OCCURRENCE OF SELECTED ANTIBIOTICS IN SEDIMENTS AND SURFACE WATER OF RIVER SOSIANI, ELDORET, KENYA AND THEIR SORPTIVE REMOVAL USING BIOCHARS

BY

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A Thesis Submitted to the School of Sciences and Aerospace Studies in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Analytical Chemistry

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DECLARATION

Declaration by the Candidate

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DEDICATION

I hereby dedicate this thesis to my family, for both emotional and financial support, without which I would not have completed this Doctorate program.

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ABSTRACT

Antibiotics as therapeutics have been widely used in both human and veterinary medicine. Their indiscriminate use and improper disposal have led to environmental pollution, exerting adverse effects on non-target organisms. Various methods have therefore been developed to remove antibiotics from environmental matrices. Adsorption using bio-based adsorbents has been a preferred method for the removal of antibiotics from water as it is eco-friendly, cost effective and relatively simple. The general objective of this study was to assess the occurrence of selected antibiotics in water sediments, and surface water of River Sosiani, Eldoret, Kenya, and their sorptive removal using modified and unmodified biochar materials derived from water hyacinth and millet husks. The specific objectives of the study were; to quantify selected antibiotics in sediments and surface water from Sosiani River in Eldoret, to prepare and characterize chemically modified and unmodified biochar materials from water hyacinth and millet husks; to determine the sorption capacity of ciprofloxacin (CIP), sulfamethoxazole (SMX) and penicillin G (PNG) antibiotics from water using the prepared biochars, and to establish sorption isotherms and kinetics for adsorption of CIP, SMX and PNG by the biochar materials. The biochars were prepared by slow pyrolysis at 350 ℃ and 500 ℃, modified using potassium hydroxide and characterized using Fourier Transform-Infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM). Batch sorption equilibrium experiments were used to determine the capacity and efficiency of adsorption and data was fitted into different isotherm and kinetic models. Quantification of the antibiotics was done using liquid chromatography tandem mass spectrometry. The results showed that 30 compounds belonging to eight antibiotics classes: fluoroquinolones, sulfonamides, macrolides, penicillins, nitroimidazoles, lincosamides, diaminopyrimidines, and salfones were present in surface water and sediments from River Sosiani. Detectable concentrations of antibiotics in water samples ranged from 0.1 - 247 ng L^{-1} and 0.01 - 974 µg kg^{-1} in the sediments. Compared to other classes of compounds, fluoroquinolones were found in higher amounts in both water (56.02 ng L^{-1}) and sediment samples (16 µg kg^{-1}). Sulfamethoxazole had the highest concentration in water (247 ng L^{-1}), whereas penicillin G showed the highest concentrations in sediments (414 - 974 µg kg^{-1}). The results of FT-IR and SEM showed that there were differences in the physical and chemical properties of the biochar pyrolyzed at 350℃ and 500 ℃; hence differences in observed sorption characteristics. Biochar prepared at high temperature (BC500) had high adsorption capacity (> 80%) for the three antibiotics, while activated biochar showed high removal efficiency (83-88%) compared to non-activated biochar (50-70%). Sorption kinetics was best described by pseudo-second-order kinetics (R^2 > 0.99), while the sorption process could be best described by the Freundlich isotherm (R^2> 0.97), and intra-particle diffusion models. Therefore, multilayer sorption of tested antibiotics on heterogeneous biochar surfaces was the most plausible sorption mechanism. The study showed a high prevalence of pharmaceuticals in surface water and sediments, and are therefore potential ecological hazards. Biochar from both feedstocks showed potential as alternatives to commercial activated carbon for the removal of CIP, PNG and SMX from water. The occurrence of antibiotics in surface water and sediments of the river differed widely according to classes of the pharmaceuticals and was mainly influenced by anthropogenic activities within the sampled location. Continuous monitoring of these pollutants is imperative to inform the public on their fate in the environment and for the enactment of policies for interventions, and sustainable mitigation strategies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistance genes
BC	Biochar
BDL	Below detection limit
CI	Confidence Interval
CIP	Ciprofloxacin
EDX	Energy Dispersive X-ray spectroscopy
EDX-SEM	Energy Dispersive X-ray-Scanning Electron Microscopy
EDTA	Ethylenediamine tetraacetic acid
FQs	Fluoroquinolones
FTIR	Fourier transform infrared
HLB	Hydrophilic lipophilic balance cartridges
LC-MS/MS	Liquid Chromatography-Tandem mass spectrometry
LLE	Liquid-Liquid extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
MBC 350	Modified Millet Husk biochar prepared at 350 ℃
MBC 500	Modified Millet Husk biochar prepared at 500 ℃
NMBC 350	Non-Modified Millet Husk biochar prepared at 350 ℃
NMBC 500	Non-Modified Millet Hyacinth biochar prepared at 350 ℃
NWBC 500	Non-Modified Water Hyacinth biochar prepared at 500 ℃
NWBC 350	Non-Modified Water Hyacinth biochar prepared at 350 ℃
NOR	Norfloxacin
OFL	Ofloxacin

pH	Potential of hydrogen ions
PLE	Pressurized liquid extraction
PNG	Penicillin G
POCIS	Polar organic chemical interactive sampler
ppb	Parts per billion
ppm	parts per million
SAX	Strong anion exchange cartridges
SEM	Scanning Electron Microscopy
SMX	Sulfamethoxazole
SPE	Solid phase extraction
TEM	Transmission Electron Microscopy
UV-Vis	Ultraviolet-visible
WBC 350	Modified Water Hyacinth biochar prepared at 350 ℃
WBC 500	Modified Water Hyacinth biochar prepared at 500 ℃
WW	Waste waters
WWTP	Wastewater Treatment plant
XRD	X-ray diffraction
PLE	Pressurized liquid extraction
PNG	Penicillin G

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

According to Catteau *et al*. (2018), antibiotics are substances that are synthetic, semi-synthetic, and natural and have antimicrobial properties against bacteria or other single- celled pathogens. The word 'antibiotic' originates from the Greek terms "anti", which means "against", and "bios", which means "life"; the word "antibiotic" means "against life." The majority of antibiotics work against bacteria by either killing (bactericidal) or inhibiting their growth i.e., bacteriostatic (Manyi-Loh *et al*., 2018). A few antibiotics have antiprotozoal activity. For both veterinary and human treatment, antibiotics are frequently administered in three different ways: (1) orally (by mouth) when in the form of capsules, pills, or oral liquids; (2) through a parenteral route (topically or dermally) when they are in form of ointments, drops, creams, and sprays or for those which are poorly or not bioavailable when taken orally and in circumstances where clinical situations require rapid or higher antibiotic concentrations to be achieved in the body, and (3) intravenously (in extreme cases of severe infections) i.e., through an injection (Hermann & Hermann, 2020).

In human history, the discovery of antibiotics was revolutionary (Davies & Davies, 2010). Antibiotics have revolutionized medicine and saved many lives. Pharmaceutical compounds belonging to this class constitute a significant therapeutic category when it comes to the treatment of bacterial infections (Schmieder & Edwards, 2012; Sriram *et al*., 2021). Antibiotics are primarily employed in the practice of veterinary medicine to avert and treat bacterial diseases in animal husbandry and to treat bacterial diseases in human therapy (Kümmerer, 2009; Thiele-Bruhn, 2003).

Antibiotics are widely used as growth promoters in animal feed, in addition to their main applications in the treatment of particular ailments in both humans and animals (Cowieson & Kluenter, 2019; Kümmerer, 2009). Antibiotics added to animal feed accelerate the animal's attainment of market weight by enhancing its absorption capacity. The proliferation of these applications has resulted in a surge in antibiotic usage (Aminov, 2009). The use of antibiotics to promote growth has been prohibited or restricted in several countries (e.g. European Union and the United States of America) countries, however, the practice is still common in many others (Manyi-Loh *et al*., 2018).

Antibiotic residues from animal and human use have the potential to infiltrate the environment through various routes. These pathways include the discharge of wastewater effluent, runoff from land that has been treated with agricultural or human waste, and leaching. Antibiotics may also find their way into the environment through pharmaceutical manufacturing facilities and the dumping of partially used medications. The prevailing consensus is that the biggest source of antibiotic contamination in surface and wastewater is home effluents (Zheng *et al*., 2012). However, effluents from pharmaceutical business setups and hospitals are also a major cause for worry (Brown *et al*., 2006). According to Hoa *et al*. (2011) and Zheng *et al*. (2012), the intensive aquaculture practices used to produce food may eventually lead to the unintentional release of antibiotics into nearby surface waters.

Antibiotics often experience partial metabolism after application, with a significant fraction excreted in their original form or as conjugated molecules that can revert back to the parent antibiotic. One prominent example is fluoroquinolones (FQs), which are mostly eliminated as unmodified molecules in urine and are recommended

at doses between 300 and 600 mg daily for therapeutic purposes. As a result, these substances end up in municipal or hospital wastewater (Lindberg *et al*., 2004), greatly increasing the amount of antibiotic residues in receiving waters. Consequently, sewage sludge that are used as fertilizers in agriculture frequently contains antibiotic pollutants (Thiele-Bruhn, 2003). These residues are subsequently introduced into environmental water systems, particularly surface water, by runoff from such contaminated agricultural lands.

Thus, the continued widespread use of antibiotics in human medicine and livestock production has led to their frequent detection in a variety of environmental matrices (Manyi-Loh *et al*., 2018). Antibiotics have been detected in various sources including hospital wastewater effluents (Burch *et al*., 2019; Diwan *et al*., 2009; Gobel *et al*., 2004; McArdell *et al*., 2003; Miao *et al*., 2004), Wastewater Treatment Plant (WWTP) effluents (Barancheshme & Munir, 2018), soils, surface waters, groundwater (Zhang *et al*., 2018), sediments, biota, WWTP biosolids, drinking water (Williams & Kookana, 2018), and natural waters (Hilton & Thomas, 2003; Reverté *et al*., 2003; Vanderford *et al*., 2003; Yang & Carlson, 2003). Antibiotic concentrations in specific wastewater effluents, sediments, and soil range from nanograms per liter (ng L^{-1}) to low levels of micrograms per liter (μg L^{-1}), and in rare situations, even greater amounts in milligrams per liter (mg L^{-1}). Hence, it is crucial to consistently monitor antibiotics in surface water and water sediments. As one of the objectives, this study determined the amounts of selected sulfonamides, fluoroquinolones and β-lactam antibiotics in sediment and surface water from River Sosiani in Eldoret town, Uasin Gishu County.

Grenni *et al*. (2018), Polianciuc *et al*. (2020), Thiele-Bruhn & Beck (2005), and Michelini *et al*. (2012) found out that the presence of antibiotic residues in the environment can affect aquatic and soil organisms in addition to plants. Antibiotic residues may alter the structure of naturally occurring bacterial populations (Allen *et al*., 2010). These alterations can have repercussions on organisms that are not the intended targets but are nonetheless crucial to ecology (Woegerbauer *et al*., 2015). These residues increase the harmful effects of antibiotics while also causing bacterial resistance, which raises the possibility of the creation of (multi)resistant infections that are harmful to both animals and human beings (Forsberg *et al*., 2012; Heuer *et al*., 2011).

In addition to the emergence of genes and bacteria resistant to antibiotics, exposure to antibiotics in the environment has been linked to a number of hazardous ecological impacts. For example, the sulfonamide sulfamethoxazole and the fluoroquinolone levofloxacin caused significant phytotoxicity in two aquatic plants, *Myriophyllum sibiricum* and *Lemna gibba* (Brain *et al*., 2004). Ciprofloxacin, a fluoroquinolone, has been shown to decrease photosynthesis by interfering with the photosynthetic pathway via DNA gyrase inhibition (Aristilde *et al*., 2010). Ciprofloxacin and its photodegradation products are genotoxic, according to Garcia-Käufer *et al*. (2012). González-Pleiter *et al*. (2013) investigated the toxicity of five different antibiotics, both singly and in mixes, to two representative aquatic organisms: the *cyanobacterium Anabaena* CPB4337 and the green alga *Pseudokirchneriella subcapitata*. The study found that the cyanobacterium was more vulnerable to exposure than the green algae, and that erythromycin (a macrolide) was extremely harmful to both organisms. In this study, the maximum ambient concentration found was compared to the anticipated no-effect value to compute the risk quotient, which

was then used to assess the ecological consequences of antibiotics (Yin, 2021).

Antibiotics contamination is therefore a major global threat due to the associated development of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in addition to other toxicological effects. Antibiotics such as sulfamethoxazole, trimethoprim, and ofloxacin have been detected in influents as well as effluents of wastewater treatment plants (Barancheshme & Munir, 2018). This is because traditional wastewater treatment plants (WWTPs) are not designed to remove antibiotics during the treatment process, which results in the release of these substances into the receiving environment, including river surface water (Halling-Sørensen *et al*., 1998; Zuccato *et al*., 2010). Therefore, there is a need to devise methods for the removal of antibiotic residues from WWTPs.

There are a lot of different physical, chemical, and biological ways to remove antibiotics out of water. Adsorption is one of the most popular methods because it is easy to use, is not harmful to the environment, and is cost effective (Dutta & Mala, 2020; Eniola *et al*., 2019). Biochar adsorbents made from farm waste have gotten a lot of attention from researchers lately because of their availability and efficiency compared to other sources (Kambo & Dutta, 2015).

Water hyacinth, or *Eichhornia crassipes,* is a floating plant that has become a valuable bioresource that could be used to clean up wastewater (WW). This plant is mostly found in Kenya in Lake Victoria and it impacts negatively the environment and the economy (Ajayi & Ogunbayio, 2012; Isichei & Okieimen, 2014; Omara *et al*., 2019a). This plant is known for growing rapidly; in just 8 months, it can produce up to 60,000 plants, which can cover an area of 0.4 ha (1 acre) of natural watery surface (Ajayi & Ogunbayio, 2012).

The millet plant can also be a good feedstock for biochar production and is readily available. In the present work, the starting materials for preparing plant residue-derived biochar were water hyacinth biomass and millet husks. The goal was to remove representative fluoroquinolone, sulfonamides, and β-lactam antibiotics from surface water and water sediments in the River Sosiani, Eldoret, Kenya.

1.2 Statement of Research Problem

The persistent and widespread use of antibiotic drugs in both human medicine and animal farming has resulted in their frequent presence in many environmental matrices (Alderton *et al*., 2021; Bombaywala *et al*., 2021; Ngigi *et al*., 2020). Following their administration, antibiotics are frequently subject to partial metabolism in humans, resulting in a substantial proportion of the antibiotic being excreted either in its original form or as conjugated compounds that can be converted back into the original antibiotic. The majority of these chemicals are eliminated in their original form in the urine and then released into hospital sewage or municipal wastewater. In the end, this results in the receiving waters accumulating antibiotic residues (Alderton *et al*., 2021).

Presence of antibiotic residues in the environment can cause adverse eco-toxicological effects in addition to the formation and development of ARGs and ARB. Antibiotics residues have been detected in diverse environmental matrices including surface water and sediments (Bombaywala *et al*., 2021; Chen *et al*., 2019; González-Pleiter *et al*., 2013; Matejczyk, 2020; Robles-Jimenez *et al*., 2021). To fully assess the effects of antibiotics in the environment, it is important to quantify and continuously monitor the residual amounts present in target places. There is inadequate information on residual antibiotics in sediments and surface water in Kenya (Ngigi *et al*., 2020;

Ngumba *et al*., 2016a), and ecological impact assessment, a knowledge gap that this study is addressing. Likewise, the environmental effects of residual antibiotics can be lowered or eliminated through enhanced sorption using appropriate adsorbent materials. Conventional WWTPs are not designed to eliminate antibiotic residues, and residual compounds in the effluents pose a contamination risk to receiving surface water and sediments, and this is of concern (Kraemer *et al*., 2019). This underscores the need for developing adsorbents that can be used in the removal of antibiotic residues from aqueous systems.

1.3 Justification of the Study

Antibiotic residues in environmental aquatic systems are of global concern (González-Pleiter *et al*., 2013; Robles-Jimenez *et al*., 2021). Residual amounts of antibiotics in surface water and sediments have been used in environmental quality management as an indicator of the extent of contamination (Hanna *et al*., 2018; Polianciuc *et al*., 2020). Establishing residual antibiotics in water sediments and surface water is important for baseline information, bridging the existing knowledge gap, and can be applicable in policy formulations. Further, the potential ecological risks were evaluated to establish the extent of health risks that residual antibiotics may pose to the environment and biota.

Adsorption process is an eco-friendly, cost effective and relatively simple method that is widely used for antibiotics removal from water (Dutta & Mala, 2020; Eniola *et al*., 2019; Shikuku *et al*., 2018). Currently, agricultural feedstock-based biochars are being explored widely for environmental (surface water, sediments among others) remediation due to biomass abundance exceeding alternative use. It is a sustainable method for management of agricultural waste biomass. Little information is available

on the adsorption of fluoroquinolones, sulfonamides and β-lactam antibiotics using novel biochar materials from plant biomass. This study evaluated the adsorption and removal capacity of selected fluoroquinolones, sulfonamides and β-lactam antibiotics from surface water using modified and unmodified biochar from water hyacinth and millet husks. This provided an environmentally sound and friendly way of removing antibiotic residues from surface water. This in turn lowers their mobility and reduces their effects in the environment.

1.4 Objectives of the Study

1.4.1 General objective

The main objective of the study was to assess the occurrence of fluoroquinolones, sulfonamides and β-lactams antibiotics in surface water and sediments, and their sorptive removal using modified and unmodified biochar materials derived from water hyacinth and millet husks.

1.4.2 Specific objectives

The specific objectives were to:

i. Quantify antibiotics in sediments and surface water from River Sosiani, Eldoret, Kenya.
ii. Evaluate the ecological risks of antibiotic residues in surface water from River Sosiani.
iii. Characterize biochar derived from water hyacinth and millet husk biomass.
iv. Determine the sorption capacity of selected fluoroquinolones, sulfonamides and β-lactam antibiotics in surface water from River Sosiani on water hyacinth- and millet husk-derived biochars.
v. Establish the sorption isotherms and kinetics for adsorption of selected

fluoroquinolones, sulfonamides and β-lactam antibiotics onto biochar derived from water hyacinth and millet husks.

1.5 Research Hypotheses (null hypotheses)

The study hypothesizes that;

Ho_1: Sediments and surface water from River Sosiani do not contain significant amounts of antibiotics.

Ho_2: Antibiotic residues in surface water from River Sosiani do not pose significant ecological risk.

Ho_3: Both, water hyacinth and millet husk biochars do not have similar physical and chemical properties.

Ho_4: There are no significant differences in the sorption capacity of water hyacinth- and millet husk-derived biochars in adsorption of the selected fluoroquinolones, sulfonamides and β-lactam antibiotics in surface water from River Sosiani.

Ho_5: The biochar materials do not possess the same sorption isotherms and kinetics for sorption of selected fluoroquinolones, sulfonamides and β-lactam antibiotics.

1.6 Significance of the Study

The study establishes the amounts of selected antibiotics in sediments, and surface water of River Sociani hence providing the basis for evaluating the corresponding eco- toxicological effects and assessing and managing the associated risks. It also prepares low-cost adsorbents from locally available materials that can be incorporated into water treatment setups to remove fluoroquinolones, sulfonamides and β-lactam antibiotics. Similarly, residual antibiotics in aquatic systems cause adverse direct and indirect effects on the environment and induce the selection for antibiotic resistance

leading to the formation of ARGs and ARBs. This work proposes organic sorbent materials for the effective removal or immobilization of antibiotics in water to hinder or minimize their environmental effects from persisting or escalating.

CHAPTER 2

LITERATURE REVIEW

2.1 Historical Perspective and Current Trends in Production and Uses of Antibiotics

Antibiotics (less commonly known as antibacterials) are among the most used drugs worldwide (Browne *et al*., 2021). Their discovery dates back to the times of the French bacteriologists Jean Paul Vuillemin, Louis Pasteur and Robert Koch (1877) who discovered that an airborne *Bacillus* was bacteriostatic against (could inhibit the growth of) *Bacillus anthracis* (Landsberg, 1949). Much later (1947), these were named antibiotics by an American microbiologist, Selman Waksman, who used the term in journal articles (Saxena, 2015).

It is easier to comprehend the history of antibiotics in two distinct contexts: (1) its early history, and (2) its modern history. Regarding the former, many texts have indicated that in the past, the Greeks and Indians used molds and other herbal treatments to treat infections (Wachtel-Galor *et al*., 2011). For example, moldy bread has long been used as a treatment for infections and wounds by the Greeks and Serbians (Sulaiman et al, 2015). It was also said that peasants in Russia treated infected wounds with warm earth (Shah, 2011). Other remarkable examples include Babylonian physicians treating eye conditions with frog bile and sour milk concoctions, and Sumerian physicians giving beer soup to patients along with adjuvants made of turtle shells and snake skins (Shields, 2020). Oil cakes were widely utilized by Sri Lankan army forces as desiccating and antibacterial preparations (Selvarajan *et al*., 2023). The use of mold as a cure was first supported by the Englishman John Parkington in 1640, marking the beginning of the current era of antibiotics. Sir John Scott Burdon-Sanderson first described the antibacterial

properties of culture fluid coated in mold in 1870 (Nikolic *et al*., 2023). *Penicillium glaucium*'s effect on human tissues was studied by Joseph Lister in 1871. In 1875, John Tyndall presented the *Penicillium* fungus's antibacterial activity to the Royal Society. Two years later, Louis Pasteur, a French scientist, proposed the theory that bacteria may, in theory, kill other germs (*Bacillus anthracis*) (Smith, 2012). After that, in 1897, Ernest Duchesne reported that giving the mold *Penicillium glaucium* to typhoid-infected guinea pigs resulted in their recovery (Duchesne, 1897). The most significant discovery regarding antibiotics is credited to Sir Alexander Fleming, who in 1921 published the first report on the enzyme lysozyme and in 1928 developed penicillin from the fungus *Penicillium notatum* (Britannica, 2023). The latter findings proved to be extremely effective in treating bacterial diseases, specifically gangrene, syphilis, and tuberculosis (Aminov, 2010). Gerhard Domagk later made the discovery of the antibiotic sulphonamide (Prontosil) in 1932 (Jesman *et al*., 2011).

With the discovery of penicillin in 1928 and streptomycin in 1943, the era in which antibiotics dominated the treatment of infectious diseases began. Between 1950 and 1960, a number of other antimicrobials had been found (Davies, 2006). Indeed, because twenty entirely new families of antimicrobials were developed and rapidly advanced during this period, the years 1930 –1962 are sometimes referred to as the "golden age" of antimicrobial inventions. For almost 60 years, many of these benefited humanity (Davies, 2006; Coates *et al*., 2011; Mantravadi *et al*., 2019). Thereafter, streptomycin - the first aminoglycoside - was introduced, which was a significant development. The US scientists Waksman, Schatz, and Bugie made this extremely potent, broad-spectrum antibiotic, which works by inhibiting the formation of bacteria proteins (Woodruff *et al*., 2014; Schatz *et al*., 2005) from *Streptomyces griseus*, and it was first employed clinically in 1944 (Krause *et al*., 2016).

In the last four decades of the twentieth century, there was an introduction of many new antibacterial chemicals that, when combined, significantly improved the quality of life for people. Four generations of quinolones were developed, each with somewhat better pharmacokinetics but identical pharmacodynamics (Andersson & MacGowan, 2003). Significant progress was also achieved in the development of aminoglycosides. Following the 1943 introduction of streptomycin, neomycin (1949), kanamycin (1957), and gentamicin (1963) were introduced. Many regions of the world still make extensive use of this. Netilmicin (1967), tobramycin (1974), and amikacin (1976) followed (Krause *et al*., 2016). These aminoglycosides all functioned similarly to streptomycin, inhibiting the production of microbial proteins by binding irreversibly to the ribosome of 30S microorganisms (Gonzalez, 1998).

Teicoplanin, a glycopeptide antibacterial discovered in the middle of the 1970s, functions by preventing the formation of cell walls, much like vancomycin. Teicoplanin is still a glycopeptide, membrane biosynthesis inhibitor, hence pharmacodynamically it does not belong to a novel class of drugs (Beauduy *et al*., 2018).

Despite being developed in 1945, cephalosporins weren't used in medicine until 1964 (Abraham, 1987). There are now five generations of cephalosporins in use in therapeutics (Pandey & Cascella, 2020), all being members of the same class of β-lactam antibiotics that work similarly to penicillin by inhibiting the formation of cell walls (Drawz & Bonomo, 2020). Cephalosporin compounds were successively introduced into the pharmaceutical business due to their growing pharmacokinetic benefits. Cefepime, a fourth-generation cephalosporin, was first brought into clinical use in 1994 to treat moderate-to-severe infectious disease conditions, such as

pneumonia, simple and complex urinary tract infections (UTIs), skin and soft tissue infections, intra-abdominal infections, and febrile neutropenia (Endimiani *et al*., 2008). It is noteworthy, nonetheless, that Cefepime resistance has subsequently been extensively documented (Chong *et al*., 2010).

The structural relationship between the carbapenems, which include doripenem, ertapenem, imipenem, and meropenem, and other β-lactam antimicrobials work by inhibiting cell walls, and they are classified as membrane- and cell-wall-active and lactam antibiotics (Beauduy *et al*., 2018). Ipenem, the first drug in the carbapenem family to be utilized in a clinical setting, was developed in the United States in 1985. Carbapenems, the most adaptable class of antibiotics, have shown exceptional effectiveness against both Gram-positive and Gram-negative bacteria. As the most dependable last-resort treatment for bacterial infections, carbapenems are saved as a potential lifesaver for patients with infections from highly resistant pathogens that could be fatal. They are less vulnerable to the majority of factors that determine beta-lactam resistance and are very effective against a broad range of bacterial species (Paterson, 2000). The first reports of *Klebsiella pneumoniae* resistance to imipenem and meropenem were published in 2001 (Yigit *et al*., 2001). But in recent times, multidrug-resistant harmful bacteria have become more widespread and evade even this last line of defense against infectious diseases (Namdari *et al*., 2021).

Among the many types of antibiotic medications are penicillins. As a penicillin antibiotic, amoxicillin has been widely successful in the market over the years, either by itself or in conjunction with clavulanic acid (Augmentin, Amoclan, Augmentin XR). Although it works similarly to other penicillins, its present uses are limited due to widespread antibiotic resistance (Beauduy *et al*., 2018). Like tazobactam and

sulbactam, clavulanic acid is a drug that is used in combination with other antimicrobials. Instead of being considered antimicrobials, these compounds are thought to be microbial β-lactamases enzyme inhibitors (Payne *et al*., 1994).

The macrolide class of antibiotics was the other significant advancement. The first antibiotic of the macrolide class, erythromycin, was made and put to use in 1952. Subsequent medications in this class, such as roxithromycin, azithromycin, and clarithromycin, showed some pharmacokinetic benefits (Bahal & Nahata, 1993). Like the other antimicrobials that had been shown to have this mode of action, the fundamental mechanism of action of macrolides is to bind to the bacterial 50S ribosomal subunit and suppress microbial protein synthesis (Dinos, 2017). Trimethoprim, which was first used in 1962, came after macrolides. Urinary tract infections are the main condition for which trimethoprim is used. By binding to dihydrofolate reductase, the compound inhibits the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF), an essential precursor in the route that leads to the creation of thymidine. This interference prevents the synthesis of bacterial DNA during the process (Gleckman *et al*., 1981). Historically, sulfamethoxazole, an inhibitor of dihydropteroate synthase, an enzyme implicated in an alternative pathway farther upstream, was often used in conjunction with trimethoprim. Through possible synergistic effects, it was believed that the two medicines would reduce the emergence of resistance (Gleckman *et al*., 1981; Eliopoulos *et al*., 2001). However, the combination became less effective with time, and the major potential adverse effects of the sulfonamide led to a greater reliance on the single medication. Drug resistance to trimethoprim is still a serious problem (Eliopoulos *et al*., 2001).

During this time, a class of antibiotics known as the quinolones was developed and steadily improved. These antibiotics are classified as bactericidal since they kill bacteria directly and interfere with the activity of DNA gyrase, topoisomerase IV, and type II topoisomerases in bacteria. This causes the enzymes to become toxic and causes long- term double-strand breaks in the bacterial chromosome. Despite the global rise in antibiotic resistance, these medications are still used. Among the beneficial innovations were new formulations; a large number of injectables from that generation are still in use today (Pham *et al*., 2019; Fàbrega *et al*., 2009).

Thus, although these forty years were responsible for a considerable incremental improvement of antimicrobial medications in classes that already existed, there was minimal fundamental innovation throughout this time. It is not surprising that when microorganisms are exposed to "me-too" agents in ways that are similar to those that have already been exploited, they soon become resistant to them. However, only two new groups of antimicrobials have been produced since 1962 (Butler & Buss, 2006; Coates *et al*., 2011). Only three of these novel classes of antibiotics were licensed for use in treating infectious infections in humans, and one class was only allowed to be applied topically (Spellberg et al., 2008). Table 2.1 displays the chronological evolution of antimicrobials. Few antimicrobial medications were developed in the first half of the twenty-first century, and the majority of those that were there had restricted uses (Cheesman *et al.*, 2017; Silver, 2011).

Table 2 1: Antibiotics development between1935 to 2015

Year of development	Antimicrobial classes
1935	Sulphonamides
1941	Penicillins
1944	Aminoglycosides
1945	Cephalosporins
1949	Chloramphenicol
1950	Tetracyclines
1952	Macrolides/Lincosamides/Streptogramins
1956	Glycopeptides
1957	Rifamycins
1959	Nitroimidazoles
1962	Quinolones
1968	Trimethoprim
2000	Oxazolidinones
2003	Lipopeptides
2015	Teixobactin

Dhingra *et al*., 2020

Consequently, an international research initiative focused on developing new tactics to counter multidrug resistance. Hospitalization, hospital stays, and healthcare costs increased significantly in the early 21st century due to infections caused by antibiotic-resistant microbes such as gram-negative *bacilli* (GNB) and gram-positive pathogens like *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacterbaumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species (Kallen *et al*., 2010; Arias & Murray, 2009; Fauci & Morens, 2012). Using advanced technology, later research on "genomics and combinational chemistry" usually failed to find new compounds with the necessary strong antibacterial action (Lewis, 2013). A number of antimicrobials that have been developed in the past 20 years have already faced drug resistance issues, such as Daptomycin, Tigecycline, Retapamulin, Fidaxomicin, Bedaquiline, and Televancin (Dhingra *et al*., 2020).

In conclusion, the manufacturing and use of antibiotics have increased exponentially since their discovery. Between 100,000 and 200,000 metric tons of antibiotics are used worldwide; out of these, beta-lactam and fluoroquinolone antibiotics are the

most widely used (Serna-Galvis *et al*., 2019). Two thirds of antibiotics used in human medicine are used throughout Europe, while usage varies by area (Ezzariai *et al*., 2018; Filippini *et al*., 2014). When it comes to the entire continent of Africa, South Africa has been the biggest antibiotic consumer. Regarding the Asian region, China (Qiao *et al*., 2018) and South Korea are the top antibiotic users (Van Boeckel & Laxminarayan, 2017).

2.1.1 Global consumption of antibiotics

Global antibiotic use rose from 21.1 to 34.8 billion defined daily doses (DDDs) between 2000–2015, a 65% rise that was linked to high levels of consumption in low- and middle- income countries (Klein *et al*. 2020). It was discovered that the Middle East, Europe, and North America had high consumption rates (Browne *et al*., 2021). Nonetheless, consumption was lower in low- and middle-income countries (LMICs) even though these nations had a greater rate of fatal bacterial diseases (GDB, 2019). Antibiotic use in LMICs is steadily rising and could soon approach high-income country levels (Klein et al., 2020). Antimicrobial consumption is known to have declined before the COVID-19 pandemic, with wealthy countries showing a larger apparent decline in antimicrobial consumption than developing ones (8.4% and 1.2%, respectively). By 2020, the consumption of antimicrobials rose by 11.2% in comparison to 2019, this due to overuse. Patients with severe or critical COVID-19 recorded the highest rate of antibiotic use. When comparing specific timeframes, the global consumption of antibiotics fell by 18.7% between April and August of 2020. In particular, the use of antibiotics declined dramatically in both developing and industrialized nations (28.0% and 16.8%, respectively). But during and after the pandemic, this trend shifted. Over the last two decades, there has reportedly been a 46% increase in the global rate of antibiotic usage (Browne *et al*., 2021).

Important public health issues include the misuse of antibiotics as well as the inability to obtain medicines (Mendelson *et al*., 2016). High income nations are thought to consume antibiotics at some of the highest rates; in the USA, for example, up to 30% of antibiotic prescriptions are reported to be unnecessary. According to projection, sub-Saharan Africa, an area marked by the highest frequency of sepsis and high prevalence of illness burden, has the lowest rate of antibiotic usage (Rudd *et al*., 2020). Even while access to and timing of antibiotics is a major contributing factor to the high death rate among children under five in low- and middle-income countries, high rates of improper antibiotic usage have also been found in many of these countries (Liu et al., 2015; Laxminarayan et al., 2016; Sulis & Gandra, 2021), particularly in South and Southeast Asia, where a recent study found significant rates of self-medication and unlicensed antibiotic sales (Do *et al*., 2021). Therefore, since improper use of antibiotics and inadequate access to high-quality healthcare commonly coexist within one health system, it is imperative to address both issues in order to ensure correct treatment of bacterial illnesses and to maintain the efficacy of antibiotics (Mendelson *et al*., 2016; Sulis *et al*., 2021).

Antibiotic usage in hospital settings is well-known for being prevalent. In order to reduce the danger of antibiotic resistance, which increases the burden of infectious diseases, treatment recommendations advise using antibiotics as prescribed (Maina *et al.,* 2021). Antibiotic usage in hospitals in low- and middle-income nations has been documented (Omulo *et al*., 2017), and the majority prefers self-medication in areas where abuse is prevalent (Do *et al*., 2021). Misuse is mostly encouraged by the ease with which antibiotics may be acquired without a prescription, a procedure that is described as less time-consuming, less expensive, and more convenient than going to medical facilities for a checkup (Do *et al*., 2021). Antibiotic use was found to be

highly frequent in a research on antibiotic use conducted in an informal community in Nairobi; 87% of respondents in the initial survey and 70% in the follow-up survey reported using antibiotics. Despite a dearth of knowledge on the proper use of antibiotics, the aforementioned high incidence of antibiotic use occurred (Omulo *et al*., 2017).

Access to pertinent usage data is essential for improving the appropriateness of antibiotic use. Regretfully, compared to other regions, data on the usage of antibiotics in African countries is lacking (Ahoyo *et al*., 2014, Versporten *et al*., 2018). Point prevalence surveys (PPS) have been widely utilized in hospitals and patient settings to monitor antibiotic use and prescription quality (Gharbi *et al*., 2016, Okoth *et al*., 2018). According to certain research, more than 80% of Kenyan patients utilize antibiotics in both inpatient and outpatient settings. The problems with prescription quality, such as the use of proprietary medicine names and incomplete prescriptions, are also highlighted by these investigations (Mulwa *et al*., 2015, Okoth *et al*., 2018).

2.1.2 Global use of antibiotics in animal production

The predicted antimicrobial use based on the amount of active ingredient used globally in 2020 for cattle, sheep, chicken, and pigs was 99,502 tonnes (95% CI: 68,535–193,052). Antimicrobial usage intensity in population correction units (PCU) (mg/PCU) is currently trending, and by 2030, it is expected that global antimicrobial use will have increased by 8.0% to 107,472 tons (95% CI: 75,927–202,661) (Mulchandan *et al.,* 2023).

Antibiotics have been utilized for both disease prevention and treatment in food animal production over the years. As a result, antibiotics have been employed for non-

medical goals such as promoting growth and improving feed proficiency (Cully *et al.,* 2014, (Rushton *et al.,* 2015). Although there are some benefits for both the animals and the producers from this application, there are also several environmental problems related to the practice (Finley *et al.,* 2013; Durso *et al.,* 2014).

Economic benefits are associated with the usage of antibiotics primarily as growth promoters and feed enhancers (Durso *et al.* 2014). In the 1950s, the practice of using antibiotics in food animals was implemented in response to the growing demand for food. Antibiotics are still used as growth promoters in several nations today due to a lack of effective laws (Founou *et al.,* 2016; Eagar *et al.,* 2012). Although their use as growth promoters have been outlawed in Europe and the USA, prophylactic and metaphylactic uses are still permitted (Woolhouse *et al.,* 2015; Argudin *et al*., 2017). Antibiotics are mostly used in food animals in the United States and Europe (Elliott *et al.,* 2017); although several developing countries have also seen a sharp rise in this application (Founou *et al*., 2016). By 2030, it is predicted that antibiotic use will rise by 67%, with China, South Africa, India, Russia, and Brazil experiencing increases nearly twice as high (Van Boeckel *et al.,* 2015).

