# APPLICATION OF A CONSTRUCTED WETLAND FOR THE REMOVAL OF ANTIBIOTIC RESIDUE, ANTIBIOTIC-RESISTANT BACTERIA, AND ANTIBIOTIC-RESISTANCE GENES FROM PHARMACEUTICALLY CONTAMINATED WASTEWATER

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**A Dissertation Submitted in Partial Fulfillment of the Requirements for the award of the Degree of Doctor of Philosophy in Environmental Science and Engineering of the Nelson Mandela African Institution of Science and Technology**

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**ABSTRACT**

The significant increases in abundance of pharmaceuticals, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) in the environment have drawn attention over public health. The presence of these contaminants in wastewaters is well-documented as a factor contributing to the decreased potency of antibiotics used in healthcare. These types of contaminants can be removed from wastewater using a number of techniques, including phytoremediation, which has demonstrated effectiveness. The removal of these contaminants by various aquatic plants has been explored, and the results are promising. The aim of this research was first, to analyze antibiotic resistance patterns of bacteria isolated from hospital wastewater effluent, which is a consequence of antibiotics occurrence in wastewater. Second, to investigate the removal of some selected antibiotics from synthetic wastewater in constructed wetland (CW) planted with *Cyperus alternifolius, Canna indica*, and one planted with both of these plant species, as well as the influence of antibiotics on microbial density and community in CW. Hospital wastewater samples were collected from the Benjamin Mkapa Hospital in Dodoma, Tanzania, where the hospital's wastewater is treated in a horizontal subsurface flow CW planted with *Typha latifolia* before being discharged into the environment. The results of hospital wastewater analysis showed that bacteria isolated from treated hospital wastewater were resistant to tested antibiotics and harbored antibiotics resistance genes. These findings demonstrate that CW can disseminate ARB and ARGs despite hospital wastewater treatment, which poses a risk to the public's health. In the pilot CW, the system planted with a single plant species (*Cyperus alternifolius*) outperformed those planted with mixed plant species or *Canna indica* alone in the removal of tested antibiotics from wastewater. This is supported by the observation of higher bacteria abundance in CW with *Cyperus altenifolius* than *Canna indica*, while the difference was not significant ($p > 0.05$). The findings of this investigation revealed that although there is a general decline in bacteria abundance, there is no significant change ($p > 0.05$) due to antibiotic presence in wastewater. It is concluded that, despite variations in performance, the plants studied play a significant role in pharmaceuticals remediation from wastewater.

## DECLARATION

I, Petro Novert Karungamye, do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and it has neither been submitted nor being concurrently submitted for degree award in any other institution.

16th July 2024

Petro Novert Karungamye — Date

The above declaration is confirmed by:

16th July 2024

Prof. Revocatus Lazaro Machunda — Date

16th July 2024

Prof. Kelvin Mark Mtei — Date

16th July 2024

Dr. Anita Mukarugaika Rugaika — Date

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# CERTIFICATION

The undersigned certify that they have read and found that the dissertation conforms to the standard and format acceptable for submission. Therefore, do hereby recommend for acceptance of dissertation entitled *"Application of a constructed wetland for the removal of antibiotic residue, antibiotic-resistant bacteria, and antibiotic-resistance genes from pharmaceutically contaminated wastewater"* in in partial fulfillment of the requirements for the award of the Degree of Doctor of Philosophy in Environmental Sciences and Engineering of the Nelson Mandela African Institution of Science and Technology.

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# DEDICATION

This work is dedicated to my father, Mr. Novert Karungamye, my mother, Mrs. Tatu Ntibansiga, my lovely wife, Uria Sanga, and my three children, Jesca, Steve, and Glory. Their prayers, love, and care gave me strength and power in my life.

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| | |
|---|---|
| AOPs | Advanced Oxidation Processes |
| AP | Algal Photobioreactor |
| ARB | Antibiotic-Resistant Bacteria |
| ARGs | Antibiotic-Resistant Genes |
| ASB | Anaerobic Sludge Blanket |
| ASP | Activated Sludge Processes |
| AZT | Azithromycin |
| BI | Biodegradability Index |
| BOD | Biochemical Oxygen Demand |
| BOD | Biochemical Oxygen Demand |
| CEF | Ceftriaxone |
| CIP | Ciprofloxacin |
| CIP | Ciprofloxacin |
| CIP | Ciprofloxacin |
| COD | And Chemical Oxygen Demand |
| COD | Chemical Oxygen Demand |
| COTECH | Commission for Science and Technology |
| CW | Constructed Wetland |
| DHPS | Dihydropteroate Synthase |
| DO | Dissolved Oxygen |
| DUWASA | Dodoma Urban Water Supply And Sanitation Authority |
| ECs | Emerging Contaminants |
| FSF | Free Surface Flow |
| GEN | Gentamycin |
| MBR | Membrane Bioreactor |
| MBR | A Membrane Bioreactor |
| MET | Metronidazole |
| PBP | Penicillin-Binding Protein |
| PPCPS | Pharmaceuticals and Personal Care Products |
| RBC | Rotating Biological Contactor |
| ROL | Radial Oxygen Loss |
| ROS | Reactive Oxygen Species |

| | |
|---|---|
| SBR | Sequencing Batch Reactors |
| SDGs | Sustainable Development Goals |
| SSF | Subsurface Flow |
| SUA | Sokoine University of Agriculture |
| SUL | Sulfamethoxazole |
| SUL | Sulfamethoxazole |
| TDS | Electrical Conductivity, Total Dissolved Solids |
| TET | Tetracycline |
| TOC | Total Organic Carbon |
| UDOM | University of Dodoma |
| UNICEF | United Nations International Children's Emergency Fund |
| WHO | World Health Organization |
| WSP | Waste Stabilization Pond |
| WSPs | Waste Stabilization Ponds |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the problem

More than 5 billion people worldwide are believed to rely on groundwater and surface water systems for a variety of purposes, including domestic, agriculture and industrial applications (Akhtar *et al.*, 2021; Mishra, 2023; Mukherjee *et al.*, 2021; Pointet, 2022). Nearly 80% of the wastewater produced by anthropogenic activities is returned to nature untreated or unreused (Torrens *et al.,* 2020). According to the World Health Organization (WHO) report of 2020 on the state of the world's sanitation, 4.2 billion people worldwide still lack access to adequate sanitation services and even the most basic sanitary facilities (Koottatep *et al.,* 2021; UNICEF & WHO, 2020). In developing countries, 25% of the urban population and 82% of the rural population do not have access to adequate drinking water and sanitary facilities (Bijekar *et al.,* 2022). According to estimates, 60% of African urban residents reside in slum areas with subpar, insufficient, and unpredictable sanitation services (Bishoge, 2021; Fotio & Nguea, 2022; Zerbo *et al.,* 2021). More than 50% of the people living in areas of Sub-Saharan Africa and several tropical islands lack access to any kind of sanitation (UNICEF & WHO, 2017). Even in countries where modern sanitation facilities are widely available, poor design and a lack of commitment to infrastructure maintenance result in significant contamination via discharge into surface and ground waters (Wear *et al.,* 2021). Tanzania is not exceptional when it comes to the problem of wastewater management. This concern impacts different cities, towns, and municipalities as a result of both natural and man-made factors such as increasing population and fast urbanization (Ringo, 2016).

Wastewater is generated by a wide range of human activities, including domestic, industrial, agricultural and commercial activities (Gavrilescu, 2021). Wastewater composition may include nutrients, radioactive materials, microorganisms, heavy metals, pharmaceuticals, and personal care products (Msaki *et al.,* 2022) and is determined by the uses to which the water was subjected (Sperling, 2007). The amount of wastewater produced is determined by a number of factors, including water availability, social and economic situation, water cost and climate (Khalid *et al.,* 2018). Wastewater discharge into natural water bodies causes increased levels of total dissolved solids, nutrients, organic matter, and other contaminants, resulting in serious environmental issues such as eutrophication, biodiversity loss, and changes in aquatic organisms and their behaviors (Akpor & Muchie, 2011; Soni *et al.,* 2022).

Water bodies are currently being impacted by Emerging Contaminants (ECs) (Gomes, 2020). The ECs are compounds that have the potential to infiltrate the environment and produce negative ecological and human health consequences, but are mostly uncontrolled and the fate and possible effects are unclear. Chemical substances that are classified as ECs include illegal drugs, hormones, sweeteners, personal care products, pharmaceuticals, pesticides and micro/nanoparticles (Flynn *et al.*, 2021). The list of ECs also includes microbial ECs, such as antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) (Vassallo *et al.*, 2021). The presence of these ECs in the environment has been associated to various impacts on the ecosystem and public health.

The primary issues associated with pharmaceuticals in aquatic environments include aquatic toxicity, bacterial resistance development, endocrine disruption, and genotoxicity (Deblonde & Hartemann, 2013; Jennifer *et al.,* 2017; Tambosi *et al.,* 2010). The toxic effects of these compounds are more likely to be chronic rather than acute since most pharmaceuticals are lipophilic and last longer in aquatic environments at low concentrations (Duarte *et al.,* 2022; Fernandes *et al.,* 2021; González-González *et al.,* 2022; Maculewicz *et al.,* 2022). The primary source of pharmaceutical residues in the aquatic environment is human excretions. These compounds are not completely removed in conventional wastewater treatment systems. Consequently, they have been detected in receiving water bodies as transformation products, metabolites, and parent compounds (Pereira *et al.,* 2020). The toxicity and fate of pharmaceutical compounds have been the subject of several published research.

Antibiotics are among the pharmaceuticals that are frequently detected in wastewater, and because of their detrimental impacts on both human health and the state of natural ecosystems, they are always given the highest priority concern (Álvarez-Torrellas *et al.,* 2017; Frascaroli *et al.,* 2021; Samal *et al.,* 2022). They are, in fact, among the most widely prescribed and used pharmaceutical class all around the world (Becker *et al.,* 2016). In Tanzania, for example, the prevalence of using antibiotics among referral hospitals in 2019 revealed that roughly 62.3% of hospitalized patients were prescribed antibiotics (Seni *et al.*, 2020). Studies have shown that the amounts of antibiotics used and the concentrations of antibiotics in wastewater are positively correlated (Lien *et al.*, 2016). This suggests that, with extensive use and a limited metabolism (excretion up to 70% unchanged), the release of antibiotics into the environment is continuously increasing (Becker *et al.,* 2016). Occurrence of antibiotics in the environments has resulted into widespread antibiotic resistance. This, particularly in aquatic environments, enhances the acquisition of ARGs by microorganisms even at low concentrations (Berglund *et* *al.*, 2014; Song *et al.*, 2018).

Bacteria are capable of developing new resistance strains by genetic mutations or by horizontal transfer of ARGs (Goulas *et al.*, 2018). Unlike antibiotics, which frequently occur as the product of a continuous release of antibiotics, ARGs can spread among various bacterial species and habitats once they are in the environment (Grenni *et al.*, 2017). Environmental bacteria that carry ARGs have the potential to act as reservoirs of resistance, which may then be transferred to pathogenic bacteria (Jian *et al.*, 2021; Skandalis *et al.*, 2021). Antibiotic resistance poses a major concern to public health because it compromises the therapeutic efficacy of antibiotics, increases the likelihood of diseases spread, cause severe illness and may even lead to death (Niu *et al.*, 2022).

The main concern is that these ECs cannot be effectively removed from wastewater by conventional wastewater treatment systems (Ortúzar *et al.*, 2022). The conventional methods are intended to remove typical components of wastewater include colloids, soluble contaminants, organic matter, and nutrients (Crini & Lichtfouse, 2018; Semreen *et al.*, 2019). It was not their intended use to get removal of ECs (Ghezali *et al.*, 2022; Ramírez-Durán *et al.*, 2019). As a result, there are significant concentrations of these ECs in the effluent generated by these techniques (Simon *et al.*, 2021; Valipour & Ahn, 2017) especially hospital effluents (Alfonso-Muniozguren *et al.*, 2021). The removal of these types of pollutants from wastewater requires effective and ecologically acceptable methods (Amiri *et al.*, 2020; Kaur *et al.*, 2019; Mahdavi & Bagherifar, 2018; Zhou *et al.*, 2021). Consequently, the focus of environmental scientists and engineers is to effectively remove the ECs from sources such as hospital, domestic and municipal wastewater systems prior to their release into the environment.

In many countries, hospital wastewater is discharged into urban drainage systems without prior treatment and co-treated with urban wastewater in conventional wastewater treatment plants (Kumari *et al.*, 2020). While conventional wastewater treatment plants are quite effective at reducing the concentration of nutrients, organic matter, and suspended particles, they are ineffective in removing ECs (Pariente *et al.*, 2022). Numerous methods and techniques have been researched for the treatment of pharmaceutical-containing wastewater most of them utilizing biological approaches including constructed wetland (CW), waste stabilization pond (WSP), rotating biological contactor (RBC), membrane bioreactor (MBR), activated sludge processes (ASP), and an algal photobioreactor (AP) (Dawood *et al.*, 2023). Among these wastewater treatment options, CWs are a viable and novel alternative for long-term, cost-effective wastewater treatment (Nyika & Dinka, 2022). A CW is a system that has been designed by humans to remove pollutants from water and wastewater by combining the influence of microbes, plants, and substrate (Huang *et al.,* 2021). A variety of kinds of wastewater have been treated with CW, including industrial effluent, contaminated river water, urban runoff, agricultural wastewater, storm water, domestic sewage, landfill leachate, and mine drainage (Agaton & Guila, 2023; Ravikumar *et al.,* 2022; Vymazal, 2022). The CW have the ability to remove a wide spectrum of organic and inorganic pollutants, including pharmaceuticals (Hijosa-Valsero *et al.,* 2010). They are able to tolerate significant fluctuations in flow and wastewater influent characteristics (Abdel-Shafy & El-Khateeb, 2013). When compared to other wastewater treatment methods, CWs are less complicated and need less operation and maintenance costs (Thalla *et al.,* 2019a).

The role of various CW components on pharmaceutical degradation, interactions among different pharmaceutical compounds in wastewater, and interactions of pharmaceuticals with microorganisms and plants remains unclear (Zapata-Morales *et al.,* 2023). However, due to intricate interactions between plants, wastewater, substrate, and microorganisms, plants in CWs play an essential role for the removal of pollutants from wastewater (Türker *et al.,* 2016). They are in responsible for uptake of nutrients, evapotranspiration, and denitrification by serving as a sustainable carbon source (Biswal & Balasubramanian, 2022; Caselles-Osorio *et al.,* 2007; Varma *et al.,* 2021). Through a process known as radial oxygen loss that takes place in the root zone, plants serve as the primary source of oxygen in CWs. This procedure improves contaminant removal through the utilization of aerobic conditions (Malyan *et al.,* 2021; Yu *et al.,* 2022). The plant roots exudates released by plants facilitate microbial-mediated degradations in the rhizosphere (Rachman, 2018; Varma *et al.,* 2021).

The plants used in CW are selected based on a number of criteria, including ecological acceptability, pest and disease tolerance, climate tolerance, pollution tolerance, hypertrophic wet conditions tolerance, ready propagation, rapid establishment, spread, and growth, and high pollutant removal capacity (Autlwetse & Kimwaga, 2022; Mburu *et al.,* 2015). Research have demonstrated that the effectiveness of CWs in removing pharmaceuticals from wastewater varies depending on the specific type of pharmaceutical and the specie of plant involved (Alsubih *et al.,* 2022; Carter *et al.,* 2014; Hu *et al.,* 2021; Ilyas & van Hullebusch, 2020a). The relationship between treatment efficiency and plant species differences, or between a single species and a polyculture, is one of the concerns being investigated (Dell'Osbel *et al.,* 2020; Du *et al.,* 2020; Herazo *et al.,* 2021; Luo *et al.,* 2023; Vymazal *et al.,* 2021). Therefore, plant species selection has important implications in CW design due to differences in their ability to absorb, accumulate, and degrade pharmaceuticals.

Tanzania is among several countries which have adopted CW technology. Given the widespread and establishment of *Canna indica* (Ali *et al.,* 2020; Maregesi & Mwakalukwa, 2015; Moshi *et al.,* 2012) and *Cyperus alternifolius* in natural wetlands in Tanzania (Kipasika *et al.,* 2016), it makes sense to evaluate their effectiveness in removing pharmaceuticals from wastewater. In addition, *Typha latifolia* is grown in numerous CWs in Tanzania (Kaseva, 2004; Kipasika *et al.,* 2016; Mashauri *et al.,* 2000), including one used to treat hospital wastewater in Dodoma. Despite being monitored, the effectiveness of this CW in removing pharmaceuticals, ARB, and ARGs has not been well documented. Insufficient information on the levels of these pollutants, particularly in hospital wastewater effluents, poses a health concern to the aquatic ecosystem and humans. Therefore, this research was done to investigate the effectiveness of hospital wastewater treatment in CW for removal of organic matter, nutrients, ARB and ARGs and to investigate the potential of the selected plant species for removal of selected antibiotics from wastewater in CW.

## 1.2 Statement of the problem

Pharmaceutical pollution of aquatic ecosystems is a serious global threat that is becoming more prevalent. Antibiotics are the pharmaceutical substances most commonly detected in wastewater; their persistent nature, incomplete metabolism, and ease of ecosystem mobility make them the most concerning (Ortúzar *et al.,* 2022). The inefficiency of conventional wastewater treatment plants to completely remove antibiotics, ARB, and ARGs has been recognized as a key contributing reason to their spread. It is important to have effective treatment methods for removing these ECs from wastewater in order to stop their release into the environment. Phytoremediation technologies, such as CWs, are being considered as an alternative secondary wastewater treatment option for pharmaceutical removal. The performance of various plant species in CWs for the removal of several classes of antibiotics from wastewater, however, is not well documented. Also, the response of various plant species and bacteria in in the rhizosphere to the presence of antibiotics in wastewater is not well documented. Moreover, research exploring the possibility that CWs may discharge wastewater into the environment that contains ARB and ARGs alongside to cleaning wastewater has not received close enough attention. Therefore, the purpose of the current research was to develop research-based evidence regarding the role of plants and the efficiency of the selected plant species in removing antibiotics from wastewater. Also, to gather evidence on the possibility that CWs may discharge wastewater containing ARB and ARGs into the environment for mitigation and future research.

### 1.3 Rationale of the study

This research investigated the performance of CW planted with selected plant species in removing antibiotics from wastewater as well as the levels of antibiotic-resistant bacteria and antibiotic resistance genes that are released from hospital wastewater effluent treated in CW. The current research provides evidence for the usefulness of the selected plants in CWs for removing antibiotics from pharmaceuticals-contaminated wastewater. This is important in the selection of CW plants for a specific target type of wastewater and local climate. A plant chosen for CW should be able to tolerate the harsh wastewater conditions, have a high pollutant removal capability, and be resistant to weed invasion. Furthermore, this work provides site-specific information on the presence of ARB and ARGs in hospital wastewater effluent treated in CW. The findings of this research can be used to develop the national policy and guidelines for hospital wastewater treatment in Tanzanian. They can also serve as evidence that pharmaceuticals, ARB and ARGs, should be monitored and controlled before treated wastewater is discharge. Effective treatment of wastewater could contribute to achieving several Sustainable Development Goals (SDGs) such as SDG 3: Good health and wellbeing, SDG 6: clean water and sanitation, SDG 11: sustainable cities and communities and SDG 14: life below water.

### 1.4 Research objectives

#### 1.4.1 General objective

To investigate the prevalence and removal of antibiotic resistant bacteria and antibiotic resistance genes from hospital wastewater treated in CW and investigate the potential of selected aquatic plants for removal of selected human antibiotics from wastewater in CW.

#### 1.4.2 Specific objectives

(i) To evaluate the performance of a full-scale CW in treating hospital wastewater and assess the prevalence of antibiotic resistant bacteria and antibiotic resistance genes in hospital effluent treated using the CW.

(ii) To investigate the performance of selected plant species on removal of the selected human antibiotics from synthetic wastewater in experimental CW.

(iii) To investigate how antibiotic exposure influences the diversity and abundance of bacteria found in the rhizosphere of various plant species in CW.

## 1.5 Research questions

(i) What is the performance of a full-scale CW in treating hospital wastewater and what are the levels of antibiotic resistant bacteria and antibiotic resistance genes in hospital effluent treated using the CW?

(ii) What is the performance of the selected plant species on removal of the selected human antibiotics from synthetic wastewater in experimental CW?

(iii) How does antibiotic exposure influence the diversity and abundance of bacteria found in the rhizosphere of various plant species in CW?

## 1.6 Significance of the study

The dissemination of research findings is an important aspect of the research process. The results and knowledge gained from the present research have been and will continue to be made available to the general public, policy makers, decision-makers and academic community through the following approaches:

(i) Publications: The findings of this research have been published in scholarly journals that offer open access. These can be freely accessed by anyone who wants to use the findings to contribute to public awareness and sustainable policymaking regarding the monitoring and controlling of pharmaceuticals in the environment.

(ii) Formal communication on the research findings will be made to the Tanzania Commission for Science and Technology (COTECH), which granted the research clearance, and the Dodoma Urban Water Supply and Sanitation Authority (DUWASA), which granted permission to collect and analyze hospital wastewater samples in Dodoma.

(iii) This dissertation, in both soft and hard copies, will be available for further reference at NM-AIST. This dissertation and all associated published papers will be deposited in the NM-AIST institutional repository, which will provide permanent storage for the documents and allow public access to the research's findings.

(iv) The author is a member of the academic staff at the University of Dodoma (UDOM). The results of this research have established a solid foundation for further research, teaching, supervision, and general academic advancement.

## 1.7 Delineation of the study

This research was done to investigate the effectiveness of hospital wastewater treatment in CW for removal of organic matter, nutrients, ARB and ARGs and to investigate the potential of the selected plant species for removal of selected antibiotics from wastewater in CW. Further, the study investigated the performance of a full-scale CW in treating hospital wastewater and levels of antibiotic resistant bacteria and antibiotic resistance genes in hospital effluent. In addition, performance of the selected plant species were invested for their potential ability of removing selected human antibiotics from synthetic wastewater in experimental CW. The present study as well focused on investigating the antibiotic exposure if influence the diversity and abundance of bacteria found in the rhizosphere of various plant species.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Pharmaceuticals in the environments

Our ecosystem has turned into a dump for both natural and synthetic pollutants as a result of poor solid waste management and inadequate wastewater treatment (Kasonga *et al.,* 2021). Among the most challenging emerging contaminants are pharmaceutical chemical substances. These chemicals can trigger biological activity in non-target organisms even at very low concentration (Rasheed *et al.,* 2019). Over 600 pharmaceutical compounds have reportedly been found in the environment on a global scale (Küster & Adler, 2014). The public awareness of how pharmaceuticals affect the environment is growing. As a result, human and veterinary pharmaceuticals, as well as their active transformation products, are considered to be significant environmental micropollutants (Bottoni *et al.,* 2010).

### 2.2.1 Sources of pharmaceuticals in the environment

Pharmaceuticals and their metabolites enter the environment via a variety of mechanisms, including direct release from manufacturing industries, excretion of human and animal wastes on the ground, veterinary and agricultural practices, and direct disposal of intact drugs into the environment (González Peña *et al.,* 2021). These sources are summarized in Fig. 1. Wastewater treatment facilities which collect discharges from households, hospitals, industries, pharmacies and veterinaries, have been identified as the primary source of these compounds into the environment (Kanakaraju *et al.,* 2018). It is generally accepted that conventional wastewater treatment processes such as coagulation, filtration, flocculation and sedimentation do not completely remove pharmaceuticals (Barbara *et al.,* 2015; Khan *et al.,* 2020). This is because these facilities were not designed to specifically remove pharmaceuticals from wastewater (Hlengwa & Mahlambi, 2020). Due to pharmaceuticals' high polarity and solubility, they have been discharged into the receiving water bodies along with effluents (Abdallat *et al.,* 2022; Lan *et al.,* 2018; Serrano *et al.,* 2011; Waleng & Nomngongo, 2022). During wastewater treatment, some pharmaceuticals have a tendency to partition in sludge, which results in their introduction into agricultural fields (Madikizela *et al.,* 2018; Waleng & Nomngongo, 2022).

Improper disposal of unused and expired pharmaceuticals is another significant source (Fekadu *et al.,* 2019; Hlengwa & Mahlambi, 2020; Kassahun & Tesfaye, 2020). Pharmaceuticals that are flushed down the toilet or sink can end up in the environment (Caban & Stepnowski, 2021; Freitas & Radis-Baptista, 2021; Gwenzi *et al.*, 2023; Rogowska & Zimmermann, 2022). Furthermore, if used test strips are not disposed of properly, some of them may end up in landfills and eventually contaminate nearby water bodies (Dar *et al.*, 2019). However, due to the lack of standard pharmaceuticals disposal guidelines in many countries, implementation of proper pharmaceutical disposal remains generally inadequate (Nilufer *et al.*, 2017).

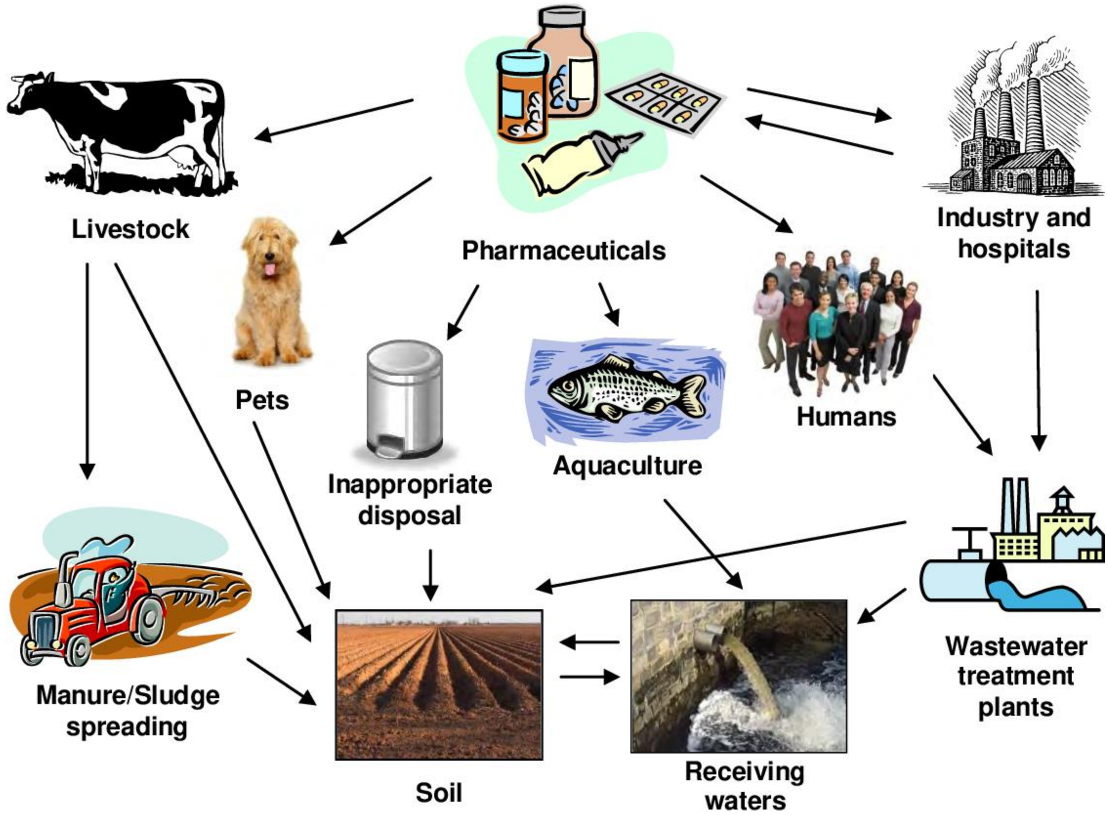



**Figure 1: Main sources and pathways of pharmaceuticals in the environment (Mceneff *et al.*, 2015)**

### 2.2.2 Effects of pharmaceuticals to the environments

Pharmaceutical compounds are intended to induce a biological response in specific organisms; however, environmental exposure may elicit a biological response in non-specific organisms as well. Given that most pharmaceutical substances are lipophilic and last longer at low concentrations in aquatic environments, toxic effects are more likely to be chronic than acute (Duarte *et al.*, 2022; Fernandes *et al.*, 2021; González-González *et al.*, 2022; Maculewicz *et al.*, 2022). The effects are summarized in Fig. 2.

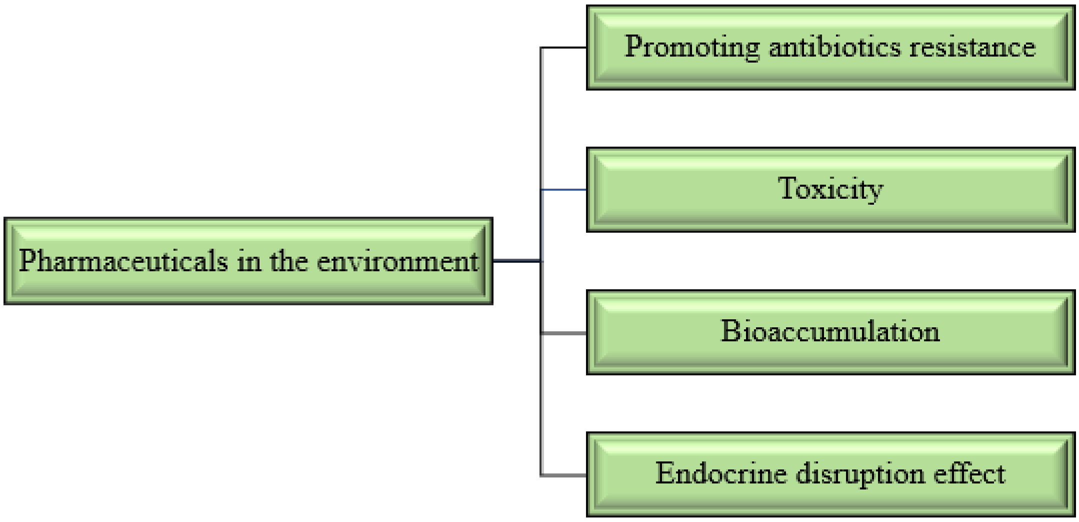



**Figure 2: Effects of pharmaceuticals in the environments**

**(i) Toxicity**

Pharmaceutical compounds have been detected in a variety of fish tissues, including the gills, muscles, blood plasma, liver, and brain. Antibiotics have also been measured in a variety of studies in aquatic invertebrates, algae, and bivalves (Gómez-Regalado *et al.*, 2023; Świacka *et al.*, 2022). These compounds are frequently detected in effluents in concentrations ranging from $ngL^{-1}$ to $\mu gL^{-1}$, and even at these low levels, their pharmacological characteristics could still pose a threat to the ecology of the receiving environment (Souza *et al.*, 2018; Świacka *et al.*, 2022). The adverse effects of pharmaceuticals are more likely to be chronic than acute because the majority of pharmaceutical compounds are lipophilic and stay longer in aquatic environments at low concentrations (Zare *et al.*, 2022). Aquatic species such as fish, algae, bacteria and crustaceans have been proven to be impacted by pharmaceuticals (Kovalakova *et al.*, 2020). Low-trophic level organisms such as cyanobacteria and algae, on the other hand, are more sensitive to pharmaceuticals such as antibiotics than higher-trophic level species such as fish (Kock *et al.*, 2023; Mojiri *et al.*, 2022). It has been shown that antibiotics affect plants as well. They have the capacity to cause phytotoxic effects both directly and indirectly. For instance, they can reduce the rate of respiration or chlorophyll synthesis (Carballo *et al.*, 2022). One study reported that a concentration of 10 mg sulfadiazine/kg soil over a 40-day exposure period had a negative effect on the root growth of maize and willow (Timmerer *et al.*, 2020). Antibiotics in the soil, decrease homeostasis and change the scaling relationships between roots and other plant organs. This could impact a plant's performance by affecting metabolic processes (Minden *et al.*, 2018).

