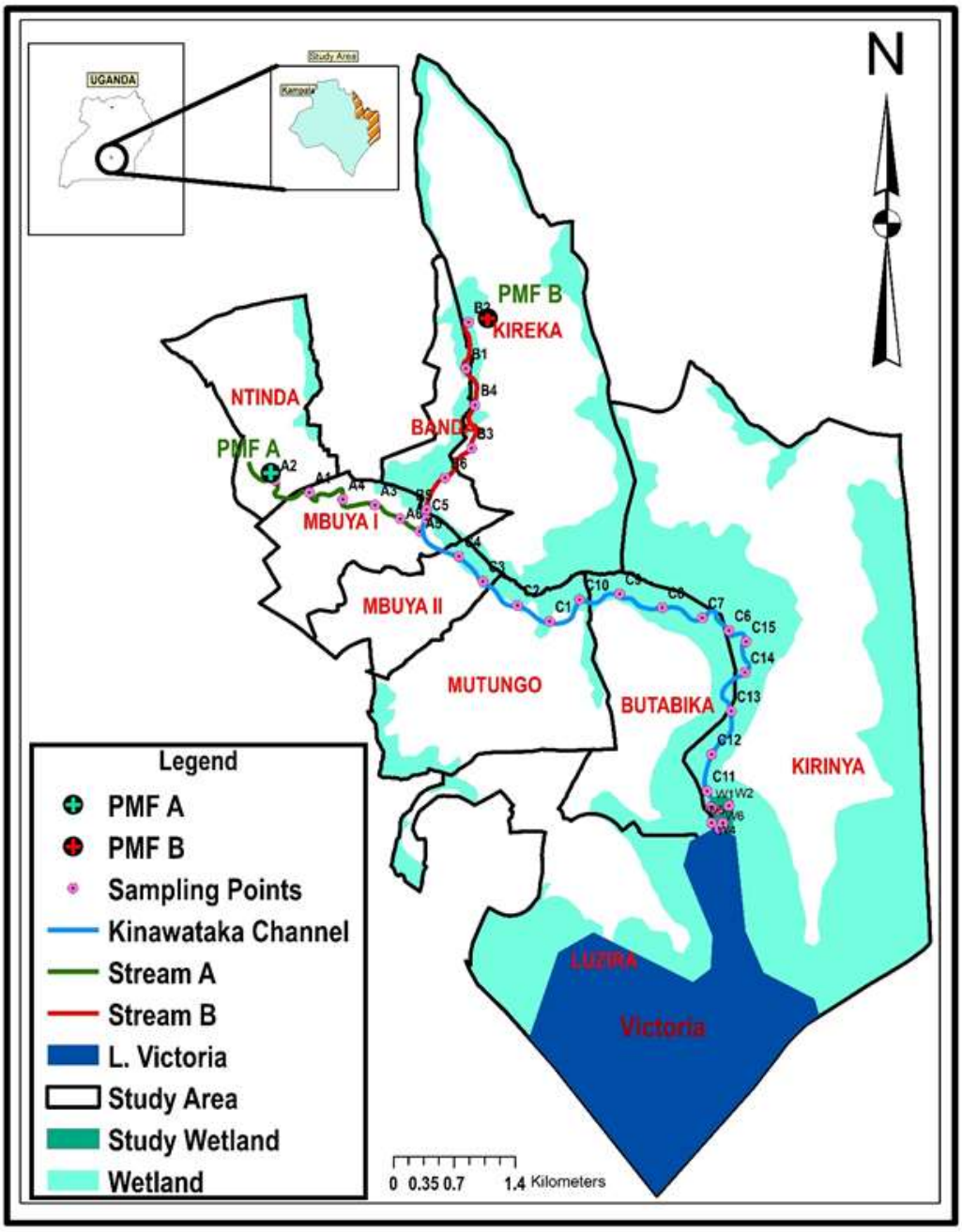
APPENDICES

Appendix 1: A detailed map of the study area highlighting the specific sample locations within the various sampling compartments, providing a clear visual representation of the distribution of sampling points across the study area.