For more than 60 years, the United States and other countries have been using antibiotics as growth-promoting drugs. In 1946 and 1950, respectively, favorable results on productivity in pigs and poultry were initially noted. Among the first antibiotics to be shown to affect young birds' growth were penicillin, oxytetracycline, bacitracin, aureomycin, and streptomycin, when added to animal feed at concentrations as low as 1– 20 parts per million. Beginning with the introduction of intensive farming practices in the early 1950s, antibiotics were approved for use in food animals with the goals of maximizing illness prevention, enhancing feed

transformation, and accelerating growth rates (Coglian *et al*., 2011). In terms of the variety of antibiotics used in food animals, between the 1950s and 1960s, the rationale for their use and the routes by which they were administered expanded. Although all antibiotics and associated medications were outlawed in the European Union in 2006 to promote livestock growth, the United States has only recently implemented regulations pertaining to the use of antimicrobial pharmaceuticals in animals. The new regulations only allow the use of these medications for therapeutic or preventative purposes under veterinary supervision, excluding the use of medically important antibiotics for the aim of promoting growth. Although this is encouraging, many developing nations lack laws allowing the use of antibiotics in cattle. This is according to the World Organization for Animal Health (Manyi-Loh, et al., 2018). The use and management of antimicrobials are still mainly unregulated in African nations. South Africa is an exception, having created and put into effect an Antimicrobial Resistance National Strategy Framework for 2014–2024 (ARNS, 2014); one of whose strategic goals is to encourage the responsible use of antibiotics for the health of humans and animals.

Antibiotic use in food animals carries several dangers, which is very concerning. Antibiotics used in the treatment of disease not only help animals stay healthy and generally well, but they also stop diseases from spreading among animals (Boamah *et al.,* 2016), which is crucial for poultry and animals alike (McEwen *et al.,* 2002). To stop the disease from spreading, some mass treatment techniques are made to medicate sick animals while attending to the remainder of the flock. Antimicrobial resistance has been determined to be largely caused by the modest doses of these drugs that are routinely given to food animals for purposes like as growth stimulation, feed proficiency enhancement, and prophylaxis. Some degree of antimicrobial

resistance has been caused by the use of antibiotic drugs in food animals, and this has resulted in the spread of resistant bacteria and resistance features among animals, their products, and the environment. For instance, according to current, openly available global data encompassing seven European nations (Chantziaras *et al*., 2014), it demonstrates that the use of antibiotics and the emergence of antibiotic resistance in *Escherichia coli* isolates from pigs, poultry, and cattle are directly correlated. Antimicrobial resistance is a result of human drug overuse, which cannot be disregarded. Moreover, there are situations where it is not possible to prove a connection between human and animal resistant strains. On the other hand, there should be alarm when it is evident that characteristics of animal-derived antibiotic resistance have been transferred to humans. There are already restrictions in place in some nations regarding the use of antibiotics in food animals. Many antibiotics used in food animals now are either identical to or substitutes for antibiotics used in human medicine. The treatment of numerous common human pathogenic bacterial infections depends on these antibiotics, which also serve as anti- infection agents during other procedures like major surgery, cancer chemotherapy, organ transplantation, and therapy for preterm babies (Sulakvelidze *et al*., 2001).

Over the years, antibiotics have been utilized in food animals more frequently for non- therapeutic than for therapeutic causes. It is estimated that 50–80% of all antibiotics manufactured are used by cattle alone in the majority of wealthy countries. In reference to the state of affairs in Africa, it was discovered that 15% of the African nations that took part in the 2016 World Organization for Animal Health assessment on antimicrobial use by region had antimicrobial growth promoters allowed for use. However, in that poll, America (about 80%) and Asia (almost 40%) reported having the highest percentage of member nations that approve the use of growth boosters.

The research also indicates that tetracyclines and macrolides were the most often used antimicrobial classes in Africa at the time of the survey, and that the majority of data on antimicrobial use across the continent had only recently been gathered (mainly since 2015) (Van *et al.,* 2020). Antibiotics are widely utilized in developing nations' food animal production to support the health and growth of animals, much like in affluent nations. Although there are some financial advantages to producers and consumers overall from this technique, there are also some drawbacks. Since a sizable portion of the antibiotics used are identical to or substitutes for antibiotics used in human medicinal treatments, there has been great worry that repeatedly exposing these animals to modest dosages of antibiotics adds considerably to antimicrobial resistance. In veterinary science, studies conducted over several decades have demonstrated a clear connection between the usage of antibiotics and antibiotic resistance (Van *et al.*, 2020).

In African nations, antibiotic resistance is a significant problem that has to be addressed, and many medications are available over-the-counter. Consequently, one of the main causes of the current antimicrobial resistance crisis is the use of antibiotics in food animal production, and they should only be administered to sick animals after a diagnosis of illness.

According to a recent study, over the three-year period (2002–2004) in South Africa, two-thirds of the 1500 tons of antibiotics supplied for animal use were used for growth promotion and included drugs that the World Health Organization (WHO) has banned (Talebi *et al*., 2019). A three-year (2010–2012) retrospective investigation on the use of antibiotics in livestock production in three states in southwest Nigeria revealed that tetracyclines, fluoroquinolones, and β-lactams/aminoglycosides were the

most commonly used antimicrobials (33.6%, 26.5%, and 20.4%, respectively). The report also revealed that between 2010 and 2012, the use of antibiotics rose by 40.4% (Adesokan *et al*., 2015). This upward tendency portends a grave risk for the management of infectious diseases in the future. Most livestock keepers in other developing nations, including Ghana, utilize antibiotics primarily for the purpose of treating and preventing illnesses in food animals. Penicillins, tetracyclines, fluoroquinolones, and aminoglycosides are among the antimicrobials that are commonly employed in this context. According to a recent study conducted in Ghana, 88% of farmers consulted a veterinarian before administering medicines to livestock, yet only 63% of farmers gave their animals the full prescribed dosage of antibiotics (Hossain *et al*., 2022).

According to the study, these farmers' use of antibiotics depended on internal variables like the size of their farms, the presence of infections, and the number of extra animals they had on site. One of the main factors driving the usage of these medications was the ease of access to antibiotics. Farmers may readily obtain information about antibiotics through veterinarians, veterinary chemical vendors, and mobile retailers.

A recent study assessing the use of antibiotics in food animals in Rwanda, Central Africa, found that up to 97% of farmers use antibiotics in their livestock with prophylactic use, that is, using the drugs to avoid disease and promote growth being the primary justification for doing so. The majority of farmers, as established from the survey, have little knowledge of using antibiotics in food animals. Furthermore, over half (55.6%) of those surveyed stated they gave their animals over-the-counter antibiotics. The study also showed that the location of farms, the kinds of animals

housed there, and the methods used to care for them (such as intensive farming) were associated with the high usage of antibiotics by farmers (Van *et al*., 2020). Data indicate that chickens have greater rates of resistance than other livestock species, presumably because they are raised in close quarters (Kariuki *et al*., 2023). It is evident that some African nations frequently use small doses of antibiotics, particularly those with broad-spectrum activity, to help manage endemic illnesses in large farmed populations of animals and birds as well as to encourage the growth and feed efficiency of food animals.

Livestock is a significant factor in socioeconomic conditions in Africa, where 250–300 million people rely on them for their income and way of life. On average, livestock contributes 30% of the agricultural Growth Domestic Product (GDP) and roughly 10% of the GDP overall. The use of antibiotics is still largely unrestricted in African countries, regrettably, as it is in many developing nations. Food derived from animals often contains antimicrobial residues due to poor handling procedures. There have been reports of antimicrobial residues in animal food in several African nations, including Egypt, Ethiopia, Ghana, Kenya, Nigeria, South Africa, Sudan, and Tanzania. For instance, a study conducted in Egypt on the prevalence of tetracycline residues (oxytetracycline, tetracycline, chlortetracycline, and doxycycline) in fresh chicken samples (meat and liver) found that 44% of samples had tetracycline residues, ranging from 38-52%, and the corresponding contamination ranged from 103 μg kg^{-1} up to 8148 μg kg^{-1}; some of these values were higher than the maximum residue limit set by Codex (200 μg kg^{-1} and 600 μg kg^{-1} for chicken meat and liver, respectively, expressed as the sum of the tetracycline group) (Salama *et al*., 2011). When it comes to antibiotic residues in foods derived from animals, tetracyclines account for the majority (41%), followed by β-lactams (18%) (Darwish *et al*., 2013).

This is hardly shocking considering how frequently these medications are used in African veterinary care. The reduced incidence of β-lactamases may be explained by their inherent hydrolysis reaction, which makes them unstable. Antibiotic abuse in food animals must be severely restricted due to the potential health concerns posed by antimicrobial residues, which include toxicity, bacterial resistance, cancer, hypersensitivity reactions, and teratogenicity (Darwish *et al*., 2013).

Antibiotics are used in animal husbandry at a higher rate than in human medicine. Approximately 37% of antimicrobials (including ionophores) utilized in animal research lack medicinal equivalents when used in humans (Argudín *et al*., 2017). As a result, drug resistance for such antimicrobials is exclusive to the veterinary world. But most other antibiotics that are used to medicate food animals also have a significant role in human medicine. As of now, human therapeutic "critically important" antibiotic drug categories like macrolides, polymyxins, aminoglycosides, and third-generation cephalosporins (Table 2 2) are also used in food animals, even though penicillin and tetracyclines are still the most frequently prescribed antibiotics for use in animals (WHO, 2016; Kaur et al, 2024).

Table 2 2: World Health Organization (WHO) antibiotic drug categories, their significance in human medicine, and some examples used in food animals.

Antibiotic class	Significance in human therapeutics	Examples utilized in food animals
Aminoglycosides	Critically important	Gentamicin, neomycin
Macrolides and lincosamides	Critically important	Erythromycin, tilmicosin[a], lincomycin[a], tulathromycin[a], tylosin[a]
β-Lactams	Critically important	Penicillin, ceftiofur[a], amoxicillin
Fluroquinolones	Critically important	Ciprofloxacin, danofloxacin[a], enrofloxacin[a]
Tetracyclines	Highly important	Chlortetracycline, oxytetracycline, tetracycline
Streptogramins	Highly important	Virginiamycin[a]
Phenicols	Highly important	Florfenicol[a]
Sulfonamides	Highly important	Many sulfonamides
Polymyxins	Important	Colistin

a - Exclusively veterinary application.

According to recent projections, the global market for antibiotics was valued at US$ 40.7 billion in 2020. From 2021 to 2028, the compound annual growth rate is expected to be 4.5% (Grand View Research, 2020). The increasing prevalence of infectious diseases and antibiotic resistance, especially for bacteria belonging to the *Enterobacteriaceae* family (such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*), which have antibiotic resistance levels averaging 40% in the developed world, is primarily due to misuse of antibiotics (Aslam *et al*., 2018; WHO, 2021). An increasing worldwide concern, antimicrobial resistance (AMR) is estimated to be the cause of over 700,000 fatalities per year (O'neill, 2014). In environments with limited resources, some conventional actions can exacerbate resistance. Antibiotic misuse is a serious issue, particularly when medicines are purchased over-the-counter without a prescription. Comparably, the availability of ingenuine or inferior medications is a serious problem (O'neill, 2014; Ayukekbong *et al*., 2017). In hospital settings, inadequate antibiotic stewardship efforts, a lack of treatment standards, and a lack of surveillance and diagnostic skills have all contributed to improper use (Petti *et al*., 2006; Elbireer *et al*., 2013; Doron & Davidson, 2011; Paterson, 2006).

According to the WHO worldwide report on antimicrobial resistance, prevalent bacteria in many regions of the world have significant prevalence levels of resistance to antimicrobials (WHO, 2014). The analysis also reveals that *Escherichia coli* and *Klebsiella pneumoniae* have substantial frequencies of resistance—up to 54%—to third- generation cephalosporins and carbapenems. Data on the usage of antibiotics in Kenya and throughout Africa are mainly lacking, despite the well-established benefits of using them appropriately to treat bacterial illnesses. Furthermore, most African nations have little capability for AMR surveillance, and there is currently little information available on AMR trends of prevalent harmful bacteria (Okeke *et al*.,

2007). In 2017, the Kenyan government implemented a nationwide strategy aimed at preventing and controlling antibiotic resistance, following the discovery of shortcomings in the monitoring of antibiotic use and consequences. The strategy aims to minimize the use of antibiotics, and this can be done by increasing national compliance with antimicrobial usage reporting regulations (Republic of Kenya, 2017).

2.1.3 Antibiotics as pollutants of emerging concern

Due to their extreme toxicity, even at extremely low concentrations, which has detrimental consequences on both humans and the ecosystem, the presence of compounds of growing concern in the environment is a global problem. Despite their high production in an effort to fulfill the expanding demand for agricultural and industrial products, researchers have paid little attention to the fate, incidence, and monitoring of these pollutants in the environment, notably in Kenya (Fatta *et al*., 2007).

Chemicals of growing concern are substances, either synthetic or natural, that are found in the environment and have negative impacts on human health and the ecosystem. They include chemicals that have lately been found in water systems, such as perfluorinated compounds, heavy metals, pesticides, heavy metals, personal care items, and surfactants. Additional substances include antibiotics, anti-inflammatory, antidiabetic, and antiepileptic medications, as well as hormone-disrupting substances (de Rezende *et al*., 2023). These are primarily used to improve agricultural productivity and other industrial activities, as well as to treat and prevent a variety of medical diseases. These substances are still bioactive, and as a result of inappropriate disposal practices and a lack of adequate technologies for total removal from wastewater, they end up in receiving water systems. They present a number of

environmental problems, including antibiotic resistance and endocrine disruption that could have an influence on the environment or public health, because of their bioactive nature and high toxicity in trace concentrations.

In Kenyan urban slums such as Kibera, a lot of people are compelled to recycle wastewater to suit their requirements. For example, they utilize the effluent from wastewater treatment plants to irrigate vegetables and other crops. This is due to inadequate water supply in informal settelements and the fact that a sizable section of the nation's territory is considered semi-arid and has a shortage of water. The majority of wastewater treatment facilities are conventional in nature, lacking the sophisticated machinery needed to efficiently eliminate organic micropollutants. Therefore, the reuse of wastewater exposes these chemicals to the environment, by polluting the irrigated crops with harmful substances (Kilingo *et al.*, 2021).

According to past research, the effluents from treated wastewater treatment plants that are released into aquatic systems include high amounts of pollutants that are becoming increasingly problematic. Whereas some can quickly biotransform, photodegrade to new compounds and metabolites upon release, depending on their chemical qualities, others can persist in the receiving water systems (Khetan & Colins, 2007). It has been documented that when persistent substances like dioxins and furans build up in the environment to a certain degree, water systems become unsafe for use (Kirkok *et al*., 2020).

A wide range of chemicals are classified as pharmaceuticals, including vitamins, nutritional supplements, prescription and over-the-counter medications for treating humans and animals. Since these substances are all beneficial for different human endeavors, it is anticipated that they will all be released into the environment in

different ways (Kandie *et al*., 2020).

Since most homes and businesses direct their wastewater into sewage systems, wastewater treatment facilities are the main point of entry for toxic pollutants into the environment. With a concentration of a wide variation, the majority of the by-products of personal care and pharmaceuticals found in municipal wastewater treatment plants come from households (Shala *et al.,* 2010). The introduction of new toxins into the environment has also been greatly facilitated by inadequate solid waste management and disposal practices. Typically present in industrial effluents, most endocrine disruptors are bioactive at trace levels that alter an organism's endocrine system's functioning (Xu *et al*., 2012). The active ingredient in birth control pills, 17α-ethynylestroidiol, nonlyphenol, nonylphenol ethoxylates, octyphenol, and bisphenol A are examples of these endocrine disruptors (EDCs) (Von Saal *et al*., 2012). Additionally, antibiotics such as tetracycline, amoxicillin, penicillin, sulfonamides, and sulfonamides have been reported as EDCs (Dutta S *et al*., 2023). Due to rising home pharmaceutical usage, the amount of pharmaceuticals released per person in residual areas' wastewater is approximately 10% of the total amount released from wastewater (Kumar *et al*., 2010). Beta-blockers, steroids, anti-inflammatory, antibiotics, and antiepileptic drugs are the pharmacological classes that are most frequently encountered (Yuan *et al*., 2014).

Antibiotics, analgesics, anti-inflammatory meds, antimalarial chemicals, and anti-viral medications are the most often used pharmaceuticals in Kenya (Kandie *et al*., 2020). However, a sizable portion of these substances are not entirely broken down or removed from the body; as a result, they are released into the environment as parent substances or as metabolites in the form of feces or urine. Due to their ineffective removal by the majority of currently operating conventional wastewater treatment

plants, pharmaceuticals have been found at ng L^{-1}, μg L^{-1} and even up to mg L^{-1}quantities in sewage sludge and effluents (K'oreje *et al*., 2018).

Antibiotic-resistant bacteria are a serious hazard to public health because of the ongoing use of antibiotics in both human and animal medicine. Furthermore, antibiotics have been found to be emergent pollutants in wastewater treatment facilities (WWTPs), where they have the ability to survive in the environment through a variety of transformation processes. Antibiotics in quantities ranging from ng L^{-1} to μg L^{-1} have been found in WWTPs across the globe. Research has indicated that the main influent and effluents have the highest quantities of antibiotics, whereas the secondary and tertiary effluents have lower values (Kümmerer, 2009; Verlicchi *et al*., 2012).

The development of antibiotic-resistant bacteria in WWTPs is a significant ecological danger associated with antibiotic use. Because drugs excert a selection pressure, antibiotic-resistant bacteria are more likely to emerge and proliferate in WWTPs. As a result, these bacteria might be released into the environment, where they might produce diseases resistant to standard antibiotic therapy and jeopardize public health even further. Additionally, bacteria have the ability to swap genes that resist antibiotics, which can spread resistance across the ecosystem (Kümmerer, 2009; Zhang *et al*., 2020).

In WWTPs around the world, antibiotics have been identified as emerging contaminants. They can also endure in the environment and undergo a range of transformations while wastewater is being treated. At amounts seen in WWTP effluent, antibiotics can be hazardous to aquatic life. Research has demonstrated that antibiotics can harm fish, algae, and other aquatic organisms both acutely and

chronically over time. Antibiotic toxicity is influenced by a number of variables, such as antibiotic type, concentration, and length of exposure. For instance, it has been demonstrated that sulfonamides can be chronically hazardous to algae at concentrations as low as 100 ng L^{-1}, whilst tetracyclines can be acutely toxic to fish at concentrations as low as 1 μg L^{-1} (Kümmerer, 2009; Liu et al., 2020).

In WWTPs, antibiotics have the potential to disturb microbial ecosystems. A diverse microbial population is used by WWTPs to break down organic materials and extract nutrients from wastewater. Antibiotics may selectively affect microbial communities, resulting in changes to microbial populations and a decrease in microbial diversity. This may have an impact on WWTP performance by lessening their capacity to filter contaminants out of wastewater (Kümmerer, 2009; Zhang *et al*., 2020).

2.2 Classification of antibiotics

There are different ways through which antibiotics are classified as indicated in Figure 2.1. Antibiotics are organic compounds broadly classified as either naturally produced by microorganisms or artificially synthesized. They have the ability to kill or slow down the growth and metabolic activity of other microorganisms through biochemical mechanisms (Thomashow & Weller, 1995).

Antibiotics are also classified based on their chemical structures because in most cases, comparable levels of effectiveness, toxicity and side effects are elicited by those antibiotics that have the same or similar functional group(s). Based on their range of microbial action, they may be broad spectrum antibiotics (those that are effective against a wide range of microorganisms) or narrow spectrum antibiotics i.e., those that work on a limited number of bacteria or microorganisms. Based on their nature of action, antibiotics may be bactericidal (directly kills bacteria) or

bacteriostatic (inhibits the growth of bacteria) as illustrated in Figure 2 1.

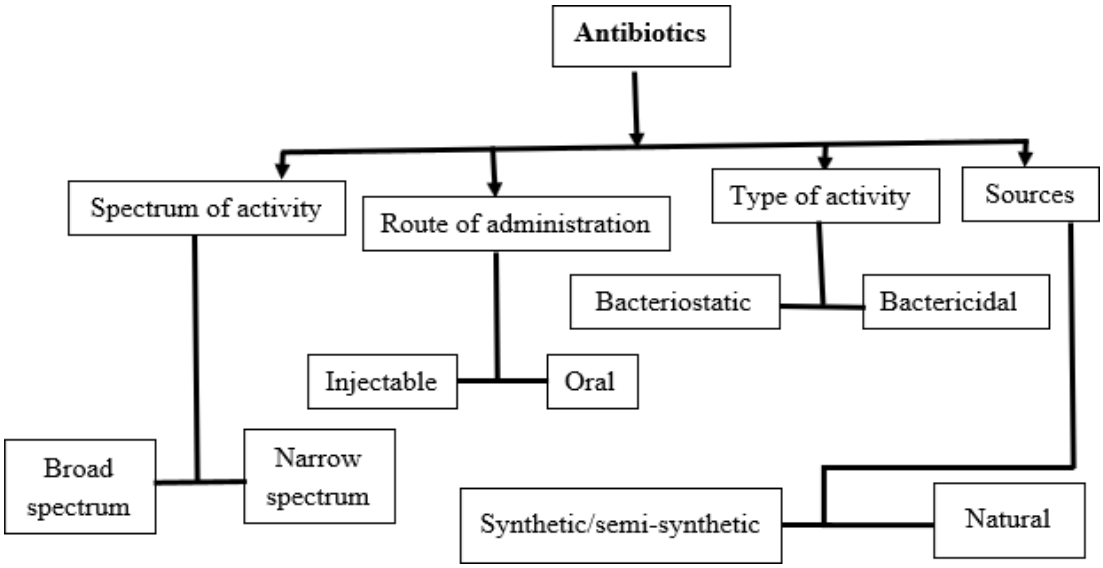


Figure 2 1: Classification of antibiotics based on different criteria.
Adapted from *https://explorable.com/history-of-antibiotics*.

The classification of antibiotics is further determined by their methods of action, which include the suppression of cell wall formation, change of cell membranes, antimetabolite activity, inhibition of nucleic acid synthesis, inhibition of protein synthesis, and competitive antagonism. The different classes of antibiotics, and a few examples in each class, are: β-lactams which include amoxillin, cephapirin, cefuroxime, ampicillin and penicillin G; quinolones (fluoroquinolones) include ciprofloxacin, difloxacin, enrofloxacin, norfloxacin, ofloxacin and levofloxacin; sulfonamides include, sulfadiazine, sulfamethazine and sulfamethoxazole; macrolides include azithromycin, clarithromycin, erythromycin and tylosin; glycopeptides, for example, vancomycin and aminoglycosides such as gentamycin, streptomycin and amikacin. Others include streptogramins, carbapenems, polymixins and cephalosporins (Zhang & Li, 2011). This study investigated the multi-residue occurrence of several antibiotics classes in River Sosiani surface water and sediment samples, and their adsorptive removal from water with specific focus on

fluoroquinolones (ciprofloxacin), sulfonamides (sulphamethoxazole) and β-lactams (penicillin G). For this reason, these antibiotic groups are further discussed in the following subsections.

2.2.1 Fluoroquinolones

Fluoroquinolones (FQs), are commonly used, broad-spectrum antibiotics that treat bacterial infections. They function by inhibiting the activity of key enzymes involved in DNA replication, specifically type II DNA topoisomerases (gyrases), which are essential for the replication of bacterial DNA and, as a result, the synthesis of mRNAs (NIDDK, 2020). At the moment, there are a number of FQs that are accessible. They include ciprofloxacin (Cipro), gatifloxacin (Tequin), moxifloxacin (Avelox), ofloxacin (Ocuflox/Floxin/Floxacin) and norfloxacin (Noroxin). Ciprofloxacin (CPF) (Figure 2 2), ofloxacin (OFL), levofloxacin (Levaquin/Quixin), gemifloxacin, levofloxacin, moxifloxacin and norfloxacin (NOR) are among the most widely prescribed FQs worldwide (Sukul & Spiteller, 2007). Nevertheless, FQs are not particularly effective against intracellular infections in the majority of cases.

Second-generation fluoroquinolones like ciprofloxacin are highly effective against the majority of Gram-positive and Gram-negative bacteria (Thai *et al*., 2021). Chemically, CPF is a quinolone, quinolin-4(1H)-one, with cyclopropyl, carboxylic acid, fluoro and piperazin-1-yl substituents at positions 1, 3, 6 and 7, respectively. The drug is a DNA anti-infective/antibacterial, topoisomerase IV and DNA synthesis inhibitor. CPF has been recognized as a problematic environmental contaminant and xenobiotic which has received the attention of researchers. Antibiotics retain their activity in wastewaters owing to their relatively stable molecular configuration, solubility in water and non-biodegradability (Aseman-Bashiz *et al*., 2021; de

Oliveira *et al*., 2019; Prasannamedha *et al*., 2021; Varsha *et al*., 2022). High levels of CPF in wastewaters has been shown to retard the growth of freshwater organisms, notably periphyton communities, duckweed and cyanobacteria (Johansson *et al*., 2014; Robinson *et al*., 2005). Thus, remediation of CPF from aquatic ecosystems is necessary as it often occur at concentrations above permissible limits, in addition to its non-biodegradable nature and its proven potential to destabilize natural ecosystems (Igwegbe *et al.*, 2021; Yi *et al.*, 2017).

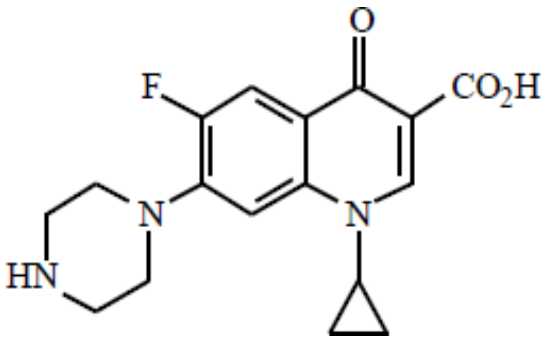


Figure 2 2: Chemical structure of ciprofloxacin, a broad spectrum fluoroquinone

2.2.2 Beta-lactam antibiotics

Beta-lactam (βL) antibiotics (or β-lactam antibiotics) are the largest group of clinically used antibiotics. They are distinguished by the presence of a reactive beta-lactam ring containing 3-carbon and 1-nitrogen atoms (a highly strained and reactive cyclic amide responsible for the antibacterial activity) in their chemical structure (Varela *et al*., 2021). In addition, β-lactam antibiotics possess different side chains which may result in variation of their pharmacological properties (Cha *et al*., 2006). Penicillin is the first β- lactam antibiotic to be used and was isolated from a rare variant of *Penicillium notatum*, currently known as *P. chrysogenum* (Pandey & Cascella, 2021). The structure of this first penicillin was proposed by Edward Abraham in 1942 but it was not until 1945 that Dorothy Hodgkin confirmed it

(Pandey & Cascella, 2021). It is worth noting that despite the narrow spectrum of the early generation (original) penicillins, many semi-synthetic penicillins have been produced since then with a better or broader spectrum of antibacterial activity (Cadelis *et al*., 2021). This class is comprised of four subclasses; penicillins, cephalosporins, monobactams, and carbapenems (Cha *et al*., 2006) and act by inhibiting cell wall synthesis. There are five relevant ring systems, including the penam, penem, carbapenem, cefem and monobactam ring structure. The most frequently detected penicillins in wastewater are penicillin G (Figure 2 3), penicillin V, amoxicillin, ampicillin, oxacillin, and cloxacillin (Zhang & Li, 2011). Beta-lactam antibiotics are bacteriostatic due to their ability to inhibit the last step in peptidoglycan synthesis through acylation of the transpeptidase that catalyzes the cross-linking of peptides to form peptidoglycan (Bush & Jacoby, 2010). Hence, these groups of antibiotics have been established to target penicillin-binding proteins (PBPs) to interrupt terminal transpeptidation and induce loss of viability and lysis, as well as through autolytic processes within the bacterial cell (Lobanovska & Pilla, 2017; Miyachiro *et al*., 2019).

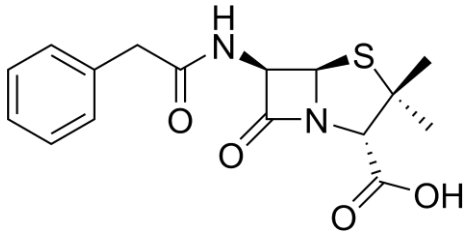


Figure 2 3: Chemical structure of Penicillin G, an antibiotic from the family of penicillins.

Penicillin G (also known as benzylpenicillin) is a narrow-spectrum, naturally occurring penicillin antibiotic that has stayed in the clinical arena since the 1940s. It is administered as a salt for oral and parenteral administration and as a repository salt for injection (Bennett, 2020; Gajdács *et al*., 2021). Penicillin G is now widely

recognized as a persistent environmental contaminant, and concerns have been raised over its continuous occurrence in surface water and wastewater treatment plants (Chavoshan *et al*., 2020b; Vieno *et al*., 2006). Thus, various physical, biological and chemical methods have been proposed and investigated for use in the removal of Penicillin G from aqueous solutions (Chavoshan *et al*., 2018; Silva *et al*., 2019).

2.2.3 Sulphonamides

Sulfonamides, often known as sulphonamides and abbreviated as SAs, are a diverse class of synthetic medications that include the sulfonamide chemical group (NIDDK, 2017). They may also be referred to as sulfa medicines. The term sulfonamide is often used inaccurately to specifically describe antibiotics that have a sulfonamide functional group in their chemical composition. Nevertheless, other non-antibiotic sulfonamides have been developed by capitalizing on discoveries made during the clinical assessment of antibiotic sulfonamides. These medications are utilized to treat various illnesses, including diabetes and to alleviate discomfort. Sulfanilamide, discovered in 1906, was not utilized as an antibacterial agent until the late 1930s (NIDDK, 2017). Sulfonamide antimicrobials exhibit bacteriostatic properties, impeding bacterial reproduction without necessarily inducing their demise. They achieve this by disrupting the synthesis of folic acid in bacteria, a vital component for nucleic acid production, including Deoxyribonucleic Acid (DNA) and Ribonucleic Acid (RNA). While humans acquire folic acid through their diet, bacteria must generate it. Sulfonamide antimicrobials can be synergistically coupled with trimethoprim to exert bactericidal effects by targeting distinct enzymes in the folic acid production pathway. The anti-inflammatory or immunomodulatory characteristics of non-antibiotic sulfonamides are believed to exist, however, the

precise mechanism by which they function in certain contexts remains unknown (Sparbier *et al*., 2012).

The commonest sulfonamide is Sulfamethoxazole bacteriostatic antibiotic that is often used in combination with trimethoprim (jointly known as Bactrim drug). Sulfamethoxazole (Figure 2 4), like other sulfonamides, is bacteriostatic due to its competitive inhibition of dihydropteroate synthase, which halts the formation of folic acid precursor, dihydropteroic acid (NIDDK, 2017). Sulfamethoxazole is chemically an isoxazole (1,2-oxazole) containing a methyl substituent at the 5-position and a 4-aminobenzenesulfonamido group at the 3-position (National Library of Science, 2022).

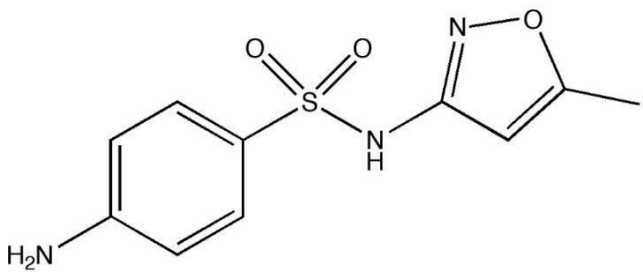


Figure 2.4: Structure of Sulfamethoxazole, a typical sulphonamide

The antibiotic has also come to the limelight as one of the persistent organic pollutant, xenobiotic and drug allergens. In the environmental context, the drug with its N-amine and carboxyl groups is chemically transformed into other oxidation, acetylated and hydrolyzed metabolites which are themselves secondary environmental contaminants (Guruge *et al*., 2019; Kurade *et al*., 2019; Li *et al*., 2020; Prasannamedha & Kumar, 2020; Straub, 2016).

2.2.4 Tetracyclines

Tetracyclines are broad-spectrum antibiotics that came into the clinical arena in the 1940s and have since been used in the prophylaxis and treatment of human and veterinary infections and sometimes at sub-therapeutic levels in animal feed as growth promoters (Chopra & Roberts, 2001). They have proven antimicrobial activity against many microorganisms such as mycoplasmas, chlamydiae, rickettsiae and protozoan parasites as well as Gram-negative and Gram-positive bacteria (Chopra & Roberts, 2001; Rusu & Buta, 2021). They are zwitterionic compounds and are highly soluble in water. Naturally occurring tetracyclines include tetracycline, oxytetracycline, chlortetracycline and demeclocycline. Other semi-synthetic members of this class include doxycycline, lymecycline, methacycline, minocycline, rolitetracycline, tigecycline (glycylcycline subclass agent) and other novel ones such as omadacycline, ervacycline and sarecycline (Shutter & Akhondi, 2022). Tetracyclines function by inhibiting the 30S ribosomal subunit, hampering the combination of the aminoacyl-*t*RNA with the acceptor site on the mRNA-ribosome complex. This causes the cells to lose their integrity and malfunction, thereby inhibiting bacterial replication and growth. Hence, tetracyclines are bacteriostatic (Bennett, 2021; Grossman, 2016; Shutter & Akhondi, 2022). They are effective against Gram-negative and Gram-positive bacteria although in the recent past, resistance towards this class of antibiotics has been recorded leading to a decrease in their use (Ahmadi, 2021). Their mechanism of action is by inhibiting protein synthesis in organisms (Zhang & Li, 2011).

Chemically, tetracyclines consist of an inflexible skeleton of four rings with various substituent groups notably the amine, alkyl and hydroxyl on their upper or lower sides of the ring. Hence, chemical modification of either the upper or lower regions of the

molecule results into the production of various therapeutic or active first and second generation tetracycline molecules (Rusu & Buta, 2021). Thus, it can be said that the tetracyclines and their analogues bore a chemical structure of tetracyclic naphthacene carboxamide ring system, with those that elicit antibacterial activity possessing a dimethylamine group at the C_4 in the ring A (Figure 2 5).

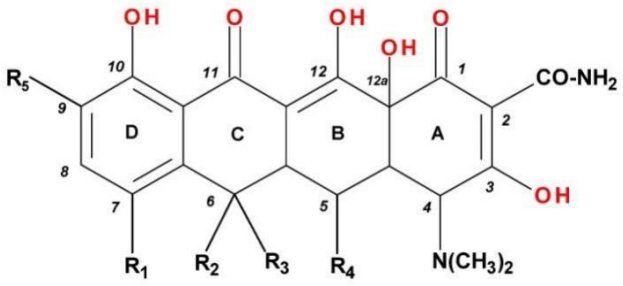


Figure 2.5: The skeletal formula of tetracyclines with atoms and four rings numbered and labeled. Adopted from Rusu and Buta (2021).

The commonest member of the tetracyclines family is tetracycline, a broad-spectrum polyketide antibiotic (Figure 2 6**)** used in the management of various bacterial infections and sometimes utilized by penicillin-allergic individuals. The drug is naturally produced by the *Streptomyces* genus of *Actinobacteria*. Chemically, tetracycline is a tertiary alpha-hydroxy ketone, a conjugate acid of a tetracycline (1-) and zwitterion molecule. Principally, it is bacteriostatic owing to its ability to bind reversibly to the bacterial 30S ribosomal subunit, thereby blocking incoming aminoacyl tRNA from binding to the ribosome acceptor site (Shutter & Akhondi, 2021). Further, it may bind to a lesser degree onto the bacterial 50S ribosomal subunit, causing changes in the cytoplasmic membrane that results in the oozing out/leakage of intracellular components of bacterial cells (Drug Online Bank, 2022).