### (ii) Antibiotic resistance

Antibiotics are a class of secondary metabolites which can be produced by microorganisms, chemically synthesized or semi-synthesized analogous compounds, which could inhibit growth and survival of other microorganisms (Ben *et al.*, 2019; Yao *et al.*, 2022). They are used to treat bacterial infections, to support surgical procedures, to cure cancer, and as a preventive measure. In addition, antibiotics are commonly used as growth promoters in aquaculture and in the veterinary treatment of domestic and livestock animals (Le Page *et al.*, 2017). Antibiotic use is quickly increasing from year to year, and it is projected that by 2030, antibiotic use will have increased by 200% (Amangelsin *et al.*, 2023).

Studies show that just a small amount of these antibiotics are completely metabolized by humans as well as animals, with 20 to 90% of them usually being removed through the urine and feces as the parent chemical compounds or metabolite (Sabri *et al.*, 2020). Because of their potential negative effects on non-target organisms and rising bacterial resistance, antibiotics are an emerging environmental contaminant that should be taken seriously (Chaturvedi *et al.*, 2021; Da Silva Freitas *et al.*, 2022; Hanna *et al.*, 2023; Serwecińska, 2020). Antibiotics can alter the prevailing flora, the structure and composition of communities, and the diversity and richness of microorganisms in an environment. Some environmental factors, however, considerably reduce the intensity of the affects (Qiu *et al.*, 2023).

Antibiotic resistance is an adaptive genetic trait that some bacterial subpopulations display or acquire that enables them to continue to grow and live despite being exposed to therapeutic dosages of an antibiotic substance that would ordinarily cause them to die or become inhibited (Carvalho & Santos, 2016). This happens because some antibiotics, which are frequently released at low concentrations into the environment, place considerable selective pressure on communities of bacteria, leading to the development of their resistance (Dires *et al.*, 2018; Konopka *et al.*, 2022).

Gene mutation or horizontal gene transfer are the mechanisms underlying the emergence of bacterial resistance. As a result, organisms can grow resistant to just one antibiotic and vulnerable to a wide range of mobile genetic elements. In addition, microorganisms could become multidrug resistant, making it difficult to treat patients in hospitals (Kunhikannan *et al.*, 2021). It is difficult to figure out the situation and quickly switch out existing antibiotics for those to which resistance has evolved. These limit the range of therapeutic alternatives, increase healthcare costs, lengthen hospital stays, produce ineffective treatments, and result in deaths (Dires *et al.,* 2018).

Conventional wastewater treatment plants are intended to remove contaminants such as total organic carbon as well as nutrients such as nitrates and phosphates. They are not specifically designed to remove micropollutants like antibiotics and antibiotic resistance genes (ARGs). The removal of antibiotics and ARGs in these systems varies by 1-2 log (Sabri *et al.,* 2020). The ARGs and antibiotics are consequently discharged in high quantities into water bodies (Ben *et al.,* 2020; Sosa-Hernández *et al.,* 2021). The ARGs may still be released into the environment and change into other bacteria, even if antibiotic-resistant bacteria have been destroyed or eliminated during wastewater treatment. Prior studies have shown that ARGs are prevalent in municipal wastewater and wastewater lagoons even after treatment (Agusi *et al.,* 2022; Monsalves *et al.,* 2022). Due to this, strategies for removing antibiotics efficiently that can also stop the proliferation of ARGs must be developed (Helt, 2012; Schwartz *et al.,* 2003).

The most significant side effect of antibiotic resistance is death. Other negative effects include a longer hospital stay, the requirement for artificial ventilation, intensive care, and the use of intrusive devices. It raises the expense of hospital nursing care, support services, diagnostic testing, and imaging (Friedman *et al.,* 2016; Sa'adatu Sunusi *et al.,* 2019). Because of the scope of the problem, global approaches and efforts by governments, relevant institutions, and stakeholders from different backgrounds are required. It is required to regulate societal antibiotic usage on the one hand, and to manage domestic and hospital wastewaters in an environmentally friendly manner on the other.

**(iii) Endocrine disruption**

The presence of pharmaceuticals, particularly endocrine-disrupting compounds, in the environment may result in endocrine disruption for a variety of reasons (Archer, 2018; Esplugas *et al.,* 2007; Söffker & Tyler, 2012). First of all, some pharmaceuticals, like contraceptives, are used to regulate hormonal functions. Second, while most pharmaceuticals are only effective for a short time in the target organism, they may stay in the environment considerably longer after excretion. Third, the hormonal systems of environmental target species may differ from those found within the organism under treatment, causing them to react differently to the presence of a pharmaceutical (Behera *et al.,* 2011; Damkjaer *et al.,* 2018; Ingerslev *et al.,* 2003; Pedersen *et al.,* 2005). These compounds, which include estriol and estrone, have an effect on hormonal regulation as well as reproductive and sexual behavior (Miraji *et al.,* 2016). Endocrine disrupting substances can be toxic to receptor organisms through continuous exposure rather than bioaccumulation when exposed to the body (Damkjaer *et al.,* 2018; Pedersen *et al.,* 2005). The evidence from the scientific literature reveals that exposure to low concentrations of endocrine disruptor compounds is related to the abnormalities and health issues that aquatic organisms experience (Kyzas *et al.,* 2015; Pironti *et al.,* 2021).

**(iv) Bioaccumulation**

There are numerous definitions of bioaccumulation. Bioaccumulation can be defined as an increase in the concentration of a chemical in a biological organism over time in comparison to the concentration of the chemical in the environment (Zenker *et al.,* 2014). The concentration of a chemical substance in or on an organism caused only by water exposure is referred to as bioconcentration (Lagesson *et al.,* 2016; Mceneff *et al.,* 2015). Bioaccumulation factors are typically estimated as the ratio of the concentration of the substance of interest in the biota sample (plants and animals) to that in the surrounding media (e.g., soil or water) (Gómez-Regalado *et al.,* 2023; Zenker *et al.,* 2014). The potential for pharmaceuticals to bioaccumulate in aquatic organisms is one of the most significant concerns regarding pharmaceutical release into water bodies.

Pharmaceuticals have a high lipophilicity, which contributes to both their bioconcentration and bioaccumulation potential (Overturf *et al.,* 2015). Some pharmaceuticals have the potential to bioaccumulate in fish and other aquatic organisms, which can cause a variety of unanticipated interactions. For instance, prolonged exposure to estrogenic contaminants in water might cause fish livers to enlarge (Badamasi *et al.,* 2020; Liney *et al.,* 2006; Topić Popović *et al.,* 2023). Environmental bioaccumulation of the pharmaceuticals has a variety of ecological impacts, including a long-term antibiotic resistance, abnormal hormonal control impairing reproduction, lower reproductive capacity, and a rise in the risk of breast and testosterone cancers (Badamasi *et al.,* 2020; Dhir, 2022; Kumar *et al.,* 2021; Leal *et al.,* 2020; Marcu *et al.,* 2023; Moreira *et al.,* 2023; Sun *et al.,* 2020; Tijani *et al.,* 2013; Topić-Popović *et al.,* 2023; Zahran *et al.,* 2020; Ziylan-Yavas *et al.,* 2022). Given their significant potential for bioconcentration and bioaccumulation, more research is needed to understand the effects that these compounds may have on aquatic organisms and humans.

### 2.2.3 Removal of pharmaceuticals from wastewater

In recent times, researchers have given much attention to removal of pharmaceuticals from the environment by employing various treatment technologies. The treatment options, such as advanced oxidation processes, adsorption, membrane processes, etc., are capable of removing these contaminants. However, such technologies are costly and energy-intensive for field-scale applications (Ravichandran & Philip, 2022). It has been proven that some conventional methods used in wastewater treatment plants degrade these compounds into environmentally inert substances, converting them into another active form of pharmaceuticals (metabolites), which is harmful to the environment in the same or more significant way (Chojnacka *et al.,* 2022).

### (i) Adsorption methods

Adsorptive removal of pharmaceuticals is one of the most promising techniques and convenience compared to other water treatment processes (Baccar *et al.,* 2012). Various adsorbents such as activated carbon, alumina, silica, mesoporous silica, functionalized mesoporous silica, zeolite, and metal–organic frameworks have been used for removal of pharmaceuticals in water treatment processes (Andrunik & Bajda, 2021; Grela *et al.*, 2021; Natarajan *et al.,* 2022; Rasheed *et al.,* 2020). For instance, the adsorption capacity of activated carbon is 221.8 mg/g amoxicillin, 90 mg/g metronidazole, 106 mg/g norfloxacin, 570.4 mg/g penicillin, 1340.8 mg/g tetracycline, 154.98 mg/g naproxen, and 638.6 mg/g quinolones (Alghamdi *et al.,* 2021; Yang *et al.,* 2021). The commercial activated carbon's adsorption capacity is 280 mg/g dimetridazole, 328 mg/g metronidazole, 394 mg/g ronidazole, and 385 mg/g tinidazole. When combined with carbon nanotubes, the capacity is even higher (Akhtar *et al.,* 2016; Carrales-Alvarado *et al.,* 2018).

The adsorption capacity of different types of clay materials have been studied. The results from studies show that montmorillonite have adsorption capacity of 2.2 mg/g ibuprofen, 3.1 mg/g goethite and 6.1 mg/g kaolinite (Abukhadra *et al.,* 2021; Show *et al.,* 2021). The removal efficiency of hydrophobic montmorillonite has been reported as 63–67% paracetamol, 95% ibuprofen and 97% carbamazepine (Kryuchkova *et al.,* 2021). Using bentonite, there is complete removal of ciprofloxacin and ibuprofen, 27.85% sulfamethoxazole, 37.30% metronidazole and 52 42% trimethoprim (Njuguna, 2018). Using silica-based adsorbents such as mesoporous silica the adsorption capacity is 160 mg/g carbamazepine, 70 mg/g clofibric acid, 410 mg/g ibuprofen and 429 mg/g Tetracycline (Akhtar *et al.,* 2016). The issue with physical methods such as adsorption is that they remove pharmaceuticals efficiently but they are non-destructive techniques that merely cause a phase transfer of contaminants (Cruz *et al.,* 2021).

### (ii) Advanced oxidation processes

A considerable amount of work has been published relating to the application of Advanced Oxidation Processes (AOPs) for the abatement of pharmaceuticals in water and wastewater. These processes may be classified into UV–hydrogen peroxide processes, Fenton and photo-Fenton, ozone-based processes, photocatalysis and sonolysis (Pal, 2018; Pandis *et al.*, 2022). The capacity of AOPs to effectively degrade a wide range of synthetic and refractory organic pollutants relies on the formation of reactive oxygen species (ROS), including hydroxyl radicals (HO•), ozone ($O_3$), chloride ($Cl^-$), and superoxide radical ($O_2^-$) (Cuerda-Correa *et al.*, 2019; Sacco *et al.*, 2019; Tufail *et al.*, 2020).

For instance, Fenton can remove more than 70% amoxicillin, ampicillin, chlortetracycline, ciprofloxacin, doxycycline levofloxacine, sulfamethoxazole, trimethoprim (Cuerda-correa *et al.*, 2020). Photo-Fenton process can remove up to 99.77% carbamazepine, 99.66% caffeine and 99.11% paracetamol (Sönmez *et al.*, 2020). Solar photo-Fenton can remove 97% amoxicillin, 88% ampicillin, 94% diclofenac and 100% paracetamol (Alalm *et al.*, 2015). Photocatalysis process can remove more than 70% metronidiazole and amoxicillin, 42% ibuprofen, 90% acetaminophen and paracetamol, and 100% asprin, diclofenac, azithromycin, ampicillin, cloxacillin, trimethoprim, ofloxacin and sulfamethoxazole (Mansouri *et al.*, 2021). Ozonation treatment can remove more than 90% of ciprofloxacin, erythromycin, metronidazole, trimethoprim, sulfadiazine, sulfamethozaxole, sulfapyridine, sulfathiazole most of them being completely degraded (Cuerda-correa *et al.*, 2020; De Wilt, 2018; De Wilt *et al.*, 2018). Treatment with UV irradiation is effective in removing atenolol (88.3%), carbamazepine (96.4%), ciprofloxacin (96.2%), clarythromycin (85.6%), cyclophosphamide (77.0%), diclofenac (99.5%), erythromycin (0.0%) and sulfamethoxazole (90.5%) (Köhler *et al.*, 2012). The AOPs can be applied as single method or combined. Besides the fact that AOPs, can originate toxic transformation products, they have higher efficiencies when compared to traditional treatments (Pereira *et al.*, 2020). However, concerns related to the considerable energy and chemical consumption necessities are considered key obstacles to the commercial application of these processes (Cruz *et al.*, 2021).

### (iii) Biological methods

Biological methods include activated sludge processes, aerated lagoons, trickling filters, sequencing batch reactors (SBR), anaerobic sludge blanket (ASB) reactors, anaerobic filters (Tatoulis *et al.*, 2017). Elimination and transformation of antibiotics during the bio- logical treatment is the result of different processes. These processes can be biotic (biodegradation, mainly by bacteria and fungi) and non-biotic or abiotic (e.g. sorption, hydrolysis, photolysis) (Michael *et al.,* 2013). It was reported in one study that the performance of a sequential membrane bioreactor was 42-64% for naproxen and erythromycin, and 71-97% for ibuprofen, roxithromycin, and fluoxetine (Serrano *et al.,* 2011). The percentage removal of pharmaceuticals in membrane bioreactor are 99.8% acetaminophen, 28% carbamazepine, 32% diclofenac, 38.5% erythromycin, 99% ibuprofen and ketoprofen, 63% mefenamic acid, 95% naproxen, 81% sulfamethoxazole, 97% tetracycline and 90% trimethoprim (Cuerda-Correa *et al.,* 2019; Hena & Znad, 2018).

Using a conventional activated sludge acetaminophen (99.1%), carbamazepine (<25%), diclofenac (50%), erythromycin (27.2%), ibuprofen (99%), ketoprofen (50%), mefenamic acid (36%), naproxen (94%), sulfamethoxazole (52%), tetracycline (71%), trimethoprim (90%) was removed (Cuerda-Correa *et al.,* 2019; Hena & Znad, 2018; Kumari & Pulimi, 2023). A Sequencing batch reactor was used to remove chiral pharmaceuticals such as alprenolol, bisoprolol, fluoxetine, metoprolol, norfluoxetine, propranolol, salbutamol and venlafaxine. The results show that the system removed the pharmaceuticals at an overall average range 48% - 63% (Amorim *et al.,* 2016). Aerobic and anaerobic processes give different pharmaceuticals degradation efficiencies in biological aerated filters. Research indicates that the removal of various pharmaceuticals in aerobic and anaerobic systems is as follows: sulfamethazine (77.3%, 23.7%), lincomycin (99.5%, 43.4%), leucomycin (99.8%, 84.1%), oxytetracycline (94.2%, 94.5%), trimethoprim (98.3%, 100%), norfloxacin (81.7%, 88.9%), ofloxacin (98.6%, 98.1%), sulfamonomethoxine (96.3%, 99.0%) and sulfachloropyridazine (93.6%, 91.8%) in aerobic and anaerobic systems, respectively (Baghapour *et al.,* 2015; Chen *et al.,* 2017). Although all these biological methods achieve relatively high pollutant removal efficiencies, their effluent quality does not usually comply with the legal limits for final disposal (Deegan *et al.,* 2011; Murugananthan *et al.*, 2011; Tatoulis *et al.*, 2017).

## 2.2 Pharmaceuticals mostly used in Tanzania

Several factors, including financial situation, knowledge, availability of pharmaceutical, demographics, and the type and severity of diseases, have an impact on the type of pharmaceuticals consumed in the community (Cronin *et al.,* 2013; Gao *et al.,* 2012; Webair & Bin-Gouth, 2013). This explains that the most common types of pharmaceuticals will always differ from one geographic region to another. The widespread use of antibiotics in both veterinary medicine and human medicine makes them one of the pharmaceuticals that cause the most environmental contamination (Khorsandi *et al.,* 2019). Antibiotics are the pharmaceuticals that are most frequently prescribed in Tanzania (Kamuhabwa & Ignace, 2015). In research done in December, 2019 at 6 referral hospitals in Tanzania, more than 60% of inpatients received antibiotic prescriptions. In this study, metronidazole, gentamicin and ceftriaxone were the most commonly prescribed antibiotics (Seni *et al.,* 2020).

Another research was carried out on the Tanzanian mainland to estimate trends in human antibiotic consumption between 2010 and 2016, utilizing data on all antibiotics imported for systemic human use. The five most commonly used antibiotics, according to this study, were cefalexin, amoxicillin, tetracycline, metronidazole and ciprofloxacin (Sangeda *et al.,* 2021a). A different investigation was done to estimate Tanzania's consumption of antibiotics from 2017 to 2019. According to the findings, the most commonly used antibiotics were amoxicillin, doxycycline and trimethoprim sulfamethoxazole (Mbwasi *et al.,* 2020). According to a point prevalence survey (PPS) carried out in a tertiary, regional, and district hospital in the Kilimanjaro region, metronidazole, ceftriaxone and other antibiotics from the penicillin class were the most often administered pharmaceuticals (Horumpende *et al.,* 2020). In the case of animal antibiotics, the surveillance carried out in Tanzania between 2010 and 2017 reveals that tetracycline, sulfonamides, and trimethoprim were the most often used antibacterial agents. Other categories of antibiotics include aminoglycosides, quinolones, penicillin and beta-lactams (Sangeda *et al.,* 2021a).

The amount of antibiotics dispensed in hospitals and the concentrations of antibiotic residue in wastewater are positively correlated. Their presence in wastewater contributes to the development of clinically relevant antibiotic resistance (Polianciuc *et al.,* 2020). Antimicrobial resistance to widely used antibiotics has been documented in some Tanzanian research (Ripanda *et al.,* 2023a). For instance, a study was conducted from October 2018 to September 2019 to evaluate the antibiotic susceptibility patterns of clinical bacterial isolates obtained from four Tanzanian referral hospitals. The data demonstrate that isolates were resistant to Trimethoprim, Gentamicin, Tetracycline, Erythromycin, Amoxicillin-Clavulanic acid, Ampicillin and third-generation Cephalosporins (Ceftazidime and Ceftriaxone) (Mnyambwa *et al.,* 2021). High levels of resistance have also been reported to streptomycin, oxytetracycline, chloramphenicol, and penicillin G (Mdegela *et al.,* 2021). This demonstrates the importance for understanding the relationship between the use of antibiotics, environmental contamination, and antibiotic resistance. Understanding this association will assist in the development of comprehensive control measures to combat the spread of antibiotic resistance.

## 2.3 Hospital wastewater

Because of improvements in medical services and products, there is now more wastewater produced in hospitals (Amouei *et al.,* 2015). A number of factors, including the water supply, the number of available beds, general amenities like air conditioning, kitchens, and laundries, the types and numbers of units or wards, and management techniques, have an impact on the hospital wastewater produced. Each of these activities adds to the overall amount of produced wastewater (Ajala *et al.,* 2022; Majumder *et al.,* 2021). In addition to pharmaceuticals and their metabolites, hospital wastewater contains disinfectants, diagnostic agents, and other substances derived from laboratory, research, and diagnostic operations, as well as patient excretion (Fatta-Kassinos *et al.,* 2011; Santos *et al.,* 2013).

Hospital wastewater contains a higher concentration of nitrogen, ammonia, biochemical oxygen demand (BOD), and chemical oxygen demand (COD) than domestic wastewater (Hocaoglu *et al.,* 2021; Pirsaheb *et al.,* 2015). It it's challenging for conventional biological systems to treat hospital wastewater because it is less biodegradable than municipal wastewater (Antonopoulou *et al.,* 2021; Imwene *et al.,* 2022; Parida *et al.,* 2022). Based on composition, the intrinsic toxicity of hospital wastewater has been shown to be 5-15 times that of municipal wastewater (Custodio *et al.,* 2022; Gómez-Herrera *et al.,* 2023; Olalla *et al.,* 2018; Parida *et al.,* 2022; Ram *et al.,* 2020). Hospital wastewater contains a diverse variety of microorganisms, including bacteria, viruses, fungi, and parasites.

Additionally, it has been discovered that hospital wastewater contains a significant number of resistant bacteria, which prevent the growth of susceptible bacteria and increase the population of resistant bacteria in the receiving water (Ajala *et al.,* 2022; Gómez-Herrera *et al.,* 2023; Yuan & Pian, 2023). It is standard procedure to test for indicator microorganisms which indicate the presence of faecal pollution due to the costly and analytical challenges involved in detecting and counting microorganisms in water samples. *Escherichia coli*, a bacterium that belongs to the faecal coliform group, is one of the most often utilized indicator organisms (Holcomb & Stewart, 2020; Motlagh & Yang, 2019; Richiardi *et al.,* 2023; Saxena *et al.,* 2015; Tambi *et al.,* 2023; Wen *et al.,* 2020).

The *E. coli* are gram-negative bacteria with rounded ends. Their primary habitat is endothermic intestines (Ishii & Sadowsky, 2008; Osińska *et al.,* 2022; Poolman, 2016). Throughout their life cycle, they alternate with different environmental habitats (secondary habitats) such as water, silt, and soils (Petersen & Hubbart, 2020). The "coliform index" is the term for the amount of *E. coli* found in potable water, which is interpreted as a symptom of contamination with human or animal waste (Percival & Williams, 2013). The *E. coli* can thrive and survive in their natural environments depending on both biotic and abiotic factors. Abiotic factors include things like temperature, the accessibility of water and nutrients, pH, and solar radiation. Examples of biotic factors include the presence of other microorganisms, their capacity to absorb resources, their ability to outcompete one another, and the development of biofilms in natural environments (Jang *et al.,* 2017). The *E. coli* may exist in a number of environments, such as wastewater, soil, and water, as well as on plants, fruits, and vegetables, raw meat, and unpasteurized milk (Osińska *et al.,* 2022). These bacteria are common in the human stomach, but they can also lead to extraintestinal and intestinal illnesses, including septicemia, meningitis, and urinary tract infections in humans, as well as colibacillosis in poultry (Osińska *et al.,* 2022).

In developing countries, hospital effluent is frequently discharged into municipal wastewater systems and released into bodies of water without treatment (Aukidy *et al.,* 2017; Reddy *et al.,* 2020; Santoro *et al.,* 2015). According to some studies, mixing hospital and municipal wastewater may prevent wastewater treatment plants from producing activated sludge (Kumari *et al.,* 2020). As a result, separate treatment of hospital wastewater is necessary to prevent contamination that may occur when hospital wastewater is mixed with municipal wastewater (Lee *et al.,* 2014). Hospital wastewater has been subjected to studies on the removal of contaminants using a variety of methods, such as functionalized membrane filtration, persulfate activated degradation, heterogeneous photocatalysis, Fenton-like degradation, and adsorption (Vieira *et al.*, 2021).

Conventional wastewater treatment methods have demonstrated their effectiveness in the treatment of wastewater (Desta *et al.,* 2014). However, these methods are ineffective in terms of pharmaceuticals removal (Otieno *et al.,* 2017; Rachman, 2018). Additionally, they are impractical for developing nations because of how expensive and energy-intensive they are (Rodríguez-Serin *et al.,* 2022; Samrot *et al.,* 2023; Valipour & Ahn, 2017). Researchers believe that there is n't actually a single universal treatment method that works for all kinds of pollutants and all sources; rather, a successful approach to treatment should involve two or more technologies in combination (Hassan *et al.,* 2021).

## 2.4 Constructed wetlands

Constructed wetlands (CWs) are artificially created systems that use the vegetations, soils, and associated microbes to treat wastewater (Makopondo *et al.*, 2020). They perform by mimicking the processes that occur in natural wetlands (Masoud *et al.*, 2022; Mekonnen *et al.*, 2015). They have been used to treat numerous types of wastewaters, including urban runoff, contaminated river, storm water, landfill leachate, mine drainage, industrial effluent, agricultural wastewater and domestic sewage (DuPoldt *et al.*, 1996; Mthembu *et al.*, 2013). Compared to conventional wastewater treatment systems, CWs have a number of inherent advantages, including very low capital costs, fewer infrastructure, reduced operating costs, simplicity of design, and ease of operation (IDNR, 2007; Prochaska *et al.*, 2007). Additionally, they are highly resilient to various environmental variability and serve as important habitat for wildlife (Knapp *et al.*, 2019; Nuamah *et al.*, 2020; Zacharia *et al.*, 2022). The CWs have been presented in the UN's Water Development Report as a promising solution for dealing with contemporary water management concerns (Kataki *et al.*, 2021).

### 2.4.1 Classifications of constructed wetlands

The CWs are classified into different types based on design parameters and system characteristics (Stefanakis, 2015). Based on hydrology, CWs can be divided into two basic categories: Free water surface flow and subsurface flow (Yalçuk & Ugurlu, 2020). Furthermore, depending on the flow direction, subsurface flow CWs can be grouped as horizontal subsurface flow CWs or vertical subsurface flow CWs (Konnerup & Brix, 2010; Mander *et al.*, 2014; Tran *et al.*, 2019; Vymazal, 2010). The type of macrophytes can also be used to classify CWs. This comprises emerging macrophytes, floating macrophytes, submerged macrophytes, and freely floating macrophytes (Valipour & Ahn, 2017). This classification is summarized in Fig. 3.

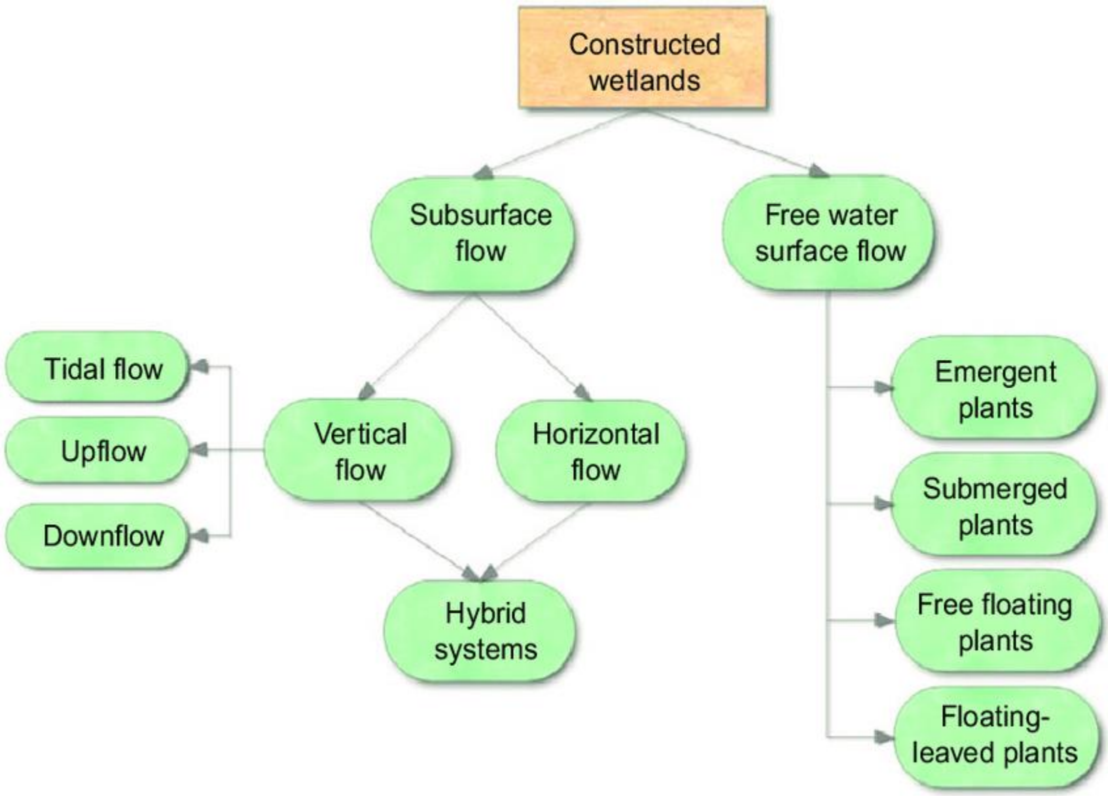



**Figure 3: Classification of constructed wetlands (Stefanakis *et al.*, 2014)**

**(i) Free water flow Vs subsurface flow constructed wetlands**

The CWs can be classified as either free surface flow (FSF) or subsurface flow (SSF) (Mihret, 2014). In FSF, wastewater flows horizontally over the wetland substrate, but in SSF, wastewater flows horizontally or vertically below the highly porous substrate (gravel, rock or soil) (Dan *et al.*, 2011; Matolisi *et al.*, 2024). On comparison between FSF and SSF constructed wetlands, the latter is preferred due to some reasons. For the treatment of an equivalent volume of sewage, FSF wetlands require a considerably greater area than SSF wetlands. The SSF systems have less complicated construction and maintenance requirements and more effective removal of organic matter, nutrients, and heavy metals (Anigrou *et al.,* 2022). Additionally, due to wastewater exposure in FSF, there is a likelihood for mosquito breeding, unpleasant odor, and human contact, all of which offer health risks (Nelson *et al.,* 2003; Stefanakis, 2020). As a result, SSF have become the most popular type of CW for wastewater treatment. The recommended design and operation parameters for FSF and SSF CWs are presented in Table 1.