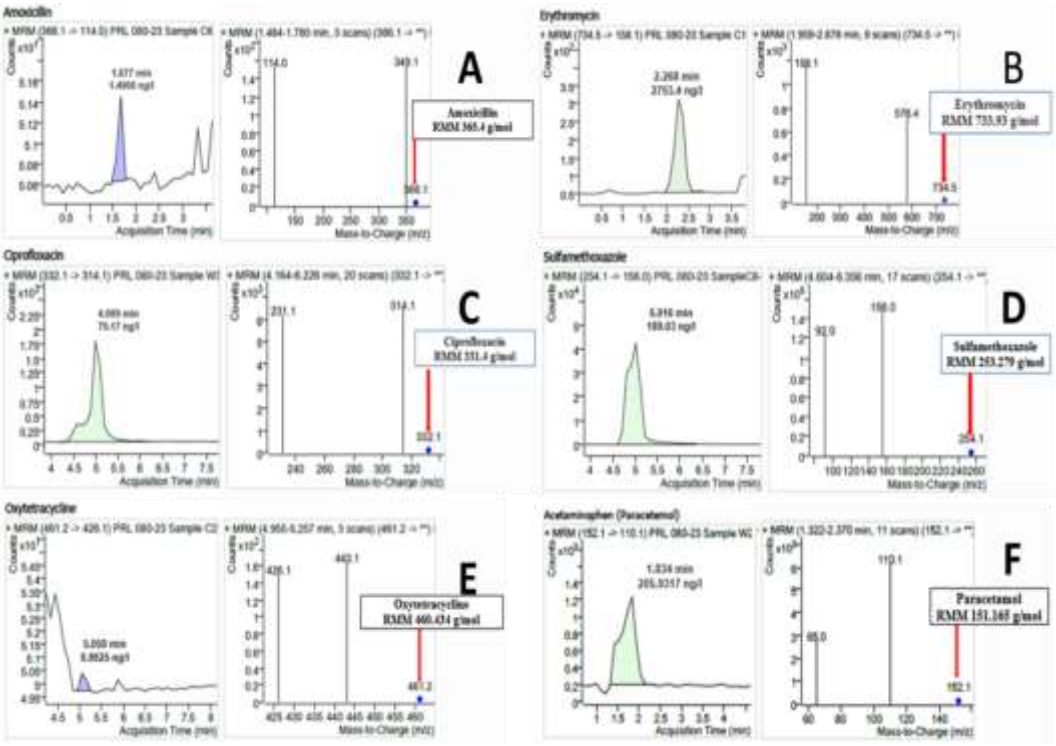
Appendix 2: The summary of water quality parameters, including their means ± standard deviations (SD), ranges, and the corresponding National Environment Management Authority (NEMA) specifications.

Parameters	Sampling Compartments	NEMA Specifications	Mean ± SD	Units	Range
PH	Channel	5.0 -8.5	7.42 ± 0.26	Units	7.08-7.82
	Jamaica stream	5.0 -8.5	7.26 ± 0.23	Units	6.93-7.58
	Kawooya stream	5.0 -8.5	7.39 ± 0.19	Units	7.12-7.68
	Wetland	5.0 -8.5	7.43 ± 0.33	Units	7.16 -7.99
TSS	Channel	50	221.80 ± 88.11	mg/L	37.00-389.00
	Jamaica stream	50	166.17 ± 45.25	mg/L	58.00-412.00
	Kawooya stream	50	241.67 ± 68.82	mg/L	133.29-312.10
	Wetland	50	105.83 ± 50.82	mg/L	20.00-145.00
COD	Channel	70	131.24 ± 72.39	mg/L	7.48-269.54
	Jamaica stream	70	98.43 ± 129.67	mg/L	11.89-334.70
	Kawooya stream	70	173.47 ± 68.82	mg/L	133.29-312.10
	Wetland	70	37.83 ± 32.92	mg/L	5.83-99.07
BOD	Channel	50	61.75 ± 25.37	mg/L	9.79-130.40
	Jamaica stream	50	65.36 ± 47.16	mg/L	34.54-159.26
	Kawooya stream	50	73.20 ± 34.12	mg/L	54.58-142.48
	Wetland	50	31.00 ± 17.52	mg/L	2.59-47.67
Phosphate	Channel	5	13.50 ± 10.46	mg/L	0.34-37.60
	Jamaica stream	5	12.66 ± 16.51	mg/L	0.49-42.60
	Kawooya stream	5	20.91 ± 12.13	mg/L	10.30-43.60
	Wetland	5	5.26 ± 5.53	mg/L	0.12-15.30
Nitrate	Channel	10	0.33 ± 0.07	mg/L	0.23-0.51
	Jamaica stream	10	0.30 ± 0.03	mg/L	0.27-0.36
	Kawooya stream	10	0.29 ± 0.08	mg/L	0.18-0.40
	Wetland	10	0.24 ± .04	mg/L	0.18-0.30

Appendix 3: Summary of quantified residual APIs and it includes the number of detects (n), detection frequency (% DF), Mean concentration (± standard deviation), range and the median of residual APIs quantified in the all the sampling compartment.