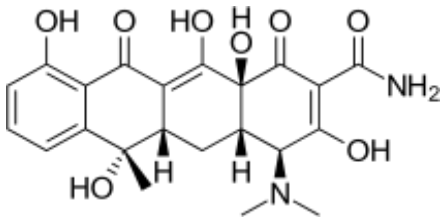


Figure 2.6: Chemical structure of tetracycline, a member of the tetracyclines group of antibiotics

Whereas tetracyclines are still in use for the management of various human and veterinary ailments, the emergence of multidrug-resistant bacterial species has limited their effectiveness. Tetracycline, as the most used member of this family has been reported in or recovered from environmental matrices such as river waters, sediments and wastewaters (Ahmad *et al*., 2021; Javid *et al*., 2016; Peng *et al*., 2020; Scaria *et al*., 2021).

2.2.5 Macrolides

The class of antibiotics known as macrolides is a broad-spectrum antibiotic that is used to treat a wide range of bacterial illnesses. Infections such as pneumonia, sinusitis, pharyngitis, and tonsillitis are typically treated with medications belonging to this class. These medications include azithromycin, clarithromycin, and erythromycin. According to Parnham *et al*. (2014), these medications are commonly used to treat infections caused by Gram-positive and fastidious Gram-negative bacteria. Macrolide antimicrobial agents are chemically characterized by the presence of at least one macrocyclic lactone ring which dictates whether they are either antifungal or antibacterial molecules (Beverly, 2022). The large 14-, 15-, or 16-membered macrocyclic lactone ring has one or more deoxy sugars (commonly cladinose and desosamine).

The macrolide antibiotic mechanism of action entails binding of the bacterial 50S ribosomal subunit, culminating into the impairment of bacterial protein synthesis

(Vázquez-Laslop & Mankin, 2018). Once bound, the drug prevents the translation of mRNA by preventing the addition of the next amino acid by the tRNA. This class of drugs are administered based on the desired medication (Vázquez-Laslop & Mankin, 2018). Thus, they are considered bacteriostatic antibiotics. Oral formulation in form of tablets for macrolides are less commonly used compared to the cream, intravenous formulations and ophthalmic preparations. This is due to their low solubility (Beverly, 2022).

The commonest member of this class of antibiotics is Erythromycin, which was the first macrolide discovered in 1952 (Figure 2 7). Erythromycin remained in the clinical arena as a substitute for penicillin for patients who are allergic to the latter or experienced penicillin-resistant illnesses. Other newer macrolides (with better absorption rates and less side effects) such as azithromycin and clarithromycin are direct outcomes of chemically modifications to erythromycin (Klein, 1997).

Erythromycin is available as tablet, capsule, delayed-release capsules, delayed-release tablet and a suspension (liquid). Resistance to macrolides has been reported (Fyfe *et al*., 2016; Miklasińska-Majdanik, 2021). In addition, this group of antibiotics has been listed as potential environmental contaminants that needs to be remediated from environmental matrices (Bai *et al*., 2014; Gomes *et al*., 2022; Voigt *et al*., 2018).

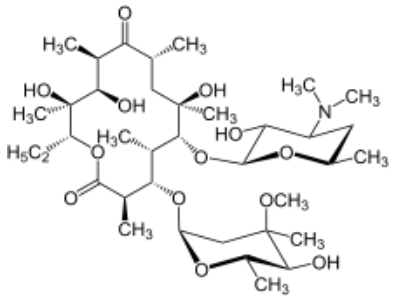


Figure 2.7: Structure of Erythromycin, a typical macrolide antibiotic.

2.3 Mode of Action and Targets for Antibacterial Drugs

The primary mechanism of action of antimicrobial agents is frequently used to categorize them for use in the treatment of bacterial infections. There are six main ways in which this compounds work: (1) blocking the formation of cell walls; (2) blocking the synthesis of proteins; (3) interfering with the synthesis of nucleic acids; (4) blocking a metabolic pathway; (5) blocking membrane function; and (6) blocking ATP Synthase. Thus, the targets of antibacterial drugs include the synthesis of proteins, nucleic acids, cell walls, membranes, and biological metabolic compounds, based on their mode of action (Kırmusaoğlu, 2019). Peptidoglycan-based cell walls envelop bacterial cells. As the primary component of the cell-wall and its outermost layer, peptididoglycan biosynthesis, is vital to the structural integrity of the cell wall. Certain antibiotics disrupt the peptidoglycan biosynthesis process, damaging the cell wall's structural integrity. Since mammalian cells lack the peptidoglycan wall structure, it is preferable to target the inhibition of cell wall peptidoglycan biosynthesis when searching for antibacterial agents, as it does not adversely affect mammalian host cells (Kırmusaoğlu, 2019; David, 2001; Brooks, 2013).

Protein synthesis is a multi-step, intricate process that requires conformational alignment and a wide range of enzymes. To prevent bacterial protein synthesis, the majority of antibiotics, however, interfere with the 30S or 50S subunits of the 70S bacterial ribosome. For instance, tetracyclines, such as doxycycline, block the 30S ribosome's A (aminoacyl) site, which prevents aminoacyl-tRNA from binding. They have the ability to prevent the synthesis of proteins in both 80S and 70S (eukaryotic) ribosomes. For the purpose of maintaining cellular homeostasis and metabolic energy transfer, the bacterial membrane offers selective permeability (Kırmusaoğlu, 2019;

David, 2001). By interacting with the bacterial membrane through a lipophilic moiety, a number of antimicrobial agents disrupt various targets, resulting in the disruption of membrane structures and functional impairment. Antibacterial agents that target the components of bacteria's cytoplasmic membrane have been reported as of late, and they can affect both Gram-positive and Gram-negative bacteria (Kırmusaoğlu, 2019; David, 2001). Antibiotics can prevent microorganisms from replicating, transcriptionally, and from synthesizing folate. Quinolone medications can disrupt the synthesis of DNA by blocking the enzyme topoisomerase, which is necessary for DNA replication. Levofloxacin, norfloxacin, and ciprofloxacin are examples of second-generation quinolone medications that are effective against both Gram-positive and Gram-negative bacteria. Certain antibiotics, like doxorubicin and actinomycin D (dactinomycin), also obstruct RNA polymerases, thereby interfering with the synthesis of RNA. As a result, they are most frequently used as antineoplastic and antitumor drugs, attacking both normal and rapidly growing malignant cells. They also interfere with bacterial and mammalian systems (Kırmusaoğlu, 2019; David, 2001).

A class of antibiotics known as "bacterial metabolism inhibitors" targets the pathways involved in the synthesis of amino acids and nucleic acids. In all living forms, tetrahydrofolic acid (TH4) is an essential coenzyme that is needed for the synthesis of nucleic acids and some amino acids. Para-aminobenzoic acid (PABA) is the precursor used by bacteria to make folic acid. By obstructing bacterial TH4 synthesis, bacterial metabolism inhibitors modify bacterial metabolic pathways (Kırmusaoğlu, 2019; David, 2001). In all organisms, from bacteria to vertebrates, ATP synthase is the primary enzyme that produces energy through either photophosphorylation or oxidative phosphorylation. Bacteria can phosphorylate fermentable carbon sources at

the substrate level or by oxidatively employing respiratory chains and ATP synthase to phosphorylate carbon sources. Certain antibiotics have been shown to prevent ATP synthase from being oxidatively phosphorylated, which impacts bacterial energy production and ultimately results in bacterial death (Kırmusaoğlu, 2019; David, 2001).

2.3.1 Sources of antibiotics in the environment

All antibiotics used in therapeutic, growth promotion, food and agricultural sectors contribute to the release of these antibiotics and their metabolites into the environment (Sosa-Hernández *et al*., 2021; Wang *et al*., 2022). These major sources can be broadly categorized into three; human antibiotics (households, industry, hospitals), agriculture and food processing and veterinary antibiotics i.e. livestock, aquaculture, poultry and pets (Binh *et al*., 2018; Goel, 2015; Polianciuc *et al*., 2020). The pharmaceutical industries make a substantial contribution to the overall concentration of antibiotics that are added to the influent of sewage treatment plants, together with home and industrial waste (Harrabi *et al*., 2018; Tang *et al*., 2022). Leakages from underground sewage systems, untreated sewage overflows during storms or system failures, and the release of treated effluents from domestic treatment plants into surface water or aquifers are significant sources of antibiotics residues in both surface and underground water systems (Boonsane, 2009; Goel, 2015; Kümmerer, 2001; Kümmerer, 2004). Unused or expired drugs are occasionally disposed of in residential drains. Consequently, antibiotic compounds are introduced into municipal sewage and subsequently enter sewage treatment plants. If these substances are not removed during the process of sewage treatment, they are released into surface water (Xu *et al*., 2021).

2.4 Occurrence of Antibiotics in Wastewater, Sediments and Surface Waters: A Global Overview, Continental and National

A research in South China found twenty-one antibiotics in sewage from two wastewater treatment plants (WWTPs), with concentrations reaching up to 5800 ng g^{-1}, with sulphamethoxazole, ofloxacin, and norfloxacin being the most prevalent (Zhou *et al*., 2013). Despite accounting for a major fraction of overall human consumption (50-70%), β-lactams are uncommon due to their unstable nature (Kümmerer, 2009; Minh *et al*., 2009).

Examination of antibiotics in Yangtze River waters in China, concluded that the concentrations of doxycycline, oxytetracycline, and tetracycline were 56.09 ng/L, 18.98 ng/L, and 11.16 ng/L, respectively (Wang et al. 2014). Ampicillin was found at the greatest quantity in both wastewater influents (104.2 g L^{-1}) and effluents (12.68 g L^{-1}) in the Yamuna River in India, with cephalosporins and fluoroquinolones present at lower concentrations (Mutiyar & Mittal, 2014).

Antibiotics from diverse structural classes have been found in variable amounts in wastewater and surface water around the world. Trimethoprim, sulfamethoxazole, erythromycin, and ciprofloxacin have all been thoroughly investigated in Italy (Verlicchi *et al*., 2012). Raw urban wastewater in Italy had the greatest absolute amounts of ofloxacin (32 g L^{-1}), roxithromycin (17 g L^{-1}), ciprofloxacin (14 g L^{-1}), sulfapyridine (12 g L^{-1}), trimethoprim (10.5 g L^{-1}) and erythromycin (10.5 g L^{-1}).

Mandaric et al. (2017), conducted a study on Alpine rivers in France and found that the concentrations of sulfamethizole, norfloxacin, and trimethoprim were 544, 163, and 45 ng L^{-1}, respectively.

Trimethoprim, spectinomycin, ampicillin, sulfamethazine, sulfadiazine, sulfanilamide, and oxacillin were found in raw hospital wastewater, wastewater treatment plant effluent, and surface water in Nairobi, Kenya, according to Ngigi *et al*. (2020). Sulfamethoxazole was found at the highest concentrations in hospital wastewater (20.6 g L^{-1}), wastewater treatment plant (7.8 g L^{-1}), and surface water (6.8 g L^{-1}).

While numerous research in industrialized nations have investigated antibiotic incidence in surface and wastewater, there is minimal evidence from developing countries, including Kenya. Kenya, which is dealing with severe water pollution, lacks data on the environmental prevalence of pharmaceuticals. The country's pharmaceutical consumption pattern, which is characterized by a high usage of antibiotics and other pharmaceuticals, highlights the need for additional research to analyze exposure and potential dangers to the ecosystem and human health in these underexplored areas.

Globally the demand for safe drinking water has increased, according to World Health Organization (WHO, 2017) about 2.1 billion people which can be equated to 29% of the global population do not have an access to clean and safe water. In addition to this, it estimated 159 million people still depend on drinking water from surface water such as rivers (Palansooriya *et al*., 2020). The contamination of the surface water by chemicals and other substances poses a health risk to a large population globally. Contaminations of aquatic life by the chemicals such as pesticides, personal care products, perfluoroalkyl, polyfluoroalkyl, industrial chemicals and pharmaceuticals, has raised a concern in the recent past because of their bioaccumulation potentiality and toxic characteristics. A million tons of these chemicals are consumed annually worldwide and they are continually released to the environment (Golovko *et al*.,

2021). These pollutants can be detected in the environmental matrices; their fate and their way to the environment is dependent on a number of factors such, disposal, removal at the wastewater treatment, production volumes, stability and consumption.

Antibiotics can undergo various transformation processes during wastewater treatment, including biodegradation, sorption, photolysis, and oxidation. The transformation products of antibiotics in WWTPs depend on several factors, including the type of antibiotic, the treatment process, and the environmental conditions.

Studies have shown that antibiotics can be transformed into various metabolites during WWTP treatment, including sulfonamides, quinolones, and aminoglycosides. These metabolites can have different properties than the parent compounds, including increased persistence and toxicity (Kümmerer, 2009).

The removal efficiency of antibiotics in WWTPs depends on several factors, including the type of antibiotic, the treatment process, and the environmental conditions. Conventional treatment processes such as primary and secondary treatment are not very effective at removing antibiotics, with removal efficiencies ranging from 0% to 50% (Kümmerer, 2009; Verlicchi *et al*., 2012). However, advanced treatment processes such as activated carbon adsorption, ozonation, and membrane filtration can be more effective at removing antibiotics, with removal efficiencies ranging from 50% to 99% (Liu *et al*., 2020; Zhang *et al*., 2020).

2.5 Effects of Antibiotics on the Environment

Antibiotics in the environment can have an effect on animals that live in water and on land (Kotzerke *et al*., 2008; Liu *et al*., 2009), change the activity and make-up of microbes in communities (Underwood *et al*., 2011), and cause bacteria to become resistant to antibiotics (Ahmadi, 2021; WHO, 2021). For example, high

concentrations of fluoroquinolones in the aquatic environment can cause genotoxic effects (Bhattacharya *et al*., 2020). Modification in a strain of the bacteria *Salmonella typhimurium* has been reported at a concentration as low as 5 μg L^{-1} for norfloxacin and 25 μg L^{-1} for ciprofloxacin in surface water. The reported concentration in hospital wastewaters of both norfloxacin (20.6 μg L^{-1}) and ciprofloxacin (236.6 μg L^{-1}) was high in some samples in a study reported by Diwan *et al*. (2009). While various mechanisms can lead to the dissipation of antibiotics in wastewater and surface water, it has been observed that even at concentrations below the level that inhibits bacterial growth, antibiotics can act as signaling molecules and promote the development of antibiotic resistance in bacteria that are exposed to them (Andersson & Hughes, 2014; Liang *et al*., 2008).

2.5.1 Emergence of antimicrobial resistance genes and antimicrobial-resistant bacteria

Bacteria have developed a variety of strategies over time for the emergence and spread of antibiotic resistance characteristics. The widespread use of antibiotics in food animals by veterinary industry has contributed to its emergence and spread. There are several reasons why this poses a significant risk to the public's health, including the encouragement of antibiotic resistance in human pathogens, potential increase of carriage and dissemination, increased human medical care expenses, reduced efficacy of human- related antibiotics, and subsequent increases in human morbidity and mortality (Boamah *et al*., 2016, McEwen *et al*., 2002, Lekshmi *et al*., 2017). Antibiotic resistance traits and antibiotic-resistant bacteria are normally transferred to the general public via food, the environment and farm workers (Heuer *et al*., 2011). Infections such as campylobacteriosis, non-typhoidal salmonellosis, and campylobacteriosis and methicillin-resistant *Staphylococcus aureus* (MRSA), which

are all capable of spread amongst animals and humans, become more difficult to treat when antibiotic-resistant strains originating from food animals are involved (Salam *et al*., 2023).

Bacteria within farm animals experience selective pressure when they are repeatedly exposed to lower doses of antibiotics. This helps bacteria become more resistant so they can continue to exist. Bacteria may obtain genes from related or unrelated strains via mobile genetic elements, specifically plasmids, as a consequence of the selective pressure that is generated. When this occurs, drugs eradicate the drug-susceptible bacteria, allowing the resistant strains to proliferate via natural selection. A combination of novel mutations and parallel gene transfer from the resistance origins mediates the occurrence of a rise in antibiotic resistance. The most common mutations result in either an increase in drug efflux or alterations to the antibiotic drug target. Other known bacterial antibiotic resistance mechanisms include reduced target expression by bacteria, gene modification and alterations to drug modification enzymes. Drug target substitution, drug change, acquisition of novel efflux pumps, resistance bypass, and protection are some of the mechanisms linked to parallel gene transfer. Genes that provide resistance to distinct antibiotics are typically associated with one another. Consequently, using a single medication could cause many medications to develop drug resistance (Nikaido *et al*., 2009). While both human and animal bacterial isolates have significant levels of antibiotic resistance, an investigation conducted in Ghana revealed that animal isolates had greater levels of resistance than human isolates (Garcia-Vello *et al*., 2020).

The observed rise in colistin resistance is largely linked with its use in food animals. Colistin is one most prescribed and commonly used antibiotics for treatment of severe

infections caused by carbapenemase-producing *Enterobacteriaceae*. High resistance to this drug was recently observed in China during regular surveillance of resistance to antimicrobials associated with commensal *E. coli* from food-producing animals (Morrill *et al*., 2015). Evidence for the potential spread of the plasmid-mediated mobilized colistin resistance gene *mcr-1* from the veterinary to human environments has been provided. Furthermore, Doumith *et al*. (2016) confirmed the first detection of this *mcr-1* resistance gene in *E. coli* and *Salmonella* spp. in the UK and demonstrated its persistence in humans in England and Wales. *Escherichia coli* containing *mcr-1* have been isolated in poultry in Algeria, South Africa and Tunisia, and the Tunisian isolates carried both *mcr-1* and $bla_{CTX\text{-}M\text{-}1}$ genes on the same IncH12 plasmid, facilitating the co-transfer of these resistance genes. Moreover, *E. coli* containing *mcr-1* were detected in livestock and hospitalised patients and outpatients in South Africa, which is of concern for public health. Since the first detection of the *mcr-1* colistin resistance gene in 2015, the variants *mcr-2* to *mcr- 9* have been reported, increasing the likelihood of resistance to colistin (Cherak *et al*., 2021).

Antibiotic use in food animals unquestionably improves the welfare of the animals overall and increases their general productivity. On the other hand, the approach causes intimicrobial-resistant bacteria and resistance characteristics to arise and spread.

2.5.2 Antimicrobial resistance in animals in the Africa region

Antimicrobial resistance in Africa has become an issue of concern due to the increase in drug resistance of different bacterial pathogens in a region where healthcare facilities are limited. Resistance to commonly available antibiotic drugs has been noted in *Mycobacterium tuberculosis, S. aureus*, *Salmonella* spp., pathogenic

E. coli and *Pseudomonas* (Jans *et al*., 2017; Zishiri *et al*., 2016). Despite limited scope to track antimicrobial resistance, the available data suggest that the African region contributes to the global trend of rising antimicrobial resistance (Ndihokubwayo *et al*., 2013).

Multidrug-resistant (MDR) *E. coli* O157 strains have been isolated from human and animal sources in central Ethiopia and the North West Province of South Africa (Beyi *et al*., 2017). There is a serious threat of transmitting antibiotic resistance along the food chain in Africa, demonstrated by the presence of MDR *Enterococcus* spp. and MDR *Salmonella* spp. in Tunisia and Algeria, respectively, MDR Shiga toxin-producing *E. coli* O157:H7, MDR *Shigella* spp. from meat and poultry products in Egypt, colistin-resistant *E. coli* strains, and carbapenemase-producing *P. aeruginosa* and *Acinetobacter baumannii* in food animals in Lebanon (Rafei *et al*., 2015). The increase in prevalence of resistance to clinically important drugs in Africa, such as quinolones and third/fourth- generation cephalosporins, in commensal *E. coli* isolated from healthy livestock is very concerning (Alonso *et al*., 2013). Antibiotic use in animal husbandry likely contributes more to the overall burden of antibiotic resistance than human usage, according to the findings of a study comparing the resistance of *E. coli* isolates from farmers and their animals in Ghana (Donkor *et al*., 2012). This was because although human and animal bacterial isolates exhibited high amounts of resistance, isolates from animals showed higher resistance than those from humans.

Studies have indicated resistance facilitation by plasmid acquisition and chromosomal assimilation of resistance genes (Wirth *et al*., 2015). In Uganda, Asiimwe *et al*. (2021) found that >90% of isolates carried at least one gene encoding enterotoxins, indicating a high risk of transmission of foodborne diseases via milk (Jans *et al*., 2017). PVL-

positive *S. aureus* is widespread in Africa. Moreover, *S. aureus* isolates from Africa show a high degree of resistance to penicillin, tetracycline and trimethoprim/ sulfamethoxazole, indicating the wide use of these drugs in African countries (Schaumburg *et al.*, 2014).

Antimicrobial resistance is spreading at the abattoir level, according to study data, and virulence and antimicrobial resistance have been found in recently recovered *Salmonella* isolates from broiler chickens in South Africa (Zishiri *et al*., 2016). A study conducted in southern Ethiopian poultry production centers revealed that the majority of the isolates were resistant to over eight antimicrobial agents, indicating the possible significance of chickens as a source of multidrug-resistant *Salmonella* infections (Abdi *et al*., 2017). Similarly, studies from different countries in Africa found that multidrug resistance was exhibited by most *Salmonella* isolates from meat and food products (Ed-Dra *et al*., 2017; Amajoud *et al*., 2017; Iwu *et al*., 2016).

Antimicrobial resistance genes encoded by *Salmonella* that have been detected in chickens and humans include $bla_{\text{PSE-1}}$ conferring resistance to ampicillin and amoxicillin, *ant(3") Ia* conferring resistance to gentamicin, and *sul1* and *sul2* conferring resistance to sulfonamides. Other *Salmonella* isolates from chickens and humans exhibited tetracycline resistance, indicated by the detection of tetA and *tetB* genes (Zishiri *et al*., 2016).

Antimicrobials are available over-the-counter without a prescription in many developing countries. Unfortunately, there is a correlation between the use of antimicrobials in animal husbandry and the incidence of antimicrobial-resistant bacteria isolated from food-producing animals (Alonso *et al*., 2017). Information on antibiotic resistance in the Africa region is still scarce, but available information

shows that it is an important sector where urgent attention is needed. The potential spread of antimicrobial resistance genes with adverse connotations for both human and animal health and welfare looms.

2.5.3 Antibiotic resistance in aquatic environment

A lot of bacterial species developed resistance to antibiotics long before people began their mass-production to treat and prevent infectious diseases (D'Costa *et al*., 2011). The constant competition between microorganisms for resources, including the natural production of secondary metabolites that are similar to many of the antibiotics used as pharmaceuticals today, is probably a major factor in the ancient and ongoing evolution of resistance mechanisms (Davies & Davies, 2010). The relatively recent introduction of antibiotics as therapeutic agents drastically altered the conditions for the evolution and dissemination of resistance by posing previously unheard-of selection pressures, particularly on domestic animal and human microbiota members and in antibiotic- polluted environments. Consequently, this selection pressure has advanced the mobilization and horizontal transfer of a large range of antibiotic resistance genes (ARGs) to many bacterial species, especially to those causing diseases (Alcock *et al*., 2020).

Antibiotics reach the environment via different pathways including excretions (urine and faeces) from humans and domestic animals (Wang *et al*., 2020), through improper disposal and/or handling of unused drugs (Anwar *et al*., 2020), through direct environmental contamination in aquaculture (Cabello *et al*., 2016) or agriculture in plant production (Tylor & Reeder, 2020), and though waste effluents from the production of antibiotics (Bielen *et al*., 2017). Hospital wastewaters represent a broad reservoir of antibiotic-resistant bacteria (ARB) and ARGs, for instance the extended-

spectrum β- lactamases (ESBLs) and carbapenemase-producing *Enterobacteriaceae* (CPE) (Hassoun- Kheir *et al*., 2020). Beyond any doubt, the most widespread discharge, and quite possibly the largest proportion of released antibiotics, are the result of use and excretion. In modern livestock farming, antibiotics are used to prevent and treat animal diseases. As a result, manure can provide a pathway for ARGs to spread into the surrounding environment. Consequently, numerous studies have shown that the abundance of resistant bacteria and/or ARGs increase after manure from antibiotic-treated animals is added as a fertilizer to farmland (Heuer *et al*., 2011).

Human and animal sources introduce antibiotic-resistant microbes into water ecosystems through anthropogenic activities. Aquatic ecosystems, are therefore regularly disturbed by human activities, and may provide favorable conditions for the horizontal transfer of antibiotic resistance genes (Marti *et al*., 2014). Lakes, rivers, streams, and even coastlines receive effluent from wastewater treatment plants (WWTPs), agricultural runoff, and other human inputs (Wang *et al*., 2020) that may either serve to raise natural background levels of antibiotic resistance genes (ARGs) and stimulate their transfer into pathogens or other organisms or as a conduit for the propagation of ARGs of concern (Ben *et al*., 2019). The usage of domestic wastewater in agricultural irrigation and recreational activities can also introduce new ARB and ARGs to the specific environment (Rodriguez-Mozaz *et al*., 2014). Therefore, it is recognized that aquatic environments are one of the key reservoirs and transmission routes for the spread of antimicrobial/antibiotic resistance (AMR/AR) (Amaya *et al*., 2012).

The presence of antibiotics in the environment is considered a main source of changes in natural environments and influence a diversity of processes, including the development of antibiotic resistance and the spread of genes through horizontal gene transfer (HGT) (Ben at al., 2019; Qiao *et al*., 2018). Thus, the aquatic environment is considered efficient for the selection of bacterial populations resistant to antimicrobials, through mobile genetic elements (Von Wintersdorff *et al*., 2016). Increased resistance to antimicrobials is often associated with a high adaptive capacity of microorganisms, with bacteria being more susceptible to changes and gene acquisition.

Antibiotic resistance has been frequently reported from freshwater sources, wastewater systems including but not limited to pharmaceutical industries, and wastewater treatment plants (WWTPs). In an aquatic environment, wastewater and WWTPs are considered one of the key potential hot spots for the spread of antibiotic resistance and transfer of ARGs (Zhang *et al*., 2021). Numerous studies across the globe have reported the prevalence of ARB and ARGs in different water bodies. Correspondingly, a large number of ARGs (tetA, tetB, sulI, qnr, aadA, tetO, ampC, etc), and ARBs of concern (*E. coli*, *Enterococcus*, *Salmonella*, *Shigella*, *Aeromonas*, *Vibrio*, etc) have been reported in water bodies including aquaculture, freshwater, wastewater and marine water (Singh *et al*., 2022). A metagenomics study of municipal wastewater and hospital wastewater showed the presence of tetracycline, β-lactam, macrolide–lincosamide–streptogramin resistance gene and multidrug resistance genes ranging from 0.06–0.98 copy/cell, and biocide/metal resistance gene ranging from 0.30–1.99 copies/cell. Municipal wastewater had more abundant and diverse ARGs than hospital wastewater (Zhang *et al*., 2021). Another study reported the presence of different ARGs in samples from seawater at 1.7×10^2

copies/giga base (Zeng *et al*., 2019).

In yet another study, high levels of ARG levels of 1.57–700.58 × 10^2 copy/ml for penicillin were reported from surface water, whereas 0.37–312.7 × 10^2 copy/ml was reported from the groundwater of Sri Lanka. Among the penicillin resistance genes, the highest percentage of blaTEM (700.58 × 10^2 copy/ml) followed by ampicillin (0.37–371.7 × 10^2 copy/ml) and OPR D (1.57 × 10^2 copy/ml) resistance genes were reported from aquatic samples, whereas tetM and tetA resistance genes at the levels of 1.35–439.88 × 10^2 copy/ml were reported from the surface water samples. Only the tetM resistance gene was reported at 215.99 × 10^2 copy/ml from the groundwater sample of Sri Lanka (Liyanage *et al*., 2021).

Different levels of erythromycin, amoxicillin, sulfamethoxazole, ampicillin, clindamycin, tylosin, vancomycin, tetracycline, chloramphenicol, among others were frequently detected from the urban canals and lakes of Vietnam (Tran *et al*., 2019). A recent study done in Kenya reported presence of ARB and ARGs to commonly used antibitics including tetracyclines, aminoglycosides, and *β*-lactam in cattle-trough water samples in addition to chicken and cattle samples (Wanja *et al*., 2023).

It is evident that the use of antibiotics has greatly contributed to the prevalence of antibiotics, ARB and ARGs in aquatic systems, which has impacted negatively to the ecosystem. This also places emphasis on the role of water bodies in the spread of antimicrobial (antibiotic) resistance (AMR), antimicrobial resistant bacteria (ARB), and antimicrobial resistance genes (ARGs) among pathogens, animals, and humans.

2.6 Issues Concerning Environment and Ethics

While manure is the primary method of introducing antibiotic drugs, antimicrobial resistance genes, and antimicrobial-resistant bacteria from animal sources into the

environment, the environment also acts as a reservoir and a channel for the emergence and spread of antibiotic resistance traits (Durso *et al*., 2007). Antibiotic-resistant genes and bacteria are primarily found in the environments of feeding animals, water, soil, and their digestive tracts (Ballard *et al*., 2015). A particular example of this is use in aquaculture. Antibiotics are often introduced into the aquatic environment for purposes of disease prevention and treatment (McEwen *et al*., 2002) which could render the water environment unsafe due to persistence of antibiotic residues.

Naturally, there are microorganisms in the environment that are resistant to some medications. Additionally, it is common for germs to get resistant to antibiotics over time. Recent metagenomics studies have demonstrated the presence of antimicrobial resistance genes in both human and animal waste (McEwen *et al.,* 2002). For instance, antimicrobial resistance is common and, surprisingly, human waste had a higher diversity of microorganisms than animal waste after studying a range of habitats (Agga *et al.,* 2015). It follows that not all antimicrobial resistance in human microorganisms is caused by the use of antibiotics in animals. On the other hand, the rate at which antibiotic resistance characteristics in bacteria emerge is undoubtedly accelerated by the extensive use of antibiotics in animals. In fact, it was proven in a very fascinating controlled study that pigs given antibiotics changed the composition of their microbiome, becoming more resistant to antibiotics on overall as well as to those that weren't given to them (Looft *et al*., 2012).

Antibiotic use in food animals raises ethical concerns regarding animal health as well as other farming procedures (Littmann *et al*., 2015). The rise of resistant strains of bacteria will lead to ineffective antibiotic therapy and negative health consequences.

The intended widespread use of antibiotics has the exact opposite effect on animal health that this detrimental effect is having. Lastly, workers in agriculture and animal husbandry are more likely to get resistant diseases (Smith *et al*., 2013) and these may then be further disseminated via a number of vehicles, including meat and fish and the staff themselves, to the general public.

Undoubtedly, numerous excreted antibiotic residues from food animals remain unaltered in the environment (Tasho *et al*., 2016), which may linger and be discovered later in sewerage installation plants next to farmlands being treated with animal manure (Zhang *et al.,* 2011). The bacteria that live in these sewage installation plants can exchange genes with one another laterally. Certain antibiotic-resistant bacteria are preferred because the antibiotic concentrations found here typically fall below the minimum inhibitory concentrations (MICs) (Andersson *et al*., 2014). The presence of an antibiotic is enough to completely or partially inhibit the growth of bacteria, causing selective pressure that will increase the prevalence of resistance (Tello *et al*., 2012). According to a study, specimens from sewage treatment facilities had higher relative levels of antibiotic resistance genes (blaCTX-M, blaSHV, and blaTEM) than those found in the effluent of an antibiotic production factory (Sidrach-Cardona *et al*., 2014).

2.7 Alternatives to Antibiotic Use in Food Animals

The problem of foodborne diseases in public health is still widespread and growing in both industrialized and developing nations (Van *et al*., 2012). Pathogen infections, including those caused by *E. Coli* O157:H7, *Campylobacter* spp., *Salmonella* spp., and *Yersinia enterocolitica*, have the ability to spread quickly and across international borders (Kadariya *et al*., 2014).

A report from the WHO estimated that 70% of diarrheal cases originate from bacterially infected foods. Therefore, in order to successfully treat bacterial infections in both human and veterinary practice, alternatives to the application of antibiotics in food animals are sought after.

Research on antibiotic alternatives for food animals is currently moving at a slow pace (Cheng *et al*., 2014). Nonetheless, some recommended alternatives to the use of antibiotics in food animals include the use of probiotics, increased vaccination rates, and updated agricultural management techniques (Reid *et al*., 2002). The use of probiotics, prebiotic-based materials, or a symbiotic combination of the two is recognized to reduce pathogen colonization since antibiotics given to food animals are assumed to interact with the animal's intestinal and associated microbiota (Patterson *et al*., 2003; Aminov *et al*., 2017).

The problem of foodborne diseases in public health is still widespread and growing in both industrialized and developing nations and continue to be an extensive and expanding public-health challenge. Therefore, altering the intestinal microbiota in food animals through food additives or fecal transplantation could be a way to prevent some infections (Musa *et al*., 2009). The effectiveness of probiotics and prebiotics as growth- promoting agents could be influenced both by environmental and stress conditions (Ben Lagha *et al*., 2017).

Furthermore, it is known that some plant-based extracts have antioxidant, antibacterial, and anticoccidial qualities, which may allow them to take the role of antibiotics in food animal production (Kumar *et al*., 2014). Red clover extract (Flythe *et al*., 2016), a plant- derived product with an abundance of active biological compounds, has recently been discovered as an alternative to antibiotic growth

promoters in food animals. Additionally, it has been demonstrated that phytogenic feed additives improve gut health and, consequently, gut function (Murugesan *et al*., 2015), and as a result, it has been advised that they be used as antibiotic alternatives in food animals. Phytogenic feed additives reduce potentially disease-causing microorganisms and modifiably alter the gut microbiota in an antibiotic growth promoter capacity (Murugesan *et al*., 2015).

Furthermore, the ability of organic acids to exhibit bactericidal and bacteriostatic characteristics based upon the physiological conditions of the organism as well as the physicochemical features of the environment (Ricke *et al*., 2003) argue for their use as alternatives to antimicrobial elements in food animals.

Phage treatment is another means of control that is being investigated. Phage therapy is one non-antibiotic alternative used in the treatment of animal diseases (Aminov *et al*., 2017) bacteriocins, phage lysins and predatory bacteria (Ben Lagha *et al*., 2017). Because bacteriophages have specialized targets within the bacterial community, they can be selected to specifically attack a disease without compromising the health of the microbiome as a whole. With varying degrees of efficacy, phage therapy has been thoroughly investigated in chicken to treat *Salmonella*, *E. Coli*, *Campylobacte*r, and *Clostridium perfringens* (Wernicki *et al*., 2017). A very pertinent study to human health examined the effectiveness of phage therapy in MRSA-infected piglets (Verstappen *et al*., 2016). Whilst killing of the bacteria was observed in vitro, no effect was seen in vivo. The challenge in phage therapy is to identify phages that have specificity for a particular target and efficacy in vivo.

Increasing the use of vaccines in food animals as alternatives to antibiotic drugs has received strong support (Allen *et al.,* 2013). There are further vaccinations against diseases that are available for use in food animals, for example, *Choleraesuis serovar, Salmonella enterica* (Allen *et al*., 2013) and *S. enterica* serovar Typhimurium (Alderton *et al*., 1991).

Lastly, farmers should consider using better biosecurity practices and hygienic farming conditions as viable alternatives to relying solely on antibiotics for disease prevention and control. These would basically function as a way to stop diseases from entering and spreading among animals (Hernández-Jover *et al*., 2016).

2.8 Global Initiatives in Tackling Antibiotic Resistance

National Action Plans (NAPs), which are currently being implemented throughout Africa, are a crucial component of the World Health Organization's (WHO) efforts to decrease antimicrobial resistance (AMR) (Fuller *et al.,* 2000). Undertaking point prevalence surveys (PPS) studies to determine existing utilization patterns as a foundation for launching focused quality improvement programmes is a crucial part of NAPs across hospitals (Siachalinga *et al*., 2023). One way to enhance future prescribing practices is to measure future prescription practices against predetermined quality indicators as part of Antimicrobial Stewardship Programmes (ASPs). Following guidelines is increasingly regarded as proving appropriate use of antimicrobials (Muniba *et al.,* 2023). But considering that several national guidelines in Africa support the prescription of antibiotics to patients even in cases when the patient has relatively few bacterial infections or co-infections, the guidelines must be strong and grounded in evidence (Chigome *et al*., 2023). Reducing the amount of antibiotics prescribed post-operatively to avoid surgical site infections (SSIs) is one of

the main goals for ASPs in hospitals, as doing so raises expenses, adverse events, and antimicrobial resistance (AMR) without improving patient outcomes (Titani *et al*., 2023). Given current concerns with high levels of inappropriate prescribing, including "Watch" and "Reserve" antibiotics, it is necessary to document current findings regarding antibiotic utilisation patterns among hospitals throughout Africa, along with documented examples of antibiotic prescribing indicators and ASPs (Siachalinga *et al.,* 2023).