**Table 1: The recommended design and operation parameters for FSF and SSF CWs**

| Parameter | Design criteria | |
|---|---|---|
| | **FSF CWs** | **SSF CWs** |
| Bed surface area ($m^2$) | Larger as possible | Less than 2500 |
| Length to width ratio | From 3:1 to 5:1 | Less than 3:1 |
| Water depth (m) | From 0.3 to 0.5 | From 0.4 to 1.6 |
| Hydraulic slope (%) | Less than 0.5 | From 0.5 to 1 |
| Hydraulic loading rate (m/day) | Less than 0.1 | Less than 0.5 |
| Hydraulic retention time (Day) | From 5 to 30 | From 2 to 5 |
| Plant density | 80% coverage | 80% coverage |

**Wu *et al.* (2015)**

#### (ii) Horizontal subsurface flow Vs vertical subsurface flow constructed wetlands

Studies shows that VSSF gives better overall performance than HSSF CWs (Raphael *et al.,* 2019; Thalla *et al.,* 2019b). The VSSF wetland system doses wastewater onto the entire surface from above, allowing it to flow vertically downward through the treatment medium (sand or gravel bed) and discharge at the bottom, in contrast to the HSSF system where wastewater flows horizontally (Sylvie & Ashton, 2021). As a result, oxygen can diffuse into the system and improve the efficiency of biodegradable organic matter mineralization (Mahmood *et al.,* 2013; Pandey *et al.,* 2013). The efficiency of HSSFCW on the removal of organic matter (COD and BOD) makes it the most used type. Despite this, it is not possible to get a totally nitrified effluent due to a low oxygen transfer capacity. Meanwhile, VSSFCW can provide favorable oxygen transfer conditions and thus favorable nitrification conditions (Vega De Lille *et al.,* 2021). However, VSSF wetlands have several drawbacks, such as being ineffective at removing solids and being clogged easily if the media selection is incorrect (Waly *et al.,* 2022).

### 2.4.2 Components of constructed wetlands

The basic components of CWs are plant species, associated microorganisms, and a support medium or substrate (such as soil, sand, or gravel) (Miranda *et al.,* 2017). These components of CWs are combined to purify wastewater by promoting organic matter breakdown, removing nutrients and suspended solids (Choi *et al.,* 2016; Saraiva *et al.,* 2019).

#### (i) Plants

The role of plants in CWs includes insulation of the filtration beds, providing rhizosphere as surface for bacterial growth, supply of oxygen to the rhizosphere, nutrient uptake and storage. They also produce root exudates which act as a carbon source for denitrifiers and thus increase nitrate removal (Li *et al.*, 2022; Vymazal, 2011a; Zhu *et al.*, 2018). The Table 2 summarizes some ways that plants may influence or contribute to the performance of CW.

**Table 2: A summary of the functions of plants in CWs**

| Process | Effect |
|---|---|
| Physical influence of the roots | Filtration, improving hydraulic conductivity, promoting sedimentation process, preventing substrate clogging Improved hydraulic conductivity |
| Influence of the roots to microorganisms | They provide surface for microbial attachment and release oxygen and roots exudates. They release phytochelatins phytometallophores and antibiotics |
| Influence of plant uptake | Phytoremediation of nutrients, metals and salts. |
| Evapotranspiration | Pollutant removal is influenced by reducing wastewater volume, which reduces outflow and concentrates contaminants, but it also increases retention time, which allows for more interaction with the wetland environment. |
| Microclimatic conditions | The plants inhibit algal growth by attenuating light. They insulate the system in various conditions. They reduce wind speed and help to stabilize the sediment's surface. |

**Brix (2003), Gorgoglione and Torretta (2018), and Shelef *et al.* (2013a)**

Plants in CW stimulate microbial richness and activity (Calheiros *et al.*, 2009, 2010). The amount of nutrients they take in during their growth cycle, which varies according to the species, the quality of the influent, and the amount of rare nutrients present, improves the quality of the wastewater (Jamwal & Shirin, 2021). The choice of plant species becomes crucial since they must tolerate the potential toxicity and fluctuations of the effluent. It should reduce energy use, greenhouse gas emissions, and unnecessary byproducts (Timotewos *et al.*, 2017).
Despite the fact that a recent study revealed that plant density could have an effect on the bacterial community in CWs, little is known regarding the effects of plant species on the bacterial community in CWs (Long *et al.*, 2016; Truu *et al.*, 2009). Unplanted CWs have also shown to have high nutrient removal efficiency. In systems without plants, the formation of biofilms on aggregate materials and microbial decomposition help remove nutrients and organic matter. The mineralization and modification of pollutants, as well as the removal of nutrients from wastewater, are all made possible by microorganisms (Cheng *et al.*, 2014; Jamwal & Shirin, 2021).

There are numerous distinct life forms or morphologies among wetland plants (Brix, 2003). The type of wastewater treatment used in a CW is determined by the plant species, whether emergent, submerged with floating leaves, floating, or submerged (Colares *et al.*, 2020). Emergent plants have been recognized as being the most frequently utilized plants in both SSF and FSF CWs (Hdidou *et al.*, 2022; Li *et al.*, 2021). The types of plants found in CWs are presented in Fig. 4.

When choosing a plant species, it is important to consider things like the site climate, the characteristics of the wastewater that needs to be treated, and the necessary effluent quality. The most appropriate vegetation for the proposed CW system should be chosen based on its ability to adapt to the saturation conditions of the terrain, the growth potential of roots and their oxygen carrying capacity, the high capacity of photosynthetic activity, the tolerance to high pollution concentrations, disease resistance, and management requirements (Gorgoglione & Torretta, 2018). Although choosing the right plant species is considered to be a crucial component of CW design, there is disagreement in the literature regarding the benefits or drawbacks of various plant species (Spieles, 2022).

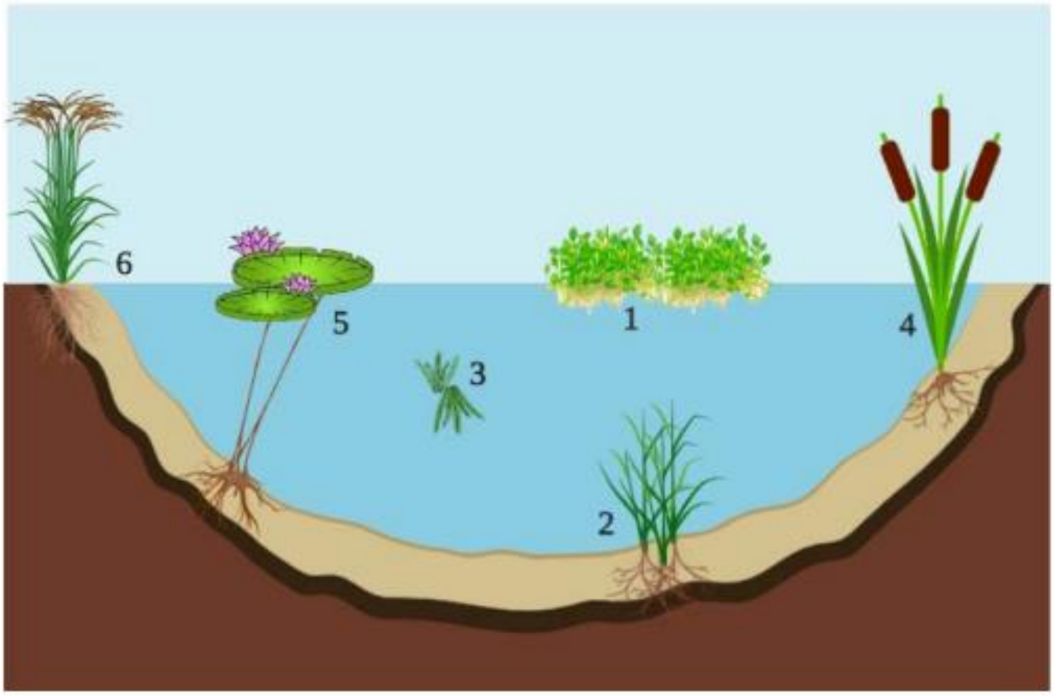



**Figure 4: Classification of aquatic macrophytes according to their biotypes: (1) Free floating, (2) Rooted submerged, (3) Submerged free, (4) Emergent, (5) Submerged with floating leaves, and (6) Amphiphytes (Kochi *et al.*, 2020)**

According to studies, *Typha latifolia, Phragmites australis, Typha angustifolia, Juncus effusus, Scirpus lacustris, Scirpus californicus,* and *Phalaris arundinacea* are the most often used plant species worldwide (Vymazal, 2011a). For instance, Phragmites australis, which is primarily utilized in northern latitudes, is not necessarily appropriate for tropical climate due to its invasive or potentially invasive behavior or because it is not a native species (Lombard *et al.,* 2017). Despite the better performance of these plants, ornamental plants are becoming more popular as a replacement for macrophytes in CWs (Macci *et al.,* 2015a).

### (ii) Substrate

The supporting matrix in the CWs has a crucial role in pollutant removal by serving as an essential media for sorption, supporting the growth of plants and microbes, and thereby inducing favorable environmental conditions (redox potential) in the porous space (Ravichandran & Philip, 2022). The particularly significant design criteria for a subsurface flow CW is the choice of a suitable permeable substrate in relation to the hydraulic and organic loads. The majority of treatment problems occur when the permeability is improperly selected for the applied load (Heike *et al.*, 2011).

The substrate in constructed wetlands not only supports plant and microbe growth, but it also directly interacts with contaminants via physical adsorption (Li *et al.*, 2014). The substrate can have a significant impact on the volume and length of the roots of the same plant. In addition to using natural materials like gravel, sand, zeolite, anthracite, volcanic rocks, granite, and quartz, man-made substrates including hollow bricks, steel slag, ceramic, activated carbon, artificial ecological substrates, and sponge iron are also used (Wang *et al.*, 2018).

Because of its ease of availability, low cost, and outstanding operating performance, gravel and sand are the most commonly utilized substrate in CWs (Waly *et al.*, 2022). The combination of gravels and sand, has been shown in some studies to provide improved hydraulic conditions and pollution removal (Shukla *et al.*, 2021). Gravel is the most commonly used substrate in horizontal SSF CWs because it does not clog easily (García *et al.*, 2010). Clogging is largely influenced by the size of the filter material and its hydraulic conductivity (Vymazal, 2018). There is still a gap of knowledge of sorption capacities of different substrates towards different forms of pollutants. The way the type of substrate influences the density and activity of microbial community in CWs requires a study.

### (iii) Microbes

Microbial degradation is regarded as the most important organic pollution elimination mechanism in the environment (Wang & Wang, 2016). Microorganisms can eliminate contaminants by using them in metabolic processes (Choudhary *et al.*, 2012). Microbes can degrade organic compounds under both aerobic and anaerobic environments. This involves the activity of diverse microorganisms such as algae, bacteria and fungi (Almuktar *et al.*, 2018; Gruchlik *et al.*, 2018; Olsson, 2011). Microorganisms grow naturally in the CW substrate. Their development and activity are primarily influenced by the hydraulic circumstances, characteristics of the wastewater and substrate, quality and availability of the nutrients, and the presence of plants (Zahui *et al.*, 2021). They also depend on environmental factors such as temperature, pH, and oxygen level. At low temperatures, activity of microorganisms and treatment efficiency decreases (Yalcuk & Ugurlu, 2009). The microorganisms living in CWs often attain their maximum activity at temperatures ranging from 15 to 25°C (Wang *et al.,* 2018). Chemical structures of organic compounds is another significant factor that significantly affects the microbial degradation process (Li *et al.,* 2014a; Spina *et al.*, 2018).

DNA analysis of CW samples reveals that bacterial species found in the wetlands belong to the *Cyanobacteria, Proteobacteria, Chloroflexi,* and *Acidobacteria* families (Xie *et al.,* 2021; Zhang *et al.,* 2016). According to literature, the majority of functional microorganisms involved in antibiotic elimination belong to the *Acidobacteria, Bacteroidetes* and *Proteobacteria* families. This is probably due to the genes for degradation present in these phyla. The phylum most significantly connected with antibiotic elimination is *Proteobacteria*, followed by *Bacteroidetes* and *Actinobacteria* (Alexandrino *et al.*, 2017; Mendes *et al.,* 2013; Sonam & Dadheech, 2024; Ye *et al.,* 2020). As a result, understanding the composition of microbes and how they vary can provide insight into how a CW system responds to changes in operational and environmental conditions.

The microbial biodiversity is reported to change as a result of exposure to pharmaceuticals such as antibiotics (Chonova *et al.,* 2016; Ding & He, 2010). Sulfonamides, for example, have been found to have an impact on the microbial community by lowering microbial diversity (Grenni *et al.,* 2017; Ohore *et al.,* 2021). Exudates from plant roots that enter the rhizosphere contain compounds that microorganisms can easily digest. They are considered to be the primary factor driving the dynamics of the rhizosphere microbial community. As a result, root exudates can moderate the effects of antibiotics on microorganisms, potentially affecting the microbial community structure of the rhizosphere (Tong *et al.,* 2020). A significant number of studies on antibiotics removal in CWs have investigated into the impacts of different types of wetland designs, plants, and treatment strategies (Bai *et al.,* 2022; He *et al.*, 2021; Liu *et al.,* 2019; Lv *et al.,* 2022). However, little is known about how antibiotics in wastewater alter microbial communities in CWs planted with various plant species (Yan *et al.*, 2018).

### 2.4.3 Removal of pharmaceuticals in Constructed Wetlands

While CWs have the potential to eliminate some pharmaceuticals from wastewater, the mechanisms by which they do so are relatively unknown. The coexistence of multiple microenvironments allows for a diverse range of microbiological communities, each of which may be capable of providing different metabolic pathways leading to pharmaceutical degradation (Hijosa-Valsero *et al.*, 2010). The studies on removal of pharmaceuticals from wastewater in CWs have been done on different classes such as antifungals, anti-hypertensives, anti-neoplastic, antiparkinsons, anti-inflammatory drugs, barbiturates, B-agonists, anti-diarrhoea drug, anti-diabetics, analgesics, antibiotics, psychiatric drugs, stimulants, receptor antagonists, antiseptics, antispasmodic, diuretic, B-blockers and lipid regulator (Verlicchi & Zambello, 2014). The effectiveness of CWs for treating hospital wastewater has been assessed by several research, and it has been established that the systems are capable of removing a range of contaminants, including TSS, COD, BOD, turbidity, nitrate, phosphate, heavy metals, and coliforms, considerably (Aukidy *et al.*, 2017; Ilyas & van Hullebusch, 2020b; Parashar *et al.*, 2022; Swarnakar *et al.*, 2022; Uluseker *et al.*, 2021). Some studies have reported the removal of pharmaceuticals in CWs with different configurations and operational parameters as presented in Table 3.

**Table 3: The removal of pharmaceuticals in CWs Modified from**

| CW | Type of plant | Country | Target Pharmaceutical (% Removal) |
|---|---|---|---|
| Full-scale SFCW used to treat 55% domestic + 45% industrial | *Phragmites australis* and *Typha latifolia* | Spain | Carbamazepine (39), clofibric acid (34), diclofenac (85), ibuprofen (96), ketoprofen (98), naproxen (72) |
| Full-scale Hybrid CW used to treat domestic wastewater | *Phragmites australis* | Spain | Acetaminophen (99), bisphenol a (>99), diclofenac (89), ibuprofen (>99) |
| Full-scale SFCW used to treat domestic wastewater | *Typha spp.*, *Phragmites spp.*, *Scirpus spp* | Sweden | Atenolol (53), carbamazepine (−19), diclofenac (30), ibuprofen (5), ketoprofen (19), naproxen (50), sulfamethoxazole (-104) |
| Full-scale HSSF-CW used to treat domestic wastewater. | *Phragmites* spp., | Italy | Analgesics/anti-inflammatory (22), antibiotics (53), anti-diabetics (24), anti-hypertensives (63), beta-agonists (16), beta-blockers (31), diuretics (35), lipid regulators (40), psychiatric drugs (2) |
| Pilot-scale VSSF-CW used to treat domestic wastewater. | *Phragmites* spp., was | Italy | Caffeine (82-99), carbamazepine (20-26), diclofenac (53-73), ibuprofen (55-99), naproxen (62-89), salycylic acid (85-98) |
| Pilot-scale VSSF-CW used to treat domestic wastewater. | *Thalia* | China | Sulfadiazine (72-46), sulfapyridine (86-86), sulfamethazine (11-31), sulfamethoxazole (24-34), trimethoprim (31-0) (summer - winter) |

**Yujie (2017)**

According to a comprehensive review of pharmaceuticals removal in CWs, the average removal efficiency of the most commonly researched pharmaceuticals ranges from 21% to 93%. The bulk of pharmaceuticals are removed through biodegradation (aerobic and anaerobic), typically in conjunction with other mechanisms (Chen *et al.*, 2016; Hijosa-Valsero *et al.*, 2011; Ilyas & van Hullebusch, 2019a). The average removal efficiencies of CWs to some studied pharmaceuticals are 93% (monensin), 89% (ofloxacin), 87% (oxytetracycline), 83% (sulfapyridine), 80% (caffeine), 79% (salicylic acid), 72% (atenolol), 72% (furosemide), 69% (doxycycline), 68% (codeine), 67% (diltiazem), 64% (acetaminophen), 62% (naproxen), 57% (ibuprofen), 56% (metoprolol), and 51% (sulfadiazine) (Ilyas *et al.*, 2020).

### 2.4.4 Constructed wetlands in Tanzania

In Tanzania, the technology of CWs started in 1995, when the University of Dar Es Salaam in collaboration with two Danish universities conducted a research project on waste stabilization ponds (WSPs). In 1998, researches on CWs started focusing on polishing WSP effluents. Among the areas researched includes modelling of nitrogen transformation and removal, selection of suitable soil media and appropriate indigenous macrophytes for CW in Tanzania (Njau *et al.*, 2011). Several pilot and full-scale CWs are presently operational in various locations throughout Tanzania (Kimwaga *et al.*, 2013). Some of reported operating CWs in Tanzania are presented in Table 4.

**Table 4: Some of the reported operating constructed wetlands in Tanzania**

| Region | Owner | Wastewater Type | Scale | Type of CW | Type of plant |
|---|---|---|---|---|---|
| Arusha | NM-AIST campus | Domestic | Full | HSSF | *Cyperus papyrus* |
| Arusha | Village of Endallah | Domestic | Full | HSSF | *Cyperus papyrus* |
| Arusha | Meat King Distributors Ltd | Industrial | Full | HSSF | *Cyperus papyrus* |
| Arusha | St. Jude Secondary School | Domestic | Full | HSSF | *Typha latifolia* |
| Arusha | BIL | Industrial | Full | HSSF | *Cyperus papyrus* |
| Arusha | Enza Zaden Africa Ltd | Agro-processing | Full | HSSF | *Cyperus alternifolius* |
| Arusha | Q-Sem industry | Industrial | Full | VSSF and HSSF | |
| Bagamoyo | Uongozi Institute | Domestic | Full | HSSF | |
| Dar es Salaam | TPCC | Domestic | Exp | HSSF | *Typha latifolia* |
| Dar es salaam | Chamazi Community | Domesstic | Full | HSSF | *Phragmites mauritianus* |
| Dar es salaam | Prof. Karoli Njau residence | Domestic | Full | HSSF | *Cyperus papyrus* |
| Dar es Salaam | Dr. Anne Outwater residence | Domestic | Full | HSSF | |
| Dar es Salaam | University of Dar es salaam | Domestic | Full | HSSF | *Phragmites mauritianus* |
| Dar es Salaam | Jangwani beach | Domestic | Full | FSF | Mangrove type *Avicennia marina* |
| Dar es Salaam | Baobab hospital | Hospital | Full | HSSF | |
| Dar es Salaam | CCBRT hospital | Hospital | Full | HSSF | |
| Dar es Salaam | International School of Tanganyika | Domestic | Full | HSSF | |
| Dar es Salaam | Majani ya chai | Domestic | Full | HSSF | |
| Dar es Salaam | Office building | Domestic | Full | HSSF | |
| Dar es Salaam | Mbagala | Domestic | Full | HSSF | |
| Dar es Salaam | Robert Mussa residence | Domestic | Full | HSSF | |
| Dar es Salaam | TBA | Domestic | Full | HSSF | |
| Dar-es-salaam | St. Anthony Secondary School | Domestic | Full | HSSF | |
| Dodoma | Centre for Community Initiatives (CCI) | Domestic | Full | HSSF | *Typha latifolia* |
| Dodoma | University of Dodoma | Hospital | Full | HSSF | *Typha latifolia* |
| Iringa | Iringa Girls Secondary School | Domestic | Full | HSSF | |

| Region | Owner | Wastewater Type | Scale | Type of CW | Type of plant |
|---|---|---|---|---|---|
| Iringa | Ruaha Secondary School | Domestic | Full | HSSF | *Phragmites mauritianus* |
| Iringa | Kleruu TC | Domestic | Full | HSSF | *Phragmites mauritianus* |
| Iringa | IRUWASA | Municipal | Full | HSSF | *Phragmites mauritianus* |
| Kilimanjaro | MUWSA | Municipal | Full | HSSF | *Phragmites mauritianus* |
| Kilimanjaro | Kibo Paper Mill | Domestic | Full | HSSF | *Phragmites mauritianus* |
| Kilimanjaro | China Paper Corporation Ltd | Industrial | Full | HSSF | *Phragmites mauritianus* |
| Mbeya | Wakulima Tea Company Ltd | Industrial | Full | HSSF | |
| Morogoro | Tanzania Tobacco Processors Ltd | Domestic and industrial | Full | HSSF | |
| Morogoro | MORUWASA | Municipal | Exp | HSSF | *Phragmites mauritianus* |
| Musoma | North Mara Mining | Acidic mine drainage | Exp | HSSF | *Phragmites mauritianus* |
| Mwanza | Mwanza City Abattoir | Abattoir | Full | HSSF | |
| Mwanza | KASHWASA | Domestic | Full | HSSF | |
| Njombe | Tanzania Wattle Company | Industrial | Exp | HSSF | |
| Pemba | Chake chake | Municipal | Full | HSSF | Macrophytes native to the area |
| Shinyanga | Shinyanga prison | Domestic | Full | HSSF | *Phragmites mauritianus* and *Typha latifolia* |
| Shinyanga | Bariadi prison | Domestic | Full | HSSF | |
| Simiyu | Malya prison | Domestic | Full | HSSF | *Phragmites mauritianus* |
| Zanzibar | Kibele | Fecal sludge | Full | VSSF | |

Kimwaga *et al*. (2013), Mahenge (2015), Mairi *et al.* (2001), Njau *et al.* (2011), Rugaika, (2020), and Sheehan (2014).

Regardless of the effectiveness of many investigated plants in Tanzania, such as *Phragmites mauritianus*, *Typha domingensis*, *Typha capensis*, *Cyperus grandis*, *Cyperus dubius*, and *Colocasia esculenta*, there is still an opportunity to investigate other available plants for better wastewater treatment in CWs.

### 2.4.5 Typha latifolia

*Typha latifolia* is one of the plant species that is most commonly grown in CWs in Tanzania. This perennial herbaceous wetland plant, sometimes known as cattail, has long, thin green stems that are capped with brown, fluffy, sausage-shaped blooming heads (Papadopoulos & Zalidis, 2019). It grows well in a wide range of climates, including tropical, subtropical, temperate, humid coastal, and dry continental conditions. Rivers, freshwater and brackish marshes, irrigation ditches, ponds, and lakes are all places where it can be found (Rana & Maiti, 2018). Additionally, this plant may grow to a height of up to 3 m, produces a lot of biomasses, and grows quite quickly (Irshad *et al.*, 2021). They usually grow as dense monocultures and can quickly colonize new wetlands with the help of wind-propagated seeds (Fitch, 2014). This plant contributes significantly to ecosystem services including bioremediation in CWs (Bansal *et al.*, 2019). Some research indicate that this plant is effective at treating wastewater in CWs (Fitch, 2014). When it comes to removing of organic matter and inorganic nutrients, it has proven to be very successful (Camacho *et al.,* 2018). It has the potential to bioaccumulate heavy metals like Zn, Ni, Pb, Cd, Se, and Cu. Additionally, it is able to tolerate and detoxify organic pollutants like synthetic pesticides (Papadopoulos & Zalidis, 2019). This plant is planted in a CW in Dodoma, Tanzania to treat wastewater from the Benjamin Mkapa Hospital (Fig. 5). This study investigated into how hospital wastewater was treated in a CW.

**Figure 5: Typha latifolia in a CW used to treat hospital wastewater in Dodoma, Tanzania (Picture taken by author)**

### 2.4.6 Cyperus alternifolius and Canna indica

*Cyperus alternifolius* and *Canna indica* (Fig. 6) were selected to be studied as CW plants for removal of the selected antibiotics from wastewater. *Cyperus alternifolius,* often known as umbrella sedge or umbrella palm, is a multi-year-old plant that thrives in moist soil or marshy environments (Ebrahimi *et al.,* 2013). It is native to northern and tropical Africa and is used as a beautiful accent in water gardens, at the edges of pools or ponds, and in a variety of landscaping (Abd-Elgleel *et al.,* 2021). It typically grows to a height of 30 to 90 cm, and in regions with the suitable environmental conditions, it can reach 150 cm (Ghamary & Mohajeri, 2021). The aerial stem of this plant is upright and unbranched, and it has strong underground roots. It is easily reproduced by employing seeds and plant pieces (Ebrahimi *et al.,* 2013).

**Figure 6: *Cyperus alternifolius* and *Canna indica* (respectively) in experimental CW at the NM-AIST campus (Pictures taken by author)**

Several studies have proven that it is effective for phytoremediation of wastewater (Rahman *et al.,* 2022). It is effective at removing several sorts of wastewater pollutants such as organic matter, nutrients, and heavy metals (Chi-Tuan *et al.,* 2021; Dolphen *et al.,* 2019). When the effectiveness of *Cyperus alternifolius* and *Phragmites australis* in municipal wastewater treatment is compared, it is found to be superior (Shahi *et al.,* 2013). When used in CWs to remove pharmaceuticals, it exhibits removal efficiencies that are equivalent to or even above those of conventional methods (Liu *et al.,* 2019; Salah *et al.,* 2023; Sánchez *et al.,* 2022).

Studies on this plant in CWs demonstrate its capacity to relate to various fluctuations in the wastewater's composition, including pharmaceuticals (Khandare *et al.*, 2021). Its widespread use in CWs is a result of its affordability. It can also be used as livestock and aquaculture feed (Nguyen *et al.,* 2021).

Several researchers are presently looking on the potential use of *Canna indica* in CW for wastewater treatment (Jamwal *et al.,* 2021). The main advantage of the Canna plant over *Phragmites australis*, a plant that is frequently used for CWs, is its high biomass production and quick pace of growth (Jamwal *et al.,* 2021). Their rapid growth rate and enormous biomass enhance the surface area of the biofilm because fast-growing plants with large roots are advantageous for nitrifying bacteria to improve nitrification (Pinninti *et al.,* 2021). Compared to ordinary wetland plants, the canna plant uses three to five times as much water. Additional advantages for its use come from the flowering and attractiveness (Zhao *et al.,* 2022).

When compared to *Cyperus alternifolius* and *Phragmites australis*, *Canna indica* outperforms them in terms of pollution reduction and greenhouse gas emissions (Chen *et al.*, 2020). *Canna indica* have a fibrous roots structure that produces high aerobic conditions throughout the CW, allowing for more removal (Sharma *et al.,* 2014). Its root system has much more root development, root number, root biomass, and root surface area than the other plant species. The flowers are showy and red or yellow. It contains a variety of spherical, glossy black seeds (Bachheti & Joshi, 2013). Although most varieties have bright green leaves, a few have glucose, brownish, maroon, or even variegated leafy (Enyoh & Isiuku, 2021). It is native to the tropical regions of America, but it may also be found in other tropical countries across the world. It is frequently used as a folkloric medicine for hepatitis, infection, and rheumatism. This plant's roots and rhizomes are thick, cylindrical, and creamy white or pinkish in colour (Kumbhar *et al.,* 2018).

This plant has a high level of pollution resistance and a lengthy root life cycle (Sharma *et al.,* 2014). The *Canna indica* plant's aerenchyma tissue makes it easier to transfer ambient oxygen to the rhizosphere, giving the ideal conditions for nitrification processes. Approximately 5–15% of the nutrients in wastewater are removed via plant absorption, but the majority of pollutants are removed by bacteria in the rhizosphere (Ayusman *et al.,* 2020; Datta *et al.,* 2021). These plants were chosen for this study due to their local availability, potential to withstand high contaminants in wastewater, and tolerance to various climatic conditions (Zorai *et al.,* 2022).

## CHAPTER THREE

## MATERIALS AND METHODS

### 3.1 Treatment of hospital wastewater

### 3.1.1 Description of the study area

This research was carried out in May 2022 at the Benjamin Mkapa Hospital (BMH) in Dodoma, Tanzania (Fig. 7). This is a tertiary public hospital located on the campus of the University of Dodoma (UDOM). This hospital was established in 2015 to deliver highly specialized healthcare services as well as to organize, supervise, and conduct research.

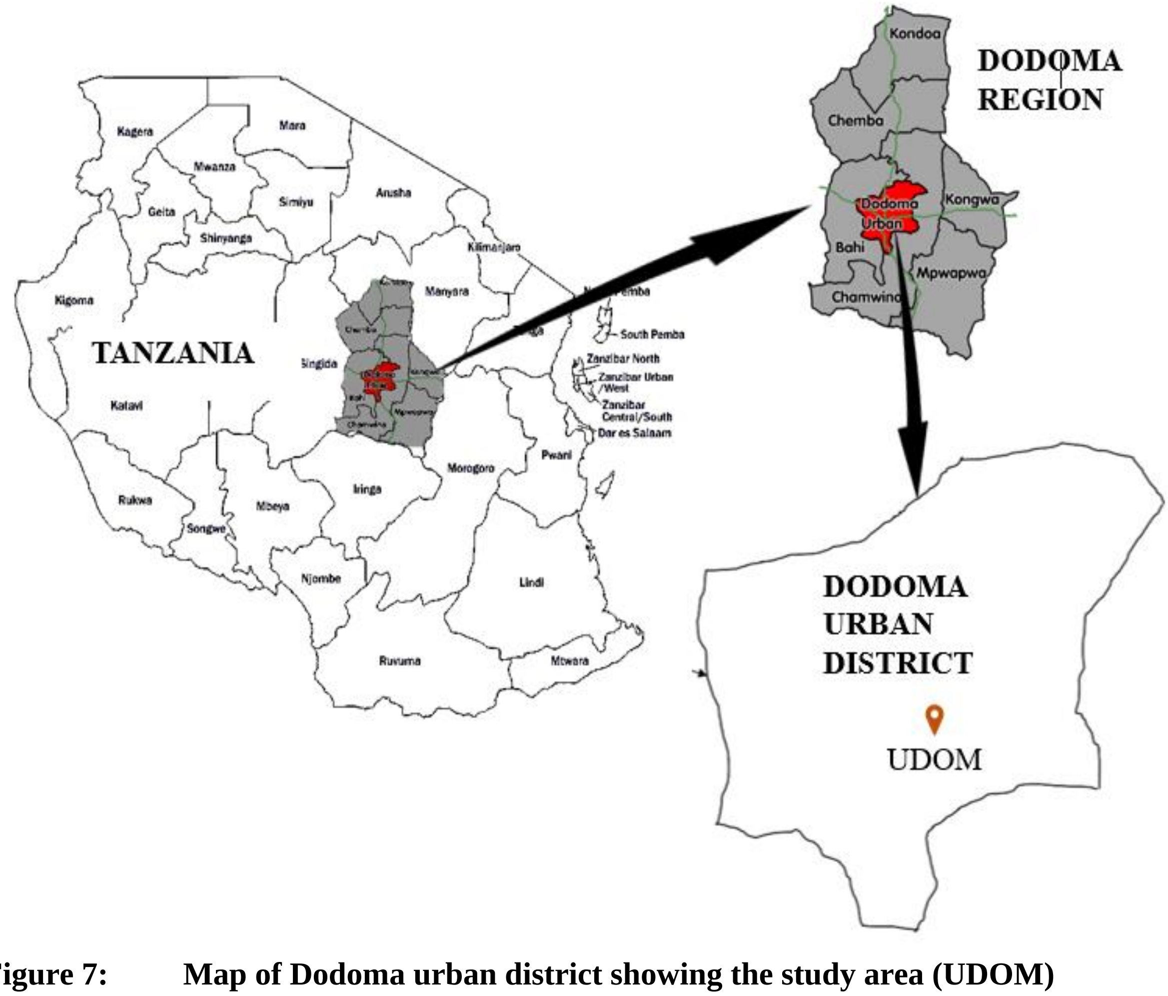



**Figure 7: Map of Dodoma urban district showing the study area (UDOM)**

The capital of Tanzania, Dodoma, has a total size of 2769 $Km^2$ and is situated between 6° 00′ and 6° 30′ South and 35° 30′ and 36° 02′ East. About 85% of the 570 mm of rainfall that falls on a given area on average between December and April. The temperature typically fluctuates between 18°C to 31°C (Mkude & Saria, 2014). Prior to being released into the environment, the wastewater from this hospital is secondary treated in the horizontal subsurface flow CW

(Fig. 8 and 9). About 3/5 of this CW's system has been planted with *Typha latifolia*, with the other portion unplanted. An impermeable concrete structure is covered with a gravel bed (about 2.5 cm) to create the CW's medium.