Analyte	Sampling Compartment	n	% DF	Mean Concentration ± SD (ng/l)	Range (ng/l)
Amoxicillin	Channel	15	100	11.00 ± 15.80	0.82 - 59.18
	Jamaica stream	6	100	5.53 ± 7.44	1.00 - 20.45
	Kawooya stream	6	100	173.91 ± 269.28	4.10 - 607.885
	Wetland	6	83.33	10.01 ± 21.21	0.00 - 53.27
Erythromycin	Channel	15	46.67	441.29 ± 804.84	0.00 – 2753.40
	Jamaica stream	6	33.33	936.55± 1503.31	0.00 - 3431.87
	Kawooya stream	6	33.33	499.30 ± 815.38	0.00 – 1905.71
	Wetland	6	16.67	49.76 ± 121.90	0.00 - 298.59
Ciprofloxacin	Channel	15	86.67	13.68 ± 19.02	0.00 - 79.17
	Jamaica stream	6	100	12.03 ± 6.26	7.95 - 23.12
	Kawooya stream	6	100	9.43 ± 1.96	7.40 - 12.79
	Wetland	6	83.33	8.55 ± 6.95	0.00 – 21.21
Sulfamethoxazole	Channel	15	86.67	189.21 ± 134.09	0.00 – 532.30
	Jamaica stream	6	83.33	154.74 ± 163.33	0.00 – 360.65
	Kawooya stream	6	100	388.91 ± 363.53	15.38 – 987.16
	Wetland	6	83.33	192.28 ± 297.59	0.00 - 760.68
Oxytetracycline	Channel	15	100	1.46 ± 0.66	0.93 - 2.71
	Jamaica stream	6	100	1.48 ± 0.97	0.79 - 3.41
	Kawooya stream	6	83.33	1.61 ± 1.88	0.00 - 5.34
	Wetland	6	100	1.28 ± 1.04	1.07 - 5.07
Paracetamol	Channel	15	100	122.18 ± 265.50	5.11 - 1070.20
	Jamaica stream	6	100	261.33 ± 376.23	22.08 – 992.99
	Kawooya stream	6	100	138.00 ± 188.40	5.79 - 514.18
	Wetland	6	100	101.58 ± 150.29	0.09 - 371.97

Appendix 4: The mass spectrum for some of the samples showing a molecular ion at a specific mass-to-charge ratio (m/z) corresponding to the respective molecular weight of each compound, thereby confirming their presence in the sample. Amoxicillin (A), Erythromycin (B), Ciprofloxacin (c), Sulfamethoxazole (D), Oxytetracycline (E) and Paracetamol (F).



Appendix 5: The details of all residual APIs in this research, their therapeutic class, formula, molecular weight, pathways, persistence in the environment and their characteristics in the environment.

API	Formula & Molecular Weight (g/mol)	Therapeutic Class	Characteristics in the Environment	Persistence in Water	Pathways into Water Systems
Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$ 253.279 g/mol	Sulfanilamide Antibiotic	It is persistent, resistant to degradation in water and can promote antibiotic resistance genes in aquatic systems.	High	Excretion, improper disposal, wastewater effluents
Ciprofloxacin	$C_{17}H_{18}FN_3O_3$ 331.4 g/mol	Fluoroquinolone antibiotic	It is persistent, binds to sediments and it bio accumulates in organisms.	Moderate to High	Excretion and hospital runoff
Erythromycin	$C_{37}H_{67}NO_{13}$ 733.93 g/mol	Macrolide antibiotic	It is persistent, accumulates in aquatic organisms, alters microbial diversity and promotes resistance.	High	Excretion, improper disposal, hospital discharge
Amoxicillin	$C_{16}H_{19}N_3O_5S$ 365.4 g/mol	Penicillin antibiotic	Moderate persistence, partially degraded in water but still contributes to resistance genes.	Moderate	Improper disposal, excretion, sewage effluents
Oxytetracycline	$C_{22}H_{24}N_2O_9$ 460.434 g/mol	Tetracycline antibiotic	It is persistent, binds to soil and water sediments, disrupts aquatic ecosystems and promotes resistance genes.	High	Agricultural use and animal excretion
Ibuprofen	$C_{13}H_{18}O_2$ 206.28 g/mol	Nonsteroidal anti-inflammatory drug	Readily degraded but can cause toxicity to aquatic organisms at high concentrations.	Low	Excretion, improper disposal, wastewater treatment
Paracetamol	$C_8H_9NO_2$ 151.165 g/mol	Analgesic and antipyretic drug	Generally non-persistent; partially degraded but can form toxic intermediates in aquatic environments.	Low	Improper disposal, sewage effluents, household wastewater

(Fatta-Kassinos et al., 2011)

Appendix 6: The pictures (1-7) indicate different field activities ranging from laboratory analysis, drug blisters dumped on stream banks, extraction of samples, samples in an ice box, sampling water samples from one of the streams as evidence of the researcher's active involvement in both fieldwork (sampling) and laboratory investigations throughout the study.