Given concerns about inappropriate antibiotic prescribing, the results can be used to guide future programmes and activities in sub-Saharan Africa aimed at reducing rising rates of antimicrobial resistance (Kariuki *et al*., 2022). The review's objective was to record the present use of antibiotics in hospitals throughout sub-Saharan Africa in order to inform future efforts to enhance the use of antibiotics in the area (Saleem *et al.,* 2022)

In order to support efforts to improve antibiotic utilisation in sub-Saharan Africa, previous reviews have provided information on current utilisation patterns, challenges, indicators, and ASP across the continent (International Monetary Fund., 2023). These reviews included the results of PPS studies conducted among various African nations.

PPS studies record the amount of antibiotics prescribed to all hospital in-patients on the morning of the survey, for example, 0800 (Bene *et al.,*2019). The authors employed quality indicators in a recent review to examine and enhance the prescription of antibiotics (Arcenillas *et al.,* 2018). To offer future direction, these were summed up and included in the cited papers. Antimicrobial stewardship teams in hospitals were able to evaluate which actions and areas to prioritise as part of ASPs

along the prescribing pathway and to evaluate their progress in order to enhance future prescribing, thanks to the establishment of prescribing or quality indicators (Rzewuska *et al.,* 2018).

All significant hospital stakeholders then came to an agreement on goals to enhance future prescription practices, which were then tracked. Numerous studies have now demonstrated that prescribing and monitoring in accordance with established indicators can reduce AMR and has a significant effect on treatment results (Massele *et al.,* 2023).

The aim of such studies was to record the existing state of affairs and model cases throughout Africa in order to offer guidance in the future. This was based on the extensive experiences of the co-authors, who have previously conducted reviews throughout Africa and beyond to record the present patterns of hospital antimicrobial utilisation, as well as the prescription practices, quality indicators, and results from antimicrobial therapy across the continent (Acam *et al.*, 2023).

Sub-Saharan African hospitals had a high rate of antibiotic use, according to the PPS research' findings (Siachalinga *et al.*,2023). The usage of broad-spectrum antibiotics, particularly cephalosporins and penicillins, is indicative of antibiotic use (Karen *et al.,* 2007). Most of the time, the justifications for the use of antibiotics are documented; but, policies and the application of microbiological testing are usually absent. Antibiotic prescriptions are therefore typically empirical. Contrary to advised standards, antibiotics administered for postoperative prophylaxis are typically prescribed for longer than 24 hours (Hungaro *et al.,* 2014). Across Africa, a variety of quality measures have been employed in an effort to enhance the future administration of antibiotics (Abubakar *et al.,* 2023). Microbiological test utilisation and the

prevalence of antibiotic use are the two most often reported quality indicators (Claudia *et al.,* 2023). Among the least often reported signs are the transition from intravenous (IV) to oral therapy and the recording of start and finish dates (Fatma *et al.,* 1999). To enhance the prescribing of antibiotics in the future, several of these markers are being employed as part of ASPs (Mertz *et al.,* 2009). The majority of research indicates that there is still need for more money, awareness, and involvement at all levels of health-care management, in addition to opportunities for improvement in the way ASPs are implemented in hospitals (Barlam *et al.*, 2016).

Antibiotic use is quite prevalent in the hospitals that were studied throughout Africa; the majority of them reported prevalence rates over 50%. There is a wide variation of prevalence rates among the hospitals surveyed, ranging from 37.7% in South Africa (Dlamini *et al.,* 2019) to 80.1% in Nigeria (Abubakar *et al.,* 2020). A patient is usually prescribed two antibiotics, the most commonly prescribed being cephalosporins, penicillins, and nitroimidazoles.

Significant worries exist over the improper prescription of antibiotics, as several studies have shown that the supporting documentation for prescriptions is either incomplete or nonexistent (Momanyi *et al.,* 2019; Horumpende *et al.,* 2020; Omuokonkwo *et al.,* 2020). 36% of the medicines prescribed in a Tanzanian research were for patients whose bacterial infection was thought to be unlikely (Horumpende *et al.,* 2020).

The studies employ different prescription and quality metrics to evaluate the standard of antibiotic prescribing in Africa. The most often reported metrics include the percentage of intravenous (IV) antibiotic prescriptions, the most commonly prescribed antibiotic class, the prevalence of antibiotic prescribing, and the use of

microbiology testing. But any subsequent indicator designed to enhance future antibiotic use in hospitals needs to be clear and practical to implement. In addition, there needs to be simple, dependable, and consistent (ideally computerised) tools to regularly gather accurate data to track any progress (Campbell *et al.,* 2002; Campbell *et al.,* 2015; Campbell *et al.,* 2011). The majority of hospital patient data collection systems in Africa are currently paper-based, which makes routine monitoring difficult. This is expected to change as more NAPs are implemented throughout the continent (Godman *et al.,* 2021).

Concerns have also been raised about the use of culture and sensitivity testing (CST), the lack of documenting of prescription justifications in patient notes, and start and stop date reviews, all of which contribute to low rates of targeted antibiotic prescribing and high rates of empirical therapy throughout Africa. Consequently, broad-spectrum antibiotics, namely cephalosporins and penicillin combos, are widely used throughout Africa (Horumpende *et al.,* 2020; Maina *et al.,* 2020). The poor utilization of CST may be caused by hospital facilities' inability to quickly conduct these tests and their high expense, particularly if they are paid for out of pocket (Afriyle *et al.,* 2020). As a result, the majority of doctors give empirical prescriptions (Momanyi *et al.,* 2019; Umeokonkwo *et al.,* 2019; Fowotade *et al.,* 2020). Moving forward, NAPs should address this issue in addition to enhancing the availability and adherence to guidelines (Godman *et al.,* 2022). This is due to data indicating that the hospitals surveyed either had no recommendations or had very low adherence (Niaz *et al.,* 2019; Niaz *et al.,* 2020) to them—as low as 4% in one research (Umeokonkwo *et al.,* 2019; Oduyebo *et al.,* 2017).

As evidenced by the WHO AWaRe classification system (World Health Organization, 2022), guidelines and studies utilising this classification are expanding to lower AMR. Antibiotics are increasingly included in these guidelines, categorised according to their potential for resistance (Godman *et al.,* 2022; Klein *et al.,* 2021; Dlamini *et al.,* 2019). The process has been made easier in part by the recent release of the WHO AWaRe antibiotic book, which offers prescribing guidelines for a variety of illnesses (World Health Organization., 2022). There are also major concerns because the breadth of antibiotic prophylaxis to prevent surgical site infections, SSIs, are frequently more than 24 hours in the published publications with multiple doses in most situations (Horumpende *et al.,* 2020; Abubakar *et al.,* 2020). This is concerning because many doses of surgical prophylaxis before surgical incision are equally effective (Mwita *et al.,* 2021; Momanyi et al, 2019). Furthermore, as previously indicated, administering additional doses raises the risk of both antibiotic adverse effects and AMR (Mwita *et al.,* 2021; Anand Paramadhas et al, 2019). As such, one of the most important areas to focus on when implementing interventions to promote future antibiotic use among hospitals in sub-Saharan Africa as part of future ASPs is the right use of antibiotics to avoid SSIs.

AMR rates are expected to rise throughout Africa (Akpan *et al.,* 2020; Siachalinga *et al.,* 2022), so there is a need to reduce them as part of agreed-upon NAPs (Godman *et al.,* 2022). A number of challenges and gaps have been identified to improve future antibiotic prescribing, leading to a number of suggestions and recommendations that can be taken into consideration moving forward. Of particular concern are rising rates of AMR throughout Africa, increasing morbidity, mortality, and costs (Godman *et al.,* 2022). A significant amount of antibiotics is typically used inappropriately, especially in cases where guidelines are lacking and there is a dearth of microbiological testing,

which results in a significant amount of empiric prescribing (Godman *et al.,* 2021; World Health Organization., 2018). Consequently, strategies are recommended to enhance adherence to evidence-based robust guidelines in hospitals which are increasingly likely to be based on the WHO AWaRe guidance (World Health Organization, 2022). The implementation of guidelines is in association with education and training of HCPs on best practices, including all aspects of successfully undertaking Antimicrobial Stewardship Programmes (ASPs) and monitoring their impact. In most cases there is currently a lack of microbiological testing across Africa. This is enhanced by the lack of microbiological infrastructures along with co-payment costs where these occur.

If proactive measures are not taken to address this as part of achieving agreed-upon NAP targets, this will result in the ongoing prescription of broad-spectrum antibiotics. Currently, a variety of quality indicators are being utilised throughout Africa to evaluate the standard of antibiotic prescription practices. Therefore, the selection of quality indicators will rely on the environment, the resources at hand, and the methods in place for regularly gathering patient-level data (Campbell *et al.,* 2015; World Health Organization, 2018). In order to accomplish NAP goals, continued ASPs can be utilised to track improvements in prescribing using agreed quality metrics.

Antibiotic use has been demonstrated to be improved by ASPs without compromising clinical results (Siachalinga *et al.,* 2022). As such, they ought to be given top priority in continuing initiatives aimed at accomplishing the goals outlined in the Nationally Adopted Plans (NAPs) of the different African nations. To start, this entails efforts such as training courses and other initiatives to remove existing obstacles. Consequently, this will guarantee that all hospital-based key health care professionals

are well-versed in and educated to carry out ASPs (Afriyie *et al.*, 2020).

As a result, sustained involvement from the government, hospital managers, medical experts, and academics has become standard procedure to guarantee the viability of important initiatives that accomplish NAP goals by means of devoted leadership, monetary backing, and multidisciplinary cooperation. Along with enhancing routine data collection, educational initiatives and training to promote awareness and adherence to standards, such as the appropriate use of SSI antibiotics targeted at healthcare professionals, have also been addressed where applicable.

In order to give additional information on the use of antibiotics, the results of interventions, and future directions, research initiatives have been supported. In conclusion, hospitals in sub-Saharan Africa prescribe antibiotics quite frequently. Because microbial testing is rarely performed, antibiotic prescriptions are typically empirical. Furthermore, guidelines are frequently absent or poorly followed when they exist. This leads to the improper prescription of antibiotics, such as those prescribed for an extended period of time in order to avoid Surgical site infections, SSIs. Antibiotic utilisation as part of Antimicorbial Stewardship Programmes (ASPs) is being improved by a number of quality measures, and this trend is expected to continue. It is important to keep funding initiatives in sub-Saharan Africa that record hospital antibiotic usage patterns and persistent issues. It is advised to carry out ongoing point prevalence surveys (PPS) research in addition to implementing and assessing agreed-upon ASPs in the wake of identified improvement gaps in order to advance practice in the future.

With its massive costs in terms of illness, mortality, and healthcare expenditures, antimicrobial resistance (AMR) presents a serious challenge to the sustainability of

ecosystems supporting human, animal, and plant health. It is a serious worldwide public health issue that has a significant impact on both the general growth of states and nations as well as the global economy and prosperity (Majumder *et al*., 2020). AMR is a multidisciplinary, cross-cutting problem that has serious negative effects on food and environmental security, human health, and the accomplishment of the Sustainable Development Goals (SDGs) (World Health Organisation, 2017).

Africa carries a significant portion of the global burden of antimicrobial resistance (AMR) due to its high prevalence of communicable and non-communicable diseases, poverty, inadequate infrastructure and health systems, poor governance, and corruption (Jasovský *et al*., 2016; Tadesse *et al*., 2017). By 2050, AMR is predicted to result in 10 million annual deaths and 2%–3.5% productivity losses at present rates (Antimicrobial Resistance Collaborators, 2022). Bacterial antimicrobial resistance (AMR) was linked to an estimated 4.95 million fatalities worldwide in 2019. Western Sub-Saharan Africa had the greatest death rate, at 27.3 per 100,000 people (Antimicrobial Resistance Collaborators *et al.*, 2022). Overuse is the main cause of antimicrobial resistance (AMR), and it is more common in low- and middle-income countries (LMICs) due to uncontrolled access, inappropriate and excessive prescribing by healthcare professionals, and a lack of use of treatment guidelines in case management (Engler *et al*., 2021).

Antimicrobial misuse in humans, animals, and plants is the primary driver of AMR. Due to inadequate regulations, low awareness and training, limited access to quality-assured antimicrobial medicines, limited diagnostic microbiology capabilities, and socioeconomic and cultural constraints, antimicrobial overuse and misuse are widespread throughout African nations (Alhaji and Isola *et al.*, 2018; Akpana *et al*.,

2020). According to a major meta-analysis, the coronavirus disease 2019 (COVID-19) pandemic is a more recent substantial cause of antimicrobial resistance (AMR). In patients with COVID-19, the estimated bacterial co-infection rate was 8.6%, but the prevalence of antibiotic prescribing was 74.6% (Langford *et al*., 2021).

Between 2000 and 2015, the world's antibiotic consumption rose significantly by 65%, with LMICs accounting for the majority of this rise (Klein *et al*., 2017). Antimicrobial stewardship (AMS) is promoted as a key tactic for maximising the use of antimicrobial medications as part of the global response to combating the problem of antimicrobial resistance (AMR), including the World Health Organization's (WHO) global action plan on AMR (World Health Organisation, 2015).

The World Health Organisation (WHO) promotes AMS programmes on all continents and created a toolkit of guidelines in 2019 to facilitate the implementation of AMS programmes in low- and middle-income countries (LMICs). The toolkit outlines the essential components of AMS at the national and healthcare facility levels. Publicly available information on the execution of AMS programmes in Africa is scarce. Only 13 studies that met the predetermined inclusion criteria were found after a systematic review of the literature across five electronic databases spanning more than 30 years (1990– 2019) was conducted. Furthermore, all of the studies were restricted to the following five countries: South Africa, Kenya, Sudan, Tanzania, and Egypt (Akpana *et al*., 2020). A distinct systematic analysis of the literature found 14 relevant studies from 11 African nations and identified challenges and facilitators of AMS programmes. The review was conducted using the Embase and Ovid MEDLINER® databases, with no constraints on the date of publication until 2020. Many of the studies were limited to certain locations within the countries, therefore they weren't

nationally representative (Porter *et al*., 2021). Only Kenya has national AMS guidelines for healthcare settings, according to a more recent scoping review of the AMS landscape in eight African countries (Ghana, Nigeria, Sierra Leone, Kenya, Tanzania, Uganda, Malawi, and Zambia). Variations in AMS programme implementation were found. The Kenyan recommendations offer guidance to those involved in AMS implementation at various service centres, such as community pharmacies, hospitals, and outpatient clinics.

2.9 Methods of Removal of Antibiotics from Environmental Matrices

Antibiotics are chemicals that may accumulate in different environmental matrices (Polianciuc *et al*., 2020). Antibiotic removal strategies have thus far included a number of different approaches. Biological treatments, electrochemical procedures, membrane filtration, reverse osmosis, ozonation, and advanced oxidation are all part of this category (Jha, et al. 2022; Ambaye, et al. 2020). According to Kraemer *et al*. (2019), the majority of these methods are either ineffective or lead to the development of undesirable by- products. The use of various sorbent materials, including plant biomass preparations, is another approach that is being considered for the adsorption-based removal of antibiotics from environmental matrices (Crini *et al.,* 2018). Adsorption is the most widely used technique because it is efficient, requires low investment, and is easy to set up (Rashed, 2013), which raises the need to develop a cost-effective and potential adsorbent material for environmental remediation.

2.10 Sorption Removal of Antibiotics from Wastewater and Surface Water

2.10.1 Removal of antibiotics by adsorption using biochar materials

Adsorption is a surface effect that depends on surface forces. It happens when a solution with an adsorptive solute comes into contact with a solid (adsorbent) that has

a porous enough surface structure. Molecular forces between liquids and solids then cause the solution to gather at the solid surface (Sims *et al*., 2019; Vital *et al*., 2016). Adsorbents are different kinds of materials that are used to get rid of organic pollutants. Waste materials containing lignin or non-lignin from agriculture, aquaculture, wood, and fibre processing industries, etc., are primarily used for the production of biochar. These mainly come from charcoal, zeolites, clays, ores, and other waste materials from plants and animals. Different types of waste have been turned into useful adsorbents, such as rice husk, bagasse from the sugar industry, seaweed and algae, sawdust, scrap tires, fly ash, fruit waste, coconut shell, fertilizer waste, oil waste, tannin-rich materials, blast furnace slag, chitosan, waste from seafood processing, and peat moss. There are two main types of adsorptions: physisorption and chemisorption. These types are based on how strongly the adsorbate and substrate interact with each other (Sims *et al*., 2019; Vital *et al*., 2016).

The direct or indirect association of liquid or gaseous chemicals with solid phases has long been utilized in environmental chemistry, and is broadly termed as sorption process. For this reason, two terminologies namely: absorption and adsorption are frequently used to describe the nature of the association between fluids and solids. There is however a line of difference between the two (Qi *et al*., 2017): the former involves molecular penetration into a three-dimensional matrix while the latter involves the attachment of the fluid molecules onto a two-dimensional matrix. The International Union of Pure and Applied Chemistry (IUPAC) in its generalization visualizes adsorption as the enrichment of particles (either atoms, molecules or ions) in the vicinity of an interface (Thommes *et al*., 2015). For context-specific use in analytical and environmental chemistry, adsorption is the process of removal of substances (called adsorbate) from one phase which is then subsequently concentrated

on another substance known as the adsorbent (Hamzezadeh *et al*., 2020). In the context of remediation of contaminants (such as antibiotics, heavy metals, dyestuffs and dyeing auxiliaries) from environmental matrices, adsorption has increasingly taken precedence as a method of choice in comparison to other remediation approaches. This is owing to the lower initial costs associated with this technique, its holistic (non-selective) remediation approach, the low technical know-how required for operation and the ease of designing batch reactors. To optimize the remediation process, various factors affecting the process of adsorption needs to be explored (Xu *et al*., 2021).

2.10.2 Synthetic Routes of Biochar

Processes namely pyrolysis, hydrothermal carbonization, torrefaction, and gasification are used to convert biomass (raw material) into biochar depending on the temperature range, conditions, and residence time. All processes produce biochar (solid) as the main product, as well as bio-oil (liquid) and biogas (gaseous) in larger or smaller quantities. The percentage of solid, liquid, and gaseous products varies from method to method. The synthetic method is selected depending on the biomass in order to obtain the maximum yield of biochar (Yaashikaa *et al*, 2020). The properties of the feedstock and the method used for biochar production have a great influence on the physical and chemical properties of biochar, so it is crucial to understand the heating rate, residence time, and decomposition temperature of the different production methods.

2.10.2.1 Pyrolysis

Exposing the feedstock to a high temperature (250–900 ◦C) in the presence of limited or no oxygen is called pyrolysis. Pyrolysis has been reported to be the preferred

method among all biochar production processes because it can be used for a wide range of biomass (Elkhalifa *et al*, 2019). During pyrolysis, the components present in biomass such as cellulose, hemicellulose, and lignin are depolymerized, fragmented, and cross linked. This leads to the formation of products in different phases such as solid, liquid, and gas. Pyrolysis conditions such as temperature, residence time, and type of biomass play an important role in biochar yield (Wei *et al*., 2019). Pyrolysis can be divided into slow pyrolysis, fast/flash pyrolysis, and microwave pyrolysis depending on the temperature and residence time.

2.10.2.2 Slow Pyrolysis

In this process, the biomass undergoes pyrolysis at 300–500 ◦C with a longer residence time of several minutes to days (Tan *et al*., 2015). Slow pyrolysis is considered the most effective because it yields a maximum of solid products (biochar) and a lower level of gaseous (biogas) and liquid products (bio-oil). In general, as the temperature increases, the yield of biochar decreases and the amount of biogas decreases. Since slow pyrolysis involves a slower heating rate and longer residence time, maximum biochar yield is obtained. According to one study, slow pyrolysis achieves a biochar yield of 35.0% from dry biomass (Tomczyk *et al.,* 2020). This is the main reason why slow pyrolysis is the most suitable method for biochar production among all pyrolysis processes.

2.10.2.3 Fast Pyrolysis

Fast pyrolysis is a thermochemical process for liquefying biomass into bio-oil, which is highly useful for energy production (Yaashikaa *et al.*, 2020). It is carried out at a temperature above 500 ◦C to 700 ◦C. This type of pyrolysis involves faster heating rates (>10–10,000 ◦C/min) and shorter residence times in seconds. As mentioned

earlier, the yield of biochar from this process is comparatively low compared with slow pyrolysis due to the increase in temperature and reduction in residence time. Instead, a maximum amount of bio-oil is obtained at the end of the fast pyrolysis. The type of pyrolysis process also affects the properties of biochar produced. A higher pyrolysis temperature results in biochar with a larger surface area, higher pH and volatiles, but lower surface functional group and cation exchange capacity (CEC) (Tomczyk, *et al.,* 2020). Ghani *et al.* (2013) demonstrated that biochar becomes more hydrophilic at temperatures below 500 ◦C (slow pyrolysis), while biochar exhibits hydrophobic behaviour and high thermal stability at temperatures above 650 ◦C (Ghani *et al.,* 2013).

2.10.2.4 Microwave Pyrolysis

Microwave pyrolysis is the decomposition of biomass by energetic microwave radiation (Jeyasubramanian *et al.,* 2021). Unlike conventional pyrolysis, in microwave pyrolysis, the temperature at the centre of the feedstock is higher than at the surface (Godwin *et al.,* 2019). This results in efficient heat transfer and shorter reaction time compared with other thermochemical methods (Nhuchhen *et al.,* 2018). Microwave pyrolysis yields a very small amount of bio-oil compared with other conventional processes. Wang *et al*. (2018) produced biochar by microwave-assisted pyrolysis from camellia (*Camellia oleifera*) peel with a yield of 37.45% and 27.45% for biochar and bio-oil, respectively.

2.10.2.5 Hydrothermal Carbonization (HTC)

In hydrothermal carbonization, also known as “wet pyrolysis”, the biomass is surrounded by water during the reaction (Libra *et al.,* 2011). In this process, the reaction temperature is applied in a range of 180–230 ◦C under low pressure in a

closed system (Cheng et al., 2021). In the HTC method, the pressure and temperature are maintained in such a way that the water remains in a liquid state throughout the process. Further, the advantages of HTC include energy efficient production of biochar in high yield, i.e., low temperature requirement (about 180 ◦C) (Hossain *et al.,* 2020). It involves a series of reactions such as hydrolysis, condensation, decarboxylation, and dehydration (Wilk and Magdziarz *et al.,* 2017). The HTC method for the production of biochar is attracting more and more attention because it offers the advantage of using wet biomass and does not require an additional drying step. To distinguish the biochar obtained by pyrolysis and HTC, the final product of HTC is called "hydrochar". The hydrochar formed has more oxygen- containing functional groups, which makes it more favourable for adsorption of heavy metals from aqueous media (Ambaye *et al.,* 2020). Reports indicate that hydrochar has more oxygen containing groups and higher cation exchange capacity (CEC) value than the biochar produced by pyrolysis, which is an important factor for the use of biochar as an adsorbent (Liu and Zhang 2009). The easy biodegradability of hydrochar limits its application for carbon sequestration (Bai *et al.,* 2013).

2.10.2.6 Torrefaction

Torrefaction is one of the new techniques for biochar production from biomass. In this process, raw material was heated for the residence time of 15–60 min within the temperature range 200–300 ◦C in an inert atmosphere (Jeyasubramanian *et al.,* 2021). Since, the operating temperature is lower than that of pyrolysis, it is also called "mild" pyrolysis (Yaashika *et al.,* 2020). In this process, biomass depolymerization occurs, the extent of which depends on the reaction time and temperature. Biochar is not the main product of torrefaction, but is suitable for adsorption of pollutants (Tan *et al.,* 2015). The biochar produced by this process exhibits hydrophobic properties because

the hydroxyl groups present on the surface of the biochar are removed during this process (Costa *et al.,* 2015). In addition, the biochar obtained from torrefaction has better ignitability, grindability, high calorific value, carbon content, energy yield, and lower moisture content, which makes it more suitable for use as a fuel (Pothomrotsakun *et al.,* 2012; Wannapeera and Worasuwannarak, 2012; Mamvura and Danha, 2020). According to a study by Pathomrotsakun *et al.* (2012), biochar produced from torrefaction of ground coffee residues has a calorific value and energy yield of 31.12 MJ/kg and 48.04%, respectively (Pothomrotsakun *et al.,* 2012).

2.10.2.7 Gasification

Gasification of biomass is shown as another alternative method for the synthesis of biochar. The biochar is synthesized in an oxidizing environment (single or mixture of gases) at a temperature of ~700◦ (Gabhane *et al.,* 2020). Further, the partial combustion of the feedstock under an oxidizing atmosphere produces syngas, a mixture of H_2, CO, CO_2, and CH_4, which is used as fuel. The reaction temperature is the key factor affecting the production of syngas (Yaashikaa *et al.,* 2020). Gasification produces the highest percentage of gaseous product and very little biochar compared with the other processes.

2.10.3 Properties of Biochar

The excellent performance of biochar as an adsorbent can be attributed to its characteristic properties. Specific properties of biochar include; porous nature, high carbon content, high specific surface area (SSA), hydrophobic nature, and ease of functionalization. Further, the composition of biochar in terms of carbon, nitrogen, sulfur, and oxygen affects the chemical properties of biochar (Jeyasubramanian *et al.,*

2021). The properties of biochar depend on several factors such as the composition of the raw material, preparation method, and pyrolysis temperature. Thus, it is important to understand how the reaction conditions influence the properties of biochar. Therefore, in order to develop biochar with desired properties or to treat the analyte of interest, it is inevitable to have a thorough knowledge of all the factors affecting its properties. In their study, Evans *et al.* (2017) demonstrated the effects of the feedstock on the properties of biochar. They produced biochar from various agricultural by-products (e.g., hardwood French fries, pine French fries, cotton litter, poultry litter, miscanthus grass, and switchgrass) as feedstocks under reaction conditions of 400 ◦C for 2 h. From this research, it was found that the mineral content of biochar varies greatly depending on the feedstock used (Evans *et al.,* 2017). Poultry litter biochar had a higher mineral content than all other types of biochar produced in the report. The pH of the resulting biochar also varied depending on the feedstock used. The pH values of the biochar produced in the study ranged from 4.6 to 9.3 (Evans *et al.,* 2017).

Kathrin *et al.* (2012) reported that temperature variation from 200 to 400 ◦C has great influence on the properties of biochar such as porosity, surface area, etc. (Kathrin and Peter, 2018). Moreover, as the temperature increases, the porosity and particle size of the biochar also increase (Kloss *et al.,* 2012). The smaller particles have larger surface area and better CEC value (Shackley *et al.,* 2013). The CEC value also depends on the type of raw material used for preparation of biochar. In addition to the raw material, the decomposition temperature in the production of biochar is also a key factor affecting the adsorption of heavy metals on biochar. It was observed that as the pyrolysis temperature increases, the functional group containing oxygen decreases thereby altering the adsorption efficiency of the biochar. Moreover, the pH of the

solution also plays a significant role in deciphering the adsorption capacity of the adsorbent; it can increase or decrease the adsorption capacity based on the model metal impurity. Chen *et al*. (2012) investigated the impact of pH on the adsorption of metal ions of copper, zinc, and lead onto biochar derived from corn straw and hardwood. Results obtained revealed that enhancement in the pH from 2.0 to 5.0 enhances the adsorption capacity of metal cations, while at pH above 5.0 the adsorption capacity decreased due to the formation of hydroxide complexes (Chen *et al.*, 2011). In contrast, a decrease in pH increased the removal of Cr, as reported by Zhang *et al*. (2013). The reason for this was thought to be the electrostatic interaction of the negative charge of the chromate ion with the positive charge of the biochar at low pH (Zhang *et al.*, 2013).

2.10.4 Adsorption Mechanism

In adsorption, the adsorbate is associated with the adsorbent's surface until equilibrium is achieved. There are certain interactions and forces that act between the target molecule and the surface of biochar. The steps involed in the adsorbation process include: (a) physical adsorption in which the adsorbate settles on the adsorbent's surface (b) precipitation and complexation in which the adsorbate deposits on the adsorbent's surface. And (c) pore filling in which the adsorbate is condensed in to the pore of the adsorbent (Fagbohungbe *et al.*, 2017). This process occurs in three stages qualifying three zones: the first is the stage in which no adsorption takes takes place; it is called the clean zone. The second stage is called the mass transfer zone in which the adsorption is in progress. The last step is the stage in which equilibrium is achieved: it is called the exhausted zone (de Ridder *et al.*, 2012). The saturated zone increases, while the clean zone decrases during the process. The mass transfer zone is affected if the adsorbate's

concentration is increased; otherwise it is not affected. The interaction between adsorbent and adsorbate majorly depends on factors such as the nature of pollutants, pore nature, pore volume, specific surface area, hydrophobic nature, surface functionalization, etc. The different adsorption mechanisms involved during biochar–pollutants interaction are shown in Figure 2 8. Based on the interactions with different organic pollutants, they were classified as coagulation, precipitation, ion exchange, electrostatic interaction, hydrophobic interaction, pore-filling interaction, and hydrogen bond formation.

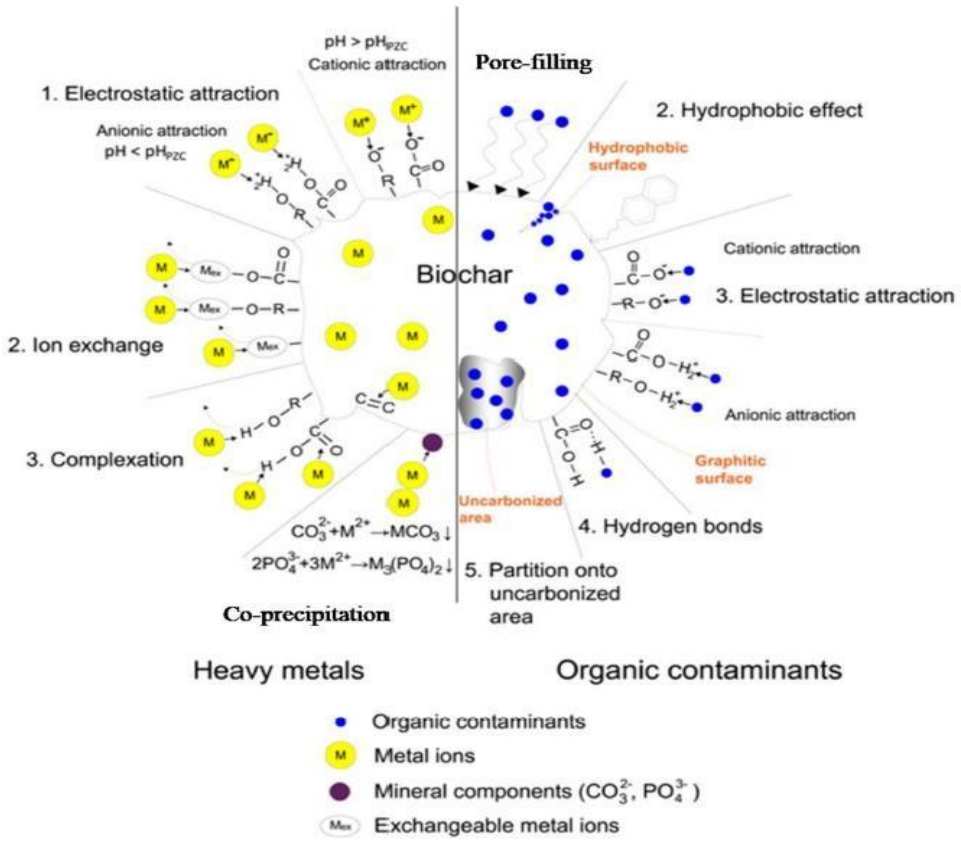


Figure 2.8: Different biochar adsorption mechanisms for organic and inorganic contaminants (Adapted from Ahmed et al. 2014)

2.10.4.1 Hydrophobic Interaction

'Hydrophobic' means water-repellent or water-phobic and arises from the presence of nonpolar groups (C-H) in a molecule (Xie et al., 2020). This mechanism is used for the adsorption of hydrophobic or neutral organic compounds through hydrophobic interactions. The hydrophobic nature of biochar can be explained by a decrease in oxygen, hydrogen, nitrogen, and sulphur content and an increase in carbon content due to carbonization (Murray et al., 2019). The increase in pyrolysis temperature during the production of biochar leads to a decrease in the number of polar groups and enhances the hydrophobic nature of biochar (Ambaye et al., 2020). The adsorption of oil on the surface of biochar occurs mainly due to hydrophobic interactions. Kandanelli et al. (2018) reported the adsorption of crude oil on the surface of biochar from rice husk by hydrophobic interaction.

2.10.4.2 Pore-Filling Interaction

The porous nature of the biochar is the key reason behind the pore-filling interaction mechanism. Biochar possess different types of pores and based on the pore dimensions, the pores that are 50 nm in diameter represent macropores (Pignatello et al., 2006). Further, the pore size of the biochar can be controlled by the number of factors which involves synthesis mechanism, pyrolysis temperature, pre- and post-treatment methods and based on the model pollutants size, removal efficiency of the adsorbent can be varied during diffusion and adsorption (Inyang et al., 2016). This mechanism also depends on the polarity of the contaminant (To achieve high adsorption by pore-filling, biochar should have a low volatile content). Qui et al. reported that sulfamethoxazole molecules were adsorbed on biochar derived from municipal sludge through pore filling effect (Qui et al, 2023)

2.10.4.3 Hydrogen Bond Formation

Numerous toxic pollutants are adsorbed by the formation of hydrogen bonds on the surface of biochar. The formation of hydrogen bonds occurs due to the negatively charged surface of biochar by the presence of -OH groups (Tan et al., 2020). Phenolic compounds, pesticides, dyes, etc., form hydrogen bonds with biochar (Tran et al., 2020). The functional groups present either on the surface of biochar or organic pollutant can participate in the formation of hydrogen bond. Groups such as amine (-NH) and hydroxyl (-OH) serve as H-donor. On the other hand, benzene rings, F, N, and O act as H- acceptors for H-bonding.

2.10.4.4 Pi-Pi (π-π) interactions

π-π interactions is a type of dispersion force from van der waals forces, it is established between unsaturated (poly) cyclic molecules. Previus studies have reported that π-π are one of the major mechanisms governing tetracycline adsorption by biochar (Xu et al, 2020; Wang et al., 2017). This interaction is attributed tobenzene ring and nitro- containing groups. It was proposed to be significant force for doxycycline and ciprofloxacin adsorption on rice straw biochar (Zeng et al, 2018).

2.10.5 Biochar regeneration

One of the primary benefits of biochar is its high-cost efficiency owing to the low cost of the feedstock and simplicity of preparation methods (Barquilha et al., 2021). Reactivation (regeneration) of biochar after the adsorption process to be used for several cycles reduces the operational and running cost of the wastewater treatment process significantly.

Chemical regeneration is one of the most popular ways to regenerate adsorbent materials (Wu et al., 2020). It relies mainly on adsorbate concentration and interaction

forces on the adsorbent (Barquilha et al., 2021). The chemical regeneration process uses solvents and chemical reagents to desorb the adsorbate from the biochar. Organic adsorbents with limited thermal stability and low boiling point are more suitable for chemical regeneration (Dai et al., 2019). Several solvents (organic/inorganic) were used to desorb both organic/inorganic adsorbates (Zubair et al., 2021; Gokulan et al., 2019; Wu et al., 2020; Zeng et al., 2019)

2.10.6 Biochar application in Adsorption

Different adsorbents have been tested for their efficacy in the removal of antibiotics contaminants in aqueous systems. For example, the use of bamboo biochar (Xu *et al*., 2012), water hyacinth biochar (Ngeno *et al*., 2016), sludge-based activated carbon (Rozada *et al*., 2003) have been previously reported. Biochar has shown promising application for the removal of organic pollutants from surface water (Hamzezadeh *et al*., 2020; Odero, 2021; Xu *et al*., 2012). Biochars from pyrolysis of biomass are highly sorptive carbonaceous materials with high porosity and surface area desirable for enhanced adsorption of pharmaceutical contaminants as already demonstrated for antibiotics (Ahmed *et al*., 2015; Dutta & Mala, 2020; Han *et al*., 2013; Teixidó *et al*., 2011). Modified biochar has improved surface area and sorption capacity than other adsorbents. Physical and chemical activation enhances the surface area or functionality of biochar. Physical activation approaches force high temperature steam through the pores of the biochar, increasing its available surface area. On the other hand, chemical activation utilizes either acidic or alkaline solutions which oxidize the biochar surface, resulting into the formation of oxygen-containing chemical groups (Bai & Hong, 2021; Rajapaksha *et al*., 2016; Sizmur *et al*., 2017; Usman *et al*., 2016). In other words, modification utilizes the biochar as a scaffold to embed new materials that produce surfaces with novel surface properties where contaminants are finally

adsorbed (Konneh *et al*., 2021; Lee, 2021; Su *et al*., 2021; Wang *et al*., 2021).