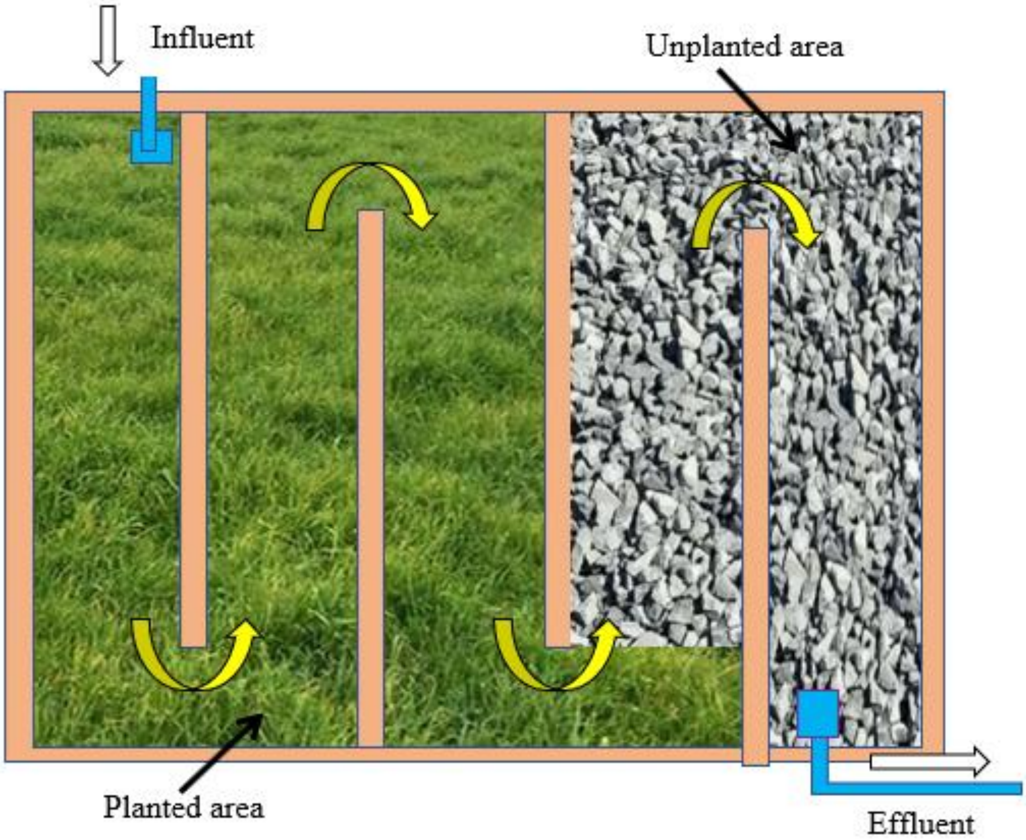



**Figure 8: Layout of the constructed wetland**


**Figure 9: Photographic appearance of (a) part of the CW used to treat hospital wastewater and (b) sampling point at the outlet (Pictures taken by author)**

### 3.1.2 Wastewater sampling

#### (i) For analysis of physico-chemical and biological characteristics

During the three-month period from the 2nd of May 2022 to the 25th of July 2022, wastewater was sampled once per week (every Monday). The water's average temperature at the time of sampling was 25.6°C, and it was the dry season. Sampling was done at a CW's inlet and outlet, where the wastewater is thoroughly mixed and at the center of the flow channel. This corresponds to around 40–60% of the water depth, where solids have the least chance of settling and turbulence is highest. Two morning and two evening grab samples were combined and put in 1.5 L plastic bottles to make daily composite samples (Kayombo & Ladegaard, 2004; Majewsky *et al.,* 2011; Paing *et al.,* 2015; Reungoat *et al.,* 2010). The sampling bottles were labeled with waterproof markers and sealed with transparent tape. Some information on the label included date and time of sampling, sampling site, and sample number. On-site measurements were taken for temperature, pH, electrical conductivity, total dissolved solids (TDS), dissolved oxygen (DO), and turbidity (Schaider *et al.,* 2017). Before being transferred to the laboratory for further analysis, the samples were initially kept up in an ice-box using ice packs to maintain them below 4°C. The analysis of the samples began as soon as they reached the laboratory, and they were maintained at 4°C throughout.

#### (ii) For antibiotics resistance testing

The CW outlet was used to collect 32 treated hospital wastewater samples over the course of four weeks, from November 7 to November 28, 2022, with 8 samples being collected once each week. Sterilized syringes were used for collecting grab samples from CW (Desta *et al.,* 2014; Imfeld *et al.,* 2010). Samples were placed in sterile 250 mL bottle and stored in ice container (Mara & Horan, 2003). The sample bottles were kept unopened until they were ready to be filled. The samples were taken to Sokoine University of Agriculture (SUA), in the Department of Microbiology, Parasitology, and Biotechnology laboratories for microbiological analysis.

### 3.1.3 Wastewater analysis

#### (i) Physico-chemical analysis

All of the analyses were performed out using Standard Methods for the Examination of Water and Wastewater, unless otherwise specified (APHA, 2017). The methods used for physicochemical analysis of synthetic wastewater are summarized in Table 5.

**Table 5: Methods used for physico-chemical analysis of wastewater**

| Parameter | Unit | Method/Instrument |
|---|---|---|
| pH | Unitless | Hanna HI98129 Combo meter |
| $NO_3$-N | mg/L | HACH test kits (NitraVer 5 Nitrate Reagent Powder Pillows) |
| $PO_4$-P | mg/L | HATCH test kit using the ascorbic acid method and PhosVer® (ascorbic acid) reagent pillows |
| Electrical conductivity (EC) | mg/L | Hanna HI98129 Combo meter |
| Chemical oxygen demand (COD) | mg/L | Reflux Titrimetric Method (Part 5220 method C) |
| Biochemical oxygen demand (BOD) | mg/L | WTW OxiTop® measurement unit in accordance with the manufacturer's instructions. |

**(ii) Enumeration of *Escherichia coli***

3M™ Petrifilm™ Select *E. coli* (SEC) Count Plates were used to count and isolate *E. coli* according to the manufacturer's instructions. After being suitably diluted in 0.1% buffered peptone water, one milliliter of the sample was inoculated onto SEC plates and incubated there for 24 hours. Most *E. coli* (about 97%) produce beta-glucuronidase which produces a blue precipitate associated with the colony indicated by the blue to red-blue colonies. The top film traps gas produced by the lactose fermenting coliforms and *E. coli*. About 95% of *E. coli* produce gas, as indicated by colonies associated with entrapped gas (within approximately one colony diameter). Blue colonies without gas are not counted as *E. coli* (Odwar, 2015; Pepper & Gerba, 2005; Perin *et al.,* 2010). All blue colonies on the SEC plates containing trapped gas, regardless of size or color intensity, were enumerated and classified as *E. coli*. The concentration was reported as less than 1 CFU/mL (detection limit), which is equal to 0 log CFU/mL, if no colony was observed on the SEC plates (Medina & Jordano, 2019; Ofred *et al.,* 2016).

### 3.1.4 Antibiotics resistance testing

**(i) Isolation and biochemical identification**

For isolation and identification of bacteria, culture was performed by enriched sample of water using Peptone Buffer water of which 2 mL of sample was inoculated into 10 ml of buffer peptone water and incubation was done at 37°C for 24 hours. Aseptically culturing was done on blood agar (Oxoid), Mac Conkey agar (Oxoid) and Nutrient agar (Oxoid), and then incubated between 24 and 48 hours at 37°C. Then subculture was done until pure culture obtained. Bacteria were stained using the gram staining technique to ascertain their microscopic features. Classical identification of bacterial colonies and bio typing were performed according to the method described (Abbott *et al.,* 2003; El Deen *et al.,* 2014). Briefly, the isolates were conventionally studied for their macro-and micro-morphological characteristics and then by biochemical assays. The assays included lactose, citrate, indole, motility, and oxidase. Triple sugar iron agar and IMViC were also used for characterization of members of the family *Enterobacteriaceae*.

#### (ii) Phenotypic antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using the disc diffusion method. The isolates were tested against Tetracycline (TET), Gentamycin (GEN), Ceftriaxone (CEF), Ciprofloxacin (CIP), Azithromycin (AZT) and sulfamethoxazole (SUL) which were all supplied by Sigma-Aldrich (St. Louis, MO, USA). The decision to utilize these antibiotics was based on their availability for testing and their frequency of use in hospitals. For antimicrobial susceptibility assays, a pool of bacterial colonies was used to prepare suspensions corresponding to 0.5 McFarland standards ($1.5 \times 10^8$ CFU/mL) using normal saline, and then bacteria were spread on top of Müller-Hinton agar using a sterile swab. Discs were placed on top of the medium, and the plates were incubated at 37°C for 24 h. Zones of inhibition were measured by means of a simple ruler, and the diameter was recorded in millimeters (mm). Isolates were defined as susceptible, intermediate, or resistant in accordance with the CLSI (CLSI, 2020) *Enterobacteriaceae* breakpoints.

### 3.1.5 Genotypic analysis

#### (i) DNA extraction

The genomic DNA was isolated from overnight growth bacterial colony using boiling method. Briefly, the colonies were put in an eppendorf tube containing 100 µl of the nuclease free water and boiled in a water bath at 95°C for 10 min and immediately transferred to the ice for 5 min. This procedure was repeated, and the suspension was centrifuged at 10 000 rpm for 10 min. Five microliters of the supernatant were taken for further process. The concentration and quality of the extracted DNA were checked by electrophoresis (1% agarose gel) and spectrophotometrically quantified using NanoDrop Spectrophotometer. All extracted DNA were stored at -20°C.

### (ii) Molecular identification of bacterial species

All isolates presumptively identified based on biochemical and phenotypic characteristics were subjected to molecular identification. The universal primers designed to give a product of approximately 1500 base pairs and are complementary to conserved regions of 16S rRNA genes were used for PCR amplification. The PCR was performed using a master mix (Bioneers premix-Korea), and the amplification was done as follows; Initial denaturation steps at 95°C for 3 min and followed by 35 cycles of denaturation at 95°C for the 30s, annealing at 58°C for 30s and extension at 72°C for 1min followed by terminal extension at 72°C for 3 min. The agarose gel (1%) stained with ethidium bromide was used to analyze PCR products by electrophoresis. Positive bands were visualized by ultraviolet trans-illumination.

### (iii) Identification of resistance genes

All isolates that expressed phenotypical resistance were screened by PCR for the presence of various recognized resistance genes to different antibiotics. Positive and negative controls were used for resistance genes. However, it was impossible to source positive controls for some screened genes. Without positive controls, optimized and previously published primers and PCR protocols were used. The amplification conditions for the *Sul 1* and *Sul 2* genes were as follows: 94°C for 5 min; 30 cycles of 94°C for 30s, 69°C for 30 s and 72°C for 45s; and one cycle of 72°C for 7 min. To detect $bla_{SHV}$, $bla_{TEM}$ and $bla_{CTX\text{-}M}$. The PCR amplification conditions were as follows: Initial denaturation step at 95°C for 5 min; 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30s. Extension at 72°C for 2 min, followed by a final extension step at 72°C for 10 min. Gel electrophoresis was performed on 1.5% agarose gels. Table 6 provides details of the primers used to detect Cephalosporins ($bla_{SHV}$, $bla_{TEM}$ and $bla_{CTX\text{-}M}$), Sulfonamides and 16SrRNA used in the present study.

### (iv) Sequencing and phylogenetic analysis

The PCR products were purified using the QIAquick PCR Purification Kit. Sequencing was performed by Macrogen Company – Korea under commercial basis. Sequence assembly was performed using CLC Main Workbench version 6.7.1 (http://www.clcbio.com). Sequence similarity was compared with published sequences in the GenBank database using the nucleotide BLAST program (Altschul *et al.,* 1990). Isolates were identified at the species level based on ≥ 99% sequence identity with type strains or reported strain. Multiple alignments were performed using Cluster W programme in the Mega7 software (Kumar *et al.,* 2016). Phylogenetic trees were inferred by the neighbour-joining method (Saitou & Nei, 1987) bootstrapped 1000 replicates based on the pdistance model (Nei & Kumar, 2000). Alignment gaps or missing data were deleted and the tree was rooted with *Rickettsia* spp ATCC VR 141 as the outgroup.

**Table 6: Primers names and sequences used in the present study**

| Primer name | Sequence | Expected band (bp) | Annealing temp. | Reference |
|---|---|---|---|---|
| **Sul1 F**<br>**Sul1 R** | CGGCGTGGGCTACCTGAACG<br>GCCGATCGCGTGAAGGTTCCG | 450bp | 55°C | Wang *et al.* (2014) |
| **Sul2 F**<br>**Sul2 R** | GCGCTCAAGGCAGATGGCATT<br>GCGTTTGATACCGGCACCCGT | 625bp | 58°C | Wang *et al.* (2014) |
| **bla SHV F**<br>**bla SHV R** | ATGCGTTATATTCGCCTGTG<br>AGCGTTGGCCAGTGCTCGATC | 862bp | 58°C | Tofteland *et al.* (2007) |
| **bla CTXM F**<br>**bla CTXM R** | SCSATGTGCAGYACCAGTAA<br>CCCGCRATATGRTTGGTGGTGGTG | 554bp | 58°C | Kallau *et al.* (2018) |
| **bla TEM F**<br>**bla TEM R** | ATGAGTATTCMCATTTCCG<br>CCMTGCTTMTCAGTGAGG | 858bp | 50°C | Tofteland *et al.* (2007) |
| **16s rDNA F**<br>**16s rDNA R** | AGAGTTTGATTCATGGCTCAG<br>TACGGYTACCTTGTTACGACTT | 1500bp | 58°C | Wang *et al.* (2014) |

### 3.1.6 Wetland removal efficiency

The removal efficiency of CW was calculated by the percent difference in values denoted as the removal percentage (r %) for all the wetland settings and was calculated by using following Equation (Eq. (1)):

$$Removal\,\% = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(1)$$

whereby, $C_{in}$ = Concentration of a parameter in influent and

$C_{out}$ = Concentration of parameter in effluent

## 3.2 Experimental constructed wetland

### 3.2.1 Description of the study area

The CW cells were built in a greenhouse (120 m$^2$) at the Nelson Mandela African Institution of Science and Technology (NM-AIST) campus in Arusha, Tanzania. The location of NM-

AIST is at 03° 24' S and 036° 47' E, and it is 1205 m above sea level. The region is defined by distinct rainy and dry seasons, as well as frigid, dry air, during much of the year. The temperature ranges from 13 to 30°C, with an average of roughly 25°C. Figure 10 shows a CW in green house used in experiments.

**Figure 10: A CW system used in experiments (Picture taken by author)**

### 3.2.2 Designing of the constructed wetland

Each of the four (4) rectangular concrete CW cells measured 1.5 m (length), 1.0 m (depth), and 0.5 m (width). For the purpose of preventing leaks, the sides and bottom of these cells were lined with polyethylene plastic sheets (Abou-Elela *et al.,* 2013; Heike *et al.,* 2011; Thathong *et al.,* 2019). Then, pre-washed gravels with a size range of 12 to 20 mm were arranged within. In order to have a better root growth and a better rhizosphere, the effective depth of the gravel column was 0.8 m in all cells (García-Ávila *et al.,* 2019; Tuttolomondo *et al.,* 2020). Based on experimental data and the relationship in Equation (2) (Kaseva, 2004), the average media porosity ($p$) was 0.35.

$$p\ (\%) = (pd - bd) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where bd is the bulk density of gravel determined as the ratio of the dry weight of the gravel sample to its volume, and pd is particle density calculated as the ratio of the dry weight of the gravel sample to the difference between the volume of gravel and the volume of waste required to replace the pores (Raphael *et al.*, 2019). The first cell (A) was left unplanted to serve as control, the second (B) was planted with *Cyperus alternifolius*, the third cell (C) with *Canna indica* and the fourth cell (D) with both *Cyperus alternifolius*, and *Canna indica* species as shown in the layout in Fig. 11.

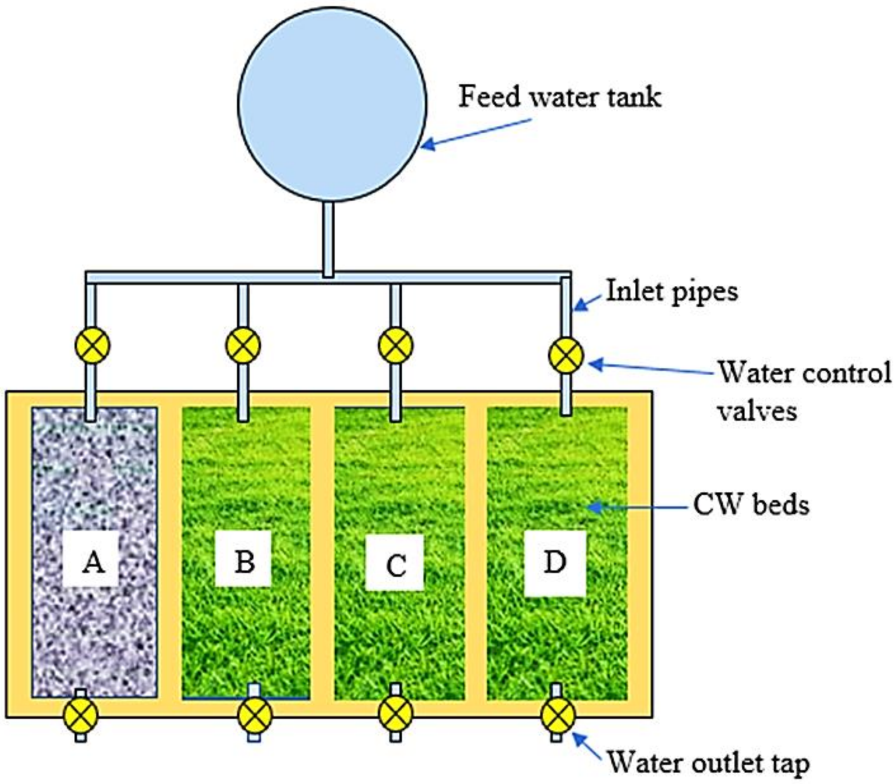



**Figure 11: Layout of the CW system used in experiments (Figure made by author)**

These plants were transplanted from natural wetland areas near the NM-AIST in March 2022 and were planted on the same day. The plants were planted in low density such as 8 plants per 0.5 $m^2$ (Rahmadyanti *et al.*, 2021; Sandoval *et al.*, 2019). To allow plants to grow, all of the CW cells were irrigated with wastewater effluent from the CW found at the NM-AIST campus's used for secondary treatment of wastewater from the student's hostels. The hydraulic retention time was five days, following which the wastewater was replaced with another batch of fresh wastewater. The wastewater used had the following characteristics: pH 7.21 ± 0.21, COD 68.32 ± 26.81 mg/L, BOD 36.45 ± 19.33 mg/L, nitrates 9.71 ± 6.80 mg/L, and phosphorus 3.66 ± 3.14 mg/L. Figure 12 (a-f) show the views of the CW at various steps of making.


**Figure 12 a-f:** **Constructed wetland cells at different stages of development (Pictures taken by author)**

### 3.2.3 Synthetic wastewater

Synthetic wastewater that had been drug-spiked was used as the experimental influent. The features of domestic wastewater were best simulated by synthetic wastewater. Even though synthetic wastewater cannot completely replicate real domestic wastewater, it does have several advantages, such as better experimental condition control, production of desirable redox conditions (Loosdrecht *et al.,* 2016), elimination of batch-to-batch variability, health risks, and storage issues (Li *et al.,* 2014; Prieto *et al.,* 2019). The synthetic wastewater should have an organic source, a source of nitrogen, phosphate, and other elements (Lima *et al.,* 2018). The synthetic wastewater used in this study was composed with 400 mg/L glucose (source of carbon) (Haritash *et al.,* 2015; Tang *et al.,* 2019), 50 mg/L acetic acid (source of organic acids), 10 mg/L $K_2HPO_4$ (source of phosphorus) and 60 mg/L Urea (source of nitrogen) (Stefanakis & Tsihrintzis, 2009). For the trace elements, 100 mg/L $MgCl_2{\cdot}6H_2O$, 0.5 mg/L $MnCl_2{\cdot}4H_2O$, 0.5 mg/L $FeCl_2{\cdot}6H_2O$ and 7.5 mg/L $CaCl_2{\cdot}2H_2O$ were used (Biplob *et al.*, 2011). All these were dissolved in tap water. 0.5 mol/L $NaHCO_3$ was used to control pH variations (Nasseri *et al.*, 2014).

**(i) For antibiotics removal and physico-chemical parameters reduction**

The formulated synthetic wastewater was spiked with metronidazole (MET), ciprofloxacin (CIP) and sulfamethoxazole (SUL). The solution formed was used in experiments.

**(ii) For microbiological analysis**

The CWs were fed with synthetic wastewater with characteristics that mimic domestic wastewater. A specified amount of Ciprofloxacin (CIP) was added to synthetic wastewater to make 100 µg/L.

### 3.2.4 Selection of antibiotics

These are some of the pharmaceuticals that Tanzanian healthcare facilities dispense the most (Mbwasi *et al.,* 2020; Sangeda *et al.,* 2021b; Seni *et al.,* 2020). Table 7 summarizes the targeted antibiotics along with their physical-chemical characteristics.

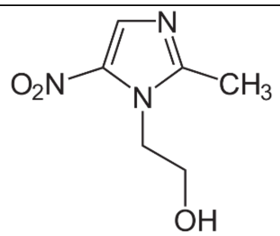
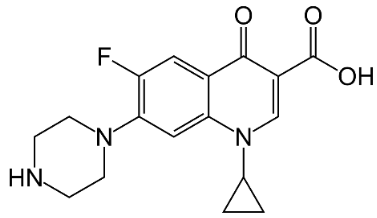
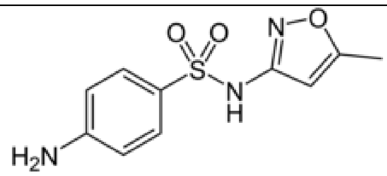
**Table 7: Physical–chemical properties of the studied antibiotics**

| Characteristics | Metronidazole | Ciprofloxacin | Sulfamethoxazole |
|---|---|---|---|
| Molecular weight (g/mol) | 171.15 | 331.34 | 253.28 |
| Water solubility (mg/L) | 11000 | 36000 | 610 |
| $\log K_{OW}$ | -0.02 | 0.28 | 0.89 |
| $pK_a$ (at 25°C) | 2.57; 15.42 | 6.1, 8.7 | 1.6; 5.7 |
| Brand used | Megyl tablets 200 mg | Aarciflox tablets 500 mg | Co-Trimoxazole tablets 400 mg |

**PubChem**

The chemical structures of these antibiotics are given in Table 8.

**Table 8: Chemical structures of the studied antibiotics**

| Antibiotic | Chemical structure |
|---|---|
| Metronidazole |   |
| Ciprofloxacin |   |
| Sulfamethoxazole |   |

### 3.2.5 Constructed wetland operation

All four CW cells were run in batch mode, which involves holding the wastewater in the media bed for a predetermined retention period before draining it out (Kiiza *et al.*, 2020; Nguyen *et al.*, 2021).

### 3.2.6 Measurement of plants developments

Plant growth was monitored for four months (weekly recordings) while being irrigated with tap water and pre-treated wastewater. Three randomly selected individual plants were used to measure the height of the plants manually using a tape measure. The height measurement was taken from the surface of the graves to the highest point of the plant. After then, the data were processed to obtain averages.

### 3.2.7 Wastewater sampling

For examining the impact of plant species at different hydraulic retention time on the removal of MET, CIP and SUL wastewater sampling was done at the outlets every morning for seven (7) consecutive days. The samples were collected at depths ranging from 0 to 20 cm using sterile syringes (Desta *et al.*, 2014; Imfeld *et al.*, 2010) put in sterile 100 mL bottle container

(Mara & Horan, 2003).The samples stored at 4°C in a refrigerator after being acidified with 2% $HNO_3$ until analysis. Sampling was done in duplicate to ensure the validity of the results (Rana & Maiti, 2018; Yadav *et al.*, 2010). To evaluate the effect of CIP on bacterial abundance, synthetic wastewater grab samples were collected before and after the addition of CIP. For antimicrobial susceptibility assay wastewater grab samples were collected after 7 days HRT with CIP. The samples were transported to the Department of Microbiology Parasitology and Biotechnology laboratories at Sokoine University of Agriculture where microbiological analysis started immediately after receiving the samples.

### 3.2.8 Wastewater analysis

#### (iii) Physicochemical parameters

Unless otherwise stated, all of the analyses were conducted using Standard Methods for the Examination of Water and Wastewater (APHA, 2017). The methods used are presented in Table 5.

#### (iv) Quantification of antibiotics

Previously optimized methods were used for analysis of Metronidazole (Naveed & Qamar, 2014) Ciprofloxacin (Naveed & Waheed, 2014) and Sulfamethoxazole (Balyejjusa *et al.*, 2002) in the inlet and outlet samples. This was done using Cary 60 UV-Vis spectrophotometer (Agilent technologies) and data collected using Cary win-UV software. The calibration graphs were produced using the standard solutions made by dissolving accurate weight of the standards antibiotics powder. Calibration characteristics are summarized in Table 9.

**Table 9: Calibration characteristics of the standards**

| Antibiotics | Conc (mg/L) | Abs (nm) | Linear equation | $R^2$ |
|---|---|---|---|---|
| MET | 10, 50, 100, 250, 500 | 340 | Abs = 0.00213×Conc + 0.05581 | 0.99916 |
| CIP | 10, 50, 100, 250, 500 | 278 | Abs = 0.00560×Conc + 0.03881 | 0.99912 |
| SUL | 10, 50, 100, 250, 500 | 238 | Abs = 0.00186×Conc + 0.21140 | 0.99921 |

#### (v) Bacteria enumaration

One milillitre of the water sample was mixed with 9 mL of sterile normal saline to determine the amount of microbiological content. The sample was then serially diluted 10-fold in sterile normal saline using 10 universal bottles. One milillitre of each dilution was poured in duplicate on the plate count medium petri plates. The plates were incubated for 24 hours at 37°C. The colonies were then enumerated, and the average colony counts were used to calculate colony forming units (CFU/mL).

#### (vi) Bacteria isolation and identification

For isolation and identification of bacteria, culture was performed by enriched sample of water using Peptone Buffer water of which 2 ml of sample was inoculated into 10 ml of buffer peptone water and incubation was done at 37°C for 24 hours. Aseptically culturing was done on blood agar (Oxoid), Mac Conkey agar (Oxoid) and Nutrient agar (Oxoid), and then incubated between 24 and 48 hours at 37°C. Then subculture was done until pure culture obtained. Bacteria were stained using the gram staining technique to ascertain their microscopic features. Classical identification of bacterial colonies and bio typing were performed according to the method described by Abbott *et al.* (2003) and El Deen *et al.* (2014) with slight modifications. Briefly, the isolates were conventionally studied for their macro-and micro-morphological characteristics and then by biochemical assays. The assays included lactose, citrate, indole, motility, and oxidase. Triple sugar iron agar and IMViC were also used for characterization of members of the family *Enterobacteriaceae*.

### 3.2.9 Genotypic analysis

#### (i) DNA extraction

The genomic DNA was isolated from overnight growth bacterial colony using boiling method. Briefly, the colonies were put in a eppendorf tube containing 100 μl of the nuclease free water and boiled in a water bath at 95°C for 10 min and immediately transferred to the ice for 5 min. This procedure was repeated, and the suspension was centrifuged at 10 000 rpm for 10 min. Five microliters of the supernatant were taken for further process. The concentration and quality of the extracted DNA were checked by electrophoresis (1% agarose gel) and spectrophotometrically quantified using NanoDrop Spectrophotometer. All extracted DNA were stored at −20°C.

#### (ii) Molecular identification of bacterial species

All isolates presumptively identified based on biochemical and phenotypic characteristics were subjected to molecular identification. The universal primers designed to give a product of approximately 1500 base pairs and are complementary to conserved regions of 16S rRNA genes were used for PCR amplification. The PCR was performed using a master mix (Bioneers premix-Korea), and the amplification was done as follows; initial denaturation steps at 95°C for 3 min and followed by 35 cycles of denaturation at 95°C for the 30s, annealing at 58°C for 30s and extension at 72°C for 1min followed by terminal extension at 72°C for 3 min. The agarose gel (1%) stained with ethidium bromide was used to analyze PCR products by electrophoresis. Positive bands were visualized by ultraviolet trans-illumination.

### 3.3 Data analysis methods

Statistical data analysis was performed using Microsoft Excel, which included computing the mean and standard deviations as well as drawing the graphs. For quality control, the experiments were done in triplicate, and two samples from each replication were collected for analysis. Values were expressed as mean ± standard deviation. The p-value was used for establishing the statistical significance of the findings. If the p-value was less than 0.05, the result was considered significant; if it was greater than 0.05, the result was considered not significant.

### 3.4 Research clearance and permission

Research clearance was provided by the Tanzania Commission for Science and Technology (COSTECH), and the permission to collect wastewater samples was provided by Dodoma Urban Water Supply and Sanitation Authority (DUWASA).

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Treatment of hospital wastewater

### 4.1.1 Physicochemical parameters

Hospital wastewater is typically characterized by a high concentration of ammonia nitrogen, organic nitrogen, nitrites, nitrates, total phosphorus, total solids, chemical oxygen demand (COD), biochemical oxygen demand (BOD), and total organic carbon (TOC) (Bhandari *et al.,* 2023; Parida *et al.,* 2022). This information served as the basis for the analysis of the physicochemical characteristics of hospital wastewater. The observed values of the physicochemical parameters are used as indicators of effluent quality in compliance with standards. The average concentrations of the physicochemical properties for the influent and effluent samples are shown in Table 1.

**Table 10: Physicochemical characteristics of wastewater in this study**

| | | **Standards** | **Influent** | | **Effluent** | |
|---|---|---|---|---|---|---|
| **Parameter** | **Unit** | | **Range** | **Mean ± SD** | **Range** | **Mean ± SD** |
| pH | Numeric | 6.5 – 8.5 | 6.5 - 7.5 | 6.93 ± 0.59 | 6.9 - 8.1 | 7.48 ± 0.63 |
| EC | µS/cm | | 1566.8 - 3236.6 | 2360 ± 918 | 1918.4 - 3001.9 | 2441 ± 623 |
| Temp | $^{0}C$ | 20 - 35 | 23.9 - 26.6 | 25.2 ± 1.58 | 23.8 - 26.0 | 25.4 ± 1.64 |
| TDS | mg/L | 3000 | 776.6 - 1477.9 | 1218 ± 479 | 1057.8 - 1634.5 | 1305.5 ± 396 |
| TSS | mg/L | 100 | 224.5 - 324.7 | 270.38 ± 66.1 | 6.9 - 102.2 | 49.17 ± 53.11 |
| Turbidity | NTU | 300 | 98.5 - 200.9 | 150 ± 57.2 | 0.8 - 32.6 | 9.1 ± 14.83 |
| DO | mg/L | | 0.5 - 1.0 | 0.75 ± 0.34 | 6.0 - 7.4 | 6.8 ± 0.94 |
| COD | mg/L | 60 | 196.9 - 446.3 | 329.2 ± 135.6 | 132.7 - 208.6 | 170.4 ± 40.6 |
| $BOD_5$ | mg/L | 30 | 74.3 - 183 | 140.9 ± 66.8 | 47.4 - 96.7 | 74.8 ± 33.5 |
| $NO_3$-N | mg/L | 20 | 75.9 - 139.6 | 108.5 ± 36.8 | 10.9 - 84.7 | 45.4 ± 39.97 |
| $PO_4$-P | mg/L | 6 (TP) | 0.9 - 2.1 | 1.55 ± 0.66 | 2.3 - 6.7 | 1.52 2.30 |

The comparison of the TSS, COD, BOD, turbidity, nitrate, and phosphate concentrations in relation to the standard values by the Tanzania Bureau of Standards (TBS) is shown in Fig. 13.

Despite decreases in many parameters, only turbidity, TSS and TDS falls within the permitted levels of wastewater discharge.

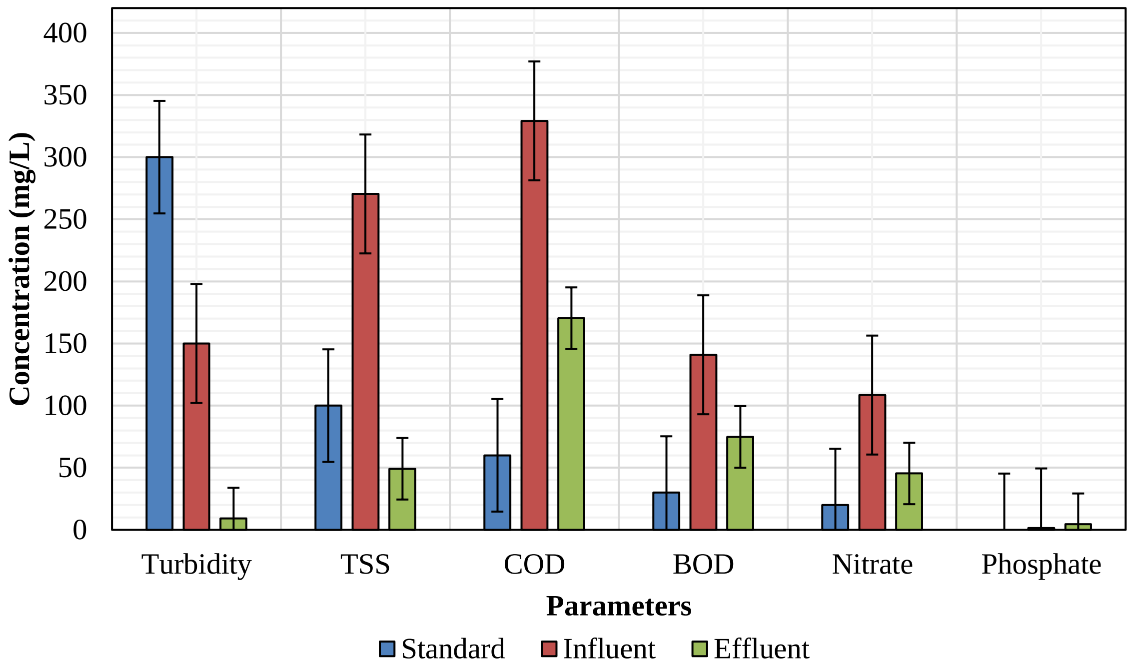



**Figure 13: Comparison between standard, influent and effluent parameters**

The physicochemical characterization of hospital wastewater includes the evaluation of different parameters (Abd El-Gawad & Aly, 2011). The results of the physicochemical characteristics in our study revealed that some parameters, such as BOD, COD, TSS, Nitrates values, were higher than the Tanzanian standards established for wastewater discharge into the environment. In the present study, the average values of pH were 6.93 ± 0.59 and 7.48 ± 0.63 for influent and effluents respectively. According to the results, the pH of the effluent has increased as compared to the pH of the influent. However, this increase was not significant ($p > 0.05$). This can be explained by the production of ammonia gas during the anaerobic breakdown of organic nitrogen (Autlwetse & Kimwaga, 2022). Additionally, plants and microalga that engage in intensive photosynthesis reduces dissolved $CO_2$ concentrations in turn, raises the pH level of water (Craggs *et al.*, 2014; Hanumantha Rao *et al.*, 2011; Kiflay *et al.*, 2021). However, the pH of the influent and effluent both fall within the permissible range of 6.5 – 8.5, which is ideal for aerobic bacteria (Permatasari *et al.*, 2018).

The EC is a unit used to describe how well a liquid conducts an electric charge. The EC is determined by the measurement temperature, ionic strength, and dissolved ion concentrations (Rusydi, 2018). The EC of water is a simple and accurate indication of salinity or total salt concentration (Corwin & Yemoto, 2020; Dahaan *et al.*, 2016; Petsetidi & Kargas, 2023).

Results in Table 10 show that, the average EC of the effluent (2441 ± 623 µS/cm) is higher than that of the influent (2360 ± 918 µS/cm) by 3%. Similar results were reported at the University of Dar es Salaam in Tanzania, where wastewater effluent from the university waste stabilization ponds was treated in a horizontal flow CW (Mashauri *et al.,* 2000). This might be because plant decomposition releases absorbed components back into the water, increasing the conductivity by raising the concentration of dissolved ions. It might also result from organic contaminants being degraded into less complex organic components (Kiflay *et al.*, 2021).

Several chemical and physical properties of water, such as gases solubility, chemicals reactivity and toxicity, and microbiological activity are all strongly influenced by temperature (Wilson & Worrall, 2021). Higher temperatures make dissolved oxygen less soluble, lowering its concentration and, consequently, its availability to aquatic species (Dallas, 2008; Miller & Young, 2022). High microbial activity at the higher temperature speeds up oxygen depletion if the organic loading is high. The habitat temperature has also an impact on the growth of aquatic organisms, reproduction, and distribution (Bhatia *et al.*, 2018; Miller, 2021). The temperature of CW water is directly related to the temperature of the air and has an effect on total treatment efficiency (Udom *et al.*, 2018). According Table 10, the temperature of influent during sampling ranged from 23.9 - 26.6°C, while effluent temperatures ranged from 23.8 - 26.0°C. There is no significant variation ($p > 0.05$) in temperature between the influent and effluent. Both influent and effluent temperatures fall in the acceptable range (25 – 35°C) which is suitable for microbial activities (Mairi *et al.*, 2001).

The TDS is a measure of the total inorganic and organic content of a liquid in ionized, molecular or microgranular (colloidal sol) suspended form. The TDS is made up of inorganic salts (mostly calcium, magnesium, potassium, sodium, bicarbonates, chlorides, and sulfates) and some organic matter that is dissolved in water (Gupta *et al.,* 2016). A high TDS affects water density, gas solubility including oxygen, osmoregulation of freshwater in organisms, and the use of water for many applications including irrigation, drinking, and industrial (Lokhande *et al.*, 2012). The TDS results in the current study were 1218 ± 479 mg/L and 1305.5 ± 396 mg/L for influent and effluent respectively. The results show increase in TDS from influent to effluent by 7%. They both fall in the acceptable levels for effluent discharge. The TDS and EC exhibit a correlation such that the TDS readings in each measurement were roughly half the EC values. The explanation for TDS observations is the same as the one provided for EC observations.

The TSS are regarded as one of the main contaminants that lead to the decline in water quality

(Verma *et al.,* 2013). Increase in water suspended solid levels hinder the efficient exchange of oxygen between water and air. They have the potential to suppress aquatic animals from breathing and lead into their suffocation. They cause an increase in turbidity, which causes oxygen level to decrease. Additionally, it may prevent the necessary light from entering the aquatic system, which would reduce the capacity of various algae and flora to produce food and oxygen. Because suspended solids can directly absorb sunlight, the water becomes warmer and has less dissolved oxygen (Wei *et al.,* 2020). The TSS removal in CWs is accomplished through a variety of mechanisms. This includes the deposition process that results from the interception of suspended solids due to the slower water flow through wetland substrate. Additionally, it comprises filtration and aggregation or flocculation (Rahmadyanti & Febriyanti, 2020). The TSS results in the current study were 270.38 ± 66.1 mg/L and 49.17 ± 53.11 mg/L for influent and effluent respectively. This shows that the system is capable of removing up to 82 % of TSS.

Turbidity is a measurement of water clarity, how deep down the water column light can penetrate (Balaji *et al.,* 2018; Scholz, 2016). Turbidity is the result of presence of suspended particles, which vary in size from large flocs to incredibly tiny colloidal particles. Infrared and visible electromagnetic radiation is scattered and absorbed by these particles (Fereja *et al.,* 2020). High water turbidity lowers the amount of light available to photosynthetic organisms (Obinnaa & Ebere, 2019). The findings of the present study demonstrated the outstanding effectiveness of CW in removing turbidity from hospital wastewater. Around 94% of the wastewater turbidity was removed by the system, from influent with 150 ± 57.2 NTU to effluent with 9.1 ± 14.83 NTU.

The DO is the amount of oxygen available in aquatic environment to aquatic organisms (Patel *et al.,* 2017). The DO is a state variable that regulates chemical oxidation, respiration, photosynthesis, and the exchange of oxygen between water masses (Carstensen *et al.,* 2012). When there is little or no DO, oxidation occurs through the reduction of inorganic salts or the action of methane-forming bacteria that produce unpleasant end products (Muttamara, 1996). The DO is generally a limiting factor in the removal of organic and inorganic contaminants such as nitrogen in CWs (Valipour & Ahn, 2017). The results from this study show that the hospital wastewater had DO of 0.75 ± 0.34 mg/L and 6.8 ± 0.94 mg/L for influent and effluent respectively. A combination of plant rhizospheric oxygen release and air-water interphase oxygen transfer from the atmosphere contribute to the increase in DO (Zhai *et al.,* 2012).

The COD is the quantity of oxygen equivalents spent during the oxidation of organic compounds by strong oxidizing agents like dichromate and permanganate (Silva *et al.,* 2009). It is a sign of the presence of reducing agents in the water, such as organics, nitrite, sulfide, ferrous salts, etc., with organics predominating. The aquatic life suffers when the oxygen in the water system is reduced significantly due to a high COD content. A high COD value indicates that there is little microbial activity, which slows down the rate at which organic matter degrades (Patel & Parsania, 2017; Udom *et al.*, 2018). The results from the current study shows removal of hospital wastewater COD by only 48 %. This is from 329.2 ± 135.6 mg/L to 170.4 ± 40.6 mg/L after the treatment process. In a similar study conducted to evaluate the efficiency of CW for hospital wastewater treatment the systems managed to remove COD by 64.9% in India (Parashar *et al.,* 2022) and by 80% in Thailand (Vo *et al.,* 2019).

The $BOD_5$ value is the amount of dissolved oxygen that aerobic biological organisms in a waterbody require to decompose organic matter present in a given water sample at a given temperature over a particular time period (Gupta *et al.,* 2016) usually 5 days (Jouanneau *et al.,* 2014; Kitalika *et al.,* 2016). It is a method of indirectly quantifying existing organic or chemical pollutants that biodegrade in the presence of oxygen in water (Maddah, 2022). The degree of oxygen depletion in the water bodies increases with increasing $BOD_5$ content. High $BOD_5$ levels have comparable implications to low dissolved oxygen levels; aquatic organisms become stressed, suffocate, and die (Aniyikaiye *et al.,* 2019). This decrease in $BOD_5$ can be driven by a range of mechanisms, including microbial degradation and physical processes such as settling, filtration, and predation of particulate organic matter (Kimwaga *et al.,* 2004). The results in this study show that the CW system achieved $BOD_5$ reduction by only 47%. This is low performance when compared to a similar study done in India where 96% of BOD was removed from hospital wastewater in HSSFCW coupled with tubesettler (Khan *et al.,* 2020). This can be associated with clogging of the substrate X

Water and wastewater contain four different types of nitrogen: Nitrite, nitrate, organic nitrogen, and ammonia nitrogen. Most nitrogen in sewage-contaminated water is present as organic and ammonia compounds, which can be converted by microorganisms into nitrites and nitrates (APHA, 2017; Samer, 2015). Nitrate, a basic nutrient for plant growth, has the potential to be a growth-limiting nutrient factor. Too much nitrate in surface water can encourage eutrophication, which degrades the water's quality (Berkessa *et al.,* 2019). The health of infants is immediately and seriously threatened by consuming water with an excessive nitrate concentration (greater than 10 mg/L) (Hassan-Omer, 2020; Kitalika *et al.,* 2016). So, discharge of nitrate into the environment should be controlled. In the current study the influent and effluent had 108.5 ± 36.8 mg/L and 45.4 ± 39.97 mg/L of nitrate respectively. This is around 58 % removal of nitrate. Despite this removal, the effluent has nitrate content above the discharge allowed level. The performance of the CW for removal of nutrients such as nitrogen can be increased by plant harvesting (Wang *et al.,* 2021). This will reduce the release of nutrients from plants decomposition and the possibility of substrate clogging (Álvarez & Bécares, 2008; Tanaka *et al.,* 2015, 2016).

High nutrients (nitrogen and phosphorus) content in wastewater has potentially adverse effects on the ecosystem. Similar to nitrogen, releasing wastewater that contains a lot of phosphorus promotes eutrophication in receiving water bodies (Autlwetse & Kimwaga, 2022; Balachandran *et al.,* 2018; Kiflay *et al.,* 2021). Water source eutrophication may also produce environmental conditions that encourage the growth of cyanobacteria that produce toxins, and human exposure to such toxins is dangerous (Edokpayi *et al.,* 2017; Nayan *et al.,* 2020). In addition, releasing organic-rich wastewater into the environment causes a rapid decline in the amount of dissolved oxygen in the water bodies it enters, which has the unintended consequences of causing aquatic life to die and disturbing the balance of the ecosystem (Egbuikwem *et al.,* 2021; Liang *et al.,* 2017). Phosphorus removal in CWs is accomplished through precipitation, bacterial removal, adsorption and plant uptake (Albalawneh *et al.,* 2016; Shukla *et al.,* 2021). Results in the current study showed that the average phosphorus in the influent and effluent were 1.55 ± 0.66 mg/L and 4.52 ± 2.30 mg/L respectively. This shows the general increase in phosphate in the wastewater after passing through the CW. This may be caused by the release of nutrients from decomposing plants and desorption from the substrates (Ávila *et al.,* 2017; Zhang *et al.,* 2007). As explained for nitrates, the solution to this includes plant harvesting (Álvarez & Bécares, 2008; Vymazal, 2011b).

### 4.1.2 Biodegradability index

The $BOD_5$/COD ratio, also known as the Biodegradability Index (BI), was calculated to assess the biodegradability of pollutants in hospital wastewater (Lai *et al.*, 2011). According to the results of this study (Fig. 14), BI for influent ranges from 0.2 to 0.9 with an average of 0.5, while BI for effluent ranges from 0.3 to 0.7 with an average of 0.4. The findings indicate that there was a weekly fluctuation in the relative effluent biodegradability.

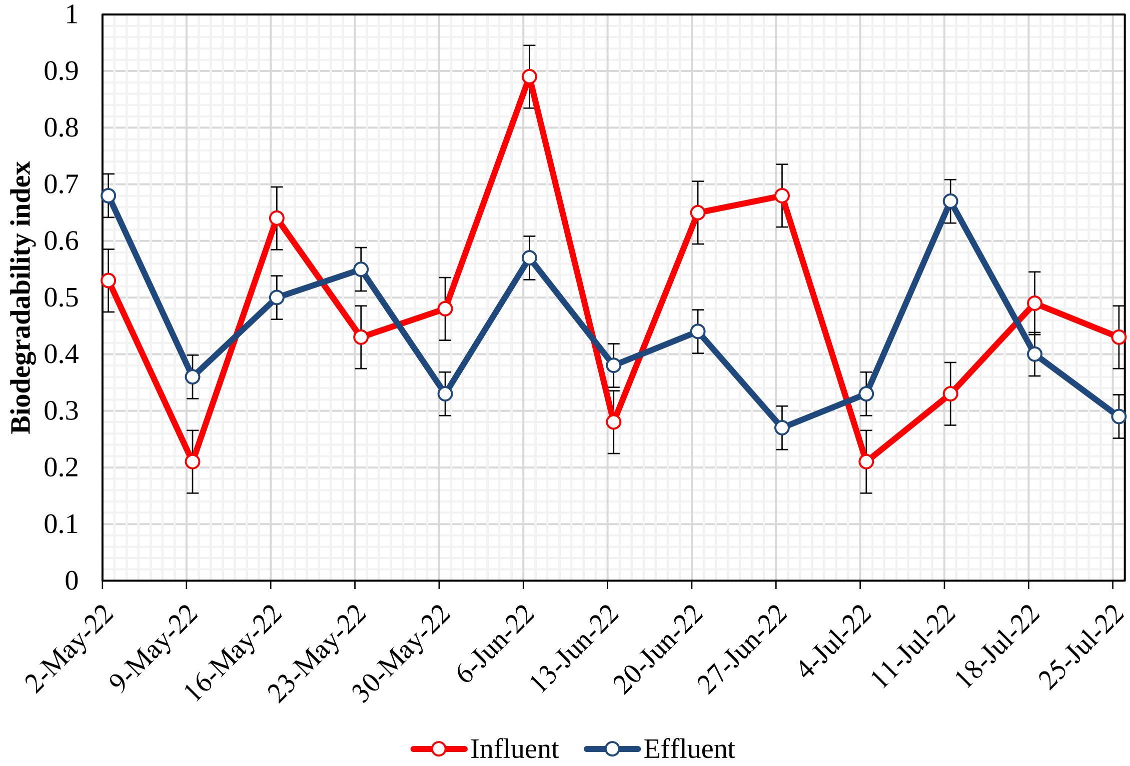



**Figure 14: Variation in biodegradability index ($BOD_5$/COD ratio) of influent and effluent**

As described before, both influent and effluent high COD and BOD values beyond the established standards for effluent discharge. The $BOD_5$/COD ratio ranged from 0.2 to 0.9 for the influent which is a normal case and this waste is easily degradable by the biological processes. As for the effluent (treated sewage) the $BOD_5$/COD ratio varied from 0.3 to 0.7. Theoretically, for domestic wastewaters, the BI ranges from 0.4 to 0.8 (Al-Sulaiman & Khudair, 2018). If the $BOD_5$/COD ratio is between 0.3 and 0.6, seeding is required to treat it biologically, since the acclimation of the microorganisms that aid in the degradation process takes time due to the slow biodegradation process. The $BOD_5$/COD ratio of less than 0.3 indicates the presence of organic compounds in the wastewater that are difficult to biodegrade, possibly toxic, and non-biodegradable. This wastewater cannot be treated biologically (Mesdaghinia *et al.*, 2015; Rim-Rukeh & Agbozu, 2013). Results from this study shows that the average effluent $BOD_5$/COD ratio is slightly low when compared to the ratios in the influent, giving clear evidence that the organic matter in the wastewater has undergone biological degradation (Zhao *et al.*, 2018). Each stage of conventional wastewater treatment results in a decrease in the $BOD_5$/COD ratio. This happens mainly because the existing bacteria first breakdown the biodegradable component of organic matter, as measured by the $BOD_5$, while the more inert fraction of organic matter often remains constant throughout treatment (Abbas *et al.*, 2022). Despite the small difference in average values of $BOD_5$/COD ratio observed in this study, in both influent and effluent, the minimum value is below 0.4. This suggests that the wastewater contains non-biodegradable organic matter which may include xenobiotic compounds such as surfactants (Prokkola *et al.*, 2022). This means that before influent can be treated via biological treatment, a pre-treatment procedure may be necessary to increase its biodegradability index (Oller *et al.*, 2011).

### 4.1.3 Enumeration of *Escherichia coli*

Figure 15 presents the results for the enumeration of *E. coli* in influent and effluent samples over a period of 13 weeks. The *E. coli* concentration in the influent samples ranged from $2.5\times10^4$ CFU/mL to $1.1\times10^6$ CFU/mL, while concentration in the effluent samples ranged from $1.1\times10^1$ CFU/mL to $1.1\times10^2$ CFU/mL. For influent and effluent, the average values were 5.21 logCFU/mL and 1.77 logCFU/mL, respectively. These data demonstrate a significant decrease in *E. coli* ($p < 0.05$) following treatment in the CW. The *E. coli* concentrations in wastewater were reduced by roughly 3.44 log CFU/mL. The amount of *E. coli* in the effluent was almost within the acceptable level for disposal of effluent.

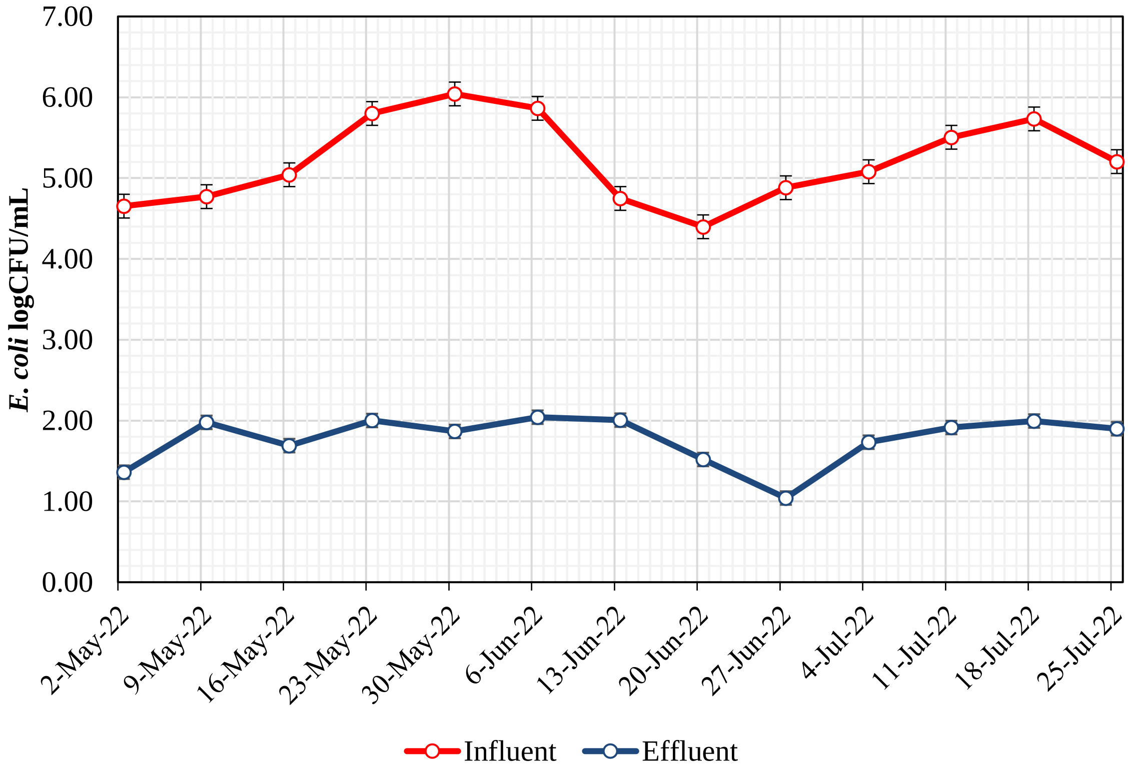



**Figure 15:** ***E. coli* enumeration**

The reduction in *E. coli* concentration in this study was 3.44 log. As a result, the effluent was within the permitted values for discharge. Some studies have indicated a 4.5 log reduction in pathogens from domestic wastewater in CW (Vega De Lille *et al.,* 2021). *Typha latifolia*-planted CW was reported to reduce *E. coli* from domestic wastewater by 3.9 log (Martinez-Guerra *et al.,* 2018). In Egypt, the effectiveness of CW integrated with septic tanks on removing fecal coliform was examined. The findings demonstrate that the fecal coliform count was decreased by almost 5 log (Abdel-Shafy & El-Khateeb, 2013). A membrane bioreactor (MBR) and disinfection with either chlorine or ozone were used in another investigation to reduce *E coli* by more than 6 log from hospital effluent (Chiemchaisri *et al.*, 2022). In a different study, carried out in Tanzania, the efficacy of fecal coliform bacteria removal from wastewater in waste stabilization ponds in Morogoro, Mwanza, and Iringa regions was assessed. The largest reduction in fecal coliforms seen in this investigation was 3.8 log (Zacharia *et al.,* 2019).

## 4.2 Antibiotics resistance patterns in hospital wastewater effluents

### 4.2.1 Isolation and biochemical identification

A total of 32 wastewater effluent samples were analyzed. 87.5% of these samples (28 samples) tested positive for one or more isolates. The 61 bacterial isolates were recovered from the samples. These isolates were identified as *Klebsiella pneumoniae* (n = 24, 39.3%), *Pseudomonas aeruginosa* (n = 11, 18.0%), *Escherichia coli* (n = 17, 27.9%). Other isolated bacteria were *Staphylococcus aureus* (n = 5, 8.2%) and *Proteus mirabilis (*n = 4, 6.6%). These data shows that *Klebsiella spp.* has the highest prevalence among the bacterial isolates, followed by *E. coli*, *Pseudomonas ssp.*, *Staphylococcus ssp.* and *Proteus ssp.*

It is worth noting that different studies have documented diverse microbial compositions in hospital wastewater. It has been reported in a similar study that the overall isolates found in hospital wastewater effluent treated in HSSFCW were 39.5% *E. coli*, 35.1% *Staphylococcus*, and 30.7% *Klebsiella* species (Dires *et al.,* 2018). In Ghana, a study was conducted to assess the presence of multidrug-resistant bacteria in hospital wastewater. The results showed that *E. coli* made up 30.6%, *Klebsiella pneumoniae* 11.2%, *Citrobacter freundii* 10.9%, *Alcaligenes faecalis* 5.8% and *Pseudomonas mendocina* 5.4% (Addae-Nuku *et al.,* 2022). Common pathogenic microorganisms found in hospital wastewater include bacteria, fungus, yeasts, viruses, algae, protozoa, parasites, and bacteriophages (Rajwinder-Kaur *et al.,* 2020). Bacteria make approximately 95% of all microorganisms in complex ecosystems and are vital for wastewater treatment (Yan *et al.,* 2021). The most common pathogen in hospital wastewater is *Staphylococcus aureus*. Other common pathogens include *Klebsiella Proteus*, *Candida albicans*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterobacter* species (Asfaw *et al.,* 2017; Oyeleke & Istifanus, 2009).

### 4.2.2 Phenotypic antibiotic susceptibility testing

Antibiotic susceptibility test was performed for *Klebsiella spp., E. coli* and *Pseudomonas ssp.* and results are presented in Table 11.

**Table 11: Antimicrobial susceptibility profile of the bacteria isolates from wastewater effluents**

| Bacteria strain | Percentage (%) Resistance to the Selected antibiotics | | | | | | |
|---|---|---|---|---|---|---|---|
| | n | TET | GEN | CEF | CIP | AZT | SUL |
| *Escherichia coli* | 17 | 35.3 | 17.6 | 23.5 | 17.6 | 23.5 | 29.4 |
| *Klebsiella pneumoniae* | 24 | 54.2 | 50 | 33.3 | 29.2 | 41.7 | 37.5 |
| *Pseudomonas aeruginosa* | 11 | 27.3 | 18.2 | 36.4 | 27.3 | 45.5 | 27.3 |

TET=Tetracycline, GEN=Gentamycin, CEF=Ceftriaxone, CIP=Ciprofloxacin, AZT=Azithromycin and SUL=Sulfamethoxazole

The results showed that *Klebsiella pneumoniae* (n = 24) had 13 strains resistant to Tetracycline, 12 were resistant to Gentamycin, 8 were resistant to Ceftriaxone, 7 were resistant to Ciprofloxacin, 10 were resistant to Azithromycin and 9 were resistant to Sulfamethoxazole. *Pseudomonas aeruginosa* (n = 11) had 3 strains resistant to Tetracycline, 2 were resistant to Gentamycin, 4 were resistant to Ceftriaxone, 3 were resistant to Ciprofloxacin, 5 were resistant to Azithromycin and 3 were resistant to Sulfamethoxazole. The *E. coli* (n = 17) had 6 strains resistant to Tetracycline, 3 were resistant to Gentamycin, 4 were resistant to Ceftriaxone, 3 were resistant to Ciprofloxacin, 4 were resistant to Azithromycin and 5 were resistant to Sulfamethoxazole.