This study evaluated the use of water hyacinth and millet husks for development of biochar for removal of Ciprofloxacin, Penicillin G, and Sulfamethoxazole from surface water from River Sosiani, Eldoret, Kenya. Water hyacinth is a major challenge in Lake Victoria, which is shared by three countries of the East African Community namely: Uganda, Kenya and Tanzania. Water hyacinth (*Eichhornia crassipes* (Mart.) Solms- Laubach) is an invasive alien species and a perennial aquatic herb of the pickerelweed family (*Pontederiaceae*) that is traced to have been native to tropical America before it was imported as an ornamental plant into Lake Victoria. It has flourished in the fresh water lake owing to suitable growth conditions, complete absence of omnivorous predators and heavy metal pollution of Lake Victoria (Omara *et al*., 2019a). It has had devastating effects as it impedes hydroelectricity generation, irrigation and water treatment operations (Opande *et al*., 2004), blocks boats and communal water points on the Victorian shorelines, kill photosynthetic phytoplankton, as well as expand the transmission window and the spread of diseases such as malaria, bilharzia, skin rush, cough, encephalitis and digestive disorders (Khabuchi *et al*., 2019; Omara *et al*., 2019a; Wanda *et al*., 2015).

Ngeno *et al*. (2016) provided a suitable alternative use of water hyacinth biomass with a dual benefit of protection of Lake Victoria ecosystem and an alternative low-cost residue- based biochar for water treatment. Water hyacinth biochar was reported to be an efficient carbonaceous adsorbent for removal of both caffeine and ciprofloxacin from aqueous solutions (Ngeno *et al*., 2016).

On the other hand, millet husks constitute one of the agricultural residues from millet production in Kenya (Farm Link, 2018; Newspot, 2021). Previous studies in Kenya

and beyond has indicated that millet husks are potential sources of biochar for remediation of contaminants from environmental matrices (Qiu, 2022).

The use of plant-derived biochar for remediation of antibiotics has been explored before. For example, efficient adsorptive removal of FQs antibiotics using bamboo biochar was reported by Lu *et al*. (2015). The maximum adsorption capacity of the biochar was 45.88± 0.90 mg g^{-1}, which suggested a great application potential of bamboo biochar for removing fluoroquinolone antibiotics.

2.11 Analytical Techniques for Analysis of Antibiotics in Environmental Matrices

Other than appropriate sampling and sample preservation, there are various analytical procedures and techniques that are central to the analysis of antibiotics in environmental matrices (Abedalwafa *et al*., 2019). In this section, these techniques and methods are discussed with much emphasis laid on those methods that were used in this research.

2.11.1 Extraction

Different methods have been employed to extract antibiotics from environmental matrices, water sediments and surface water. For example, Solid Phase Extraction (SPE) was used to extract sulphonamides from wastewater (Al Aukidy *et al*., 2012; Bhandari *et al*., 2008; Gomes *et al*., 2022; Minh *et al*., 2009; Ngigi *et al*., 2020). Pressurized Liquid Extraction (PLE) has also been used to extract sulphonamides from surface water (Sabourin *et al*., 2009; Tso *et al*., 2011). Polar organic chemical interactive sampler (POCIS) has been used to extract fluoroquinolones from wastewater (Alvarez *et al*., 2005), whereas liquid-liquid extraction (LLE) has been used to extract macrolides from wastewater (Alvarez *et al*., 2005; Bartelt-Hunt *et al*., 2009; Bhandari *et al*., 2008).

Ultrasonic solvent extraction (USE) and solid phase extraction (SPE) are also utilized in the sample preparation and pre-treatment due to their simplicity and fast, and there are usually employed in the extraction and enrichment of micro-contaminants from different environmental matrices (Lindberg *et al*., 2005)

2.11.2 Methods for detection and quantifications of antibiotics

Methods have been devised to identify and measure antibiotics from various categories (Goel, 2015; Pamreddy *et al*., 2013). The identification and quantification of antibiotics in various matrices are facilitated by their structural and chemical features, as well as their abiotic modifications (Bouki *et al*., 2013; Yan *et al*., 2018). Environmental matrices are typically intricate, and only mass spectrometry can provide the necessary precision and specificity to overcome the significant interferences present in these matrices. While simpler detectors like UV/Vis, fluorescence, and DAD are occasionally employed, the primary detection techniques used to identify trace amounts of antibiotics (in the parts per billion range and below) in complex environmental samples are mass spectrometry (MS) or tandem MS (Sparbier *et al*., 2012). Antibiotics ranging from a few hundred to several thousand ng L^{-1} have been identified in municipal wastewater (Gomes *et al*., 2022; Li *et al*., 2018).

Various techniques are used for the analysis of antibiotics. These include high-performance liquid chromatography (HPLC), fluorometry, electrochemical sensors, surface plasmon resonance, capillary electrophoresis, enzyme-linked immunosorbent assay (ELISA), immunochemical detectors, liquid chromatography-tandem mass spectrometry (LC-MS/MS), Raman spectroscopy and colorimetric sensors. Of these, colorimetric sensor techniques built on polymers, microbiological, immunoassays,

metal ions and gold nanoparticles have proven high sample throughput, rapid detection, highest sensitivity, low cost and ease of use (Abedalwafa *et al*., 2019). As this study aimed at detection and quantification, the common methods used in quantification of antibiotics in environmental matrices are discussed as follows.

Liquid Chromatography-Mass Spectrophotometry (LC-MS) is an hyphenated analytical technique aimed at achieving instrumental efficiency, sensitivity and lower detection limits (Rambo *et al*., 2019; Trubetskaya *et al*., 2021). Individually, mass spectrometry (MS) is a typical high-throughput technique that relies on the measurement of the mass- to-charge ratio (*m/z*) of ions from ionization and fragmentation of molecules in samples present in either the gas or liquid phases. As molecules tend to fragment following unique patterns, fragmentation patterns for molecules can be harnessed in their identification, quantification, or structural elucidation (Baghel *et al*., 2017). For the most part, the portion of the mass spectrometer is coupled with either a gas chromatograph (GC-MS) or liquid chromatograph system (LC-MS) in which case the former is used as a detector.

As a technique, Liquid Chromatography-Mass Spectrophotometry normally produces a single ion in the source, typically the molecular ion plus or minus a hydrogen: $(M+H)^+$ if in the positive ionization mode, or $(M-H)^-$ if in the negative ionization mode. However, the generation of a single ion is also a restriction of LC/MS, because without more than one ion for identification, analytes in complicated environmental matrices can be misidentified. As a result, approaches for more specific identification must be applied when utilizing LC/MS, tandem MS, or MS/MS. The hyphenation of MS with LC has been a sort of obvious extension but the progress to realize the same remained limited for some time owing to the relative incompatibility of the previously

existent MS ion sources (Mengting *et al*., 2019).

The precursor ion produced in the MS source (typically the $(M+H)^+$ or $(M-H)^-$ ion in LC/MS) is activated and collides with a triple quadrupole, ion trap, or magnetic sector mass spectrometer during tandem mass spectrometry (MS/MS), leading to the formation of product ions. Studies have shown that these product ions generally indicate the removal of different functional groups from the analytes, including $(M+H-OH)^+$ and $(M+H-CH_3)^+$ (Bouki *et al*., 2013; Yan *et al*., 2018).

Mass spectrometry has experienced a progression with respect to its acquisition and ionization techniques. Significantly, in recent years, there has been a proliferation of techniques such as electrospray ionization (ESI), matrix-assisted laser desorption/ionization, and time-of-flight, quadrupole, and orbitrap mass selection methods (Cotter *et al*., 2020).

In addition, tandem mass spectrometry (MS/MS) affords enhanced sensitivity and precision in compound identification (Chu & Metcalfe, 2007). Other than this, the use of high-performance liquid chromatography (HPLC) and ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) system has also been reported (Chen & Zhou, 2014; Gros *et al*., 2013; Zhou *et al*., 2012).

Pagajeva *et al*. (2017) explored the chromatographic separation of pharmaceutical residues using a UHPLC system with promising results. The study demonstrated good linearity, repeatability, and recovery, with a detection range of 10 to 100 pg L^{-1} for various pharmaceuticals, including caffeine, acetaminophen, ciprofloxacin, xylazine, ketoprofen, naproxen, ibuprofen, and diclofenac.

Ngumba (2016) investigated antibiotics and antiretroviral drugs in the Nairobi River Basin, employing solid-phase extraction followed by liquid chromatography-electrospray ionization tandem mass spectrometry (SPE-LC-ESI-MS/MS). The method exhibits excellent linearity, with recoveries ranging from 57.51% to 96.36%. The limit of quantification varied between 8 and 122 ng L^{-1} across different samples, providing valuable insights into the occurrence of these compounds in the studied area

2.12 Method Validation

Quality assurance and quality control are used together to ensure that all actions, checks and procedures within a process, with quality assurance establishing in standards and guidelines to ensure that analytical results are reliable and accurate while quality control ensures the effectiveness of procedures put in place (Mull *et al*., 2013). All background contaminations are minimized to reduce signal to noise ratio. In every step of analysis, a blank using LC grade water are prepared and all the blanks analyzed in the same condition as those of the sample.

There are so many factors that are considered in validating the method efficiency. These factors include Limit of quantification LOQ, limit of detection LOD, ruggedness, robustness, accuracy, selectivity, specificity, precision and linearity range (Araujo, 2009). The ratio of signal to noise is often used to determine the LOD and LOQ, where LOD is the signal that is thrice greater than the standard deviation (STD) of the signal of the blank while LOQ is ten times greater than the STD of the signal of the blank. The correlation coefficient (r^2) of the calibration curve is used to determine the linearity where an r^2 of 1 indicates a perfect linearity. The precision represents the degree of agreement of replicate measurements. Precision is achieved by analyzing samples in appropriate replicates and assessed as either reproducibility or

repeatability of the method using the standard deviation.

2.13 Sources, Properties and Modification of Biochars

The common word ‘‘biochar’’ is a 20th-century English neologism with roots in the Greek words βίος (bios)-meaning life, and ‘‘char’’ alluding to charcoal obtained through carbonization of biomass. Thus, biochar is widely appreciated as ‘‘charcoal which is a participant in the biological processes occurring in the soil, aquatic and animal digestive systems (Khalid *et al*., 2022). Biochar is defined as a carbon-rich material prepared through pyrolysis in limited air or oxygen to confer upon it a compact structure with surface functional groups leading to high adsorptive capacity (Kyi *et al*., 2020). Biochar and its potential use in the remediation of environmental matrices form an area of intensive research globally. This is explained by its potential role in carbon sequestration, which may afford a cheap way to mitigate the impacts of global warming and decadal climate change (Lehmann *et al*., 2015; Werner *et al*., 2018; Yousaf *et al*., 2016).

Several factors influence the properties of biochar, some of them include the nature of feedstock and pyrolysis temperature. The composition of the starting materials (feedstock) to be pyrolyzed and/or hydrothermal carbonized for biochar production impacts the morphology and the absorbent capacity and quality of the resultant biochar (Oleszczu *et al*., 2016; Taskin *et al*., 2019). Biochars are made from a range of materials that are carbonaceous in nature. Currently, there has been a shift with a specific focus on invasive alien species (noxious weeds) and agricultural wastes such as sugarcane bagasse, rice and wheat straws, corn stover, rape and cotton stalks, coffee and millet husks, peels, maize cobs and tassels (Gaffar *et al*., 2021; He *et al*., 2018). The use of such affordable sources of biochar feedstock has boosted the choice

of cleaning up environmental matrices using adsorbents with logistic and availability advantages, yet delivering high sorption capacity (He *et al*., 2018; Wang & Wang, 2019).

One of the greatest factors affecting the properties of biochars during production is pyrolysis temperature. For instance, biochar produced at relatively higher pyrolysis temperatures i.e., 300 ℃ to 700 ℃ or higher has been reported to be rich in carbon and also possess higher surface area as high temperatures lead to the creation of micropore volumes following the expulsion of volatile organic compounds (Gang *et al*., 2015; Wang & Wang, 2019). On the other hand, biochars produced at much lower temperatures have higher yields and are known to possess more organic functional groups than those obtained at higher pyrolytic temperatures. It is of the utmost importance that a trade-off between the temperature at which the biochar is prepared, the intended yield, and the intended purpose be met (Taskin *et al*., 2019; Wang & Wang, 2019). This is due to the fact that the sorption potentials of the biochars are a direct function of the functional groups that are present in the biochar.

2.14 Analytical Techniques for Characterization of Biochar Materials

Biochar materials are usually characterized for their physical, morphological, chemical and physico-chemical characteristics. Nevertheless, the characterization techniques are usually dictated by the end user's choice and accessibility to the relevant analytical instruments (Igalavithana *et al*., 2017). These may range from simple conventional analytical equipment for basic physical properties such as moisture (moisture analyser), volatile matter, ash content and fixed carbon (i.e. proximate analyses using hot air ovens), electrical conductivity (using electrical conductivity/total dissolved solids meters), pH (pH meters), elemental

characterization (ultimate analyses) and cation exchange capacity (CEC) to more advanced techniques such as Fourier Transform infrared spectroscopy, X- ray powder diffraction, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) (Amin *et al*., 2016; Mašek *et al*., 2020). For example, mercury porosimetry has for a long time been utilized in the establishment of biochar porosity, surface area and density (Brewer *et al*., 2014). For elemental characterization, X-ray fluorescence (XRF) spectroscopy has been adopted. In the same stride, pyrolysis hyphenated with gas chromatography-mass spectrometry (Py-GC/MS) has been utilized in the characterization of organic molecules contained in biochars (Kaal *et al*., 2012; Rombolà *et al*., 2016). Other less commonly used techniques in this area include thermogravimetric and derivative thermo-gravimetric techniques which focus primarily on unveiling the changes in the physical and chemical properties of materials that occur following biochar heating processes (Liu *et al*., 2014; Missaoui *et al*., 2017). For the purpose of this study, this section is focused on the main (advanced) analytical techniques used in chemical characterization of biochar materials.

2.14.1 Fourier transform infrared spectroscopy (FT-IR)

Fourier Transform Infrared (FTIR) spectroscopy is a powerful, less intuitive and versatile tool for surface characterization. As an analytical technique, it is used for both structural and compositional analysis to obtain the infrared spectrum of absorption, emission and photoconductivity of solids, liquids and gases. It is capable of simultaneously collecting high-resolution (of 4 cm^{-1}) spectral data over a wide spectral range, usually 400-4000 cm^{-1}. This is called the multiplex or Fellgett's advantage, when compared with scanning (dispersive) spectrometry (Griffiths & de Hasseth, 2007). Furthermore, FTIR provides additional advantages, such as

throughput advantages such as Jacquinot's advantage, where interferometer throughput is mostly determined by the diameter of the collimated beam from the source. It also delivers Connes' advantage of wavelength precision since the instrumental wavelength scale is calibrated by a laser beam of known wavelength traveling through the interferometer. Furthermore, as Griffiths and de Hasseth showed in 2007, FTIR has a lower sensitivity to stray light.

The technique utilizes a mathematical process called Fourier transform to translate interferograms into sample spectra. It is useful for the identification of chemical bonds and/or functional groups in a sample (Deena *et al*., 2019). Appreciation of the chemical bonds/groups in the sample material is based on the interpretation of its molecular fingerprint which is usually scanned at high spectral resolutions (Deena *et al*., 2019). In the case where biochar materials were prepared at different temperatures, FTIR spectroscopy can be harnessed to establish whether some changes occurred in the functional groups as temperature was increased (Odero, 2021; Ray *et al*., 2020; Zhang *et al*., 2020). This technique is also frequently used in combination with other analytical techniques. For example, thermogravimetric analysis-infrared spectrometry is used for quantifying gases evolved during the heating of materials (Jindo *et al*., 2014; Krauklis *et al*., 2018; Shabanian *et al*., 2020).

2.14.2 X-ray powder diffraction

X-ray powder diffraction, or XRD, is a quick, strong, and non-destructive crystallographic method used to check samples' crystalline and amorphous properties as well as their atomic spacing (Barbosa *et al*., 2021; Bunaciu *et al*., 2015). Bragg's law says that the sample to be examined and the monochromatic X-rays that are diffracted at certain angles from each set of lattice planes in the sample will interfere

positively. Therefore, it is possible to say that XRD works because X-rays are both waves and particles, which gives information about the structure of solid materials (Holder & Schaak, 2019). The peak levels that are obtained are carefully studied by looking at where the atoms are in the lattice planes, since both positive and negative diffractions follow Bragg's law in crystalline materials (Ameh, 2019). The method gives detailed information about the crystal structures, stages, and features of the structure, such as crystal defects, average grain size, and strain (Toso *et al*., 2021). XRD is a faster, more accurate, and in situ analytical method that can search an online standard database for powder diffraction patterns. This lets scientists quickly identify phases, which is especially helpful when testing samples with different crystal structures (Holder & Schaak, 2019). XRD is one of the most important tools for studying biochar because it can show changes in the amount of carbon in the biochar, as well as its porosity (Cuixia *et al*., 2020; Pandey *et al*., 2021).

2.14.3 Transmission Electron Microscopy

Dating as far back as 1931, transmission electron microscopy (TEM) has been a vital microscopic technique used for establishing the sizes of biochar-nanoparticle composites, and in investigation of the morphology and crystalline structures of materials as far as the atomic level (Tang & Yang, 2017). Vis-à-vis scanning electron microscopy, TEM possesses a higher magnification power which confers upon it the ability to examine both the molecular composition and crystallographic structure of samples. Using electrons as the excitation source, TEM functions similarly to SEM. It requires more time than scanning electron microscopy (SEM) to prepare the specimens for analysis since they must be extremely thin to be semi-transparent to electrons. Strides in the advancement of TEM have equipped it with the capacity to examine the aspect of nano-reinforcement dispersion that is not possible when SEM is

used (Scuderi *et al*., 2021).

2.14.4 Scanning Electron Microscopy and hyphenated techniques

Scanning Electron Microscopy (SEM) is one of the analytical techniques commonly utilized in the characterization of materials, specifically to establish their surface characteristics, that is the topographical visualization of structures (Czerwińska-Główka & Krukiewicz, 2021; Pongchaiphol *et al*., 2021). The technique creates elaborate high- resolution micrographs of the analytes by rastering an electron beam across the sample surface and detecting secondary or backscattered electron signals (Abd Mutali *et al*., 2017; Khare *et al*., 2019).

A complimentary technique, Energy Dispersive X-ray spectroscopy (EDS or EDX), is often coupled with SEM to afford Energy Dispersive X-ray-Scanning Electron Microscopy (EDX-SEM). The hyphenation exploits the benefits of EDX, which is capable of performing a non-destructive, qualitative and elemental examination of samples based on their characteristic X-rays (Guo *et al*., 2022; Scimeca *et al*., 2018). Because it affords *in situ* sample (minor or no sample preparation), EDX in EDX-SEM is either quantitative, qualitative or semi-quantitative but is also routinely used to obtain information on the spatial distribution of elements through mapping i.e. both image analysis and elemental mapping of samples are possible (Scimeca *et al*., 2018). It measures the relative abundance of emitted X-rays versus their energy (Mutalib *et al*., 2017). Like other techniques, the combination of the two analytical instrumentation may be insufficient to provide adequate information for precise identification of the sample composition, hence other powerful multi-pronged techniques notably FTIR and Raman microscopy, Surface Analysis (using either X-ray photoelectron spectroscopy or Time-of- Flight secondary ion Mass Spectrometry)

and Nuclear Magnetic Resonance spectroscopy may be harnessed to gain better insights on the composition of the sample (Abd Mutali *et al*., 2017; Schu *et al*., 2021).

In this study, the main analytical techniques used for the characterization of the biochars after preparation and after being used for adsorption experiments were FTIR, SEM, and LC-MS/MS.

CHAPTER 3

MATERIALS AND METHODS

3.1 Research Philosophy

The present investigation employed a positivist paradigm of research. Positivism is a paradigm that operates on the principles of measurement and the logical deduction that knowledge can be discerned through the impartial and quantifiable observation of an action, reaction, or activity. Experimental qualitative and quantitative analyses were conducted for the purpose of elucidating the observed phenomena in this study.

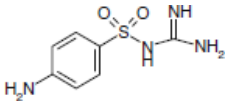
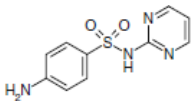
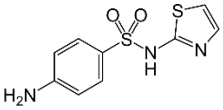
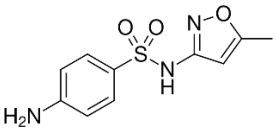
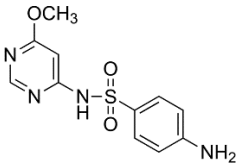
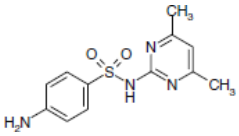
3.2 Apparatus and Reagents

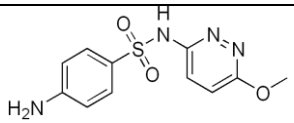
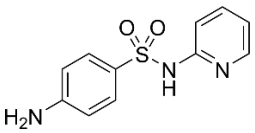
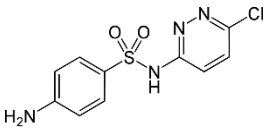
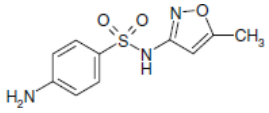
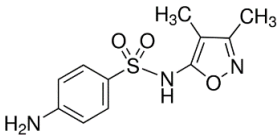
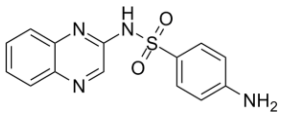
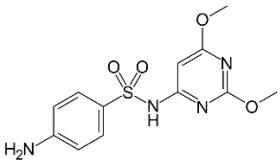
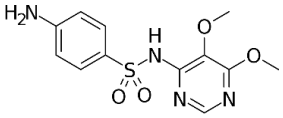
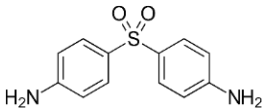
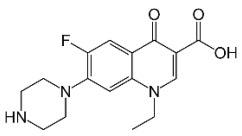
3.2.1 Chemicals and reagents

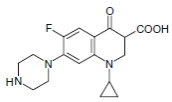
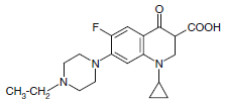
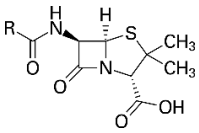
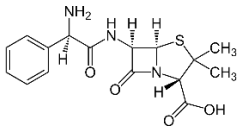
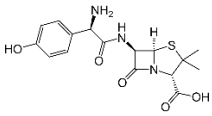
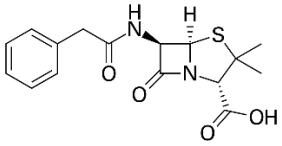
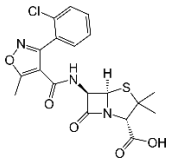
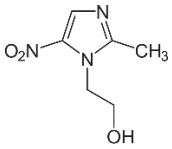
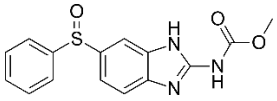
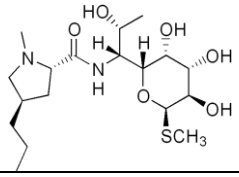
Standard pharmaceutical reference chemicals with a purity level of more than 98% and liquid chromatography water were acquired from Merck in the United States of America through the Kenya branch of Scientific Laboratory Supplies Ltd. Basic properties and abbreviations for the compounds utilized in this study are listed in Table 3 1. Methanol (MeOH), acetonitrile (ACN), and formic acid (HCOOH) of the high-performance liquid chromatography (HPLC) grade were procured from Kobian Scientific Ltd., located in Nairobi, Kenya. Other chemicals, including buffers, potassium hydroxide (KOH), sodium hydroxide (NaOH), hydrochloric acid (HCl), and ethylenediaminetetraacetic acid (EDTA) were acquired from the same supplier and were of analytical grade. Glass microfiber filters (0.45 µm and 0.22 µm) and Oasis hydrophilic-lipophilic balance cartridges (HLB, 200 mg/6 mL; 60 mg, 3 mL) were obtained from Waters, USA, located in Milford, MA (Waters, USA). The remaining reagents were obtained from Kobian Scientific Ltd. and were all of analytical grade. In order to make singular standard stock solutions with a

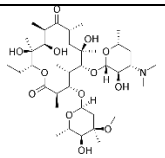
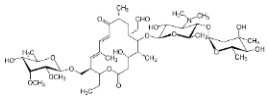
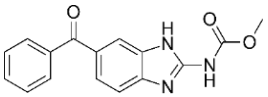
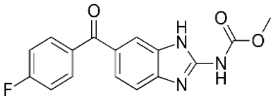
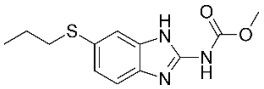
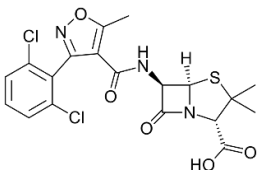
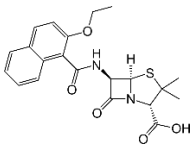
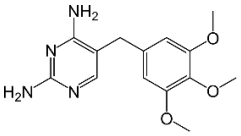
concentration of 1000 mg L^{-1}, a solution was prepared by dissolving 10 mg of the reference standard in 10 mL of an appropriate solvent.

Table 3 1: Molecular formulae, chemical structures and characteristics of targeted compounds

Name	Empirical	Chemical structure	pKa	Log Kow	Solubility in water (mg L^{-1})
Sulfaguanidine (SFG)	C7H10N4O2S		1.55;11.24	-1.22	2200
Sulfadiazine (SDZ)	C10H10N4O2S		2.10;6.28	-0.09	77
Sulfathiazole (SFT)	C9H9N3O2S2		2.04;6.93	0.05	373
Sulfamethizole (SMT)	C10H11N3O3S		1.95; 6.71	0.54	611
Sulfamonomethox ine (SMM)	C11H12N4O3S		2.0; 6.0	0.7	4030
Sulfamethazine (SMZ)	C12H14N4O2S		2.28;7.42	0.19	1,500

Name	Empirical	Chemical structure	pK_a	Log K_{ow}	Solubility in water (mg L^{-1})
Sulfamethoxypyridazine (SMP)	$C_{11}H_{12}N_4O_3S$		2.02;6.84	0.51	325.0
Sulfapyridine (SPY)	$C_{11}H_{11}N_3O_2S$		2.63;6.24	0.35	268
Sulfachlorpyridazine (SCP)	$C_{10}H_9ClN_4O_2S$		2.02;6.60	0.97	133
Sulfamethoxazole (SMX)	$C_{10}H_{11}N_3O_3S$		1.83;5.57	0.89	610
Sulfisoxazole (SSZ)	$C_{11}H_{13}N_3O_3S$		2.17; 5.80	1.01	300
Sulfaquinoxaline (SFQ)	$C_{14}H_{12}N_4O_2S$		2.13; 6.79	1.24	76.10
Sulfadimethoxine (SDT)	$C_{12}H_{14}N_4O_4S$		2.1; 6.3	1.63	343
Sulfadoxine (SDX)	$C_{12}H_{14}N_4O_4S$		2.55; 6.12	0.70	296
Dapsone (DPS)	$C_{12}H_{12}N_2O_2S$		2.39; 2.41	0.97	380
Norfloxacin (NOR)	$C_{16}H_{18}FN_3O_3$		5.77; 8.68	-1.03	101.0

Name	Empirical	Chemical structure	pK_a	Log K_{ow}	Solubility in water (mg L^{-1})
Ciprofloxacin (CIP)	$C_{17}H_{18}FN_3O_3$		6.68; 8.63	0.28	1350
Enrofloxacin (ENR)	$C_{19}H_{22}FN_3O_3$		5.69;6.68	0.58	612
Penicillin V (PEV)	$C_{16}H_{18}N_2O_4S$		-4.90; 3.39	2.79	454
Ampicillin (AMP)	$C_{16}H_{19}N_3O_4S$		3.24; 7.44	1.35	605
Amoxicillin (AMX)	$C_{16}H_{19}N_3O_5S$		3.23; 7.43	0.87	958
Penicillin G (PNG)	$C_{16}H_{18}N_2O_4S$		-2.60; 3.53	1.83	210
Cloxacillin (CLX)	$C_{19}H_{18}ClN_3O_5S$		-0.41; 3.75	2.48	53.2
Metronidazole (MET)	$C_6H_9N_3O_3$		3.09; 15.44	-0.02	5920.0
Oxfendazole (OXF)	$C_{15}H_{13}N_3O_3S$		3.57; 9.26	2.62	387
Lincomycin (LIC)	$C_{18}H_{34}N_2O_6S$		7.97; 12.37	0.56	293

Name	Empirical	Chemical structure	pK_a	Log K_{ow}	Solubility in water (mg L^{-1})
Erythromycin (ERY)	$C_{43}H_{75}NO_{16}$		8.38; 12.44	2.60	2000
Tylosin (TYL)	$C_{46}H_{77}NO_{17}$		7.20; 12.45	1.46	211.0
Mebendazole (MEB)	$C_{16}H_{13}N_3O_3$		3.93;8.44	2.83	71.30
Flubendazole (FLB)	$C_{16}H_{12}FN_3O_3$		3.42;9.17	3.40	28.80
Albendazole (ALB)	$C_{12}H_{15}N_3O_2S$		4.27;9.51	2.70	22.80
Dicloxacillin (DCX)	$C_{19}H_{17}Cl_2N_3O_5S$		0.71; 3.75	2.91	296.0
Nafcillin (NFC)	$C_{21}H_{22}N_2O_5S$		- 1.9; 3.31	3.33	172.0
Trimethoprim (TMP)	$C_{14}H_{18}N_4O_3$		7.12; 17.33	1.26	400

Source: Based on previous studies reported by Ngigi *et al*. (2020).

3.2.2 Equipment

The equipment used in this study included liquid-chromatography tandem mass spectrometer (LC-MS/MS), FT-IR spectrometer, heating oven, 50 mm and 180 mm mesh size Sieves, Furnace, pH meter, SPE manifold, and scanning electron microscope.

3.3 Description of the Study Area

The water and sediment samples analyzed for antibiotics in this study were obtained from River Sosiani in Eldoret, Kenya. The river lies between the coordinates 00°–03' S and 00°–55' N and 34°–50' E and 35°–37' E. It is one of the nine core tributaries of the larger River Nzoia and is ideally a sub-catchment of river Nzoia sub-basin, which directly drains its water into Lake Victoria, the largest fresh water lake in Africa, that is shared by many East African countries (Chepkemboi, 2013; Chibole, 2013). The tributary flows from Kaptagat forest in the highlands of the Keiyo escarpment, traverses through Eldoret Municipality in the South East towards the North West where it joins the main River Nzoia (Figure 3 1). Geologically, the area is composed of basic rocks of weathered Uasin-Gishu Phonolites (Owen *et al*., 2018).

River Sosiani is the main source of water and fish to the riparian population. However, there has been dumping of wastes accruing from various sources such as hospitals, the Jua Kali sector, agriculture and industries (Chepkemboi, 2013), which could potentially pollute the river. With rainfall averaging 900 mm (Ministry of Water and Irrigation, 2009), dumped wastes and those from Eldoret Municipal Council landfill at Huruma, are sometimes washed down to the river in storm runoffs (Khazenzi, 1996).

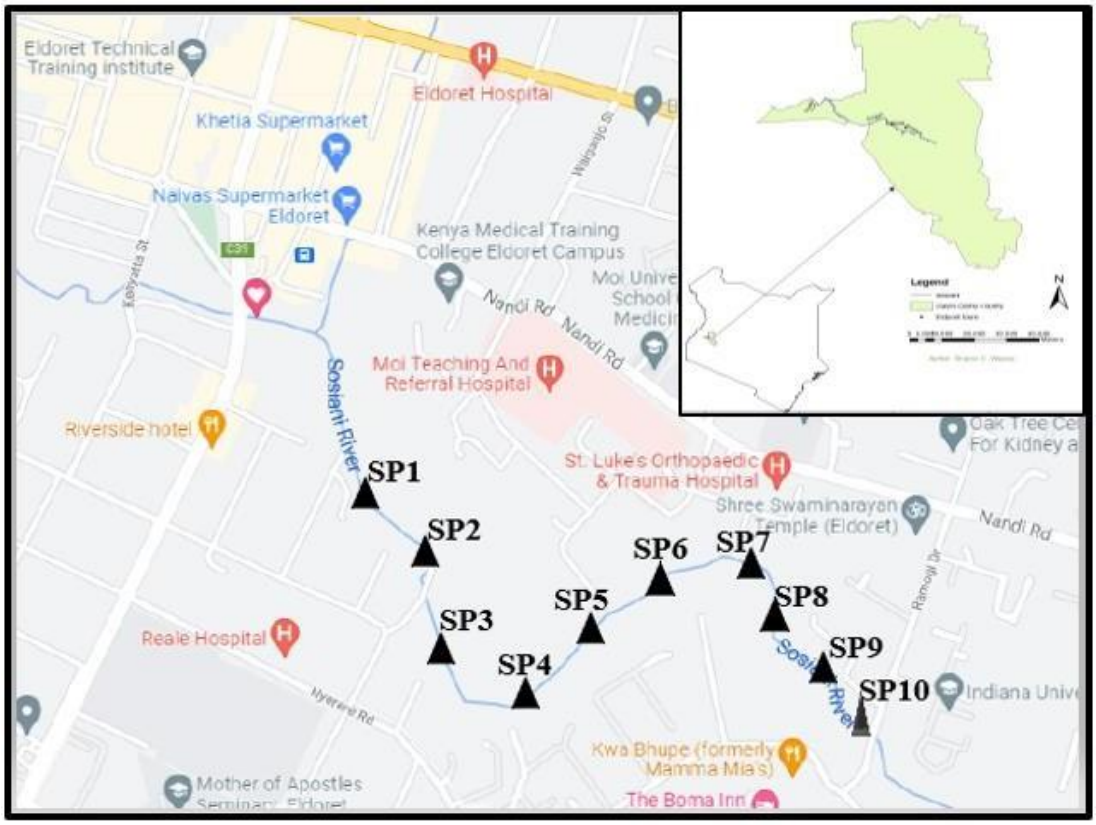


Figure 3.1 Map of River Sosiani showing the location of the sampling stations, inset is the location of Eldoret in Kenya. SP1 to SP10 are the 10 selected sampling points along the river

3.4 Sample Collection and Preparation

3.4.1 Sample Collection

Surface water samples and sediments were collected from the River Sosiani in Eldoret Municipality during both the rainy season (June 2020) and the dry season (February 2021). Ten sampling places were selected (Table 3 2) in both the suburban and urban sections of the river. These sampling points were located in close proximity to urban settlements, hospitals, the city's sewer system, or locations that discharge wastewater. For the purpose of collecting water samples, amber glass bottles were utilized. These bottles were first cleaned in a sequential manner with a saturated MeOH- EDTA solution, then rinsed with distilled water, and then dried in an oven.

Before the samples were collected, the bottles were pre-rinsed with sample water three times when they were being used for the sampling process. A composite sample was obtained by combining five grab water samples, each of which was one liter in volume, that were obtained at each sampling station. Temperature and pH readings were taken in the field, and the results showed that the pH range was between 7.0 and 8.0, with the bulk of the samples lying in the neutral range.

Moreover, five grab samples of sediments, each weighing approximately one hundred grams, were taken at each sampling position. These samples were then thoroughly mixed together to generate a composite sample. Sodium azide, at a concentration of 0.5 g L^{-1}, was added to each water sample immediately after the sampling process in order to prevent any potential biodegradation. The samples were carried in a cooler box and then stored in a cold chamber at a temperature of 4 ^{o}C (or -20 degrees Celsius for sediments).