Gram negative bacteria including *Klebsiella pneumoniae*, *E. coli*, and *Pseudomonas aeruginosa* were tested for antibiotic resistance. The results showed that different levels of antibiotic resistance exist among the studied bacteria and antibiotics. Bacteria as a group or species are not necessarily equally susceptible to or resistant to a certain antimicrobial agent. Even among closely related bacterial groups, resistance levels might differ significantly (Reygaert, 2018). Bacteria have several defensive mechanisms to defend against the effects of antimicrobials and to endure environmental stress. The most prevalent antibiotic resistance mechanisms include inhibiting drug absorption, modifying drug targets, making drugs inactive, and active drug efflux (Grehs *et al.,* 2021; Lépesová *et al.,* 2019; Mutuku *et al.,* 2022). The sorts of mechanisms utilized by gram negative bacteria on antibiotics resistance differ from those used by gram positive bacteria due to variations in the structure of the cell wall. Gram positive bacteria lack the ability for some forms of drug efflux mechanisms and less frequently employ limiting the absorption of a drug, whereas gram negative bacteria use all four of the basic mechanisms (Reygaert, 2018).

The major cause of Gram-negative bacteria resistance to a variety of antibiotics is their outer membrane. To reach their targets, most antibiotics must cross the outer membrane. Resistance can be produced by Gram-negative bacteria altering the outer membrane in any way, including by mutating porins, modifying the hydrophobic characteristics, or altering other variables. Because Gram-positive bacteria lack this crucial layer, Gram-negative bacteria are more resistant to antibiotics than Gram-positive bacteria (Breijyeh *et al.*, 2020; Gupta & Datta, 2019). Results from the current study showed that *Klebsiella* ssp were more resistant than *Pseudomonas* ssp for TET, GEN, CIP and SUL. *Pseudomonas* ssp. were more resistant that *Klebsiella* ssp. for CEF and AZT. Studies shows that *Staphylococcus aureus* is the most common pathogenic gram-positive bacterium with high level of multidrug resistance (Rajwinder *et al.,* 2020). The results from the current study suggest that disposing hospital wastewater effluent with high intensity of these resistant bacteria poses a health risk to the general public.

### 4.2.3 Molecular identification of bacterial species

A total of 12 Gram-negative bacterial isolates were amplified using universal primers targeting the 16SrRNA gene. Results showed that all positive isolates (Amplicons) appeared at 1500 bp, as shown in Fig. 16. Whereby, M is a 100 bp marker, lane 1, 5,7 and 10 are *klebsiella species,* and lane 2, 3, 4 and 11 are *pseudomonas species* and lane 6, 8, 9 and 12 are *E. coli* species. Positive products are located at 1500 bp as shown in Fig. 16.

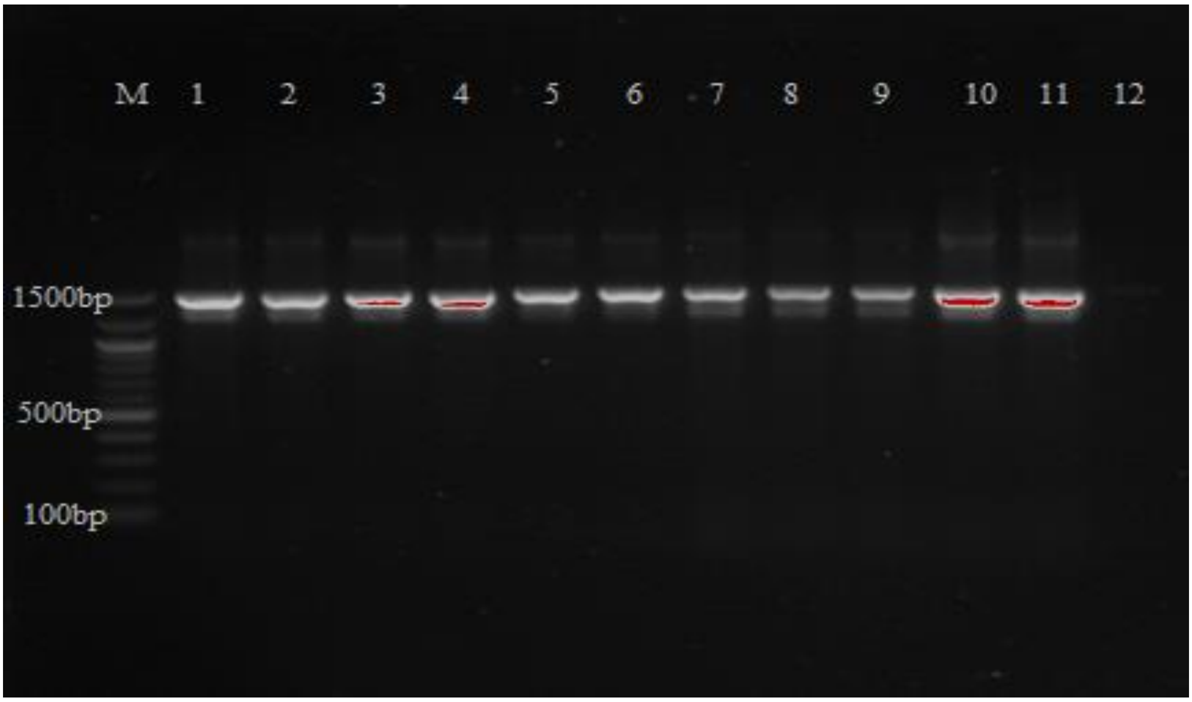



**Figure 16: Polymerase chain reaction amplification of 16SrRNA**

### 4.2.4 Identification of resistance genes

The detection of antibiotic-resistant genes on 12 bacterial isolates showed that *Klebsiella spp* harboured more resistance genes (40%), followed by *Pseudomonas spp*., (35%) and *E. coli* (20%) as shown in Table 12. Six (6) isolates out of 12 contained sulphonamide resistant genes as follows: Sul 1 (n = 4) and Sul 2 (n = 2), making up 50% of the total resistant gene analyzed in this study (Fig. 16 and Table 12). β-lactamases (*bla* $_{CTX-M}$ , *bla* $_{TEM}$ *and bla* $_{SHV}$) were found in all isolates, with *Klebsiella* harbouring more resistance genes than others as shown in Table 12 and Fig. 16, 17 and 18.

**Table 12: Antibiotic-resistant genes on bacterial isolates**

| **Bacterial spp** | **Number ( N)** | ***Sul 1* (n)** | ***Sul 2* (n)** | ***SHV* (n)** | ***CTXM* (n)** | ***TEM* (n)** | ***MDR genes* (%)** |
|---|---|---|---|---|---|---|---|
| ***Pseudomonas spp*** | 4 | 3 | 0 | 3 | 0 | 1 | 35 |
| ***Klebsiella spp.*** | 4 | 1 | 1 | 1 | 5 | 0 | 40 |
| ***E. coli spp.*** | 4 | 0 | 1 | 1 | 0 | 2 | 20 |
| **Resistance genes (%)** | | 33 | 17 | 42 | 42 | 25 | |

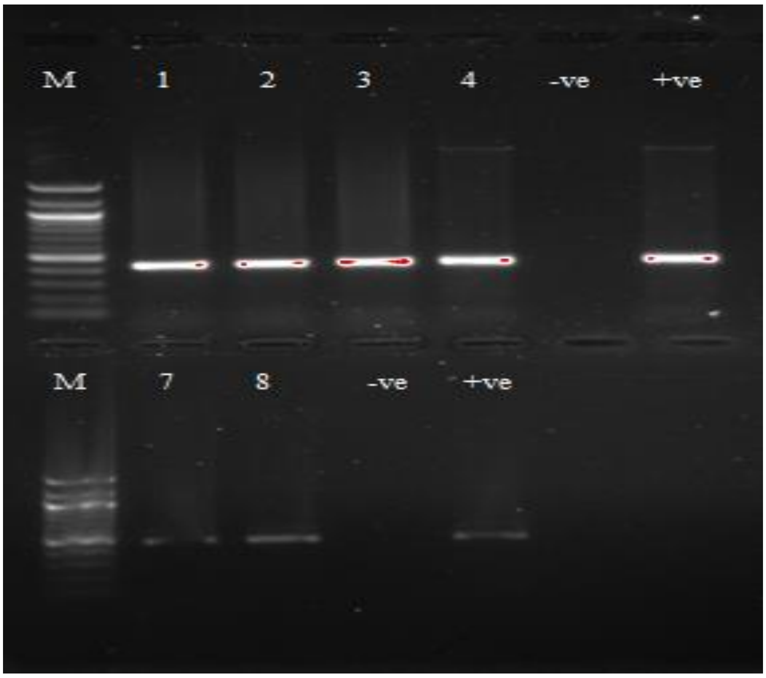



**Figure 17:** **Polymerase chain reaction amplification of Sulfonamide 1 and 2 (Sul1 and Sul2) resistance gene. M is a 100bp marker and lane 1-4, 7, 8 are samples, where lane 1-4 are positive for Sul1 resistance gene located at 450bp and lane 5 and 6 are negative and positive controls for Sul1 gene respectively and lane 7, 8 are positives for Sul2 resistant gene located at 625bp and lane 9 and 10 are negative and positive controls for Sul2 respectively**

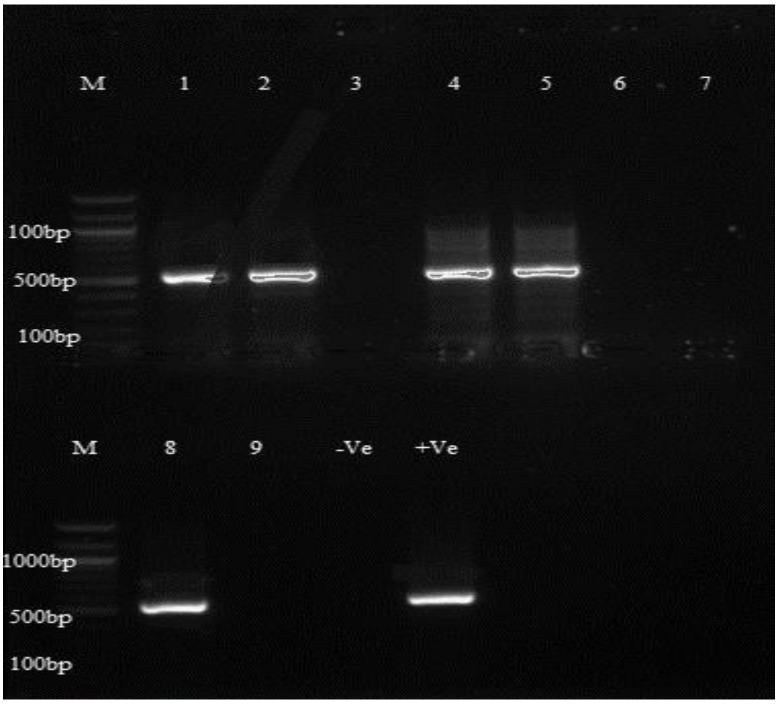



**Figure 18:** **Polymerase chain reaction amplification of bla$_{CTXM}$ resistance gene. M is a 100bp marker and lane 1-9 are samples, where lane 1, 2, 4, 5 and 8 are positives located at 554bp. Lane 10 and 11 are negative and positive controls respectively**

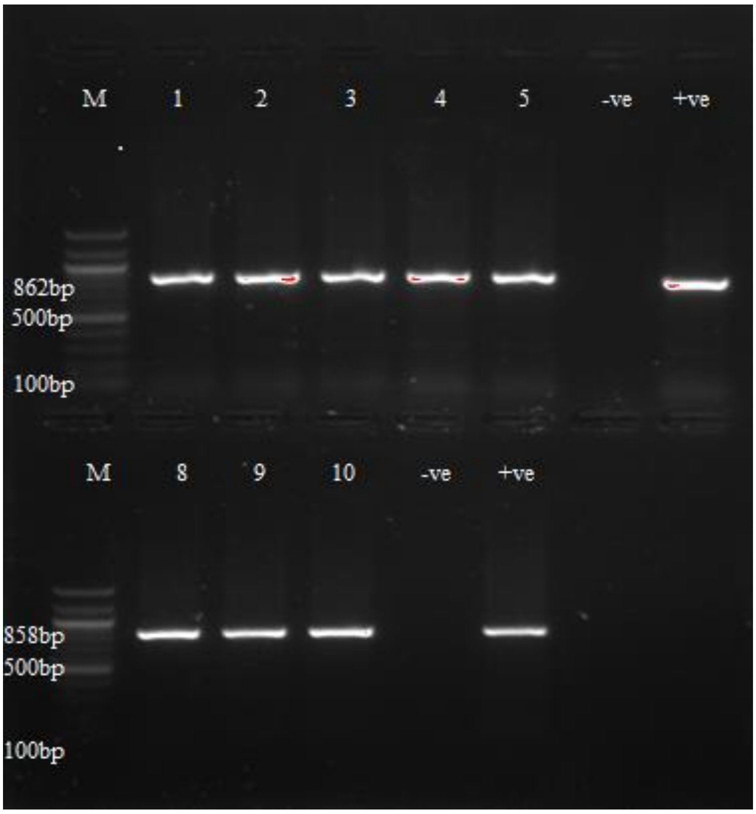



**Figure 19: Polymerase chain reaction amplification of bla $_{SHV}$ and bla$_{TEM}$ resistance gene. M is a 100bp marker and lane 1-5, 8 -10 are samples, where lane 1-5 are positive for bla$_{SHV}$ resistance gene located at 862bp and lane 6 and 7 are negative and positive controls for bla$_{SHV}$ respectively and lane 8-10 are positives for bla$_{TEM}$ resistant gene located at 858bp and lane 11 and 12 are negative and positive controls for bla$_{TEM}$ respectively**

### 4.2.5 Sequencing and Phylogenetic analysis

The 16S rRNA gene sequences of all isolates were subjected to BLAST analysis to identify the closest reference sequences available in GenBank. The BLAST analysis revealed that all isolates belonged to the family *Enterobacteriaceae*. The *E. coli* from this study showed similarity with other *E. coli* species from the GeneBank database. While other members like *Klebsiella spp* and *Pseudomonas spp* revealed high similarity (99 – 100%) to their respective genus as shown in Fig. 19. These results confirm the findings obtained from convectional diagnosis (Culture and Biochemical tests) obtained earlier in the present study.

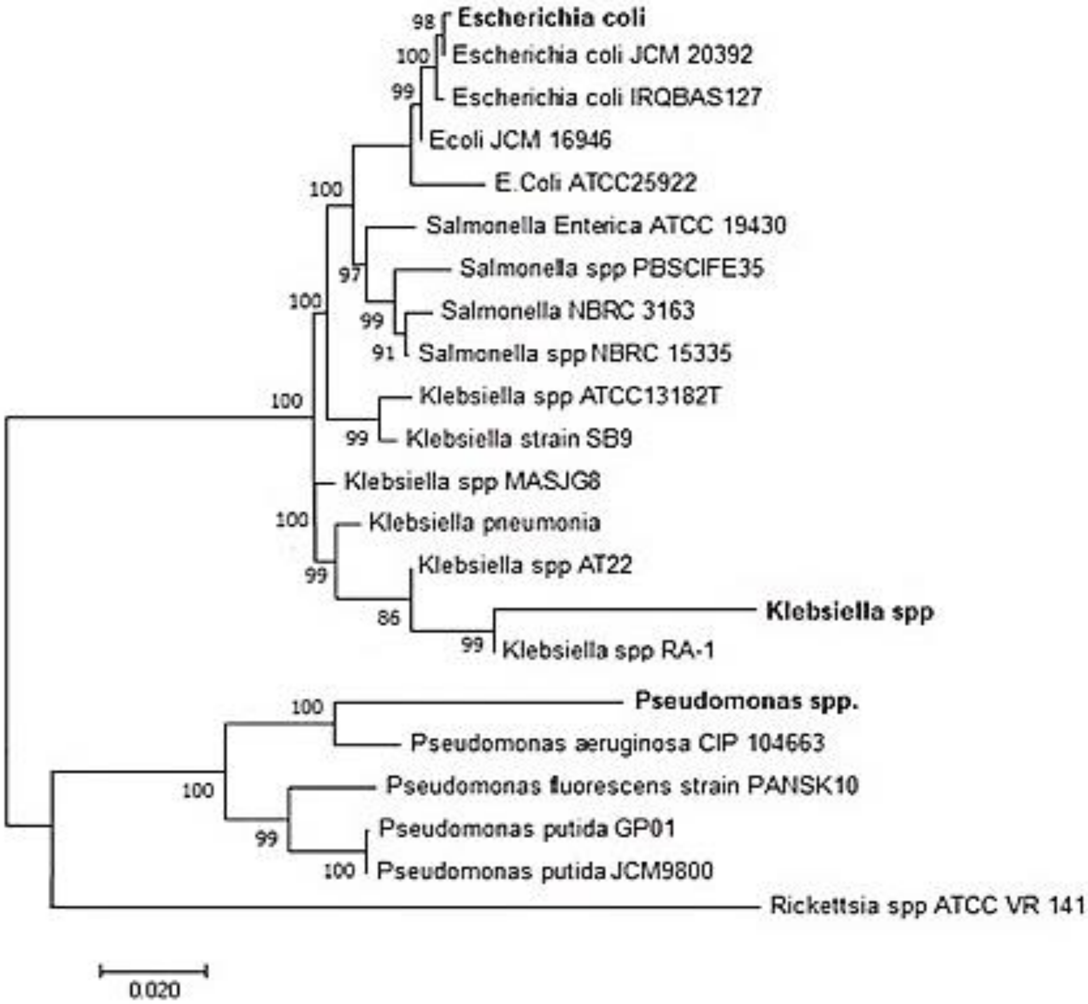



**Figure 20: Neighbour-joining phylogenetic trees based on 16S rRNA gene partial sequences of Escherichia coli, Klebsiella spp, Pseudomonas spp (Bolded) obtained from this study clustered together with the closely related genera of the family Enterobacteriaceae retrieved from GenBank database. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Rickettsia species was used as an outgroup to root up the tree**

Antibiotic resistance genes (ARGs) are currently regarded as an emerging contaminant and an ecological issue since they are present in practically every ecosystem. The ARGs found in the environment may operate as a reservoir and spread horizontally to bacteria that are linked with humans, contributing to the emergence of new antibiotic-resistant strains (Rozman *et al.*, 2020). Compared to other wastewater systems, such as municipal sewage systems, hospital wastewater has higher risks of ARGs spread (Verlicchi *et al.*, 2015; Zheng *et al.,* 2018). More global awareness should be given to the role that hospital wastewater plays in the spread of ARGs in the environment. The present study shows that *Klebsiella spp* harboured more resistance genes (40%), followed by *Pseudomonas spp*., (35%) and *E. coli* (20%).

Sulfonamides (SN) or sulfanilamides are a significant family of synthetic antimicrobial drugs that are used pharmacologically as broad-spectrum antibiotics to treat bacterial infections in both humans and animals. A distinctive 6- or 5-membered heterocyclic ring and the presence of the sulfanilamide group define Sulfonamides structures, which are organo-sulfur compounds containing the $-SO_2NH_2$ and/or $-SO_2NH-$ group (Ovung & Bhattacharyya, 2021). Bacterial resistance to sulfonamides typically results from mutations in the *folP* gene, which encodes the dihydropteroate synthase (DHPS) enzyme involved in nucleotide biosynthesis, or from the acquisition of alternative DHPS genes (*sul1, sul2, and sul3*), whose byproducts have low affinity for sulfonamides. Since *sul* genes are frequently found in plasmids, they serve as the most prevalent mechanism of sulfonamide resistance and have been found in a variety of bacterial species from a variety of habitats, including agricultural soils and wastewaters (Kim *et al.,* 2019). The results of the present study indicate that, of the 12 isolates tested, six (6) included sulfonamide resistant genes, specifically *Sul1* (n = 4) and *Sul2* (n = 2), which together accounted for 50% of the total resistance genes examined in this study.

The class of antibiotics known as β-lactams all have the same basic structure, the beta-lactam ring (three carbon and one nitrogen ring). Due to their bactericidal action and low toxicity, beta-lactam antibiotics are the most often used antibacterial drugs for treating bacterial infections, with the exception of individuals who have allergies (Balsalobre *et al.,* 2019). Examples of β-lactam antibiotics include penicillins, cephalosporins, carbapenems, and monobactams (Worthington & Melander, 2013). β-lactam antibiotics work by inhibiting the formation of bacterial cell walls, which results in cell lysis and death. The enzyme necessary for the building of the bacterial cell wall, penicillin-binding protein (PBP), is specifically bound and acylated by beta-lactam antibiotics (Bush & Bradford, 2016). The most prevalent mechanism of resistance in Gram-negative bacteria, and very rarely experienced by Gram-positive bacteria, is the formation of β-lactamases, enzymes that hydrolyze β-lactam antibiotics, rendering them ineffective (Waśko *et al.,* 2022). Numerous Gram-negative bacteria, such as *Acinetobacter, Aeromonas caviae, Proteus mirabilis, Providencia spp., Escherichia coli,* and *Klebsiella pneumoniae*, have also been reported to have several AmpC β-lactamase genes (Lin *et al.*, 2021). The results from this study show β-lactamases (*bla* $_{CTX\text{-}M}$, *bla* $_{TEM}$ *and bla* $_{SHV}$) were found in all isolates, with *Klebsiella* harbouring more resistance genes than others. 1 *Klebsiella pneumoniae* isolate contained *bla* $_{SHV}$ and 5 isolates contained *bla* $_{CTX\text{-}M}$. The 1 *E. coli* isolate contained *bla* $_{SHV}$ and 2 isolates contained *bla* $_{TEM}$. 3 isolates of *Pseudomonas aeruginosa* contained *bla* $_{SHV}$ *and 1 isolate contained bla* $_{TEM}$. The presence of β-lactamases genes in hospital wastewater effluent isolates implies that the effluent still harbors these ARGs despite CW treatment, posing a health concern.

#### 4.2.6 Plant growth in experimental Constructed wetland

The rate of plant growth was monitored for 4 months (Recording done weekly) while irrigated with tap water and pre treated wastewater. The results are presented in Fig. 21.

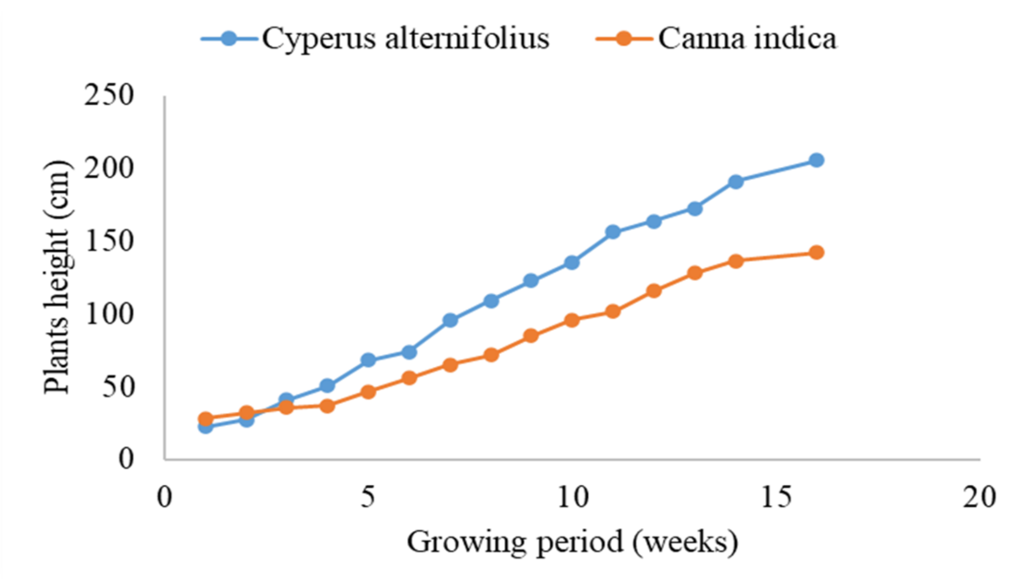



**Figure 21: Growth rate of *Cyperus alternifolius* and *Canna indica* in Constructed wetlands**

The findings demonstrate that both *Cyperus alternifolius* and *Canna indica* grow faster in greenhouse environments. However, as the plants became older and matured, the rate of growth for both plants decreased, indicating that the cycle of development had come to an end.

### 4.3 Removal of physico-chemical parameters in experimental constructed wetland

The treatment of some physico-chemical parameters from synthetic wastewater in CWs planted with different plants are summarized in the Table 13. According to these results, there is small change in electrical conductivity in all cells. The CW with mixed plants had highest removal of EC (10.2%) and phosphate (66.3%). The highest removal rate of $NO_3$-N (76.6%) and COD (79.5%) was observed in CW planted with *Cyperus alternifolius*. The CW planted with both *Canna indica* and *Cyperus alternifolius* was the best for removal of BOD (72.7%).

**Table 13: The removal of physico-chemical parameters from synthetic wastewater in CWs**

| Parameter | Unit of measure | Influent | HRT (Days) | % Removal: Without plants | *Canna indica* | *Cyperus alternifolius* | Mixed plants |
|---|---|---|---|---|---|---|---|
| EC | mg/L | 1690 ± 16.77 | 3 | 1.2 | 4.1 | 4.2 | 7.1 |
| | | | 5 | 1.1 | 4.4 | 4.3 | 8.5 |
| | | | 7 | 2.4 | 4.4 | 4.7 | 10.2 |
| $NO_3$-N | mg/L | 34.7 ± 3.48 | 3 | 13.5 | 57.4 | 62.2 | 64.2 |
| | | | 5 | 28.6 | 62.6 | 67.9 | 66.1 |
| | | | 7 | 34.6 | 71.5 | 76.6 | 74.7 |
| $PO_4$-P | mg/L | 3.84 ± 0.71 | 3 | 22.7 | 55.2 | 54.9 | 59.6 |
| | | | 5 | 27.6 | 62.2 | 58.1 | 66.4 |
| | | | 7 | 36.7 | 65.4 | 60.7 | 69.5 |
| BOD | mg/L | 86.67 ± 12.47 | 3 | 34.6 | 63.1 | 59.6 | 62.9 |
| | | | 5 | 41.9 | 66.6 | 65.5 | 67.7 |
| | | | 7 | 46.2 | 70.1 | 71.0 | 72.7 |
| COD | mg/L | 156.29 ± 19.56 | 3 | 37.2 | 63.6 | 62.9 | 61.7 |
| | | | 5 | 45.6 | 69.2 | 67.6 | 68.1 |
| | | | 7 | 49.2 | 77.7 | 79.5 | 77.9 |

There was a generally low decrease in EC where the trend in different CW is mixed > *Cyperus alternifolius* > *Canna indica* > without plants. The trend for $NO_3$-N is *Cyperus alternifolius* > Mixed > *Canna indica* > without plants and for $PO_4$-P is Mixed > *Canna indica* > *Cyperus alternifolius* > without plants. The trend for BOD is mixed > *Cyperus alternifolius* > *Canna indica* > without plants and for COD is *Cyperus alternifolius* > mixed > *Canna indica* > without plants. This is well presented in Fig. 22.

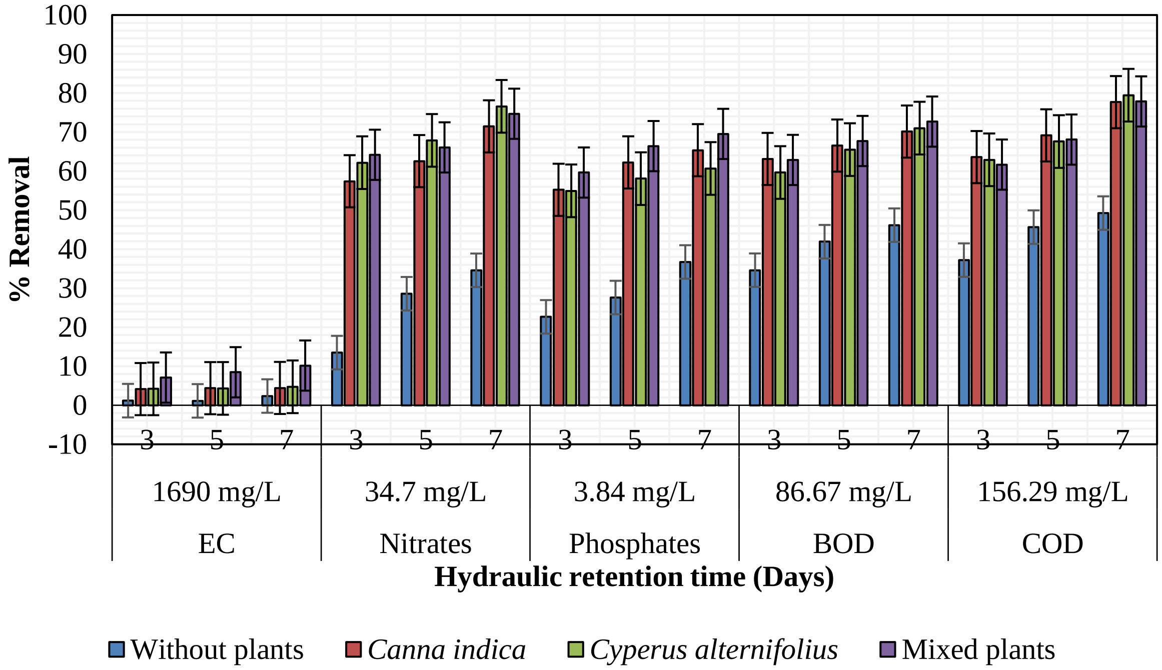



**Figure 22: Removal of chemical oxygen demand from synthetic wastewater**

### 4.3.1 Removal of nitrates and phosphates

The removal of nitrates and phosphates in all CW cells increase with increase in HRT. Nitrogen is removed from CW through both aerobic and anaerobic conditions in nitrification and denitrification processes (Khan *et al.,* 2020; Wang *et al.,* 2022). Nitrification is the microbial oxidation of ammonium ($NH_4$) to nitrite ($NO_2$) and finally nitrate ($NO_3$) (Vymazal, 2008). Because this process requires oxygen, it happens in aerobic wetland regions. The nitrate then dissipates into the wetland's anaerobic regions, where it may be denitrified (Jones *et al.,* 2016; Shelef *et al.,* 2013b). This process is the rate-limiting process in the nitrogen removal. Nitrate ($NO_3$) is transformed into gaseous nitrous oxide ($N_2O$) and nitrogen gas ($N_2$) during the denitrification process, and these gases are then discharged into the atmosphere (Turpie *et al.,* 2010). The removal of nitrate in CW planted with *Canna indica* (71.5%), *Cyperus alternifolius* (76.6%) and mixed plants (74.7%) are in agreement with efficiency reported by other researchers such as 62.1% (Sudarsan *et al.,* 2015). The larger root surface area of *Cyperus alternifolius*, which is greater than *Canna indica*, is believed to be responsible for the higher removal rate of nitrate in CW planted with *Cyperus alternifolius* than *Canna indica*.

The presence of plants in CW facilitates for phosphorus elimination by plant uptake. The organic form of phosphate, which cannot be removed by plants can be transformed to the inorganic form through the action of enzymes. It has long been recognized that one key route to remove phosphorus in CWs is through plant growth metabolism (Abou-Elela *et al.,* 2013). Phosphorus can also be eliminated by sorption and deposition on the substrate (Ravichandran & Philip, 2022). The results obtained in this study fall in the range of phosphates removal (50% - 80%) reported by other researchers (Pinninti *et al.,* 2021). In comparison to an unplanted CW (36.7%) or a monoculture of *Canna indica* (65.4%) or *Cyperus alternifolius* (60.7%) there was high significant phosphate removal in CW with mixed plants (69.5%).