Table 3 2: Sampling points and geographical positioning system coordinates of surface water and sediment samples from River Sosiani, Eldoret, Kenya

Sampling point	**Season**	**Co-ordinates**	**pH**	**Altitude (ft)**	**Temperature (^{o}C)**	**Location**
SP1	W& D	0° 30’ 35.1” N35° 16’ 41.1” E	7.4	200	19.3 ;21.4	Forest
SP2	W& D	0° 30’ 32.7” N35° 16’ 43.4” E	7.2	50	22.6; 23.0	Hospital
SP3	W& D	0°30’ 33.2” N35° 16’ 42.0” E	7.2	50	21.8;23.1	Hospital
SP4	W& D	0° 30’ 46.8” N35° 16’ 32.8” E	7.8	100	22.7; 23.6	Hospital
SP5	W& D	0° 30’ 46.5” N35° 16’ 34.9” E	7.8	200	23.7; 24.4	Bridge
SP6	W& D	0° 30’ 45.7” N35° 16’ 34.6” E	7.4	200	22.8; 24.1	Hospital
SP7	W& D	0° 30’ 42.9” N35° 16’ 39.3” E	7.8	83.3	21.5; 22.7	Hospital
SP8	W& D	0° 30’ 38.9” N35° 16’ 43.4” E	7.5	200	21.5; 21.9	Forest
SP9	W& D	0° 30’ 31” N 35° 17’ 2” E	7.9	83.3	20.4; 22.3	Residential
SP10	W& D	0° 30’ 31” N 35° 16’ 59” E	7.8	83.3	19.7; 22.5	Bridge

Note: W represent wet season and D the dry season

3.4.2 Sample Preparation

One liter of the sample was filtered through a 0.45 m Millipore filter before being cleaned using solid-phase extraction (SPE). Oasis hydrophilic-lipophilic balance (HLB) cartridges (200 mg, 6 mL) were preconditioned with 6 mL of methanol (MeOH) and 6 mL of LC water. Following that, a triplicate set of 1-L water samples was run through the cartridge at an 8 mL/min flow rate using a SPE Vacuum Manifold. The cartridges were rinsed with 6 mL of a 10% MeOH in LC water solution and air-dried for 10 minutes. Elution was performed using 3 mL of MeOH, and the volume was lowered to 100 µL with a moderate nitrogen flow. Before LC-MS/MS analysis, the sample was reconstituted to 1 mL using a 1:1 MeOH: LC water solution (acidified with 0.1% HCOOH), filtered through a 0.22 m glass membrane filter, and kept at 4°C.

River sediments, 2 g, were Freeze-dried and weighed into a 50 mL centrifuge tube. 6 mL of extracting solvent (methanol: water, 80:20) was added to the sample and vortexed for one minute. After a 20-minute sonication, the mixture was centrifuged for 10 minutes at 4500 rpm, and the supernatant was collected in a 15 mL glass test tube. After a repeat extraction, the extracts were mixed in a 15 mL tube with 6 mL of 100% methanol. The extract was rotary evaporated to about 1 mL and reconstituted to 10 mL with LC water. This extract was filtered through a 0.45 m glass microfilter and cleaned using the SPE method with Oasis (HLB) cartridges (60 mg, 3 mL), using the same process as water samples.

3.5 Instrumental Analysis of Antibiotic Residues

The samples were analysed using an Agilent LC-1290 infinity II system from Germany, which was connected to an API 6460c triple Quad Mass Spectrometer

from Applied Biosystems/MDS Sciex Instruments in Toronto, Canada. The system was equipped with a Zorkax Eclipse Plus C_{18} RRHD column measuring 50 mm × 2.1 mm × 1.8 microns, as well as a guard column (3.0 x 4 mm) made of the same material. The separation was accomplished by maintaining the oven temperature at 35°C, using a binary solvent gradient elution program with a flow rate of 0.30 mL min^{-1}, and injecting a volume of 10 µL. The mobile phase A consisted of LC water with 5 mM ammonium formate and acidified with 0.1% HCOOH, whereas the mobile phase B was composed of acetonitrile. The gradient first consisted of 98% mobile phase A for a duration of 2 minutes. It then decreased to 60% during the following 3 minutes, followed by a further decline to 50% within the subsequent 3 minutes. Finally, there was a rise to 95% mobile phase B for a duration of 2 minutes. A 3-minute interval was established between sample runs to allow for column re-equilibrium. The mass spectrometer (MS) was used in positive electrospray ionization (ESI) mode, employing the subsequent source parameters: a gas temperature of 325 °C, a gas flow rate of 1 L/min, a nebulizer gas pressure of 45 psi, and a capillary voltage of 4000V. Compound identification involved the utilization of a precursor ion and two or three product ions (Table 3 3).

Table 3 3: Mass Spectrometry acquisition parameters for individual compounds

Compound	Precursor ion	Production ions	Cell Acc (V)	Frag (V)	CE (V)	R_t (min)
Sulfaguanidine	215.0	156, 108, 92.1	60	10	4	0.66
Sulfadiazine	251.1	155.9, 108	96	12	3	4.29
Sulfathiazole	255.9	156, 92	90	15	4	4.23
Sulfamethizole	271.0	155.9, 108.9	150	12	5	4.47
Sulfamonomethoxine	281.1	155.9, 80	120	16	3	4.51
Sulfamethazine	279.1	186.1, 92	132	12	3	4.53
Sulfamethoxypyridazine	281.1	155.9, 108.1	120	16	6	4.51
Sulfapyridine	250.1	108, 65	150	20	3	4.62
Sulfachlorpyridazine	284.9	155.9, 108.1, 91.9	90	10	4	4.67
Sulfamethoxazole	254.1	156, 108	108	12	4	4.74
Sulfisoxazole	268.1	113.1, 92.1	100	12	4	4.82
Sulfaquinoxaline	301.1	156.1,108.1	118	12	6	4.99
Sulfadimethoxine	311.1	156, 108	128	20	3	5.01
Sulfapyridine	311.1	92.1, 65	126	36	4	4.99
Dapsone	249.1	156.1, 108.1	151	8	6	4.62
Norfloxacin	320.1	302.1, 231.1	90	15	4	4.27
Ciprofloxacin	332.2	314.1, 231	110	15	4	4.29
Dapsone	360.1	342, 286.1	90	15	4	4.35
Penicillin V	351.1	113.9, 53.2	123	40	4	4.58
Ampicillin	350.1	114, 106.1	120	40	5	4.57
Amoxicillin	367.0	208.1, 114	110	5	4	5.12
Penicillin G	335.1	160.1, 114.1	120	8	4	5.09
Cloxacillin	436.1	160.1, 114	120	14	4	6.06
Metronidazole	172.1	127.9	90	10	4	3.32
Oxfendazole	315.7	190.7, 158.9	110	15	4	4.75
Lincomycin	828.5	174.2, 109	150	35	4	5.4
Erythromycin	734.6	576.4,158.1, 116.1	150	15	4	4.76
Tylosin	916.6	174.2, 101.2	150	35	4	4.84
Mebendazole	295.8	263.7, 127.9	110	25	4	5.27
Flubendazole	313.7	281.7, 122.9	110	15	4	5.44
Albendazole	265.9	233.9, 190.8, 158.8	110	15	3	5.9
Dicloxacillin	470.0	160.1, 114	113	20	3	6.06
Nafcillin	446.5	199.1, 171.1	110	30	4	6.5
Trimethoprim	291.1	123.1, 80.9	60	30	4	4.24

Note: CE = collision energy, Cell Acc = Cell acceleration, Frag= fragment voltage, Rt = Retention time

3.6 Method validation, Quality assurance and Quality control

3.6.1 Quality control and quality assurance

As a precaution against contamination during sampling and laboratory procedures, each batch of sample events included a field blank and a procedural blank with high-purity water. Before analyzing each sample sequence, a solution containing only the solvent (methanol) and a solution containing only the instrument's cleaning water (redistilled water) were injected into the instrument to evaluate any possible interference in the analysis. A calibration solution with a moderate concentration was added at the beginning, middle, and end of each sample sequence to assess the sensitivity and consistency of the instrument's performance. To prevent any cross-contamination, methanol was injected and passed through the column after analyzing a sample with potentially high-level concentration. For standard measurements, samples were tested twice, and the average values were calculated. Furthermore, the method's thresholds for detecting and quantifying substances were determined.

3.6.2 Method validation

The SPE clean-up method was tested by spiking mixed standards into triplicate 0.5-liter matrix-matched blank water samples at two levels (0.01 g L^{-1} and 0.5 g L^{-1}). Following that, the samples were submitted to the same SPE method as the original water samples. Additionally, 1.0 g duplicate sediment samples (without residual analytes) were spiked to final concentrations of 0.1 g kg^{-1} and 0.5 g kg^{-1}, extracted, and SPE cleaned in the same manner as the sediment samples. The measured amount for each analyte compound was compared to the spiked amount, and the ratio was multiplied by 100% to determine recoveries (%).

As external calibration standards, reference standard substances at concentrations ranging from 0.0 g mL^{-1} to 2.0 g mL^{-1} in acetonitrile: LC water were prepared and injected into the LC-MS/MS to assess the linearity of the quantification method. In addition, six replicates of the lowest calibration mixed standard were injected into the LC-MS/MS equipment to determine the Limit of Detection (LOD) and Limit of Quantification (LOQ) for each compound. The LOD and LOQ were evaluated using *Equation 3. 1* and Equation 3. 2**.**

$$LOD = 3.3 \times \sigma/s \qquad \textit{Equation 3. 1}$$

$$LOQ = 10 \times \sigma/s \qquad \textit{Equation 3. 2}$$

Where σ = standard deviation of the replicates and s = the slope.

3.7 Ecological risk assessment

The ecological risk associated with urbanization is of great concern where multiple stressors and risk receptors co-exist. Probabilistic risk characterization methods were applied to assess the ecological risks of urban surface water environment in Eldoret.

The potential ecological risk of the pharmaceutical residues in water was evaluated using risk quotient (RQ_w) which was evaluated based on maximum measured environmental concentration (MEC) to the predicted no-effect concentration (PNEC, ng L^{-1}) using *Equation 3. 3* (EC 2003; Yin 2021).

$$RQ_w = \frac{MEC}{PNE\ C_W} \qquad Equation\ 3.3$$

The $PNEC_w$ (maximum drug concentration with no adverse effect on the microorganisms or the ecosystem in the environment) was determined using the evaluation (or assessment) factor, AF (*Equation 3. 4*).

$$PNECw = \frac{NOEC \text{ or } EC_{50}}{AF} \qquad \textit{Equation 3. 4}$$

where EC_{50} is the half-maximum effect concentration (ng L^{-1}). EC_{50} and $NOEC_{50}$ values (for the most sensitive species) were obtained from literature (Oh *et al*. 2004; Sharma *et al*. 2021; Chen *et al*. 2021a). $NOEC_{50}$ values were used in cases where EC_{50} could not be obtained. An AF value of 1,000 was used when acute toxicity (EC_{50}) data was used, and 100 when chronic toxicity data, $NOEC_{50}$ was used (Jiang *et al*. 2014). From the commonly used criteria of ranking the RQ values, RQ range of 0.01 to 0.1 is considered as low risk, 0.1 > RQ < 1 medium risk, and RQ > 1 as high risk, and this criterion was adopted in this study (Yan *et al*., 2013).

3.8 Preparation, characterization and modification of biochar materials

Water hyacinth *(Eichhornia crassipes*, WH), 2 Kg in weight, was harvested from Lake Victoria, Kenya (0.1448° S, 34.7367° E). The plant's roots were chopped into pieces, thoroughly washed with de-ionized water to remove any dirt, and subsequently air-dried in the shade. The preparation of water hyacinth biochar (WHB) involved a slow pyrolysis process conducted at 350 °C and 500 °C, with a heating rate of 10 °C per minute and a dwell time of 60 minutes in a furnace. The resulting sample was further washed with de- ionized water until the effluent was neutral to litmus and then subjected to oven-drying at 100 °C for 2 hours.

Millet husks, 2 Kg in weight, were sourced from a farm in Uasin Gishu County, Eldoret, Kenya (0.36° N 34.17° E) and air-dried under shade conditions. The preparation of millet husk biochar (MHB) also involved a slow pyrolysis process at 350 °C and 500 °C, with a heating rate of 10 °C per minute and a dwell time of 60 minutes in a furnace. The resulting pyrolyzed sample underwent multiple washes with de-ionized water until the effluent reached a neutral litmus reading. Subsequently, it was oven-dried at 100 °C for 2 hours.

Biochar yield from the two feedstock biomasses was defined as the amount of biochar (g) produced at each pyrolysis temperature and was computed using *Equation 3. 5* (Gaffar *et al*., 2021).

$$Biochar\ yield\ (\%) = \frac{Mass\ of\ biochar\ produced}{Mass\ of\ feedstock\ used} \times 100 \qquad Equation\ 3.\ 5$$

From which the masses of both the biochar and the feedstock are in grams, both measured on a dry weight basis.

The two prepared biochars were labeled as Water Hyacinth biochar (WBC) and Millet Husk biochar (MBC).

3.8.1 Chemical modification of water hyacinth and millet husk biochars

Pyrolyzed biochars were sieved with between 50 mm and 180 mm mesh size sieves, and were then activated using potassium hydroxide (KOH) as follows: 10 g of MBC and WBC biochars were placed in separate 100-mL beakers in which 100 mL of 2M KOH solution was added and stirred on a magnetic stirrer for five hours at 65 ℃. The mixture was filtered to obtain the activated biochars and recovered on Qualitative Filter Paper. After that, the filtered biochars were washed 10 times (0.5 h per time) in 500 mL warm deionized water (50 ℃) with stirring until a stable pH was obtained,

dried at 60 ℃ for 24h and stored in a desiccator (Guy *et al*., 2022; Bai & Hong, 2021). Activated Water Hyacinth and Millet Husk Biochar prepared at 350 ℃ and 500 ℃ were labelled WBC350, WBC500, MBC350 and MBC500, respectively. The non-activated water hyacinth and millet husk biochar prepared at 350 ℃ and 500 ℃ were labelled NWBC350, NWBC500, NMBC350 and NMBC500, respectively.

3.8.2 Determination of biochar properties

The identification of the functional groups present in biochar was accomplished using Fourier Transform Infrared (FT-IR) spectroscopy. The spectral resolution was configured to 4 cm^{-1}, and the scanning range spanned from 500 cm^{-1} to 4000 cm^{-1}.

3.8.2.1 Moisture Content

Determination of moisture content was done using a moisture analyzer (Mettler Toledo MJ 33, U.S.A). A 2 g biochar aliquot was placed in a dried, pre-weighed porcelain crucible, transferred to an oven set at 115 ℃, heated for 24 hours, and the constant weight recorded. Moisture content (%) was obtained as shown in Equation 3. 6.

$$Moisture\ Content = \frac{Initial\ biochar\ weight}{Final\ biochar\ weight}\ x\ 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 3. 6}$$

3.8.2.2 Ash Content

Ash content was determined as per the ASTM D2584-18 (2018) and ISO 3451 (2017) protocols. Briefly, 2 g of biochar was placed in a dried, pre-weighed porcelain crucible, and burned in an air atmosphere at temperatures of 700 ℃ for 5 minutes. The ash content in the crucible was left to cool at room temperature in a desiccator, and then weighed. The percentage ash content was calculated as follows (Equation 3. 7):

$$Percentage\ Ash\ Content = \frac{Weight\ of\ Ash}{Weight\ of\ Biochar}\ x\ 100 \ldots\ldots\ldots\ldots\ldots\ldots Equation\ 3.7$$

3.8.2.3 Bulk Density

Bulk density was determined as per VDLUFA-Method A 13.2.1 (EBC, 2012). Briefly, a 100 mL volumetric flask was weighed and its mass was recorded. Then, the 100 mL volumetric flask was filled with the biochar, and its weight was recorded. The difference in weight between the biochar-filled volumetric flask and the empty volumetric flask was divided by 100 mL.

3.8.3 Chemical Characterization of the produced biochars

3.8.3.1 Biochar pH

The biochar's pH value was determined following DIN ISO 10390 (2021) ($CaCl_2$) method. Concisely, 2.0 grams of the activated BC were mixed with 100 milliliters of deionized water. A volume of 5 mL of the mixture was transferred into a 100 mL glass beaker. Following the addition of 25 mL of a 0.01 M $CaCl_2$ solution, the suspension was agitated for one hour using an overhead shaker. The obtained suspension underwent a direct pH measurement (Metrohm 712 Conductometer, Switzerland).

3.8.3.2 Determination of functional groups in the biochar

The functional groups in biochar were identified using a Fourier Transform Infrared (FT- IR) Spectrophotometer (Jasco-4700, Tokyo, Japan) at ambient temperature in the temperature range 4000-400 cm^{-1}. To make the FTIR analyses, 1 mg of the samples was coarsely crushed, passed through a 150 m filter, and mixed with 200 mg KBr. Using a hydraulic press, the resultant mixture was crushed into a pellet.

3.9 Determination of surface morphology

The surface morphology of the biochar was studied using High-resolution Scanning Electron Microscopy (HRSEM) in a (JEOL -7600F, Japan), with an acceleration of 30 kV and theoretical resolution of 1 nm. Biochar sample's surface morphology was determined before adsorption and labelled as NA-Non-activated, A-Activated, BA-Before adsorption, and AA-After Adsorption.

3.10 Batch Sorption Experiments

All glassware used for sorption experiments was washed thoroughly with distilled water and 3.0 M nitric acid solution, then rinsed with EDTA-saturated methanol followed by deionized-distilled water before drying. Stock solutions (1000 mg L^{-1}) were prepared for each antibiotic reference standard (Ciprofloxacin, Penicillin G and Sulfamethoxazole) and stored at -4.0 ℃. The pH of the solutions was measured using a pH meter (Metrohm 712 Conductometer, Herisau, Switzerland) and regulated by adding either 0.1 M sodium hydroxide (NaOH) or 0.1 M hydrochloric acid (HCl). Preliminary experiments on equilibration time indicated that 24 hours were sufficient for the equilibrium uptake of these antibiotics and that loss during the equilibrium tests were negligible, and no interference was found.

3.11 Batch Equilibrium Adsorption Experiments with Modified and Unmodified biochar

The adsorption capacity of modified and unmodified biochar was evaluated on ciprofloxacin (CIP), penicillin G (PNG) and sulfamethoxazole (SMX). Batch equilibrium experiments were conducted at initial antibiotic concentration range of 2.5-80 mg L^{-1}, pH of solution varied from 2-12, and biochar dosage in the range 0.1–0.6 g L^{-1} for the different treatments. Adsorption experiments were carried out (in

triplicate) in 50 mL of 0.01 mol L^{-1} calcium chloride solution to maintain a constant ionic strength and addition of 0.2 g L^{-1} of NaN_3 to inhibit microbial activities. Method blank was also prepared consisting of 0.4 g L^{-1} of biochar mixed with 50 ml of distilled water. Control samples contained only the antibiotic-spiked $CaCl_2$ solutions. The flasks were covered with parafilm and allowed to equilibrate for 24 hours at room temperature on a shaker at 120 rotations per minute. The initial and equilibrium concentrations of antibiotics in aqueous solutions were determined using UV/Vis spectrophotometer (Beckman Coulter, DU 720, U.S.A) at 360, 570 and 276 nm for PNG, SMX and CIP, respectively. The amount of biochar adsorbed per gram of biochar (mg g^{-1}) known as the sorption capacity (q_e) and efficiency of adsorption (R) were determined by use of Equation 3. 8 and Equation 3. 9**,** respectively. Identity and quantity of the antibiotic compounds were confirmed for representative solutions from sorption studies for the different experiments using LC- MS/MS using the method described in section 3.5.

$$q_e = \frac{(C_0 - C_e)V}{M} \qquad \textit{Equation 3. 8}$$

$$R = \frac{(C_0 - C_e)}{C_0} \times 100 \qquad \textit{Equation 3. 9}$$

Where C_0 represents the initial standard antibiotic in mg L^{-1} (Penicillin G, Tetracycline and Ciprofloxacin) concentration in the solution, Ce is the standard antibiotic (Penicillin G, Tetracycline and Ciprofloxacin) concentrations in the solution at equilibrium (mg L^{-1}), m the biochar mass (g), v is the volume of the solution (L), qe is the adsorption capacity (mg g^{-1}) and R is the adsorption percentage.

3.11.1 Effect of initial antibiotic concentration on adsorption

In order to evaluate the amount of ciprofloxacin, penicillin G and sulfamethoxazole

antibiotics adsorbed by biochar prepared from millet husk and water-hyacinth at temperatures of 350 °C and 500 °C, batch equilibrium experiments were conducted at initial antibiotic concentration range of 2.5-80 mg L^{-1}. Adsorption experiments were carried out (in triplicate) in 50 mL of 0.01 mol L^{-1} calcium chloride solution with addition of 0.2 g L^{-1} of NaN_3. A measured 0.4 g L^{-1} of the biochar was added to the solutions. Method blank was also prepared consisting of 0.4 g L^{-1} of biochar mixed with 50 ml of distilled water. Control samples contained only the antibiotic-spiked $CaCl_2$ solutions. The flasks were covered with parafilm and allowed to equilibrate for 24 hours at room temperature on a shaker at 120 rotations per minute.

The residual antibiotic concentrations in aqueous solution were determined in a pre-filtered (0.45 µm polysulfone, PSF, membrane filters) 4 mL aliquot using UV-Vis spectrophotometer. The amount of antibiotic adsorbed per gram of biochar (mg g^{-1}) known as the sorption capacity (q_e) and efficiency of adsorption (R) were determined by use of Equation 3. 8 and Equation 3. 9**,** respectively (section 3.10).

3.11.2 Effect of pH

To determine the effect of pH on antibiotic uptake by biochar, 50 mL of the 0.01 mol L^{-1} $CaCl_2$ solutions in beakers were spiked to a final concentration of 5 mg L^{-1}. The pH of the solutions was adjusted to 2, 4, 6, 8, 10, 12 and 0.4 g L^{-1} of adsorbent was added into each of the beakers. The solutions were kept at room temperature and shaken in a thermostatic shaker at 120 rotations per minute for 24 hours (this period of time was determined on the basis of preliminary studies which indicated that 24 hours was sufficient for the equilibrium uptake of these antibiotics). Afterwards, the adsorbent was separated from the solution and antibiotic residues quantified by UV-Vis spectrophotometer at specific antibiotic wavelength.

3.11.3 Effect of biochar dosage on adsorption

In order to ascertain the most effective dosage of the adsorbents, various amounts (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 g L^{-1}) of the adsorbent under investigation were introduced to a 50 mL equilibration solution containing 5 mg L^{-1} of each antibiotic. The solution was agitated at 120 revolutions per minute for 24 hours while being agitated in a thermostatic shaker with the pH set to its optimal value of 8. The quantity of antibiotics remaining was subsequently calculated. Then, the efficiency and amount of the adsorption were determined using Equation 3. 10 and Equation 3. 11, respectively.

$$q_t = \frac{(C_0 - C_e)V}{M}$$ *Equation 3. 2*

$$R = \frac{(C_0 - C_e)}{C_0} \times 100$$ *Equation 3. 3*

3.12 Adsorption Kinetics

To study the kinetics of the sorption of CIP onto the biochars, batch sorption experiments were conducted by mixing 5 mg L^{-1} of the antibiotic with 0.4 g L^{-1} biochar at pH 8.0 and incubating for 72 h at 25 ^{o}C (on a shaker at 120 rpm). Sampling was done at 0, 6, 12, 24, 48 and 72 h time period and residual antibiotic (Penicillin G, Tetracycline and Ciprofloxacin) determined in the solutions.

To obtain the kinetics parameters, the kinetics data was fitted into two widely used kinetics models; pseudo-first order and pseudo-second-order (Equation 3. 12 and Equation 3. 13) (Ho, 2006). To determine the diffusion mechanisms involved, the data was further fitted into the intra-particle diffusion model (Weber and Morris, 1963) represented in Equation 3. 14.

$$q_t = q_e(1 - e^{-k_1 t})$$ *Equation 3. 4*

$$\frac{t}{q_t} = \frac{1}{K_2 (q_e)^2} + \frac{t}{q_e}$$ *Equation 3. 5*

$$q_t = K_p t^{0.5} + C$$ *Equation 3. 6*

Where;

t (min) and q_t (mg g^{-1}) are time and amount adsorbed at equilibrium time, respectively; q_e (mg g^{-1}) is the equilibrium adsorption capacity; k_1, K_2 and K_p are rate constants for pseudo-first order, pseudo-second-order and intra-particle diffusion kinetic models, respectively.

3.13 Adsorption isotherm studies

The Langmuir and Freundlich adsorption isotherms (Equation 3. 15 and Equation 3. 16) were used to describe the sorption of ciprofloxacin, penicillin G and tetracycline onto the different biochar materials.

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e}$$ *Equation 3. 7*

$$q_e = K_F C_e^{1/n}$$ *Equation 3. 16*

Where q_e is sorption capacity of the sorbent and q_{max} is the maximum sorption capacity in mg g^{-1}, K_L (L g^{-1}) is Langmuir equilibrium constant, C_e (mg L^{-1}) is the equilibrium concentration, n is the heterogeneity factor, and K_F ($g^{1-1/n}$ $L^{1/n}$ kg^{-1}) is the Freundlich equilibrium constant (Table 3 4).

Table 3 4: Sorption isotherm models used in the study

Isotherm model	Equation	Parameters
Langmuir	$\frac{1}{q_e} = \frac{1}{Q_0} + \frac{1}{Q_0 K_L C_e}$	$Q_0 (mg.g^{-1}), K_L = (L.g^{-1})$ $R_L = \frac{1}{1 + K_L C_0}$
Freundlich	$log\ q_e = log\ K_f + \frac{1}{n} log C_e$	K_f, n

Where:

The variables q_e (mg g^{-1}) and C_e (mg L^{-1}) represent the solute adsorption and solution concentration at equilibrium, respectively. Q_o (mg g^{-1}) refers to the monolayer adsorption capacity. C_o (mg L^{-1}) and R_L represent the initial solution concentration and Langmuir constant, respectively. The K_L and K_f represent the Langmuir and Freundlich constants, respectively, while 1/n is associated with the adsorption affinity or surface heterogeneity (Galhetas *et al*., 2014; Swenson & Stadie, 2019).

3.14 Statistical analysis of data

In this study, all experiments were done in triplicates. Quantitative data were expressed as means ± standard deviations of replicates. Descriptive and other data analysis were done using Microsoft Excel 2016 (Apple Inc., USA) computer software. Pearson's correlation (r, ρ = 0.05) was used for the comparison of pharmaceutical residues in river surface water and in sediments. Pseudo-partitioning coefficients ($K_{p,s}$) of test compounds between water and sediments were used to estimate the sorption capacity of these compounds and were calculated using the equilibrium: $K_{p,s} = C_s/C_w$, where C_w is the mean concentration of the target compound in the water sample (µg L^{-1}), and C_s is the mean concentration of the target compound in the sediment sample (ng g^{-1}).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Occurrence of Pharmaceuticals in Surface Water and Sediments of River Sosiani

4.1.1 Method validation

The assessment of the analytical method's performance involved evaluating linearity, accuracy, and repeatability. These parameters were expressed through the correlation coefficient (R^2) of calibration curves, percentage recoveries, Limit of Detection, Limit of Quantification, and the standard deviation for replicate measurements of both samples and spiked samples (Table 4: 1). The calibration curves exhibited good linearity throughout the calibration range, with correlation values ≥0.93 obtained for all tested compounds. The percentage of recoveries ranged from 83% to 114% for water samples and from 80% to 108% for sediments. The Limit of Detection of the adopted method ranged from 0.003 ng L^{-1} to 0.317 ng L^{-1} whereas the Limit of Quantification varied from 0.010 ng L^{-1} to 0.908 ng L^{-1}. All values reported were above the Limit of Quantification for each corresponding analyte (Table 4: 2).

These validation results obtained in this study are in agreement with preceding analytical methodologies for the same matrices (Eugenia *et al*., 2021; Gros *et al*., 2019; Gros *et al*., 2013; Huerta *et al*., 2016; Jelic *et al*., 2009; Ngumba *et al*., 2016b; Santos *et al*., 2019). For example, method detection limits of 0.2 ng L^{-1} to 31 ng L^{-1} for water samples and 0.1 mg kg^{-1} to 6.0 mg kg^{-1} dry weight for sediments, with recoveries ranging from 21% to 156% reported by Eugenia *et al*. (2021). It is worth mentioning that some of the recoveries were lower than 95%. In the quantification of antibiotics, this often results from high solubility of some groups of antibiotics in water which limits their retention during solid phase extraction (Ngumba *et al*.,

2016a). Further, the relatively higher Limit of Quantification of some of the detected antibiotics can be attributed to their zwitterionic nature (Ngumba *et al*., 2016a). Overall, the investigated pharmaceuticals were prone to matrix effects at various extents either as signal suppression or signal enhancement.

Table 4:1: Parameters for validation of the analytical method used for the analysis of the 34 pharmaceutical compounds detected in water and sediment samples from River Sosiani, Eldoret, Kenya

Compound	**R^2**	**% Recovery in water**	**% Recovery in sediments**	**Limit of Detection (ng L^{-1})**	**Limit of Quantification (ng L^{-1})**
Sulfaguanidine	0.999	90.0	93.4	0.028	0.084
Sulfadiazine	0.998	113.8	108.0	0.023	0.069
Sulfathiazole	0.988	98.4	98.9	0.119	0.359
Sulfamethizole	0.996	92.9	89.5	0.006	0.019
Sulfamonomethoxine	0.995	99.0	99.8	0.006	0.018
Sulfamethazine	0.991	104.8	100.2	0.014	0.043
Sulfamethoxypyridazine	0.997	93.3	80.1	0.006	0.018
Sulfapyridine	0.999	98.2	93.3	0.054	0.162
Sulfachlorpyridazine	0.990	87.6	99.4	0.016	0.050
Sulfamethoxazole	0.989	109.0	100.5	0.056	0.170
Sulfisoxazole	0.998	98.7	97.9	0.040	0.122
Sulfaquinoxaline	0.982	97.7	97.4	0.022	0.068
Sulfadimethoxine	0.999	101.9	81.1	0.005	0.016
Sulfadoxine	0.999	100.7	96.6	0.014	0.041
Dapsone	0.999	97.3	93.4	0.006	0.018
Norfloxacin	0.928	96.5	94.6	0.092	0.280
Ciprofloxacin	0.984	101.3	97.7	0.044	0.133
Enrofloxacin	0.955	95.1	97.8	0.109	0.330
Penicillin V	0.998	96.8	98.2	0.317	0.960
Ampicillin	0.993	88.2	82.8	0.020	0.059
Amoxicillin	0.998	80.8	82.8	0.014	0.043
Penicillin G	0.997	91.7	98.8	0.113	0.341
Cloxacillin	0.997	85.7	90.7	0.300	0.908
Metronidazole	0.999	89.0	97.4	0.053	0.162
Oxfendazole	0.999	98.3	99.0	0.003	0.010
Lincomycin	0.998	91.7	96.2	0.015	0.045
Erythromycin	0.993	83.0	100.4	0.082	0.247
Tylosin	0.993	99.0	96.6	0.018	0.055
Mebendazole	0.999	89.1	98.8	0.011	0.035
Flubendazole	0.999	92.0	94.3	0.004	0.011
Albendazole	0.999	92.4	93.2	0.014	0.043
Dicloxacillin	0.999	104.0	95.9	0.089	0.270
Nafcillin	0.999	103.1	90.1	0.008	0.023
Trimethoprim	0.998	86.5	81.0	0.027	0.082

4.1.2 Pharmaceutical residues in surface water

This study examined a total of 30 antibiotic pharmaceuticals, encompassing eight categories: sulphonamides (SAs), penicillins (PNs), fluoroquinolones (FQs), macrolides (MLs), lincosamides (LINs), nitroimidazoles (NIs), diaminopyrimidines (DAPs), and salfones. Additionally, four compounds from the benzimidazoles (BZs) class, classified as anthelmintics, were also included in the investigation. Out of the total of 34 compounds analysed, residues of varying quantities were identified for 28 compounds in surface water, constituting 82.3% of the analytes. The six compounds that were not detected in the samples were sulfaguanidine, sulfamonomethoxine, sulfachlorpyridazine, sulfaquinoxaline, dapsone, and flubendazole. Detectable amounts of sulfonamides (0.1 ng L^{-1} to 247.0 ng L^{-1}), fluoroquinolones (13.7 ng L^{-1} to 56.0 ng L^{-1}), penicillins (0.2 ng L^{-1} to 10.4 ng L^{-1}), macrolides (3.3 ng L^{-1} to 9.0 ng L^{-1}) and β-lactams (3.1 ng L^{-1} to 8.0 ng L^{-1}) were obtained. The concentration of nitroimidazoles (Metronidazole) ranged from 3.1 ng L^{-1} to 5.6 n L^{-1}, oxfendazole (2.0 ng L^{-1} to 2.2 ng L^{-1}), lincosamides (Lincomycin) from 3.0 ng L^{-1} to 7.7 ng L^{-1}, anthelmintics (Mebendazole and Albendazole) from 2.7 ng L^{-1} to 4.6 ng L^{-1}, whereas Trimethoprim ranged from 0.2 ng L^{-1} to 67.3 ng L^{-1} (Table 4: 1 to Table 4: 3). Of the ten (10) sulfonamides detected, Sulfamethoxazole had the highest concentration of 247 ng L^{-1} followed by Sulfamethazine with 23.3 ng L^{-1}. Sulfathiazole was detected at only one sampling point (SP4) at a concentration of 7.4 ng L^{-1} (Table 4: 1). The frequency of detection of sulfonamides varied among determined compounds and ranged from 0.05 - 100%. Concentrations of fluoroquinolones were generally higher than the other classes of compounds determined (Table 4: 4).

There was a 100% occurrence frequency of the three fluoroquinolones (Ciprofloxacin, Enrofloxacin, and Norfloxacin) at all sampling points. Similar to what was seen with Sulfathiazole, ampicillin was only found at one test point, SP4 (0.2 ng L^{-1}). Penicillin G was found in four samples, which is 2% of the total. Amoxicillin and Cloxacillin, on the other hand, were found at all sampling points. There was a 100% detection rate for all chemicals that were found except for sulphonamides and penicillins.

Table 4 2: Concentration (ng L^{-1}) of sulfonamides in surface water samples from River Sosiani, Eldoret, Kenya

Site	Sulfadiazine	Sulfathiazole	Sulfamethizole	Sulfamethazine	Sulfamethoxypyridazine	Sulfapyridine	Sulfamethoxazole	Sulfisoxazole	Sulfadimethoxine	Sulfadoxine
SP1 W	ND	ND	0.70(0.06)	ND	0.33(0.05)	2.53(0.06)	ND	2.51(0.05)	1.65(0.07)	3.04(0.01)
SP1 D	ND	ND	0.94(0.15)	ND	0.73(0.13)	2.92(0.26)	ND	2.74(0.07)	1.94(0.01)	3.19(0.13)
SP2 W	ND	ND	0.70(0.00)	ND	0.33(0.03)	2.61(0.13)	ND	2.54(0.09)	1.64(0.02)	3.06(0.03)
SP2 D	ND	ND	0.99(0.13)	ND	0.35(0.02)	2.80(0.04)	ND	2.82(0.35)	1.91(0.02)	3.34(0.11)
SP3 W	ND	ND	0.78(0.19)	ND	0.39(0.05)	2.52(0.08)	ND	2.61(0.11)	1.66(0.00)	3.05(0.00)
SP3 D	0.00	ND	0.98(0.26)	ND	0.44(0.06)	2.89(0.20)	ND	2.63(0.21	1.76(0.02)	3.66(0.04)
SP4 W	0.44(0.05)	ND	0.83(0.22)	0.73(0.20)	0.71(0.07)	2.82(0.01)	10.11(0.50)	2.55(0.02)	1.69(0.03)	3.17(0.07)
SP4 D	2.93(0.28)	7.38(0.06)	1.13(0.07)	16.33(0.55)	1.16(0.09)	3.27(0.11)	247.0(5.59)	5.95(0.55)	3.69(0.06)	4.90(0.03)
SP5 W	ND	ND	0.77(0.14)	ND	0.45(0.02)	2.61(0.11)	ND	2.65(0.19)	1.68(0.06)	3.02(0.01)
SP5 D	ND	ND	0.70(0.05)	0.80(1.13)	0.48(0.09)	3.74(0.15)	9.00(0.35)	4.72(0.12)	2.59(0.11)	4.18(0.04)
SP6 W	ND	ND	0.75(0.11)	ND	0.36(0.03)	2.45(0.03)	ND	2.67(0.23)	1.63(0.02)	3.10(0.09)
SP6 D	0.24(0.03)	ND	0.78(0.15)	10.71(0.82)	0.65(0.20)	5.83(0.26)	4.21(0.14)	2.70(0.27)	2.56(0.03)	4.15(0.02)
SP7 W	ND	ND	0.88(0.06)	ND	0.36(0.00)	2.74(0.32)	ND	2.69(0.11)	1.64(0.03)	3.05(0.04)
SP7 D	0.08(0.00)	ND	0.76(0.09)	5.50(0.48)	0.68(0.01)	4.51(0.08)	1.09(0.18)	3.10(0.41)	1.91(0.01)	4.10(0.04)
SP8 W	ND	ND	0.84(0.19)	ND	0.53(0.28)	2.57(0.04)	ND	2.60(0.07)	1.62(0.01)	3.04(0.04)
SP8 D	0.11(0.02)	ND	0.74(0.01)	5.67(0.21)	0.71(0.15)	5.62(010)	1.29(0.33)	4.98(0.16)	2.70(0.04)	3.94(0.06)
SP9 W	ND	ND	0.75(0.14)	ND	0.57(0.12)	2.57(0.02)	2.55(0.58)	2.47(0.01)	1.65(0.02)	3.07(0.05)
SP9 D	0.39(0.02)	ND	0.71(0.02)	23.64(0.37)	1.23(0.03)	5.68(0.16)	8.59(1.04)	4.13(0.54)	1.70(0.02)	3.10(0.00)
SP10 D	0.42(0.02)	ND	0.73(0.02)	23.38(1.37)	1.05(0.01)	6.57(0.0)	6.35(0.90)	4.14(0.09	2.61(0.01)	4.07(0.01)

Note: For sampling sites**,** codes ending with W represent Wet Season and D represents Dry season. ND = Below method detection limit. Values are presented as Means (Standard deviations) of triplicates.