### 4.3.2 Removal of biochemical oxygen demand and chemical oxygen demand

The BOD and COD express the organic carbon content in wastewater. In this study, high rates of BOD and COD removal were achieved. The performance in COD and BOD removal increase with increase in HRT. In all cells, the highest removal percentage is observed at 7 days HRT. The obtained results for COD and BOD removal agree with results reported by other researchers which is more than 70% (Pinninti *et al.,* 2021; Sudarsan *et al.,* 2015). The removal of COD and BOD can be explained by action of both aerobic and anaerobic microorganisms attached to the roots and rhizome, as well as the porous substrate (Papaevangelou *et al.,* 2016).

Under aerobic conditions COD acts as an electron donor utilized in denitrification process under anaerobic condition (Ding *et al.,* 2014). The COD removal can also be accomplished using suspended particles filtration or sedimentation (Rahmadyanti *et al.,* 2020). All these mechanisms require time for the organic matter to interact with the CW components. There is significant difference ($p < 0.05$) in performance between CW with plants and without plants. This shows a role of plants in performance of CWs. For removal of COD, the CW planted with *Cyperus alternifolius* (79.5%) had the highest performance and for removal of BOD the CW planted with both *Canna indica* and *Cyperus alternifolius* performed better (72.7%) than CWs planted with only *Canna indica* (70.1%) or *Cyperus alternifolius* (71.0%). This can be the outcome of effective partitioning of the root zone in a system with diverse species of plants (Karathanasis *et al.,* 2003).

## 4.4 Removal of antibiotics in experimental constructed wetland

The performance of CWs without plants, with *Canna indica*, *Cyperus alternifolius* and mixed plants on antibiotics removal from synthetic wastewater is presented in Table 14.

**Table 14: The removal of antibiotics from synthetic wastewater treated in CWs**

| | | **Without plants** | | ***Canna indica*** | | ***Cyperus alternifolius*** | | **Mixed plants** | |
|---|---|---|---|---|---|---|---|---|---|
| **Ant** | **HRT (Days)** | Av | % R | Av | % R | Av | % R | Av | % R |
| MET | Inf | 135.89 | | 137.57 | | 138.38 | | 131.73 | |
| | 1 | 96.21 | 29.2 | 73.24 | 46.8 | 77.93 | 43.7 | 80.22 | 39.1 |
| | 2 | 87.26 | 35.8 | 68.98 | 49.9 | 65.78 | 52.5 | 71.89 | 45.4 |
| | 3 | 82.23 | 39.5 | 60.17 | 56.3 | 59.14 | 57.3 | 62.33 | 52.7 |
| | 4 | 79.92 | 41.2 | 58.11 | 57.8 | 49.89 | 63.9 | 54.66 | 58.5 |
| | 5 | 74.69 | 45.0 | 54.14 | 60.6 | 46.11 | 66.7 | 52.12 | 60.4 |
| | 6 | 66.54 | 51.0 | 42.89 | 68.8 | 38.95 | 71.9 | 47.78 | 63.7 |
| | 7 | 59.66 | 56.1 | 36.89 | 73.2 | 30.37 | 78.1 | 42.28 | 67.9 |
| CIP | Inf | 65.97 | | 65.03 | | 66.17 | | 65.93 | |
| | 1 | 50.66 | 23.2 | 37.33 | 42.6 | 34.67 | 47.6 | 36.78 | 44.2 |
| | 2 | 47.23 | 28.4 | 33.22 | 58.1 | 30.98 | 53.2 | 29.81 | 54.8 |
| | 3 | 45.37 | 31.2 | 24.93 | 61.7 | 22.87 | 65.4 | 25.49 | 61.3 |
| | 4 | 43.17 | 34.6 | 22.99 | 63.1 | 20.09 | 69.6 | 22.84 | 65.4 |
| | 5 | 40.33 | 38.9 | 22.1 | 66.0 | 18.11 | 72.6 | 20.71 | 68.6 |
| | 6 | 39.01 | 40.9 | 21.07 | 69.1 | 15.98 | 75.9 | 18.99 | 71.2 |
| | 7 | 37.23 | 43.6 | 18.71 | 74.3 | 15.17 | 77.1 | 18.46 | 72.0 |
| SUL | Inf | 45.76 | | 45.43 | | 46.31 | | 45.94 | |
| | 1 | 41.27 | 9.8 | 32.34 | 28.8 | 30.23 | 34.7 | 32.98 | 28.2 |
| | 2 | 40.02 | 12.5 | 31.09 | 31.6 | 29.18 | 37.0 | 29.71 | 35.3 |
| | 3 | 38.22 | 16.5 | 27.98 | 38.4 | 27.11 | 41.5 | 28.95 | 37.0 |
| | 4 | 37.18 | 18.8 | 26.72 | 41.2 | 26.07 | 43.7 | 27.91 | 39.2 |
| | 5 | 35.52 | 22.4 | 25.91 | 43.0 | 23.13 | 50.1 | 25.8 | 43.8 |
| | 6 | 33.89 | 25.9 | 24.71 | 45.6 | 20.83 | 55.0 | 22.99 | 50.0 |
| | 7 | 32.78 | 28.4 | 23.82 | 47.6 | 19.22 | 58.5 | 21.35 | 53.5 |

The results showed decrease in concentration of MET, CIP and SUL as HRT increase in all CW cells. The variations in the decrease of concentrations with HRT were observed among planted and unplanted cells, between single and mixed plants, between plant species and among the studied antibiotics. These variations can be viewed in Fig. 23, 24 and 25.

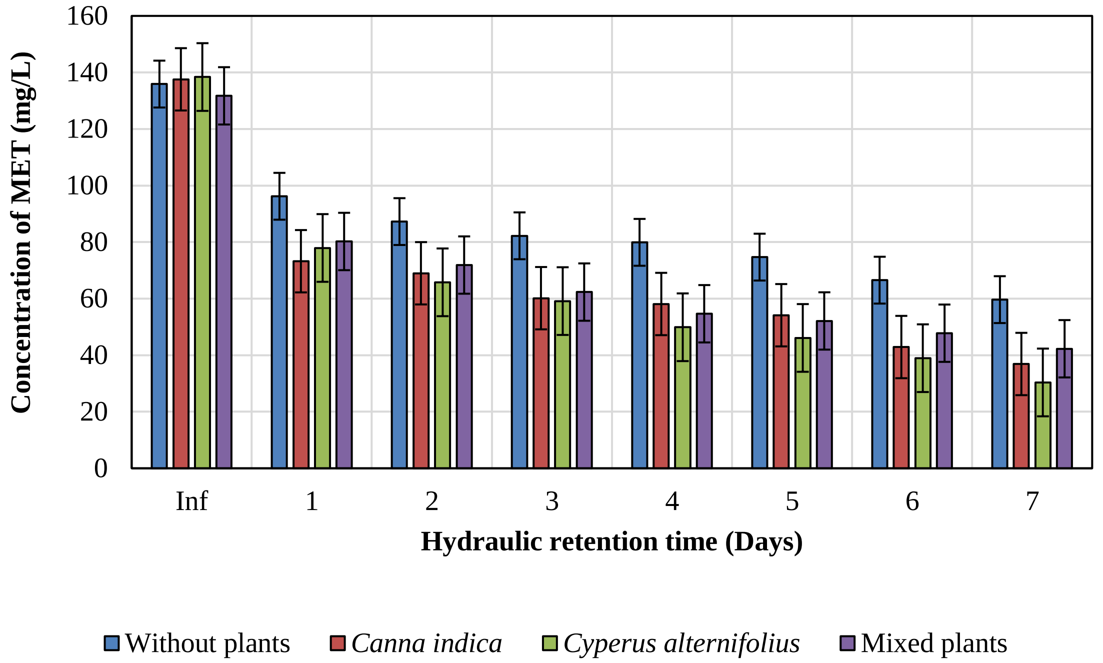



**Figure 23:** **Decrease in metronidazole concentration from synthetic wastewater treated in constructed wetlands**

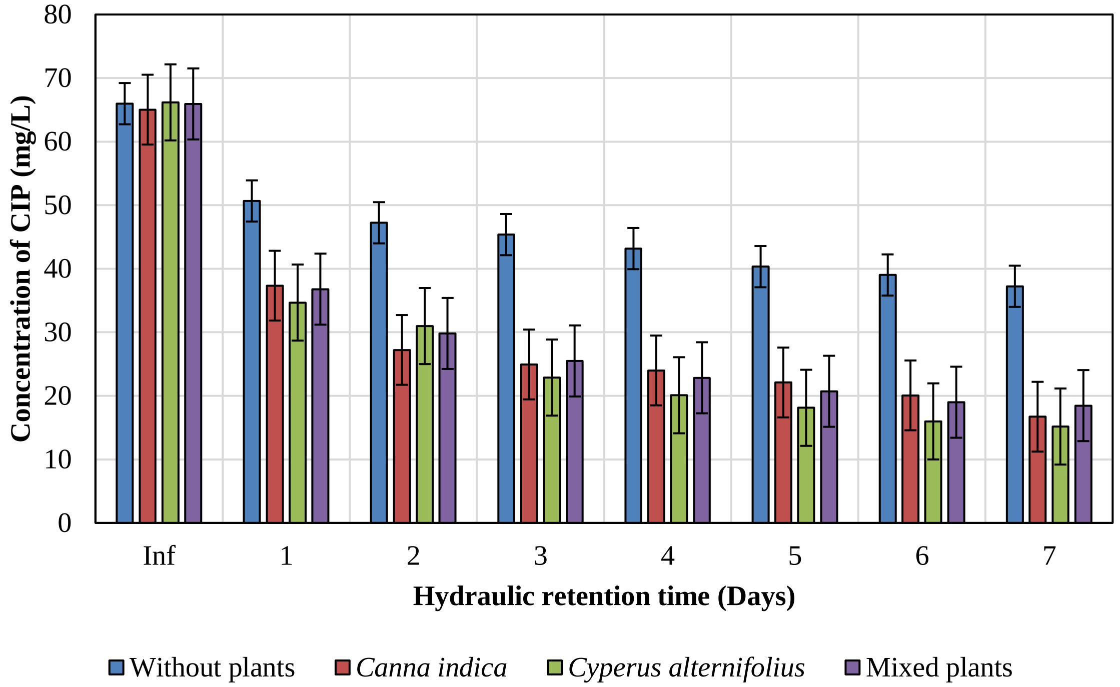



**Figure 24:** **Decrease in Ciprofloxacin concentration from synthetic wastewater treated in constructed wetlands**

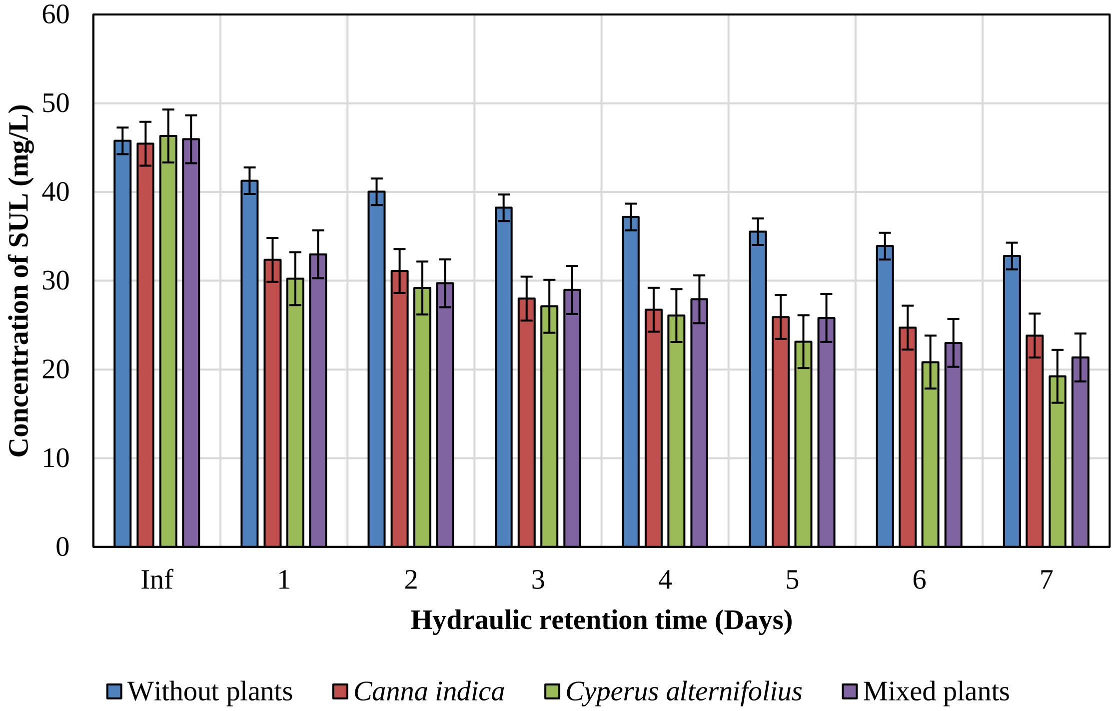



**Figure 25:** **Decrease in SUL concentration from synthetic wastewater treated in constructed wetlands**

The results show increase in removal efficiency of MET, CIP and SUL as HRT increase in all CW cells. The variations in % removal was observed among planted and unplanted cells, between single and mixed plants, between plant species and among the studied antibiotics. These variations can be viewed in Fig. 26, 27and 28.

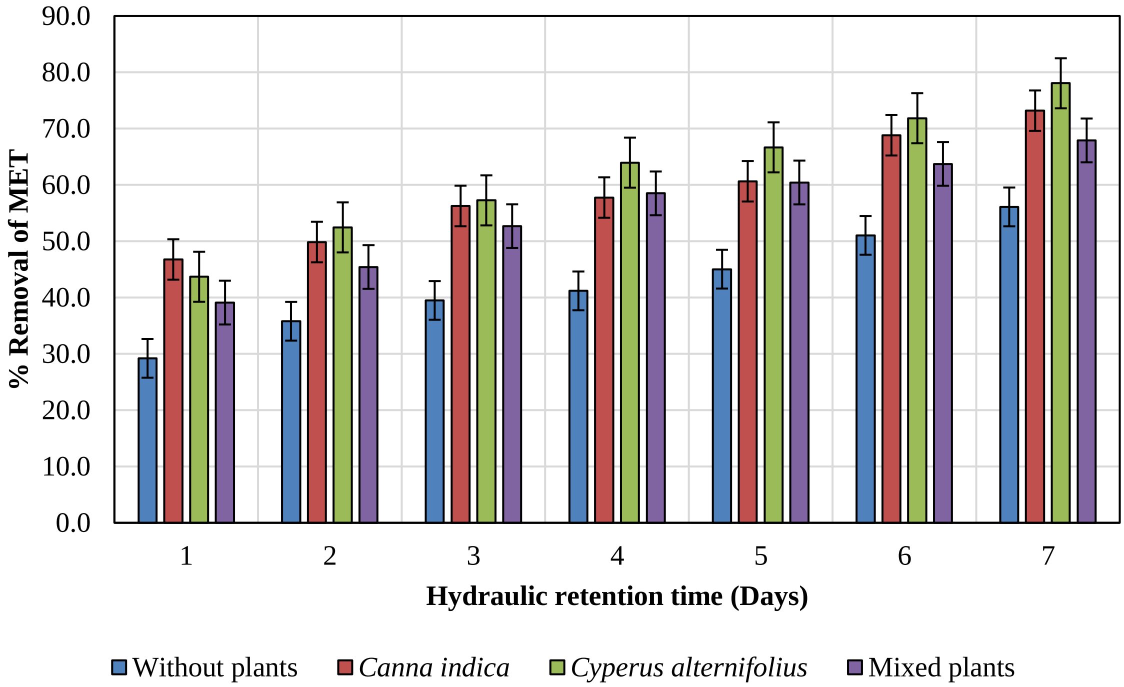



**Figure 26:** **Percentage removal of metronidazole from synthetic wastewater treated in constructed wetlands**

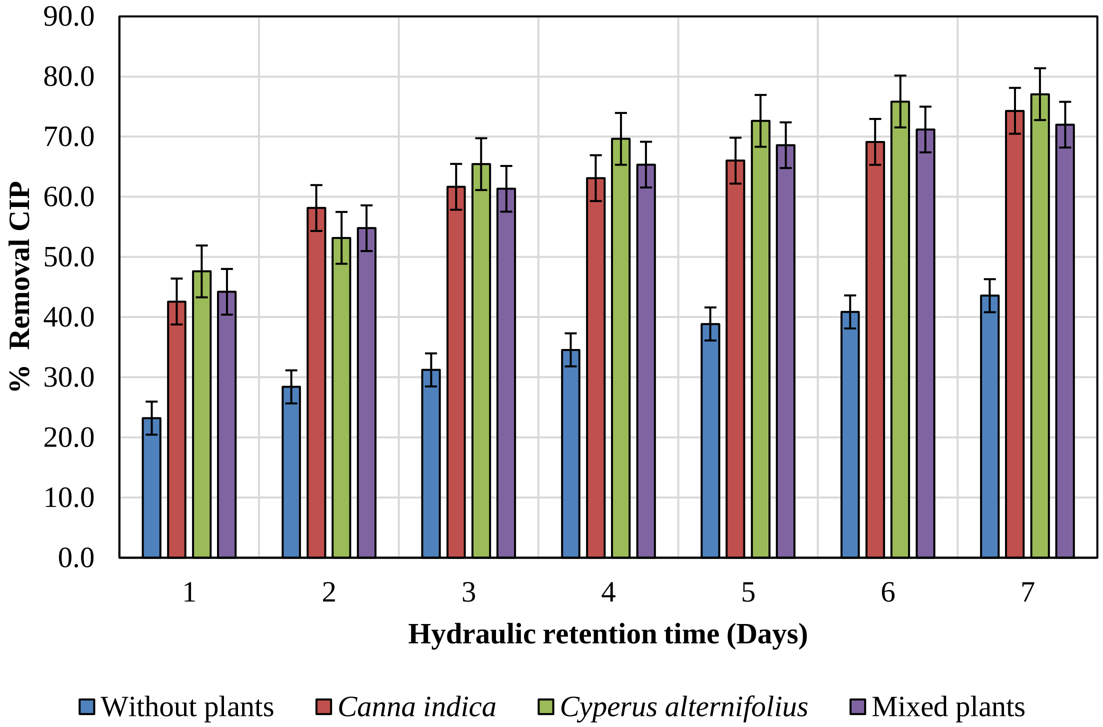



**Figure 27:** **Percentage removal of CIP from synthetic wastewater treated in constructed wetlands**

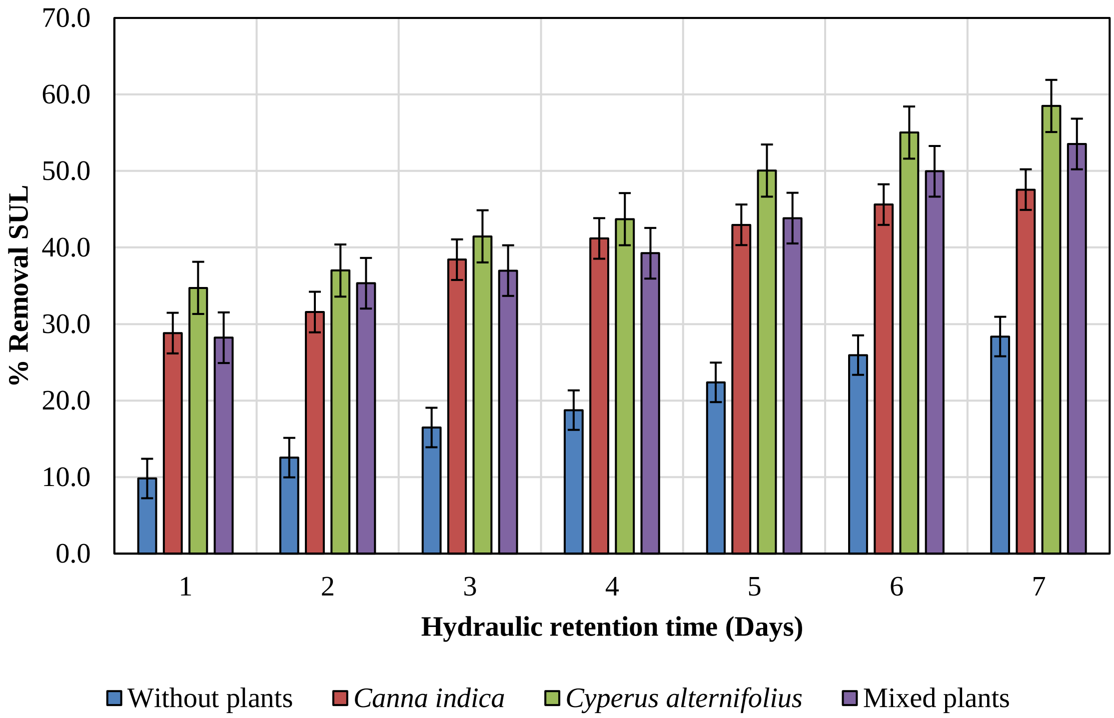



**Figure 28:** **Removal of Sulfamethoxazole from synthetic wastewater treated in constructed wetlands**

## 4.5 Effect of plants in constructed wetlands

In all experiments, the CWs with plants gave higher performance than CWs without plants. For instance, for unplanted CW, the lowest removal (18.4%), was observed in Sulfamethoxazole in 3 days HRT and the highest (56.1%) was observed in MET in 7 days HRT. This can be compared with planted CWs where the lowest removal (35.2%) was observed in CW planted with *Cyperus alternifolius* for removal of SUL in 3 days HRT while the highest removal (78.1%) was observed in CW planted with *Cyperus alternifolius* for removal of MET in 7 days HRT. This shows the role played by plants in CWs in removing contaminants from wastewater. Plants provide the majority of the oxygen in CWs via a mechanism known as radial oxygen loss (ROL), which occurs in the root zone (Jiang *et al.,* 2020). Aerobic conditions in CWs support aerobic respiration pathways, which are more effective than anaerobic pathways at removing contaminants (Zhang *et al.,* 2012). In the rhizosphere, where there is extensive microbial activity, plants produce root exudates that enhance the ability of wetland to degrade the pollutants (Punyapwar & Mutnuri, 2020). Antibiotics can also be removed from wastewater in CWs by plant uptake (Decezaro *et al.,* 2018). Despite the good performance of both plants in the removal of antibiotics, the results showed superior performance of *Cyperus alternifolius* than *Canna indica*. Only few exceptions were observed such as for removal of Sulfamethoxazole at 3 days HRT where *Canna indica* performed better (36.9%) than *Cyperus alternifolius* (35.2%).

### 4.5.1 Effect of single and mixed plants species in constructed wetlands

In this study CWs planted with single plant species (*Cyperus alternifolius*) performed better in wastewater treatment than the one with mixed plant species. However, in many experiments the system with mixed plants performed better than the system planted with only *Canna indica*. Because of this, it is difficult to say if mixed plants are preferable than single plants in CWs. Based on the investigated antibiotics and HRT, some variations in the removal of antibiotics were observed. The lack of a distinct pattern in CW performance in single and mixed plants makes plant selection an important factor in CW design. Single plant systems are thought to be more vulnerable to plant death due to predation or disease than mixed and native plant systems, which are thought to be more resilient (Zhang *et al.,* 2007). The reduced effectiveness in the system with mixed plants could be attributed to significant plant competition and other interactions (Leiva *et al.,* 2018). Because the plants planted in CWs have the same or comparable growth patterns, individual size, and light demand competition in CWs may be more intense than in other plant communities (Liang *et al.,* 2011a). The capability of plants to absorb nutrients is constrained by competition. It also has a negative impact on the ongoing purification process of the stable plant community in CWs (Liang *et al.,* 2011; Vymazal, 2013).

More study is required to determine the nature of dominance in CWs and how it affects growth and performance due to the likelihood that plant dominance can change with the seasons. This is because changes in the composition of plant species takes some years to become apparent due to the slow process of competition between plant species (Vymazal, 2022). Further research is necessary to identify optimized plant species matching, which will result in a good appearance of the CWs, less interspecific competition, and incredible performance in removal of pollutants.

### 4.6 Effect of Hydraulic Retention Time

The HRT is one of the important hydraulic parameters influencing the removal efficiencies of pharmaceuticals in CWs (Merino-Solís *et al.,* 2015; Nyieku *et al.,* 2020; Özengin & Elmaci, 2016). This is a measure of the average length of time that wastewater remains in a CW (Cui *et al.,* 2010). The HRT has a distinct effect on different CWs with different plant species and communities. From the results for all cells and antibiotics, the removal efficiency increases with increase in HRT. This is expected due to the fact that at smaller HRT the compounds have less contact time with the microbials and thus, lower removal efficiency (Wu *et al.*, 2015). At 7 days HRT (maximum HRT in this study), the CW planted with *Canna indica* removed 71.2% CIP, 73.3% MET and 50.2% SUL. At the same HRT, the CW planted with *Cyperus alternifolius* removed 74% CIP, 78.1% MET and 56.0% SUL. Also, the CW planted with mixed plant species removed 72.0% CIP, 70.2% MET and 53.5% SUL. It is likely that adding more HRT than what was tested in this study would result in a higher removal efficiency. This is owing to the fact that antibiotics removal in CWs involves a number of mechanisms, including adsorption, photodegradation, aerobic and anaerobic biodegradation and plant uptake, all of which are slow processes (Ilyas & van Hullebusch, 2019b). However, if wastewater is left in CW for a prolonged period of time, the removal rate will decline since there will not be as much nutrients for the microbial growth (Rani *et al.*, 2011). Additionally, a considerable amount of land will be needed, increasing the capital and operating costs (Kümmerer, 2009). Efficiency and cost must therefore be balanced when designing of CWs.

### 4.7 Performance with different antibiotics

The studied antibiotics were removed at different rates in different CWs. The CIP was removed with the highest rate (74.0%) and this was observed in a CW planted *Cyperus alternifolius* in 7 days HRT. The other cells removed 72% (Mixed plants), 71.2% (*Canna indica*) and 43.6% (Without plants). For removal of MET, the highest rate (78.1%) was observed in a CW planted with *Cyperus alternifolius* in 7 days HRT. The other cells removed 70.2% (Mixed plants), 73.2% (*Canna indica*) and 56.1% (Without plants). For removal of SUL, the highest rate (56.0%) was observed in a CW planted with *Cyperus alternifolius* in 7 days HRT. The other cells removed 53.5 % (Mixed plants), 50.2% (*Canna indica*) and 28.4% (Without plants). These results show that there is low removal of SUL in all CWs. This observation maybe associated with low sorption coefficient (Prasannamedha & Kumar, 2020) and low degradability of SUL in aqueous systems (Wang & Wang, 2018). These findings are consistent with those of previous studies which examined the elimination of CIP and SUL in CW, where CIP was removed by more than 77% (Nas *et al.,* 2021) and SUL was removed by 24-30% (Nowrotek *et al.,* 2016).

## 4.8 Influence of antibiotics exposure on bacterial communities in constructed wetlands

### 4.5.1 Bacteria enumeration

The results for bacteria abundance in each CW cell are presented in Table 15. The log values of average microbial abundance in CW cells without CIP were 3.34, 4.60, 4.50 and 4.91 for control, CW planted with *Cyperus alternifolius, Canna indica* and mixed plants respectively. The bacteria abundance with 100 µg/L CIP were 3.06, 4.39, 4.03 and 4.64 for control, CW planted *Cyperus alternifolius, Canna indica* and mixed plants respectively.

**Table 15: Bacteria abundances in constructed wetland cells**

| Experiment | log (CFU/mL) without CIP | | | | log (CFU/mL) with 100 µg/L CIP | | | |
|---|---|---|---|---|---|---|---|---|
| | **C** | **CA** | **CI** | **M** | **C** | **CA** | **CI** | **M** |
| 1 | 2.88 | 4.56 | 4.43 | 3.63 | 4.11 | 4.91 | 3.00 | 4.41 |
| | 2.65 | 5.21 | 5.80 | 5.33 | 2.60 | 4.64 | 4.87 | 4.85 |
| | 3.96 | 3.58 | 4.57 | 5.37 | 3.57 | 3.91 | 3.08 | 5.19 |
| 2 | 3.92 | 5.13 | 3.23 | 4.76 | 3.24 | 4.70 | 4.75 | 4.79 |
| | 4.15 | 4.38 | 4.98 | 5.40 | 2.23 | 3.88 | 3.29 | 4.41 |
| | 3.36 | 4.14 | 3.55 | 4.16 | 3.30 | 4.16 | 3.80 | 4.20 |
| 3 | 3.20 | 4.10 | 4.20 | 5.23 | 3.58 | 3.81 | 4.86 | 3.95 |
| | 3.16 | 4.64 | 4.10 | 4.76 | 2.43 | 4.93 | 3.87 | 5.13 |
| | 2.79 | 5.66 | 5.61 | 5.50 | 2.52 | 4.61 | 4.75 | 4.81 |

C=Control, CA= *Cyperus alternifolius*, CI= *Canna indica* and M=Mixed plants

These results show significant difference ($p < 0.05$) in microbial abundance between CW without plants and those with plants. This shows the role of plants in microbial composition and activity in CWs. They accomplish this by dispersing exudates and oxygen into the rhizosphere, which in turn has an indirect impact on enzymatic activity (Haichar *et al.,* 2014). The breakdown of aged tissue in the root and healthy plant tissues are the principal sources of root exudates (Wang *et al.,* 2018). By releasing inorganic ions and different organic compounds, these root exudates can act as electron (Hussain *et al.,* 2018; Macci *et al.,* 2015b). They can also increase microbial activity in the rhizosphere by acting as an organic carbon source (Zhai *et al.,* 2016). Some plant exudates may act as catalysts for the degradation of organic molecules in addition to the biological processes (Chen & Liu, 2024; Jones *et al.,* 2004; Mavrodi *et al.,* 2021; Rohrbacher & St-Arnaud, 2016; Williams *et al.*, 2021). There is also the possibility that root exudates may play a role in the stimulation of specific metabolic activities, providing the ability to breakdown certain pharmaceuticals or enhance bioavailability of pharmaceuticals by serving as surfactants or transporters (Zhang *et al.,* 2012). However, it is confirmed that root exudates of some plants such as *Thalia dealbata* can inhibit the growth and development of the Cyanobacteria (Kumar *et al.,* 2020). This means the root exudates may both stimulate and inhibit bacterial growth (Stottmeister *et al.*, 2003).

Again, the plants in CWs provide root oxygenation which have a significant impact on enhancement of microbial activity (Di *et al.,* 2020; Shelef *et al.,* 2013b). The presence of aerenchyma in roots and stems transport oxygen to roots in a process known as radial oxygen loss (ROL). The role of ROL in wetland microbial ecology is probably essential because it has the potential to affecting the rhizosphere microbial community (Tian *et al.*, 2015). By means of a wind-driven venture mechanism or a humidity-induced pressurized flow through, the oxygen is moved from the shoot to the root (Srivastava *et al.,* 2017). All these roles make the CWs with plants have high CFU than those without plants.

The result from this study showed high bacteria abundance in CW cell planted with *Cyperus altenifolius* than *Canna indica* although the difference is not significant ($p > 0.05$). This observation was similar to previous reports in which the abundances of bacteria and total cells in the rhizosphere of *Cyperus altenifolius* was higher than those in *Canna indica*. Aquatic species may have varied microbial compositions and activities on the biofilm of root or rhizome surfaces due to variations in both root exudates and oxygen release levels (Zhang *et al.*, 2014). This distinction demonstrates that the exudates and chemicals secreted by plant roots attract specific microbial populations (Shahid *et al.,* 2020). Different species and even subspecies have different exudates with different chemical compositions (Kumar *et al.,* 2020; Stottmeister *et al.,* 2003). The supply of oxygen in CWs is mostly determined by plant species and the redox potential of water, which accounts for 90% of rhizospheric oxygen and promotes the growth of aerobic bacteria (Srivastava *et al.*, 2017). The difference in this study may be related to the larger underground biomass and denser roots of *Cyperus altenifolius* than those of *Canna indica* which promoted the aerobes reproduction (Wu *et al.,* 2019). While *Canna indica* has a larger root type but is more delicate, *Cyperus alternifolius* has a strong, extended rhizome type, small size, and adventitious roots. Roots of *Cyperus alternifolius* can reach deeper layers of the media than those of *Canna indica*. Due to its ability to successfully reach a larger region of media, *Cyperus alternifolius* has increased oxygen delivery (Trifando *et al.,* 2022).

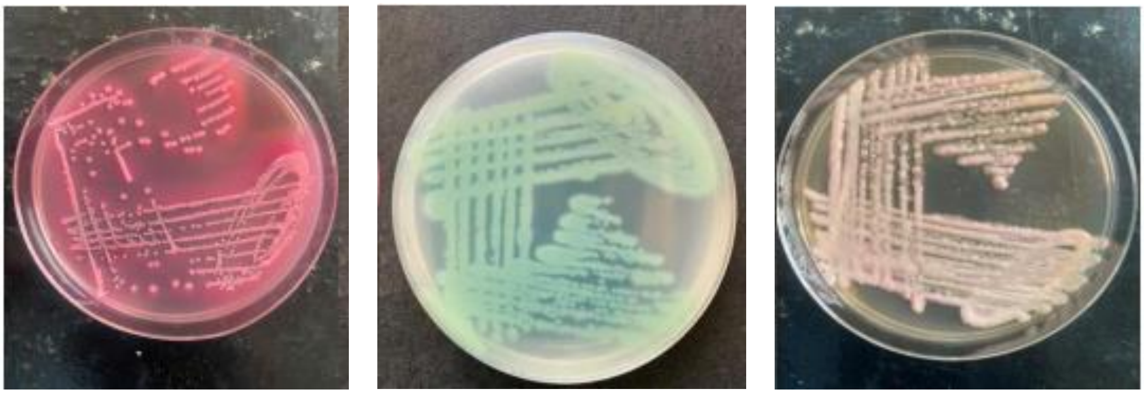
The results from this study showed high bacteria abundance in CW cell with mixed plants than CW cells with either *Cyperus altenifolius* or *Canna indica* only although the difference is not significant ($p > 0.05$). According to previous research, CWs with mixed plants have more diversified microbial communities than CWs with single plant species (Liang *et al.,* 2011b; Zhu *et al.,* 2017). These results support those earlier findings. The establishment of different microbial communities is generally regarded to be a consequence of high resource environment heterogeneity caused by high plant species richness or functional variation (Zhang *et al.,* 2010). The relationship between changes in the microbial community and how well CWs remove contaminants like antibiotics is not well understood. More research is required.

The results of this study showed that no significant difference ($p > 0.05$) in bacteria abundance caused by presence of CIP although there is a general decrease. This might be as a result of plants and microbial communities' adaptation to CIP presence without substantially changing their diversity (Fernandes *et al.,* 2015). Antibiotic-related selective pressures can change the composition of the general microbial community by altering its microbial makeup or by lowering the variety of its taxa. This is accomplished by two effects on the bacterial community: the selection of antibiotic-resistant bacteria and the degradation of microbial physiological processes (Wang *et al.,* 2022).

In general, exposure to antibiotics favors a growth in Gram-negative bacteria rather than Gram-positive bacteria. Those that lack an outer cell membrane are more susceptible to antibiotics and disinfectants. The abundance of microbes in the environment can, however, also be stimulated by antibiotics (Kraemer *et al.,* 2019). The results from this study are different from the results obtained in previous study on the effect of CIP on the development, function and stability of bacterial communities in wetland systems where CIP decreased the total number bacteria and the overall diversity of bacterial operational taxonomic units (Weber *et al.,* 2011). The change in the structure of microbial community affects the performance of the CW on removal of pollutants (An & Qin, 2018).

### 4.5.2 Bacteria isolation

A total of 64 water samples were obtained from the CWs, with 8 samples collected from each cell before and after spiking with CIP. The 79.69 % of these samples (51 samples) tested positive for one or more isolates. The 117 bacterial isolates were recovered from the samples, with 69 from samples before CIP and 48 from samples after spiking with CIP. These isolates were identified as *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Escherichia coli*. Fig. 28 depicts the macromorphological properties of the detected microorganisms. The distribution of the isolates is given in Table 16. These bacteria were confirmed by molecular identification shown in Fig. 28.



a) b) c)

**Figure 29: Macro-morphological characteristics of identified bacteria; *Klebsiella pneumoniae* (a), *Pseudomonas aeruginosa* (b) and *E. coli* (c)**

**Table 16: Rates of isolation of *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *E. coli* from various samples without and with CIP**

| | Samples without CIP | | | | Samples with CIP | | | |
|---|---|---|---|---|---|---|---|---|
| | C | CA | CI | M | C | CA | CI | M |
| Total samples collected | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| Samples positive for one or more isolates | 6 | 7 | 7 | 7 | 5 | 7 | 6 | 6 |
| Number of isolates | 13 | 19 | 18 | 19 | 7 | 14 | 12 | 15 |
| *Pseudomonas spp.* isolates | 4 | 5 | 6 | 5 | 1 | 2 | 3 | 4 |
| *Klebsiella spp*. isolates | 6 | 7 | 7 | 7 | 3 | 7 | 5 | 6 |
| *E. coli* isolates | 3 | 7 | 5 | 7 | 3 | 5 | 4 | 5 |
| % *Pseudomonas spp*. | 30.77 | 26.32 | 33.33 | 26.32 | 14.29 | 14.29 | 25.00 | 26.67 |
| % *Klebsiella spp*. | 46.15 | 36.84 | 38.89 | 36.84 | 42.86 | 50.00 | 41.67 | 40.00 |
| % *E. coli* | 23.08 | 36.84 | 27.78 | 36.84 | 42.86 | 35.71 | 33.33 | 33.33 |

C=Control, CA= *Cyperus alternifolius*, CI= *Canna indica* and M=Mixed plants

According to Table 16, *Klebsiella* spp. was the most prevalent isolate (n=48; 41%), followed by *E. coli* (n=39; 33.3%) and *Pseudomonas* (n=30, 25.6%). All bacterial strains experienced an overall decrease in the number of isolates under the influence of CIP. Klebsiella are Gram-negative bacteria that can be found singly, in pairs, or in chains. The genus *Klebsiella* is part of the order Enterobacteriales and belongs to the family Enterobacteriaceae. These opportunistic bacteria are naturally occurring and can be found in the environment and the gastrointestinal systems of many different animals (Gundogan, 2014). Their adaptability to various habitats is believed to be a result of separate sublineages acquiring niche-specific adaptations and corresponding metabolic adaptations that make them more adapted to a given environment. The majority of these organisms are found as saprophytes in soil, surface water, sewage, and plants. They can survive in the dust, water, and feaces of animal and human (Samanta & Bandyopadhyay, 2020). *Klebsiella* bacteria are major causes of nosocomial infections (Cooney *et al.,* 2014). They are the second-most prevalent cause of Gram-negative bacteraemia after Escherichia coli, and they frequently result in urinary and respiratory tract infections (Shi *et al.,* 2020).

The *E. coli* are Gram-negative bacteria with rounded ends. Their major habitat is the intestines of endotherms (Ishii & Sadowsky, 2008; Jang *et al.,* 2017; Osińska *et al.,* 2022; Poolman, 2016) which they alternate with other environmental habitats (secondary habitats) such as water, silt, and soils throughout their life cycle (Petersen & Hubbart, 2020). As a result, the presence of *E. coli* in drinkable water is utilized as a sign of contamination with human or animal excrement and is known as the "coliform index" (Percival & Williams, 2013). Both biotic and abiotic factors have the potential to affect the growth and survival of *E. coli* in their natural habitats. Temperature, the availability of water and nutrients, pH, and sun radiation are examples of abiotic variables. The existence of other microbes, as well as the capacity of *E. coli*' to consume resources, outcompete other microbes, and build biofilms in natural habitats, are all examples of biotic factors (Jang *et al.,* 2017). The *E. coli* may live in a variety of habitats, including wastewater, soil, and water, as well as plants, fruits and vegetables, raw meat, and unpasteurized milk (Osińska *et al.,* 2022). Although these bacteria are gut commensals, they can also cause intestinal and extraintestinal diseases, such as septicemia, meningitis, and urinary tract infections in humans and colibacillosis in poultry (Osińska *et al.,* 2022).

The *Pseudomonas* genus is naturally available in the environment. It has low growth requirements, employs a variety of carbon sources for growth, can endure concentrated salt solutions, and can withstand temperatures between 20$^{o}$C and 42$^{o}$C (Silva *et al.,* 2019). Several species can be found in human and animal waste, and many can multiply in water with adequate nutrition. They could cause a general decline in the microbiological quality of drinking water and increase customer complaints about taste and odor. As an opportunistic pathogen, *Pseudomonas aeruginosa* can infect people, especially those whose natural defenses may be compromised, such as the very young, the very aged people, or the immunosuppressed (Brandt *et al.,* 2017). A further significant fact is that they have established colonies in medical environment owing to their capacity for survival in different environments (Silva *et al.*, 2019). The pathogen *Pseudomonas aeruginosa* is a leading cause of nosocomial infections. Infections caused by *P. aeruginosa* are more severe and sometimes fatal. They are challenging to treat because their low antibacterial susceptibility and the frequent emergence of antibiotic resistance (Diaz *et al.,* 2018). The principal antibiotic resistance pathways of *P. aeruginosa*'s can be categorized as intrinsic, acquired, or adaptive (Plantin *et al.,* 2012). The inherent resistance of *P. aeruginosa* is comprised of low outer membrane permeability, development of efflux pumps that expel drugs from the cell, and the creation of antibiotic-inactivating enzymes. The *P. aeruginosa* acquired resistance can be established through horizontal transfer of resistance genes or mutational alterations. The adaptive resistance of *P. aeruginosa* involves the development of biofilm in the lungs of infected patients, where the biofilm acts as a diffusion barrier to prevent the bacterial cells from being exposed to antibiotics (Naveed *et al.,* 2019; Pang *et al.,* 2019). They have been used in traditional wastewater treatment for removal of different types of pollutants including antibiotics and achieved high removal rates (Zhong *et al.,* 2022).

Several researchers have found these bacteria species in wastewater samples. In a Tanzanian investigation that looked at wastewater from wastewater treatment ponds and receiving waters, 57 recovered isolates were found, and of them, 59.65% were *E. coli*, 47.37% were *Klebsiella spp*., 8.78% were *Proteus spp*., and just 1.75% were *Pseudomonas aeruginosa* (Ripanda *et al.,* 2023b). Another study was done to identify the determinants of resistance produced by Gram-negative bacteria in the influent and effluent of two wastewater treatment plants where the most frequent isolates were *Escherichia* spp. (57.1%,), followed by *Aeromonas* spp. (16.1%) and *Klebsiella* spp. (12.7%) (Mesquita *et al.,* 2021). In general, there are many different types of microbes that can exist in wastewater, with the type and quantity being greatly influenced by the socioeconomic factors and traditions of the people (Gupta & Ali, 2013; Okoh *et al.,* 2007).

Under the influence of CIP, there was a general decrease in the number of isolates for all bacterial strains. From 69 isolates recovered from samples before addition of CIP, *Klebsiella spp*. was the most prevalent isolate (n=27; 39.1%), followed by *E. coli* (n=22; 31.9%) and *Pseudomonas ssp* (n=20, 29.0%). Of 48 recovered isolates from samples after CIP addition, *Klebsiella spp*. was the most prevalent isolate (n=21; 43.8%), followed by *E. coli* (n=17; 35.4%) and *Pseudomonas* (n=10, 20.8%). These results showed that despite the decrease in number of isolates for all strains, there was a variation in relative number of isolates. Samples collected from control CW had less isolates compared to the CWs with plants. Growth of bacteria in CWs following CIP application indicates that bacteria may have acquired antibiotic resistance while breaking down antibiotics (Wang *et al.,* 2022). Similar conclusion was made by other researchers in a study to remove Sulfamethoxazole from synthetic wastewater in CWs (Zhang *et al.,* 2020). Multi-resistant strains of *Pseudomonas aeruginosa* were discovered in wastewater treatment facilities of a hospital complex in a Brazilian study. Some of the bacteria tested positive for resistance to all antibiotics tested, including gentamicin, ciprofloxacin, cefepime, imipenem, and aztreonam (Santoro *et al.*, 2015).

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

The first objective was to evaluate the performance of a full-scale CW in treating hospital wastewater and assess the prevalence of antibiotic resistant bacteria and antibiotic resistance genes in hospital effluent treated using the CW. Despite treatment in the CW, an analysis of the effluent for antibiotic resistance revealed a significant level of ARB and ARGs in the hospital wastewater. All bacteria tested positive for antibiotic resistance, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Furthermore, all bacteria contained ARGs such as *sul1, sul2, sul3,* $bla_{CTX\text{-}M}$, $bla_{TEM}$ *and* $bla_{SHV}$ where with *Klebsiella spp.* harbouring the most resistance genes, followed by *Pseudomonas spp*. and *E. coli.* Analysis of influent and effluent samples demonstrated a considerable decrease in physicochemical and biological properties, demonstrating the system performance in wastewater treatment. However, based on the parameters analyzed, the system does not generate acceptable effluent. The only physico-chemical parameters that were within the acceptable limits were turbidity and TSS. The number of colonies of *E. coli* in the wastewater discharged was within the allowed limits. The effluent was released with amounts of COD, $BOD_5$, and nitrates that exceeded the permissible limits. The presence of non-biodegradable organic matter, which may include xenobiotic chemicals such as surfactants, is suggested by a small variation in average $BOD_5$/COD ratio values between influent and effluent. The findings presented here show that, despite the removal of several physico-chemical parameters, the CW was not effective in removing ARB and ARGs from hospital wastewater. This may necessitate combining of CW with advanced wastewater technologies in order to completely remove ARB and ARGs which will prevent the spread of antibiotic resistance.

The second objective was to investigate the performance of selected plant species on removal of the selected human antibiotics from synthetic wastewater in experimental CW. Investigation on the removal of CIP, MET, and SUL from synthetic wastewater was conducted in an experimental CW planted with *Cyperus alternifolius*, *Canna indica*, and a mixture of these two plant species. It has been determined that CWs that include plants can remove more antibiotics than those without. Despite the fact that both plants performed well in terms of antibiotic removal, the data suggest that *Cyperus alternifolius* outperformed *Canna indica*. There was no statistically significant difference in antibiotics removal caused by combining the two plants in a CW. However, mixing plants, particularly ornamental plants, in CWs improves the visual appearance of the systems while treating wastewater. While CIP and MET were removed by more than 70%, SUL was removed by as much as 56%. It should be mentioned however, that this study involved batch experiments using synthetic wastewater and antibiotics concentrations that were higher than those found in real wastewater. Again, the experiments were conducted out in a greenhouse. Therefore, the same plants and antibiotics may produce different outcomes in real environments and real wastewater.

The third objective was to investigate how antibiotic exposure influences the diversity and abundance of bacteria found in the rhizosphere of various plant species in CW. The CIP was studied for its effect on microbial abundance and diversity in CWs. The findings of this study show that plants play an influence on microbial composition in CWs ($p < 0.05$). The CW with mixed plants had the greatest CFU, followed by CWs planted with *Cyperus altenifolius* and finally *Canna indica*, however the difference was not significant ($p > 0.05$). Microbial diversity changed substantially in CWs with mixed plants in response to CIP, however, the decrease in microbial abundance was not statistically significant ($p > 0.05$). This is related to bacteria adapting to the surroundings containing CIP. It generally accepted that microorganisms are in responsibility for the degradation of antibiotics. Antibiotics alter microbial density and diversity while also stimulating antibiotic resistance genes. This is an emerging challenge in wastewater treatment systems: Removing antibiotics from wastewater without introducing antibiotic resistance into the environment.

## 5.2 Recommendations

(i) Based on the effluent physicochemical parameters, the CW does not efficiently treat wastewater. The performance of the system may be impacted by substrate clogging, plants degradation, and insufficient wastewater pretreatment. Improving the efficacy of wastewater treatment requires intervention in the wastewater treatment system. It is recommended to recover the clogged CW, which may be accomplished by either cleaning the clogged substrate materials or returning them to the CW, entirely or partially replacing the clogged substrate with a fresh one, or exposing the clogged substrate to an oxidising agent such as $H_2O_2$. The typical approach of restoration is to replace clogged substrates with clean ones. The effect of clogging treatment, treatment costs, restoration time, and operation complexities should all be considered.

(ii) It has been established that harvesting plants in constructed wetlands has a favorable impact on pollutants removal and microbial abundance. According to the findings, it is recommended to plan for the optimal season and carry out harvesting as an operation and management approach to enhance the system performance. Furthermore, harvested biomass should be disposed of rather than left in the constructed wetland system because their decomposition releases carbon, nutrients, and elements into the system, which contributes to the composition of effluent and causes substrate clogging.

(iii) The presence of ARB and ARGs in wastewater effluents demonstrates the CW system's shortcomings in removing these newly emerging contaminants. It is recommended that hospital wastewater treatment systems (CW) be improved in order to properly remove antibiotics, ARBs, and ARGs and preserve public health. Since these pollutants are linked to the presence of antibiotics in the environment, it is recommended that actions be taken to limit the release of antibiotics into the environment. These include reducing unnecessary antibiotic use as well as improving antibiotic disposal. It is also advised to conduct monitoring and surveillance to track the existence and spread of ARB and ARGs in aquatic ecosystems in order to show the effects of antibiotic pollution.

(iv) We recommend key stakeholders to be involved in the science-policy interface to identify the occurrence of antibiotics in the environment, establish potential consequences, monitor, and control the discharge of antibiotics into the environmental systems. The scientific evidence should be used in revising the regulations and policies for monitoring and surveillance of antibiotics, ARB and ARGs, as well as in joining other international efforts to combat the problem.

(v) Considering that only *Cyperus alternifolius* and *Canna indica* were used to plant the experimental CWs in this study, it is recommended that many more native aquatic plants be researched for their capacity to remove pharmaceuticals from wastewater. Different plants should be evaluated to see whether they are more efficient in a single or mixed condition. This would be helpful in figuring out the best plant varieties for the removal of pharmaceuticals from CWs in Tanzania's climate. In addition, research should be conducted to determine how native plants react in habitats contaminated with pharmaceuticals such as antibiotics.

(vi) The pharmaceutical compounds detected in the wastewater sites belong to a wide range of therapeutic pharmaceuticals classes. This research investigated only three antibiotics, CIP, MET, and SUL. More research into the removal of other pharmaceuticals from other classes in single and mixed component mixtures is recommended. Long-term ecotoxicological effects of individual pharmaceuticals and/or their mixes, and environmental issues connected with the existence of pharmaceutical compounds and their metabolites in the environment should all be investigated in future research.

(vii) The studies were carried out in an experimental CW, in a green house, with synthetic wastewater for a short period of time. It is recommended to conduct large-scale testing with real wastewater samples collected from the environment. Future research should focus on pharmaceutical removal mechanisms, pharmaceutical toxicity to constructed wetland plants and the effects of key characteristics such as operating circumstances, hydraulic mode, configuration design, and environmental factors. Future research should consider the feasibility of integrating CW with other technologies to create a low-cost and effective means for removing antibiotics, ARBs, and ARGs from wastewater.

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# APPENDICES

## Appendix 1 Institutional Approvals: Introduction letter to DUWASA

THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY (NM-AIST)

OFFICE OF THE DEAN MATERIALS, ENERGY, WATER AND ENVIRONMENTAL SCIENCE (MEWES)

**In reply please quote:**
**Ref. No: NM-AIST/P.006/T20/6**

**Date: 2nd March, 2022**

Managing Director,
Dodoma Urban Water Supply and Sanitation Authority (DUWASA),
Chimwaga road No. 9, Block B,
P.O BOX 431,
**DODOMA.**

**INTRODUCING MR. PETRO KARUNGAMYE**

Dear Sir/Madam

The Nelson Mandela African Institution of Science and Technology (NM-AIST) is one in a network of Pan-African Institutions of Science and Technology located across the continent. This institution is located in Arusha Tanzania which solely offers Masters and PhD Degree Programmes. In this letter, the institution is humbly introducing Mr. Petro Karungamye who is a PhD student at NM-AIST with Reg. No. P006/T.20 in the program of Environmental Science and Engineering specializing in Environmental Science.

Petro is doing research on the potential of aquatic plants on removal of pharmaceuticals from wastewater in constructed wetlands. As part of the study, Petro will sample wastewater from selected treatment systems and analyse them to determine the occurrences and levels of frequently used pharmaceuticals in wastewater treatment facilities in Tanzania. Finally he will recommend the best way to optimize their treatments.

Kindly accord him any necessary assistance so that he can accomplish his research study.

Yours sincerely,

Prof. Revocatus Machunda
**Dean, School of Material Energy Water and Environmental Science**

**Appendix 2: Introduction letter to COSTECH**

THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

THE NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY (NM-AIST)

OFFICE OF THE DEAN MATERIALS, ENERGY, WATER AND ENVIRONMENTAL SCIENCE (MEWES)

**In reply please quote:** **Date: 2nd March, 2022**
**Ref. No: NM-AIST/P006/T20/07**

Tanzania Commission for Science and Technology (COSTECH),
P.O. Box 4302,
Ali Hassan Mwinyi Road,
Dar es Salaam, Tanzania.

**INTRODUCING MR. PETRO KARUNGAMYE**

Dear Sir/Madam

The Nelson Mandela African Institution of Science and Technology (NM-AIST) is one in a network of Pan-African Institutions of Science and Technology located across the continent. This institution is located in Arusha Tanzania which solely offers Masters and PhD Degree Programmes. In this letter, the institution is humbly introducing Mr. Petro Karungamye who is a PhD student at NM-AIST with Reg. No. P006/T.20 in the program of Environmental Science and Engineering specializing in Environmental Science.

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Kindly accord him any necessary assistance so that he can accomplish his research study.

Yours sincerely,

Prof. Revocatus Machunda
**Dean, School of Material Energy Water and Environmental Science**

## Appendix 3: Permission from DUWASA

THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF WATER

DODOMA URBAN WATER SUPPLY AND SANITATION AUTHORITY (DUWASA)

**Ref . No.: DUWASA/T.10/11/VOL.II/367** **16/03/2022**

Petro Karungamye,

College of Natural and Mathematical Sciences,

P.O.Box 338,

**DODOMA.**

**RE: PERMISSION TO CONDUCT REASERCH IN DODOMA WASTE WATER STABILISATION PONDS**

Refer to the above heading and your dated 3rd March, 2022 by which you requested for treated waste water sample.

2. We are glad to inform you that your request has been accepted, more instruction shall be given after reporting to the Human Resource Office at our main Office DUWASA.

3 Please note that the Authority will not be responsible for any cost incurred during the field work.

4. Yours faithfully,

Godfrey Kilolelo

**For: MANAGING DIRECTOR**

Tambukareli Ward, Salmin Street, DUWASA Building, Block B-Plot No. 9, Mkapa/A.H. Mwinyi Road, P.O. Box 431, Dodoma, Tel: +255262324245, Fax: +255262320060, Toll Free Call No.: 0800110078, Email: md@duwasa.go.tz, Website: www.duwasa.go.tz

## Appendix 4: Research permit from COSTECH

**UNITED REPUBLIC OF TANZANIA**

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

**TANZANIA COMMISSION FOR SCIENCE AND TECHNOLOGY**

***RESEARCH PERMIT***

| | |
|---|---|
| **Permit No.** | 2023-043-NA-2023-0297 |
| **Date Issued** | March 14, 2023 |
| **Researcher's Name** | PETRO NOVERT KARUNGAMYE |
| **Nationality** | Citizen of Tanzania |
| **Research Title** | POTENTIAL OF AQUATIC PLANTS FOR PHYTOREMEDIATION OF PHARMACEUTICALS |
| **Research Area(s)** | Arusha Dodoma |
| **Validity** | From March 14, 2023 To March 13, 2024 |
| **Contacts of local Collaborator (With Affiliated Institution)** | NM-AIST |

.............................................................
**Director Of Research Cordination And Promotion**

.............................................................
**Director General**

**IMPORTANT REQUIREMENTS**

- **A PI who wishes to continue with a research beyond the expiry date of the research permit should write to COSTECH two months before the operational permit's expiry date, to request for an extension or renewal of the permit.**
- **Research permit that involves collecting human, plant or animal materials / data that will be exported outside Tanzania must submit a signed Material Transfer Agreement (MTA), Data Transfer Agreement (DTA) between Tanzania host institution and the foreign counterpart. The MTA/DTA will indicate terms for collecting, storing/managing, transporting, disposal or returning of the materials/DATA to Tanzania after the closure of the research project.**
- **Any patent or intellectual property and royalty emanating from any research approved by the National Research Clearance Committee shall be owned as stipulated in the research proposals and in accordance with the IP policy of the respective research institutions.**
- **All researchers are required to report to a Regional Administrative Secretary (RAS) of the study area and present the introduction letter and activity schedule(plan) prior starting any research activity.**
- **All researchers are required to submit semi-annual, annual and final reports and all relevant publications made after completion of the research.**
- **All communications should be addressed to COSTECH Director General through rclearance@costech.or.tz; dg@costech.or.tz or +255 (022) 2700749; +255 (022) 2771358. Terms and conditions of the permit are found at www.costech.or.tz**

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Tanzania Commission for Science and Technology, Ali Hassan Mwinyi Road, P.O. Box 4302, Dar Es Salaam.
General line: +255(022) 277 1358, Fax: COSTECH, E-mail: dg@costech.or.tz,
Website: http://www.costech.or.tz

## Appendix 5: Introduction letter from COSTECH

**UNITED REPUBLIC OF TANZANIA**

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

**TANZANIA COMMISSION FOR SCIENCE AND TECHNOLOGY**

In reply please quote: **2023-043-NA-2023-0297** **Date: March 14, 2023**

Permanent Secretary,
President's Office,
Regional Administration and Local Government,
P.O. Box 1923,
**DODOMA.**

Dear Sir/Madam,

**INTRODUCTION LETTER ON RESEARCH PERMIT**

1. I wish to introduce **PETRO NOVERT KARUNGAMYE** , a citizen of **Tanzania** has been granted Research Permit No. 2023-043-NA-2023-0297 dated **March 14, 2023**
2. The permit allows him/her to conduct research titled "**Potential of aquatic plants for phytoremediation of pharmaceuticals** " under the terms and conditions as per the National Research Registration and Clearance Guidelines of 2022. The research will be conducted in **Arusha Dodoma**
3. COSTECH is therefore kindly requesting you to introduce the researcher(s) to relevant Regional Administrative Officer(s) and support with any necessary assistance and guidance under national laws and regulations.
4. Thank you for your cooperation.

.......................
**Dr. Amos M. Nungu**
**DIRECTOR GENERAL**

CC: Regional Administrative Secretary: **Arusha Dodoma**

Tanzania Commission for Science and Technology, Ali Hassan Mwinyi Road, P.O. Box 4302, Dar Es Salaam.
General line: +255(022) 277 1358, Fax: COSTECH, E-mail: dg@costech.or.tz,
Website: http://www.costech.or.tz

**Appendix 6: List of Plates**

**Plate 1: A green house at NM-AIST in which CW was made**

**Plate 2: Labor work, lining of the CW**

**Plate 3: Transplanted plants in CW**

**Plate 4: Irrigating the young plants**

**Plate 5: Monitoring the rate of plants growth**

**Plate 6: Field supervision by one of the supervisors (Prof. Mtei)**

**Plate 7: Dissolving of chemicals to make synthetic wastewater**

**Plate 8: Sample collection**

**Plate 9: CW at the Benjamin Mkapa Hospital in Dodoma**

**Plate 10: The CW outlet where effluent samples were collected**

**Plate 11: Wastewater observed above the gravels indicating clogging of the substrate**

**Plate 12: Harvested plants (dry) left in the CW**

**Plate 13: Harvested plants in the CW**

**Plate 14: A tortoise observed during wastewater sampling showing wetland services**

**Plate 15: Wastewater sampling at the inlet point to the CW**

**Plate 16: Wastewater effluent sampling along the stream**

## RESEARCH OUTPUTS

### (i) Publications

Karungamye, P. N. (2022). Potential of Canna indica in Constructed Wetlands for Wastewater Treatment: A Review. *Conservation*, *2*(3), 499–513. MDPI AG. Retrieved from http://dx.doi.org/10.3390/conservation2030034

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Karungamye, P., Rugaika, A., Mtei, K., & Machunda, R. (2022). The pharmaceutical disposal practices and environmental contamination: A review in East African countries. *HydroResearch*, 5, 99-107, ISSN 2589-7578

Karungamye, P., Rugaika, A., Mtei, K., & Machunda, R. (2023). The removal of ciprofloxacin from synthetic wastewater in constructed wetland. *HydroResearch*, 6, 138-146, ISSN 2589-7578 https://doi.org/10.1016/j.hydres.2023.04.001

Karungamye, P., Rugaika, A., Mtei, K., & Machunda, R. (2023). Antibiotic Resistance Patterns of Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa Isolated from Hospital Wastewater. *Applied Microbiology*, *3*(3), 867–882. MDPI AG. Retrieved from http://dx.doi.org/10.3390/applmicrobiol3030060

Karungamye, P., Rugaika, A., Mtei, K., & Machunda, R. (2023). Physicochemical and microbiological characterization of hospital wastewater in Tanzania. *Total Environment Research Themes*, 100075, ISSN 2772-8099,

### (ii) Posster Presentation