Table 4 3: Concentration (ng L^{-1}) of sulfonamides in surface water samples from River Sosiani, Eldoret, Kenya

Site	Norfloxacin	Ciprofloxacin	Enrofloxacin	Penicillin V	Ampicillin	Amoxicillin	Penicillin G	Cloxacillin	Mebendazole	Albendazole
SP1 W	22.40(0.14)	14.02(0.15)	19.70(0.45)	3.34(0.36)	ND	1.23(0.01)	1.22(1.72)	2.75(0.14)	2.77(0.01)	2.89(0.05)
SP1 D	42.93(3.91)	21.40(7.57)	29.73(5.05)	4.07(0.22)	ND	2.10(1.19)	ND	3.27(0.71)	2.74(0.07)	2.82(0.01)
SP2 W	22.32(0.06)	14.55(0.90)	19.32(0.57)	2.90(0.04)	ND	1.30(0.00)	1.59(0.07)	2.89(0.27)	2.97(0.03)	2.84(0.02)
SP2 D	25.31(0.50)	14.40(0.35)	19.78(0.35)	3.82(0.34)	ND	1.76(0.08)	ND	2.96(0.83)	2.69(0.01)	2.80(0.00)
SP3 W	22.41(0.16)	13.94(0.12)	19.01(0.15)	3.14(0.25)	ND	1.24(0.07)	ND	2.21(0.17)	3.26(0.19)	2.83(0.01)
SP3 D	22.32(0.02)	16.84(2.44)	25.95(9.03)	3.41(0.43)	ND	1.28(0.08)	ND	2.81(0.75)	2.67(0.00)	2.82(0.01)
SP4 W	25.58(2.64)	13.82(0.11)	20.45(0.97)	4.08(1.53)	ND	1.28(0.03)	ND	3.42(0.25)	3.98(0.01)	4.55(0.57)
SP4 D	52.19(2.84)	56.02(1.16)	21.53(3.13)	4.86(0.58)	0.19(0.09)	1.40(0.14)	3.74(0.08)	10.37(0.48)	2.71(0.01)	3.48(0.03)
SP5 W	22.98(0.97)	14.08(0.42)	19.79(1.04)	2.99(0.06)	ND	1.39(0.20)	ND	2.32(0.36)	3.55(0.04)	2.81(0.01)
SP5 D	22.19(0.14)	14.02(0.26)	19.76(0.43)	3.090.28	ND	1.71(0.57)	ND	3.40(1.57)	2.77(0.14)	3.04(0.00)
SP6 W	22.16(0.09)	13.93(0.21)	19.60(0.27)	2.89(0.01)	ND	1.21(0.01)	ND	2.90(0.62)	3.12(0.01)	2.81(0.00)
SP6 D	22.84(1.07)	13.92(0.22)	19.16(0.08)	3.09(0.23)	ND	1.35(0.22)	0.46(0.03)	2.14(0.06)	2.69(0.02)	3.07(0.27)
SP7 W	27.24(1.78)	14.04(0.08)	19.28(0.44)	3.03(0.20)	ND	1.21(0.01)	ND	2.35(0.27)	3.75(0.07)	2.77(0.01)
SP7 D	22.48(0.27)	15.40(1.81)	21.46(0.37)	3.22(0.08)	ND	1.30(0.06)	ND	2.47(0.09)	2.69(0.01)	2.95(0.03)
SP8 W	22.38(0.15)	13.88(0.02)	19.67(0.04)	3.28(0.36)	ND	1.26(0.10)	ND	2.70(0.37)	3.74(0.01)	2.77(0.01)
SP8 D	23.01(1.11)	16.78(1.76)	21.04(1.59)	3.37(0.13)	ND	1.47(0.01)	ND	2.82(0.45)	2.70(0.01)	3.05(0.06)
SP9 W	22.23(0.13)	13.85(0.25)	21.42(3.25)	3.00(0.01)	ND	1.32(0.09)	ND	2.60(0.75)	2.69(0.02)	2.80(0.02)
SP9 D	22.62(0.45)	13.68(0.02)	21.97(2.37)	3.17(0.20)	ND	1.35(0.04)	ND	2.93(0.14)	2.77(0.01)	5.18(0.05)
SP10 D	23.33(1.27)	14.72(1.08)	19.52(0.39)	3.78(0.33)	ND	1.23(0.02)	ND	3.08(0.69)	3.00(0.04)	21.39(7.81)

Note: For sampling sites**,** codes ending with W represent Wet Season and D represents Dry season. ND = Below method detection limit. Values are presented as Means (Standard deviations) of triplicates.

Table 4 4: Concentration (ng L-1) of macrolides, beta-lactams, nitroimidazoles, benzimidazoles and lincosamides in surface water from River Sosiani, Eldoret, Kenya

Site	Metronidazole	Oxfendazole	Lincomycin	Erythromycin	Tylosin	Dicloxacillin	Nafcillin	Trimethoprim
SP1 W	3.13(0.00)	2.21(0.02)	3.67(0.00)	3.53(0.06)	5.46(0.01)	3.16(0.02)	3.99(0.11)	0.50(0.05)
SP1 D	3.12(0.01)	1.99(0.05)	6.43(0.36)	3.97(0.00)	5.82(0.11)	3.49(0.01)	3.66(0.01)	1.53(0.35)
SP2 W	3.12(0.02)	2.02(0.04)	3.70(0.02)	3.47(0.14)	5.46(0.04)	3.15(0.01)	3.89(0.01)	0.17(0.05)
SP2 D	3.11(0.00)	2.14(0.15)	6.69(0.02)	3.98(0.01)	5.51(0.12)	3.39(0.05)	3.62(0.00)	0.72(0.09)
SP3 W	3.12(0.00)	2.10(0.06)	4.93(0.37)	3.32(0.07)	5.50(0.08)	3.17(0.02)	3.79(0.06)	0.29(0.13)
SP3 D	3.12(0.01)	2.06(0.02)	6.47(0.71)	3.97(0.00)	6.63(0.08)	3.34(0.03)	3.64(0.01)	0.66(0.05)
SP4 W	3.22(0.12)	2.30(0.32)	5.66(0.00)	4.13(0.05)	8.49(0.11)	3.19(0.04)	3.77(0.01)	2.40(0.15)
SP4 D	5.56(0.20)	2.47(0.05)	6.95(0.11)	6.73(0.44)	9.00(0.76)	8.03(0.17)	3.65(0.03)	67.32(2.87)
SP5 W	3.14(0.01)	2.09(0.05)	5.70(0.05)	4.00(0.05)	8.83(0.43)	3.16(0.00)	3.76(0.06)	0.66(0.32)
SP5 D	3.15(0.04)	1.97(0.03)	7.32(0.08)	4.01(0.02)	8.52(0.06)	3.30(0.02)	3.63(0.01)	2.87(0.00)
SP6 W	3.14(0.03)	2.02(0.00)	5.71(0.02)	4.00(0.01)	6.96(0.58)	3.15(0.02)	3.74(0.00)	0.44(0.06)
SP6 D	3.30(0.27)	2.11(0.02)	7.63(0.10)	4.03(0.07)	8.57(0.05)	3.26(0.00)	3.62(0.00)	1.52(0.24)
SP7 W	3.14(0.02)	2.14(0.01)	6.17(0.71)	3.98(0.00)	8.71(0.37	3.14(0.01)	3.68(0.01)	0.46(0.20)
SP7 D	3.15(0.00)	2.09(0.04)	6.83(0.22)	4.10(0.03)	8.85(0.18)	3.20(0.03)	3.61(0.01)	0.64(0.13)
SP8 W	3.12(0.01)	2.12(0.07)	6.32(0.57)	3.98(0.02)	8.49(0.03)	3.13(0.00)	3.70(0.03)	0.95(0.14
SP8 D	3.17(0.01)	2.02(0.02)	7.77(0.14)	4.05(0.04)	9.08(0.34)	3.22(0.05)	3.62(0.00)	2.25(0.75)
SP9 W	3.14(0.03)	1.99(0.04)	5.99(0.00)	3.83(0.07)	8.49(0.10)	3.12(0.02)	3.68(0.01)	0.30(0.01)
SP9 D	3.14(0.00)	2.06(0.09)	7.68(0.01)	4.09(0.10)	8.92(0.07)	3.18(0.01)	3.61(0.01)	1.41(0.10)
SP10 D	3.25(0.02)	2.16(0.06)	7.69(0.01)	4.13(0.06)	9.02(0.05)	3.13(0.01)	3.61(0.00)	1.49(0.20)

Note: For sampling sites**,** codes ending with W represents Wet Season and D represents Dry season. ND = Below method detection limit. Values are presented as Means (Standard deviations) of triplicates.

In the case of fluoroquinolones, ciprofloxacin was detected at the concentrations that were lower than 560 ng L^{-1} and 346 ng L^{-1} reported for surface waters in Nairobi River basin, Kenya (Ngumba *et al*., 2016a) and Laizhou Bay, China (Zhang *et al*., 2012). The obtained values were comparable to concentrations of 25 ng L^{-1} and 37.5 ng L^{-1} in Finland waters (Vieno *et al*., 2006) and water samples from River Arno, Italy (Zuccato *et al*., 2010).

The high frequency of sulfonamides in the water samples is because of their high mobility. This is in turn explained by their low chelating ability and sorption coefficients (Boxall *et al*., 2005; Halling-Sørensen *et al*., 2002; Ngigi *et al*., 2020; Tolls, 2001). The concentrations of the antibiotics quantified in the surface water samples from River Sosiani were within the ranges of those reported in Kenya and some parts of the world. For example, Ngigi *et al*. (2020) reported a concentration of 6,800 ng L^{-1} for Sulfamethoxazole while K'oreje *et al*. (2012) obtained 23,350 ng L^{-1} for Sulfamethoxazole and 9,480 ng L^{-1} for Trimethoprim in surface water samples from rivers in the Nairobi River basin, Kenya. In the Kenyan context, the high concentrations of these two antibiotics reported in the Nairobi river basin may be due to the prevalence of HIV/AIDS in such informal settlements (Ngumba *et al*., 2016a). Sulfamethoxazole is reportedly one of the most used antibiotic in Kenya (K'oreje *et al*., 2012), particularly for the prevention and treatment of opportunistic infections in HIV patients usually in combination with Trimethoprim at a dose ratio of 5:1 (Ngumba *et al*., 2016a). Additionally, the antibiotic Sulfamethoxazole is resistant to degradation in aquatic ecosystems (Al-Ahmad *et al*., 1999; Radke *et al*., 2009; Straub, 2016), which makes it recalcitrant and the most frequently detected antibiotic.

Elsewhere, Boxall *et al*. (2005) reported a mean concentration of 4,130 ng L^{-1} for Sulfadiazine whereas Zhang *et al*. (2012), Fick *et al*. (2009) and Yao *et al*. (2017) had 13,680 ng L^{-1} and 5875 ng L^{-1} for Trimethoprim and 1.6 µg L^{-1} for Erythromycin in surface water samples.

In a recent study in Brazil, Sulfamethoxazole was detected at higher concentrations (range: 332.78 ng L^{-1} to 7,112.44 ng L^{-1}) than Sulfadiazine (range: 3.55 ng L^{-1} to 61.44 ng L^{-1}) (Beatriz *et al*., 2020). In other rivers in the propinquity of the Brazilian city of Curitiba, Sulfamethoxazole was found at concentrations of 1859 ng L^{-1}, while Sulfadiazine was reported at concentrations of 27 ng L^{-1} (Beatriz *et al*., 2020). In the Republic of South Africa, Sulfamethoxazole was detected in surface water at concentrations of 7,300 ng L^{-1} (Matongo, Birungi, Moodley, & Ndungu, 2015) and 14,000 ng L^{-1} (Ngumba *et al*., 2016a), while Sulfadiazine has been detected in concentrations of up to 40 ng L^{-1} in rivers from Nigeria (Oluwatosin *et al*., 2016). In China, up to 764.6 ng L^{-1} of Sulfamethoxazole was detected in river water samples (Chen & Zhou, 2014). In Turkey and Italy, the compound Sulfamethoxazole was previously reported at maximum concentrations of 322 ng L^{-1} in Buyukcekmece Watershed (Aydin & Talinli, 2013) and 11.4 ng L^{-1} in River Arno (Zuccato *et al*., 2010).

In regards to macrolide antibiotics, Erythromycin was observed at a concentration range of 3.32 to 6.73 ng L^{-1}. In comparison to a previous study in Kenya (Ngigi *et al*., 2020), the antibiotic Erythromycin was recovered at most sampling points from River Sosiani due to the existence of agricultural activities such as poultry and livestock farming near this River, which are known to lead to such contamination with erythromycin. Interestingly, the concentrations reported in this study were much

lower than the 1.9 μgL^{-1} that was recently reported in the Nairobi River basin (Ngigi *et al*., 2020). The amounts obtained for Erythromycin were also lower than those previously reported in surface waters in Nigeria and South Africa of 1,000 ng L^{-1} and up to 20,000 ng L^{-1}, respectively (Matongo *et al*., 2015; Oluwatosin *et al*., 2016).

In the context of beta-lactams, Amoxicillin was reported in some Brazilian rivers at concentrations of up to 1,284 ng L^{-1} in rivers from the state of Sao Paulo (Locatelli *et al*., 2011) and 1,570 ng L^{-1} in Curitiba (Beatriz *et al*., 2020), which are higher than the concentrations found in water samples from River Sosiani in this study. Cha *et al*. (2006) indicated a range of 9.0 ng L^{-1} to 11.0 ng L^{-1} for five selected beta-lactam antibiotics that were quantified in surface water samples from China.

Sulfonamides are typically bacteriostatic in their action and based on the infections they treat; they are usually reported at relatively higher concentrations in rivers in the proximity to cities (Gomes *et al*., 2022). They are also the predominant class of antibiotics reported in rivers in China, the leading producer of antibiotics. This is illustrated by the report of Chen and Zhou (2014) who detected Sulfadiazine and Sulfamethazine at 100% at average residual concentrations of 259.6 ng L^{-1} and 7.6 ng L^{-1}, respectively.

Further, beta-lactam antibiotics (such as Amoxicillin, Ampicillin and Cloxacillin) and macrolides (notably Erythromycin and Azithromycin) are bacteriostatic through their binding to penicillin-binding proteins (Bush & Jacoby, 2010; Lobanovska & Pilla, 2017; Miyachiro *et al*., 2019) and inhibitory activity on microbial protein biosynthesis, respectively. These groups of antibacterial medicines have been utilized in both veterinary and human medicine.

It was noted that sampling site SP4 had the highest determined amount (88%) for almost all of the pharmaceutical compounds detected. No general order of determined pharmaceuticals could be established, but fluoroquinolones were generally detected in higher amounts than other classes of pharmaceuticals. Some compounds were, however, detected at relatively comparable levels. For example, Sulfamethizole and Sulfamethoxypyridazine; Sulfapyridine, Sulfisoxazole, Cloxacillin, Mebendazole and Oxfendazole; Sulfadoxine, Penicillin V and Dicloxacillin. The results of this study are comparable to a previous study on samples from Nairobi River Basin, Kenya in which three antibiotics (ciprofloxacin, sulfamethoxazole and trimethoprim) and antiretroviral drugs: zidovudine, lamivudine and nevirapine) were detected, with the highest frequency of 97.5% being for sulfamethoxazole and the lowest being for ciprofloxacin at 60% (Ngumba *et al*., 2016a). The maximum residual concentrations in the samples were 509, 13,800 and 2650 ng L^{-1}, for ciprofloxacin, sulfamethoxazole and trimethoprim, respectively. The drugs occurred at higher concentrations in river water samples than in wastewater treatment plant effluents, pointing out that such contaminations were from anthropogenic sources (Ngumba *et al*., 2016a). Overall, sulfamethoxazole is often detected in environmental matrices owing to its frequent utilization either singly or in combination with Trimethoprim as co-trimoxazole (National Library of Science, 2022). This is because it is more efficacious against a wide spectrum of infections as well as infections caused by methicillin-resistant *Staphylococcus aureus* triggered by their multidrug-resistance (Genç *et al*., 2008). It is also worth noting that the higher concentration of sulfamethoxazole could explained by deconjugation reaction of its metabolites to the parent antibiotic molecule when sulfamethoxazole-contaminated wastes are dumped in aquatic environments (Dinh *et al*., 2017).

It is also evident from Table 4.3 that the penicillins were undetected or detected in very low concentrations at some of the sampling points. This has been reported in other surface water samples from Argentina (Eugenia *et al*., 2021), and this is usually due to the chemical instability of penicillins (Rodríguez-Mozaz *et al*., 2015).

Whereas environmental factors and physicochemical parameters of the river water may influence the concentration of antibiotics in water systems (Gomes *et al*., 2022) and it is expected that the observed concentrations of the antibiotics may decrease over time, it should be emphasized that the levels of antibiotics detected in this study are of environmental concern. This is because the current global disease burden stemming from multidrug (antimicrobial) resistance implies that there is continuous use of the antibiotics, which can be translated into continuous loading of antibiotics residues into River Sosiani water systems. Furthermore, antibiotics occurring at lower (including sub-inhibitory concentrations) are known to act as signaling molecules that can induce resistance in bacteria. This is because such antibiotics tend to induce natural selection in favor of drug- resistant bacteria through growth inhibition or bactericidal activity against susceptible bacteria (Grenni *et al*., 2018; Ngigi *et al*., 2020). The resultant resistant bacterial pathogens may then get better adapted to environmental conditions which expands the transmission window of antibiotic resistance (Quillin & Seifert, 2018).

4.1.3 Concentration of pharmaceuticals in sediments (sedimentary phase)

Sediments were collected at each corresponding sampling point for water from River Sosiani. The residual pharmaceuticals varied in the range of below the detection limit (BDL) to 26.4 µg kg^{-1} (Figure 4 1). However, for two compounds Penicillin G (a penicillin drug) and Albendazole (an anthelmintic), the concentration ranged from

414.0 µg kg^{-1} to 974.0 µg kg^{-1} and 3.0 µg kg^{-1} to 125.0 µg kg^{-1}, respectively (Table 4: 3). Residual concentrations in sediments were significantly higher than in surface water (in orders of 10 to 1000 times). Out of the 15 sulfonamides analyzed, 8 were detected in the sediments. Concentrations of sulfonamides ranged from 0.01 µg kg^{-1} to 4.13 µg kg^{-1}. As observed with water samples, fluoroquinolones were detected in higher concentrations (ranging from 13.2 µg kg^{-1} to 26.4 µg kg^{-1}) compared to other classes of compounds other than the two aforementioned compounds (Peniccilin G and Albendazole). The concentration of Penicillins ranged from 0.8 µg kg^{-1} to 3.4 µg kg^{-1} (except Penicillin G), macrolides (4.0 µg kg^{-1} to 11.6 µg kg^{-1}) and beta-lactams (3.1 µg kg^{-1} to 5.2 µg kg^{-1}). For the remaining classes, nitroimidazoles ranged from 2.0 µg kg^{-1} to 4.2 µg kg^{-1}, benzimidazoles from 2.0 µg kg^{-1} to 5.1 µg kg^{-1}, lincosamides from 4.3 µg kg^{-1} to 6.5 µg kg^{-1}, anthelmintics from 2.7 µg kg^{-1} to 25.8 µg kg^{-1} and trimethoprim ranged from 0.1 µg kg^{-1} to 3.0 µg kg^{-1}. It was also observed that the sampling SPD4 had relatively higher concentrations compared to other sampling points (Figures 4 1, Figures 4 2, and Figures 4 3).

In sediments, seven compounds which included Sulfathiazole, Sulfamonomethoxine, Sulfachlorpyridazine, Sulfaquinoxaline, Dapsone, Ampicillin and Flubendazole were below the method detection limits. Except for the sulfonamide Sulfathiazole, which was measurable in water but not in sediments, all pharmaceuticals that were not detected in water were also absent in sediments.

SDZ = Sulfadiazine, SMT = Sulfamethizole, SMZ = Sulfamethazine, SMP = Sulfamethoxypyridazine, SPY= Sulfapyridine, SSZ = Sulfisoxazole, SDT = Sulfadimethoxine, SDX = Sulfadoxine.

Figure 4.1: Concentrations of sulphonamides in sediment samples from River Sosiani, Eldoret, Kenya

Table 4 5: Mean concentration of Penicillin G and Albendazole (µg kg^{-1}) in sediment samples from Sosiani River, Eldoret, Kenya

Drug	**SD1**	**SD2**	**SD3**	**SD4**	**SD5**	**SD6**	**SD7**	**SD8**	**SD9**	**SD10**
Penicillin G	483.8	545.7	502.0	616.7	641.5	604.7	973.8	414.1	413.6	450.5
Albendazole	3.0	3.0	2.8	124.6	73.4	8.2	4.3	5.4	6.4	70.4

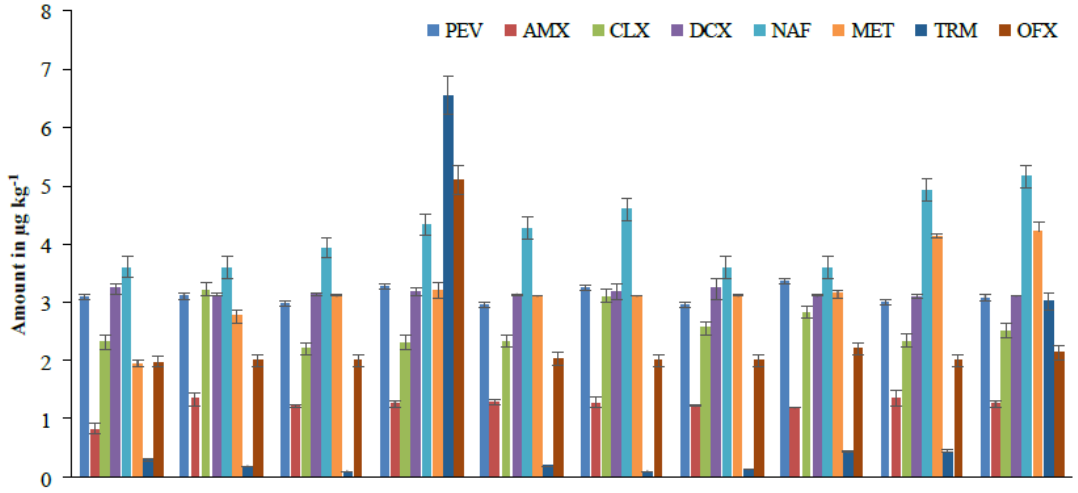


Figure 4.2: Concentration of Penicillins, Metronidazole, Trimethoprim and Oxfendazole in sediment samples from River Sosiani, Eldoret, Kenya

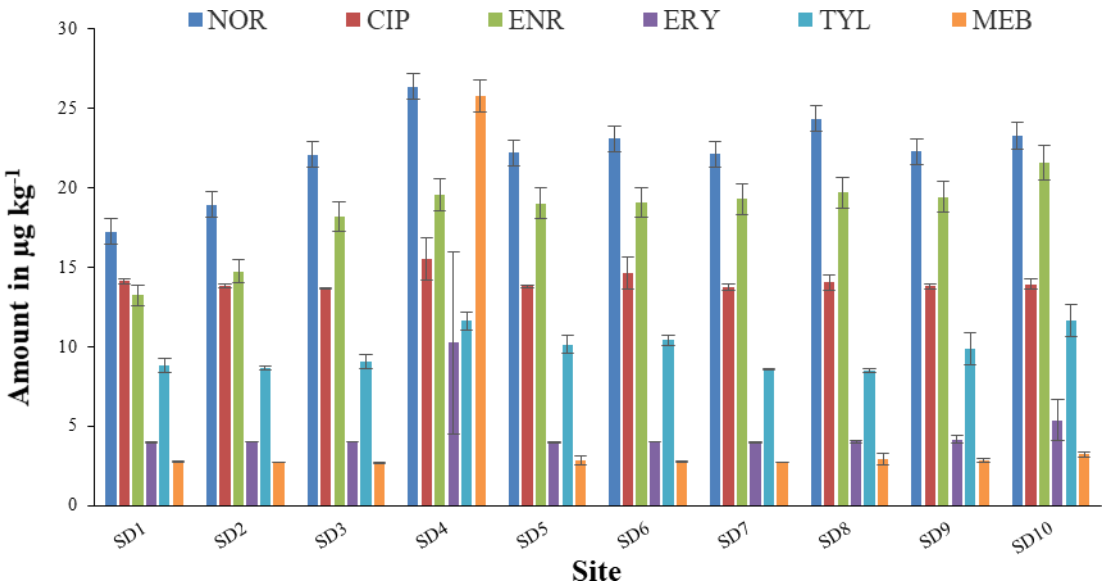


Figure 4.3: Concentration of Fluoroquinolones, Macrolides and Mebendazole in sediment samples from River Sosiani, Eldoret, Kenya

Pharmaceuticals are widely present and are one of the primary man-made contaminants in the environment. Previous studies have documented the existence of antibiotics and other pharmaceutical substances in river sediments across the globe. Detected amounts of antibiotics from the classes: sulphonamides, fluoroquinolones, penicillins, macrolides and beta-lactams in water were within the previously reported values. The environmental concentration of antibiotics in sediments ranges from a few

ng kg^{-1} to several hundred µg kg^{-1} (Chen & Zhou, 2014; Chen *et al*., 2019; Kimosop *et al*., 2016), hence supporting observed results in this study where the quantified amount ranged from 0.01 µg kg^{-1} to 974 µg kg^{-1}. A previous study in River Sosiani analyzed two samples taken from upstream and downstream and reported concentrations of antibiotics (ciprofloxacin, ampicillin, amoxicillin, sulfamethoxazole and chloramphenicol) ranging from below the limit of quantification to 0.054 ng kg^{-1} and below the limit of quantification to 0.094 ng kg^{-1}, respectively (Kimosop *et al*., 2016). In the Chinese Huangpu River, antibiotic compounds were reported to have had high detection rates, particularly for sulfonamides, macrolides, and fluoroquinolones (Chen & Zhou, 2014), with total concentrations ranging between 12.4 µg kg^{-1} and 101.0 µg kg^{-1} and following the ascending contribution order: chloramphenicols (3%) < sulfonamides (11%) < fluoroquinolones (16%) < macrolides (30%) < tetracyclines (40%).

Nitroimidazoles are a class of veterinary pharmaceuticals commonly used for the treatment and prevention of certain bacterial and protozoal diseases in poultry, and dysentery in swine. Although not as widely studied in the environment as antibiotics, nitroimidazoles have also been reported in sediments. Wagil *et al*. (2015) reported a concentration of 12.0 ng g^{-1} in sediments. The benzimidazoles are a large chemical family used to treat nematode and trematode infections in domestic animals. Anthelmintics are used to treat parasitic worms (for example flukes and tapeworms) in animals.

For sulfonamides, the concentrations obtained in this study were low. This is in agreement with previous studies in Chinese rivers and lake sediments (Chen & Zhou, 2014; Li *et al*, 2012) wherein sulfonamides occurred at concentrations ranging

between 0.05 to 0.9 µg kg^{-1}. Considering fluoroquinolones, the concentrations recorded were lower than previously reported in Chinese rivers (Chen & Zhou, 2014; Li *et al*., 2012; Xu *et al*., 2009).

As observed, concentrations of the determined pharmaceuticals from seven different classes varied in surface water and river sediments. Also observed were the comparable concentrations of individual analytes from the 10 sampling sites, in both water and sediments, except for a few exceptions, notably sampling site 4 (SP4) for most of the samples. This trend is attributable to the closeness of the sampling points, hence low locational diversity. The observed spike in concentrations of pharmaceuticals at some sampling sites (eg SP4) could be due to some anthropogenic activities in the proximity of the river. As noted from the area description, the site is close to a dumping site for wastes including expired drugs, resulting in elevated concentrations of pharmaceuticals especially during the dry season.

It is worth noting that environmental contaminants (such as heavy metals and antibiotics) sometimes transfer from water to sediments. For this reason, superficial sediments are known to act as the sink for contaminants of aquatic ecosystems, enhancing the accumulation of such contaminants in benthic invertebrates, which ultimately transfer the pollutants to higher levels of the food chain (Matongo *et al*., 2015; Omara *et al*., 2019b; Xu *et al*., 2009).

4.1.4 Comparison of pharmaceutical residues in river surface water and in sediments

For those pharmaceutical compounds that were quantified in both compartments, Pearson's correlation was used to determine whether there was any association between the concentrations found in surface water and sediments. The target

chemicals' pseudo- partitioning coefficients ($K_{p,s}$) between water and sediment were also assessed. Although $K_{p,s}$ is not usually a relevant parameter in aquatic systems since the sorption is not in equilibrium, it can be useful in evaluating the sorption capacity of compounds that are of interest.

According to Pearson's correlations ($\rho = 0.05$), there was a positive and significant association between the component concentrations in water and solubility ($r = 0.71$), whereas there was a negative correlation ($r = -0.02$) between the concentrations in sediments and solubility (Table 4:*6*). Additionally, there was a weak negative correlation ($r = -0.09$) between the concentrations of the chemicals in the water and the sediments, suggesting that one parameter was comparatively larger than the other. The $K_{p,s}$ values ranged from 52 - 943, 797 - 322190, 870 - 919, 1171 - 1262, and 1111 - 7372 L Kg^{-1} for sulfonamides (SAs), penicillins (PNs), fluoroquinolones (FQs), microlides (MLs) and benzamides (BZs), respectively.

SAs were among the compounds with low $K_{p,s}$ values, indicating their low adsorption in sediments. $K_{p,s}$ values of over 2000 L Kg^{-1} correspond to highly adsorbed compounds. Hence the BZs, albedazole, and penicillin G (PNs) were strongly adsorbed in sediments, with potential of accumulation. Other compounds were moderately adsorbed (FQs, MLs, Lincomycin, LIN, and Metronidazole, MET). However, for such $K_{p,s}$ values, data should be obtained for a considerable period of time for fair representation of sorption equilibrium with relation to sources of the pollutants.

Table 4. 6: Pearson's correlation parameters of the test pharmaceuticals

	Concentration water (µg L^{-1})	**Amounts in sediments (µg kg^{-1})**	**Log Kow**	**Solubility**
Concentration water (ug L^{-1})	1			
Amounts in sediments (ug kg^{-1})	-0.086	1		
Log K_{ow}	-0.208	0.051	1	
Solubility	0.713	-0.022	-0.342	1

4.1.5 Ecological risk analysis

Based on the risk quotient (RQ_w) values, the majority of the chemicals exhibited a moderate ecological risk in aquatic systems. The antibiotics used in the study were Penicillin V, Ampicillin, Penicillin G (referred to as PNs), Norfloxacin and Enrofloxacin (referred to as FQs), Erythromycin and Tylosin (referred to as macrolides), and Lincomycin (included in Table 4:1). The sulfonamide compound, sulfamethoxazole (SMX), and the fluoroquinolone compound, ciprofloxacin (CIP), present a significant threat to aquatic organisms, with RQ_w values of 1.11 and 3.24, respectively. No risk was found with the remaining assessed substances. Several medications present an ecological hazard due to their ongoing use (some in significant quantities) and long-lasting presence in the environment. According to a previous ecological risk assessment conducted by Chen and Zhou (2014), SMX was found to have the potential to cause moderate harm to *daphnia* in the aquatic ecosystem. Additionally, research by García-Galán *et al*. (2011) identified SMX as the sole sulfonamide that posed a risk to algae in effluent water, with a risk quotient greater than 7. The study conducted by Qin *et al*. (2020) found that sulfachloropyridazine had high risk quotients (RQs) of 335.5 for green algae and 152 for *Daphnia magna* in ditch water. Furthermore, the study revealed that the combined dangers posed by sulfonamide mixes were greater than the risks associated with individual sulfonamides, both in terms of ecological and human health impacts. Duan *et al*. (2022) revealed that the sulfonamides SDZ, SMX, and SMZ caused a significant

threat to the aquatic ecosystem. Tang *et al*. (2015) found that SMX, ofloxacin, CIP, and ENR in the surface water of Lake Chaohu and its inflowing rivers could potentially have a significant negative impact on algae and plants. Enoxacin, CIP, and SMX exhibited significant ecological hazards (RQ>1) to the aquatic species *Vibrio fischeri*, *Microcystis aeruginosa*, and *Synechococcus leopoliensis*, correspondingly, in aquatic habitats (Zhang *et al*., 2012). According to Chen *et al*. (2021), the benzimidazole ALB was found to pose a moderate risk to certain organisms in the Tuojiang River and its water source in Sichuan, China. Ecological risk studies clearly indicate that drugs, when present at specific concentrations, constitute a significant threat to aquatic habitats. Additional research is required to provide a comprehensive understanding of the dangers posed by these pollutants, particularly in light of the potential health concerns to humans caused by antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) found in aquatic environments (Khan *et al*., 2019).

Table 4.7 Risk quotients for the target antibiotics and benzimidazoles in surface water

Antibiotic	**Mean (ng L^{-1})**	**Organism**	**EC50 (mg L^{-1})**	**NOEC50**	**AF**	**PNEC mg L^{-1}**	**RQw (mean)**
Sulfadiazine (SDZ)	7.380	*Scenedesmus vacuolatus*	13.1	-	1000	0.013	0.001
Sulfamethizole (SMZ)	10.529	*Scenedesmus vacuolatus*	19.52	-	1000	0.020	0.001
Sulfapyridine (SPY)	3.713	*Chlorella vulgaris*	1	-	1000	0.001	0.004
Sulfamethoxazole (SMX)	29.919	*Synechococcus leopoliensis*	0.027	-	1000	0.000	1.108
Sulfadimethoxine (SDT)	1.912	*Lemna minor*	0.248	-	1000	0.000	0.008
Penicillin V (PEV)	3.477	*Microcystis aeruginosa*	0.006	-	1000	0.000	0.579
Ampicillin (AMP)	0.190	*Microcystis aeruginosa*	0.0002	-	1000	0.000	0.950
Amoxicillin (AMX)	1.420	*Synechococcus leopoliensis*	0.00222	-	1000	0.000	0.639
Penicillin G (PNG)	1.753	*Microcystis aeruginosa*	0.006	-	1000	0.000	0.292
Norfloxacin (NOR)	24.596	*Vibrio fischeri*	-	0.01038	100	0.000	0.237
Ciprofloxacin (CIP)	16.215	*Microcystis aeruginosa*	0.005	-	1000	0.000	3.243
Enrofloxacin (ENR)	20.007	*Vibrio fischeri*	-	0.00288	100	0.000	0.695
Erythromycin (ERY)	4.068	*Microcystis aeruginosa*	0.023	-	1000	0.000	0.177
Tylosin (TYL)	7.701	*Microcystis aeruginosa*	0.034	-	1000	0.000	0.226
Lincomycin (LIN)	6.279	*Anabaena Raphidocelis*	0.01	-	1000	0.000	0.628
Metronidazole (MET)	3.281	*Chlorella Raphidocelis*	3.22	-	1000	0.003	0.001
Oxfendazole (OFX)	2.108	*Vibrio fischeri*	2.21	-	1000	0.002	0.001
Albendazole (ALB)	4.088	*Vibrio fischeri*	0.77	-	1000	0.001	0.005

4.2 Percentage yield, physical and chemical properties of the prepared biochar

4.2.1 Biochar yield

Biochar is a by-product of thermal pyrolysis of carbon-rich biomass (Gaffar *et al*., 2021; Mandal *et al*., 2017; Rajapaksha *et al*., 2014). Biochars can be produced from nearly any organic feedstock. The use of materials such as obnoxious plants or weeds (invasive alien species), agricultural wastes and other non-food residues have been proposed and utilized for the production of biochars (Domingues *et al*., 2017; Gaffar *et al*., 2021; Lehmann & Joseph, 2009). In this study, a noxious ecological weed that doubles as an invasive alien species (water hyacinth) and an agricultural waste (millet husk) were used as feedstock for preparing biochar. There were average yield reductions of about 18% for biochars produced from water hyacinth and millet husks with increasing pyrolysis temperature. A previous study in India (Masto *et al*., 2013) also indicated that biochar yield decreased with an increase in temperature from 300 °C to 500 °C, with the yield decreasing more sharply at temperatures beyond 400 °C. This is usually expected due to the dehydration of hydroxyl (OH) groups and thermal degradation of lignocellulose structures (Gaffar *et al*., 2021; Novak *et al*., 2009). Therefore, high pyrolysis temperatures result in increased rates of dehydration and release of volatile components of the biomass (Mašek *et al*., 2011).

In comparison to a previous study on water hyacinth, the biochar yield obtained in this study is lower than the 46.8% yield reported for the same biomass harvested for India pyrolyzed at 300 °C for 30 minutes (Najmudeen *et al*., 2019). Overall, biochar yield is contingent on the fixed carbon amount of the feedstock (Yaman *et al*., 2021). For millet husks, no previous reports are available on its biochar yields.

In comparison to water hyacinth, millet husk biochar had lower percentage reductions

in yield probably because the feedstock contained little water as it was initially dry. Conclusively, the higher biochar yields obtained from water hyacinth which is considered as the "world's worst and recalcitrant aquatic weed" on Lake Victoria (Nakweya, 2019) positions it as an excellent source of feedstock for bulk production of biochar for the removal of contaminants from environmental matrices in a cost-effective way. This conversion of water hyacinth into biochar affords a more sustainable approach for the management of this noxious weed.

4.2.2 Physicochemical properties of prepared biochar materials

In remediation, the sorption characteristics of ionizable organic contaminants onto biochar is largely contingent on the properties of the biochar itself (Li *et al*., 2020; Ma *et al*., 2020; Min, 2020). The physicochemical properties and quality of biochar are on the other hand influenced by various factors including the temperature, speed, residence time and the nature of the material being pyrolyzed (Brewer *et al*., 2009; Chen *et al*., 2016; Mohd Hasan *et al*., 2019; Tsai & Chang, 2021; Videgain *et al*., 2021). This study investigated the effect of pyrolysis temperature on the quality of biochar. To draw conclusions, various physicochemical parameters of the produced biochar were determined. Biochar was characterized by Scanning Electron Microscope (SEM) to establish its surface properties and Fourier Transfor-Infrared (FT-IR) spectroscopy to establish the functional groups present.

The physicochemical characteristics of the prepared biochars varied for the different feedstock and at different pyrolysis temperatures (Table 4: *8*). For example, the average moisture content was reduced by 1.59% and 1.34% with increasing pyrolysis temperature for water hyacinth biochar and millet husk biochar, respectively. It is well established that higher pyrolysis temperatures can potentially increase the degree of

aromatization, resulting in the loss of gaseous products, tar oil as well as other low molecular weight hydrocarbons, which reduces the moisture content and volatile matter content of biochars. This phenomenon is associated with more pore formation on the biochars at higher pyrolysis temperatures (Gaffar *et al*., 2021). In a similar study characterizing biochar from water hyacinth harvested from India, Najmudeen *et al*. (2019) reported a moisture content of 16.20%, which is higher than was obtained in this study.

Table 4:8: Physical and chemical properties of biochar from water hyacinth and millet husks prepared at 350 ^{o}C and 500 ^{o}C

Biochar	**Moisture (%)**	**Ash (%)**	**Bulk density (g cm^{-3})**	**pH**
WBC 350	6.51	33.26	0.125	8.43
WBC 500	4.92	31.21	0.112	8.19
MBC 350	7.49	31.18	0.121	8.55
MBC 500	6.15	30.53	0.108	8.51

Note: WBC and MBC represent Water hyacinth and Millet husk biochars, respectively. Values presented are averages of results obtained in triplicate.

There was a small decrease in the ash content of the biochars with increasing pyrolysis temperature. This is explained by the fact that the ash primarily remains in the solid fraction. Thus, increasing pyrolysis temperature in this study did not increase the concentrations of minerals and combusted organic residues (Cao & Harris, 2010). There was a decrease of 2.05% and 0.65% in the ash content of biochars derived from water hyacinth and millets husks, respectively, as the pyrolysis temperature increased from 350°C to 500 °C (Table 4:8). Najmudeen *et al*. (2019) reported an ash content of 20.23% for water hyacinth from India, which is lower than values obtained for biochar at both temperatures experimented in this study. Ash is considered to be an important factor in the remediation of environmental contaminants using biochars because it influences the sorption behavior of hydrophobic organic compounds that

have the potential to either block surface sorption sites in the produced biochars or limit access to the same owing to their interactions with inorganic moieties (Deng *et al*., 2014; Gaffar *et al*., 2021; Zhang *et al*., 2013).

All the biochars were alkaline with pH values ranging from 8.19 to 8.55 (Table 4: *8*). This pH is higher than the pH of 6.4 previously reported for water hyacinth biochar from India (Najmudeen *et al*., 2019). There was, however, a reduction in the pH of the biochars at higher temperatures. Usually, an increase in biochar pH with increased pyrolysis temperature is expected due to the gradual elimination of acid functional groups such as carboxylic, phenolic and carbonyl groups from the biochar surface and the increase in ash content (Gaffar *et al*., 2021). This is the reverse in this study as the ash contents were also observed to have reduced. Nevertheless, biochars with pH in the alkaline range have the potential to neutralize or increase the pH of acidic media (Deng *et al*., 2014; Novak *et al*., 2009).

4.2.3 Fourier transform-infrared spectra of the unmodified and modified biochars

The FTIR spectrums obtained after the adsorption process are in reference to ciptofloxacin (CIP), which was taken as a representative compound.

The FT-IR spectrum of the non-activated millet husk biochar prepared at 350 ^{o}C before adsorption (NA-MB350-BA) is presented in Figure 4 1 (a). The band at 2946.70 cm^{-1} was assigned to aliphatic C-H, principally CH_2 stretching (Ashokkumar & Ramaswamy, 2014; Bautista-Hernández *et al*., 2021). On the other hand, the stretch at 1898.58 cm^{-1} corresponds to C = O stretching or aromatic N=O (Lindblom, 2014). The stretch at 1556 cm^{-1} could be attributed to C = N stretching while the stretch at 1387.53 cm^{-1} was due to O-H stretching of carboxylate groups in a typical

heterogeneous material (Ji *et al*., 2011). The frequency (1251.58 cm^{-1}) could be related to aromatic C O or C-N stretching. These functional groups could potentially bond with CIP. The peaks in the fingerprint region at 1009.55 cm^{-1}, 880.35 cm^{-1}, 829.24 cm^{-1}, 754.99 cm^{-1}, 691.36 cm^{-1} and 500.44 cm^{-1} can be attributed to bending of functional groups, especially out-of-plane deformation of aromatic compounds (Chen *et al*., 2008).

After sorption, NA-MB350-AA (Figure 4 2 b), there were significant changes to the spectrum and the peak at 1898.58 cm^{-1} (C = O stretch) was the only one that was retained. Some peaks disappeared including those at 1387.53 cm^{-1}, 1251.58 cm^{-1}, 829.24 cm^{-1} and 754.99 cm^{-1}. A number of peaks were enhanced and shifted, with new peaks being observed at 3893.57 cm^{-1}, 3700.73 cm^{-1}, 563.11 cm^{-1}, and 525.11 cm^{-1} (Figure 4 3 b). The peaks at 3893.57 cm^{-1} and 3700.73 cm^{-1} can be attributed to the O–H stretching vibration of water or H-bonded hydroxyl groups. This is possible given that CIP was in an aqueous solution and, therefore, water molecules were sorbed during sorption. After interaction of biochar with CIP, a number of peaks were enhanced and shifted: 2946.70 cm^{-1} to 2972.73 cm^{-1} and 2869.56 cm^{-1}, 1898.58 cm^{-1} to 2074.07 cm^{-1}, 1556 cm^{-1} to 1549.52 cm^{-1}, 1000.99 cm^{-1} to 1054.87 cm^{-1}, 1043.30 cm^{-1} and 1024.02 cm^{-1}, 880.35 cm^{-1} to 874.56 cm^{-1}, 691.36 cm^{-1} to 679.78 cm^{-1}, and 500.1 cm^{-1} to 507.19 cm^{-1}.

The FT-IR spectrum of the activated millet husk biochar, prepared at 350 ^{o}C before (A- MB350-BA) and after (A-MB350-AA) adsorption, are presented in Figure 4 5 (a) and Figure 4 5 (b), respectively. From Figure 4 5 (a), the peaks at 3730 -3859 $cm^{-1`}$ can be attributed to the O–H stretching vibration of water or H-bonded hydroxyl groups. Chemical activation using aqueous KOH solution that was used for biochar

modification can explain the presence of water molecules before sorption. They could also be attributed to overtone of C = O, or N-H stretch associated with amines (The functional groups for the other peaks present have already been assigned). There were fewer functional groups in the activated biochar (Figure 4 5 a) relative to non-activated biochar (Figure 4 4 a).

After adsorption (Figure 4.4 b), two peaks at 3730.62 cm^{-1} and 1054.87 cm^{-1} were retained, but the others shifted their positions. The peak at 3859.83 cm^{-1} shifted to 3842.47 cm^{-1}, while the following peaks were both enhanced and shifted: 2944.77 cm^{-1} to 2972.73 cm^{-1}, 1549.52 cm^{-1} to 1550.49 cm^{-1}; 870 cm^{-1} to 873.60 cm^{-1}; 500.44 cm^{-1} to 505.26 cm^{-1}. These shifts could be attributed to electronically induced effects, resulting in a possible sorption mechanism through p–p interactions involving the functional groups of antibiotics and biochar graphene sheet. The new peaks at 2869.56 cm^{-1} and 537.08 cm^{-1} were not pronounced and there were therefore no significant changes in the spectrum after interaction of antibiotics with biochar. This implies that, unlike the non-activated biochar (NA-MB350-BA), direct complexation between antibiotics and the biochar functional groups was highly unlikely, and that antibiotics sorption via hydrophobic partitioning did not therefore occur (Mackay and Canterbury, 2005).

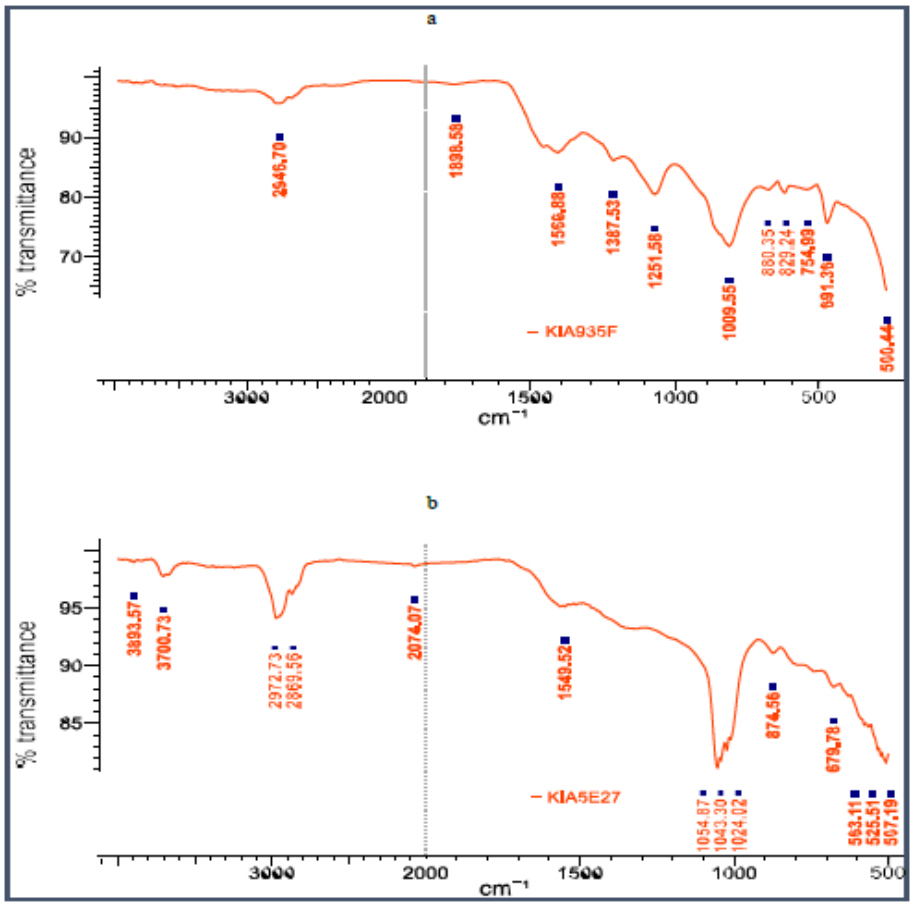


Figure 4.4: FTIR spectrum of non-activated millet-husks-derived biochar produced at 350 °C before adsorption, NA-MB350-BA (a) and after adsorption, NA-MB350-AA (b)

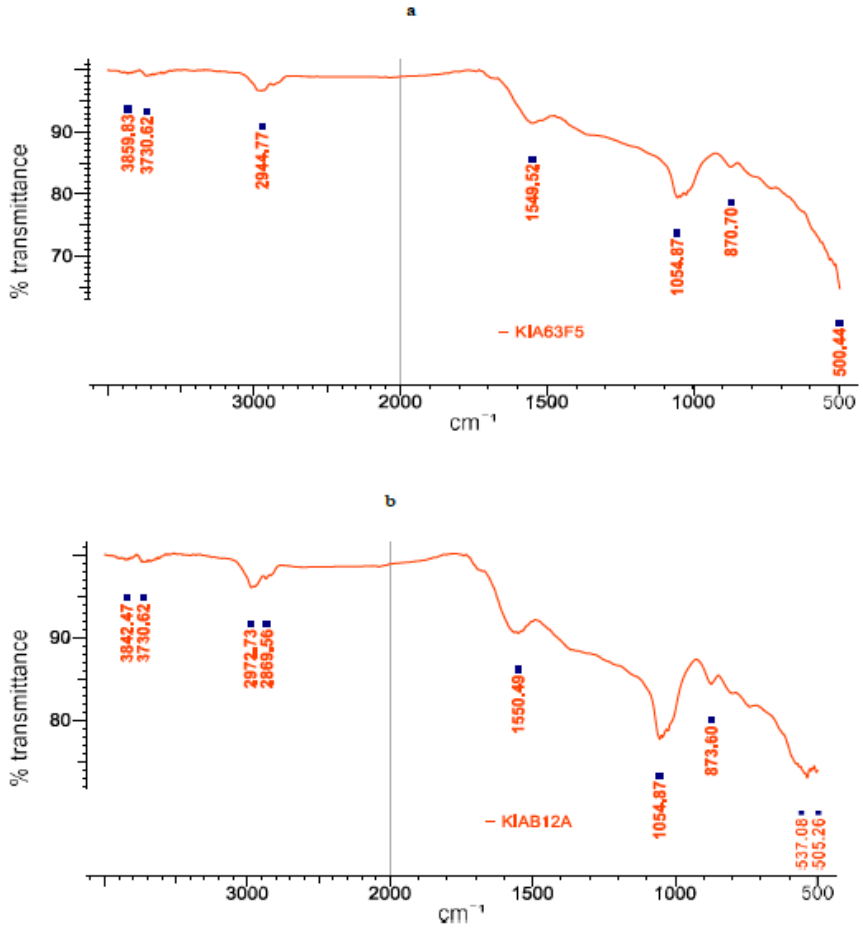


Figure 4.5: FTIR spectrum of activated millet-husks-derived bio char produced at 350 ^{o}C (MBC350) before adsorption, A-MB350-BA (a) and after adsorption, A-MB350- NA-MB350-AA (b).

The FT-IR spectrum of the non-activated water hyacinth biochar, prepared at 500 ^{o}C before (NA-WB500-BA) and after adsorption (NA-WB500-AA) are presented in Figure 4.6 (a) and Figure 4.6 (b), respectively.

From Figure 4.4 a, there were peaks at 3716.16 (O–H stretching of H_2O or H-bonded OH groups or overtone of C = O, or N-H stretch associated with amines), 2972.73 (aliphatic C-H stretching), 1405.85 (C = O or aromatic N=O stretching), 1054.87 (C-N stretching) (Jia *et al*., 2013), and bending of aromatic functional groups: 871.67 (aromatic C bending), 690.39 (aromatic S-O bending) and 500.44 (bending of C-Cl) (Deng *et al*., 2020).

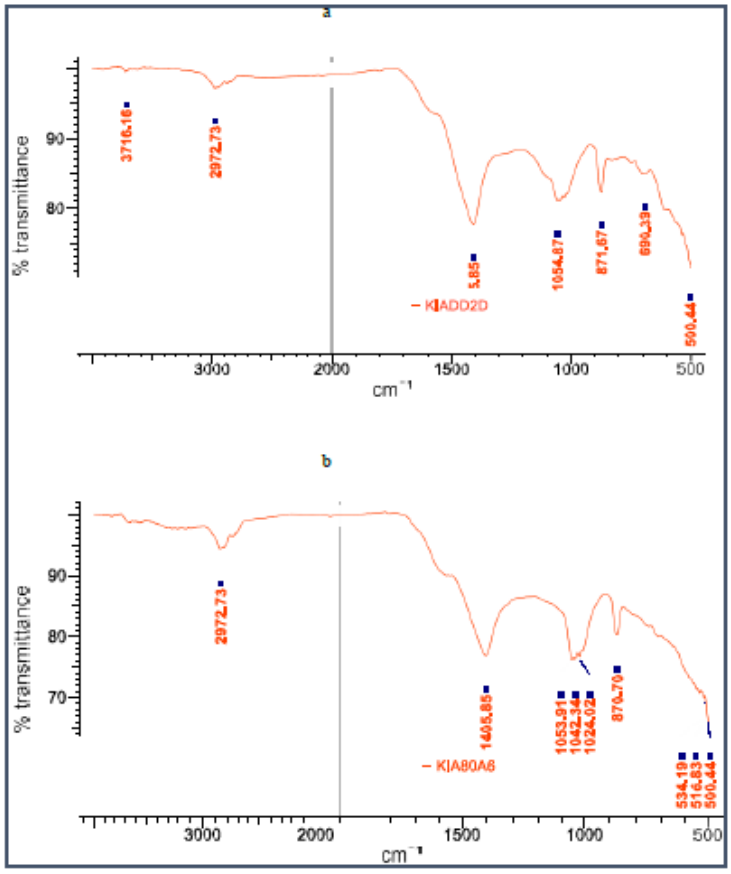


Figure 4.6: FTIR spectrum of non-activated water-hyacinth-derived biochar produced at 500 °C before adsorption, NA-WB500-BA (a) and after adsorption, NA-WB500-AA (b).

After adsorption in Figure 4.6 a, the weak peak at 3716.16 cm^{-1} (overtone of C = O, or N-H stretch associated with amines) disappeared but the more prominent peaks at 2972.73 cm^{-1} (C-H stretch) and 1405.85 (C = O or aromatic N=O stretching) were retained and enhanced. The were no new peaks, but largely enhancement of existing peaks, and the two peaks at 3716.16 cm^{-1} and 690.39 cm^{-1} (aromatic S-O bending) were not prominent. The shifts included 1054.87 cm^{-1} (C-N stretch) which appeared as shifted and enhanced to the bands at 1053.91 cm^{-1}, 1042.34 cm^{-1} and 1024.02 cm^{-1}; 871.67 cm^{-1} shifted to 870.70 cm^{-1}; 500.44 cm^{-1} (C-Cl bend) shifted and enhanced to 534.19 cm^{-1} and 516.83 cm^{-1}. There were thus no major changes in the spectrum after the interaction of CIP with biochar implying that direct complexation and CIP

sorption via hydrophobic partitioning were highly unlikely to occur (Mackay and Canterbury, 2005). The shift of peaks, attributable to electronic-induced effects, suggests a sorption mechanism involving p–p interactions of CIP functional groups and biochar graphene sheets (Jia *et al*., 2013).

The FT-IR spectrum of the activated water hyacinth biochar prepared at 500 ^{o}C, before (A-WB500-BA) and after (A-WB500-AA) adsorption is presented in Figure 4 7. From Figure 4 7 (a), there were peaks at 3356.50 cm^{-1} (O–H stretching of H_2O or H- bonded OH groups or overtone of C = O, or N-H stretch associated with amines), 2974.66 cm^{-1} (aliphatic C-H stretching), 1576.52 cm^{-1} (N-O stretch), 1407.78 cm^{-1} (C=O or aromatic N=O stretching), 1023.05 (C-N stretching) (Jia *et al*., 2013: Jie *et al*., 2020), 871.67 cm^{-1} (aromatic C bending), 690.39 cm^{-1} (aromatic S-O bending) and and bending of aromatic functional groups (535.15 cm^{-1}, 525.51 cm^{-1}, 505.26 cm^{-1}) (Deng *et al*., 2020).

After adsorption (Figure 4 7 (b), the weak stretches at 3716.16 cm^{-1} (overtone of C = O, or N-H stretch associated with amines) disappeared but the more prominent peaks at 2972.73 cm^{-1} (C-H stretch) and 1405.85 cm^{-1} (C = O or aromatic N=O stretching) were retained and enhanced. The peaks at 1576.52 cm^{-1} and 871.67 cm^{-1} were retained, but a number of peaks shifted: 3356.50 cm^{-1} to 3362.28 cm^{-1}; 2974.66 cm^{-1} to 2973.70 cm^{-1}; 1407.78 cm^{-1} to 1405.85 cm^{-1}; 1023.05 cm^{-1} to 1024.02 cm^{-1} and 1042.34cm^{-1}; the band 535.15 cm^{-1}, 525.51 cm^{-1} and 505.26 cm^{-1} was enhanced and shifted to 534.19 cm^{-1} and 514.90 cm^{-1}. There were no new peaks and the major effect of sorption was shifting and enhancement of existing peaks. This implies that the interaction of antibiotics with biochar did not involve direct complexation and antibiotics sorption via hydrophobic partitioning did not occur (Mackay and

Canterbury, 2005). The shift of peaks, attributable to electronic-induced effects, suggests a sorption mechanism involving p–p interactions of antibiotics functional groups and biochar graphene sheets (Jie *et al*., 2020).

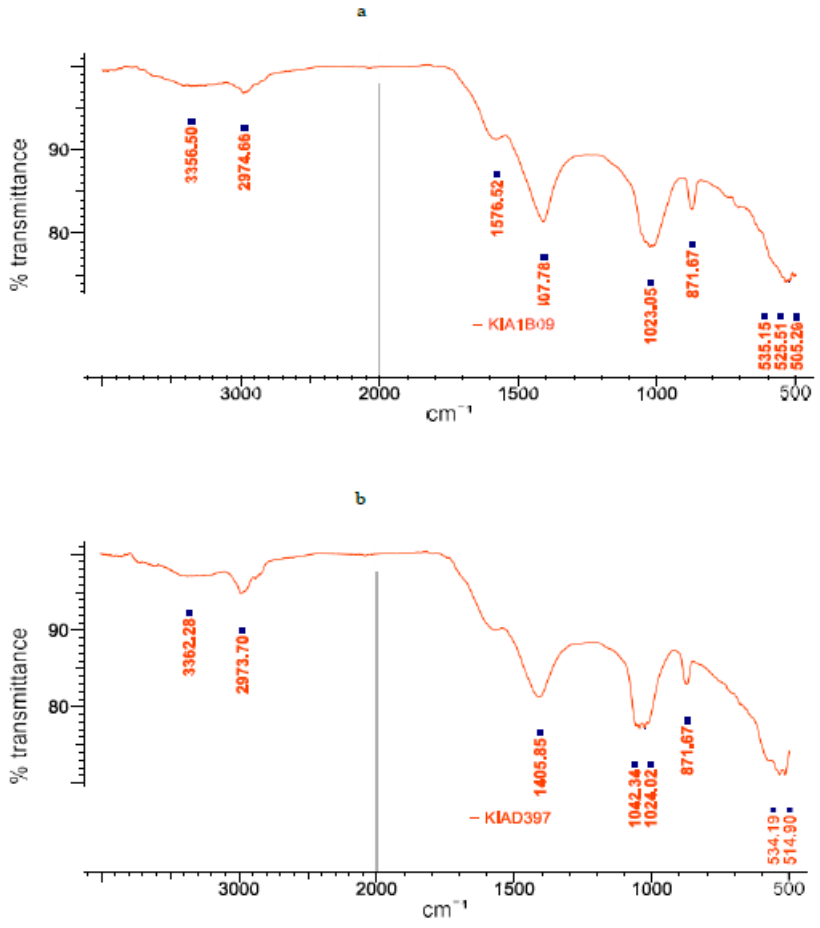
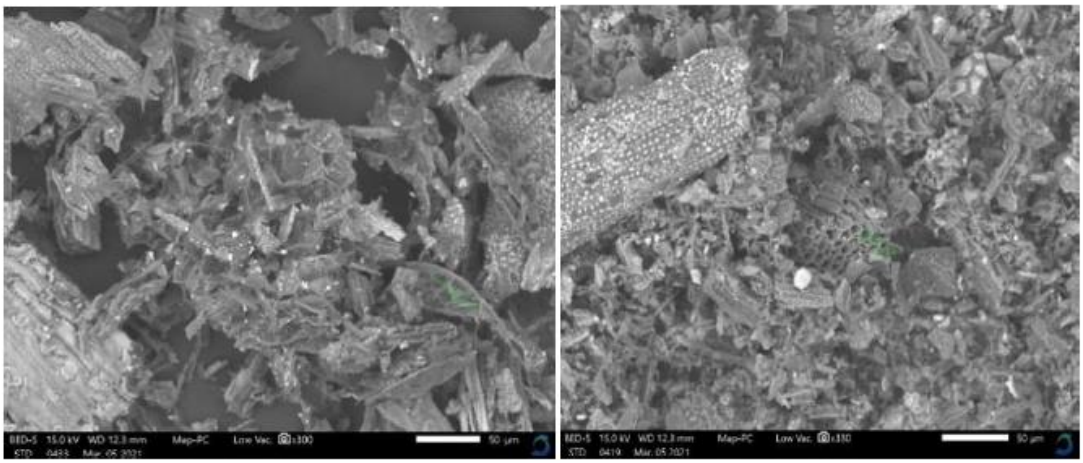


Figure 4.7: FTIR spectrum of activated water-hyacinth-derived biochar produced at 500 °C before adsorption, NA-WB500-BA (a) and after adsorption, NA-WB500-AA (b)

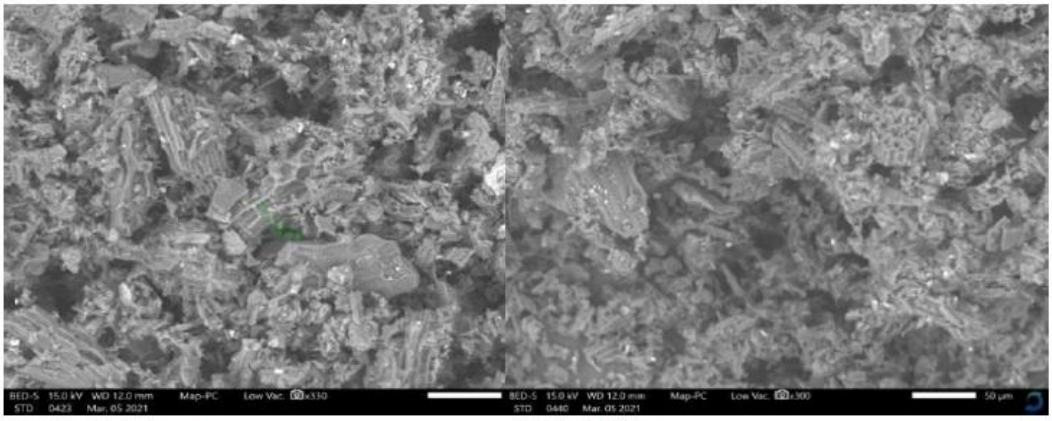
4.2.4 Scanning Electron Microscopy (SEM) of the biochars

To provide further insights into the sorption processes, SEM analysis of the selected biochar samples was done before and after adsorption. SEM was used to examine the surface morphology and porosity of the biochars, and the effect of CIP, PNG and SMX sorption on these properties. Figure 4 8 (a-h) presents the SEM images of

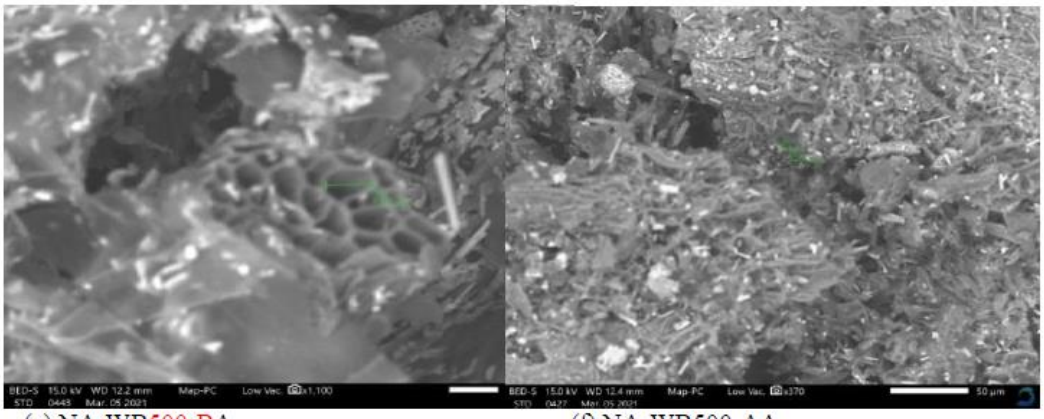
biochar derived from millet husks and water hyacinth that was prepared at 350 ^{o}C and 500 ^{o}C, respectively.



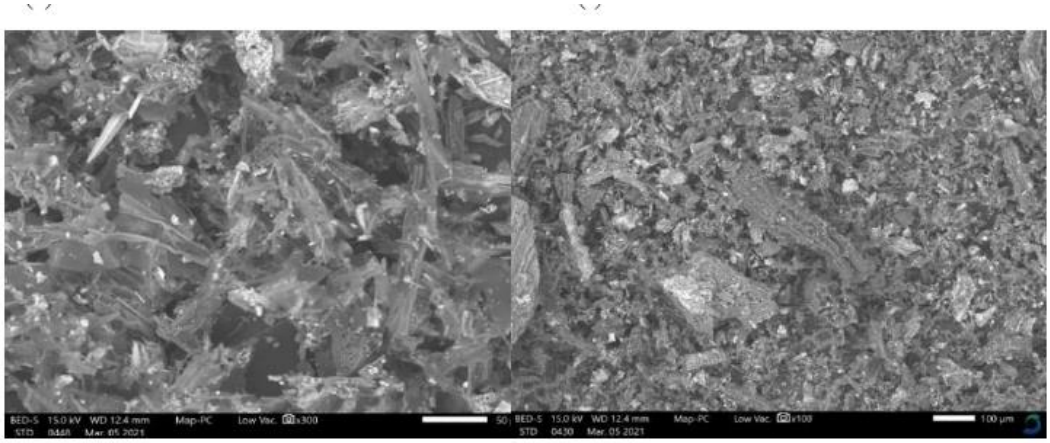
(a) NA-MB350-BA (b) NA-MB350-AA



c) A-MB350-BA (d) A-MB350-AA



(e) NA-WB500-BA (f) NA-WB500-AA



(g) A-WB500-BA (h) A-WB500-AA

Figure 4.5: SEM micrographs of non-activated millet-husks-derived biochar produced at 350 ^{o}C before adsorption, NA-MB350-BA (a) and after adsorption NA-MB350-AA (b); activated millet-husks-derived biochar produced at 350 ^{o}C before adsorption, A- MB350-BA (c) and after adsorption A-MB350-AA (d); non-activated water hyacinth- derived biochar produced at 500 ^{o}C before adsorption, NA-WB500-BA (e) and after adsorption NA-WB350-AA (f); activated millet-husks-derived biochar produced at 500 ^{o}C before adsorption, A-WB500-BA (g) and after adsorption A-WB500-AA (h).

The non-activated biochars obtained based on millet husks (Figure 4.8 a and b) have poorly developed porous structures. The surface of biochar prepared at low temperatures is known to be heterogenous with lactonic, phenolic and carboxylic functional groups (Gusiatin *et al*., 2016) that could potentially bond to antibiotic residues. The non- activated biochars obtained based on water hyacinth (Figure 4.8 e and f) were characterized by slightly better developed porosity. Although there could be differences due to the original feedstock, the main factor might be temperature. Studies have shown that increasing pyrolysis temperature results in generation of more pores and cracks (Hao *et al*., 2013; Masto *et al*., *et al*., 2013; Li *et al*. 2020). Apart from development of pores in biochar, higher temperatures may also result in significant improvement of biochar pore properties, increase in crystallinity of mineral

components, and ordering of aromatic structures in biochar (Gang *et al*., 2015; Ji *et al*., 2011). The observations of this study are congruent with those of Masto *et al*. (2013), whose SEM micrographs showed that biochar derived from water hyacinth had large pores on the surfaces. A plausible explanation for this is that heating of the water hyacinth biomass causes volatile matter to be released (which promote the formation of vascular bundles in the biochar), thereby creating pores on the surface, whereas the volatiles trapped inside the same biomass expand its microstructure. Therefore, the resulting biochar had high surface area and porosity (Masto *et al*., 2013; Najmudeen *et al*., 2019). This was particularly evident for the WH biochars prepared at 500 ^{o}C that exhibited rougher surfaces and more porous structures.

Modification of biochar through activation with KOH caused the development of more porosity (Figure 4.8 c and e). In all cases after the KOH activation, a layered structure was observed, which can indicate the order of the tested biochars. Alkalis and inorganic compounds (such as potassium hydroxide, sodium hydroxide and potassium carbonate) used for activation are known to etch on the structure of biochar, broadening it into an array of mesopores or macropores from the initial micropore structures (Zhong *et al*., 2021). Previous studies have indicated that KOH activation of biochar derived from millet husks and sludge led to increased surface area of the biochar (Bai & Hong, 2021; Mian & Liu, 2020; Shang *et al*., 2020). However, there were no substantial changes in the surface morphology of the original particles; they largely retained the macroscopic shape (Figure 4 7) (a to h). It can be stated that the KOH activation affects the microporous structure of the obtained biochars including the size and volume of micropores but does not affect the mesoporous structure (Li *et al*., 2020). Therefore, the net effect of activation is an increase in heterogeneity of biochar surfaces. This is expected to, ultimately, result in

better adsorption of contaminants. This is consistent with other results in this study showing that the Freundlich isotherm model, which assumes heterogenous sorption, best fitted the sorption data. Given that there were fewer functional groups in the activated biochar relative to non-activated, it means that the enhanced sorption capacity (K_f and K_L) by the activated biochars was as a result of increased etching of the biochar surface via activation (Zhang *et al*., 2020; Zhong *et al*., 2021) and not due to addition of functional groups.

4.3 Adsorption kinetics and isotherms and effects of experimental conditions on the adsorption of the studied antibiotics

4.3.1 Adsorption of Ciprofloxacin

4.3.1.1 Effect of contact time on CIP adsorption

The adsorption dynamics of ciprofloxacin (CIP) by the eight unmodified and modified biochars as a function of time are shown in Figure 4 9. The best adsorption was achieved after 24 hours, with the modified millet husk biochar produced at 500 °C (MBC500) having the best removal capacity of 88.4%. This was followed by modified water hyacinth biochar produced at 500 °C (WBC500) at 82.8%, then unmodified millet husk biochar (NMBC 500) and water hyacinth biochar (NWBC500) with adsorptions of 69.6% and 64.8%, respectively. The lowest percentage removal was 49.8% by the unmodified water hyacinth biochar from pyrolysis at 350 °C. Ngeno *et al*. (2016) reported a higher value (80.4% removal) of CIP by unmodified water hyacinth biochar produced at 350 °C.

In comparison with unmodified biochars (NWBC350, NWBC500, NMBC350 and NMBC500), removal rates for the modified biochars (WBC350, WBC500, MBC350 and MBC500) after 24 hours were higher. This confirms the influence of pyrolysis

temperature and chemical modification on CIP removal by the biochars (Appendix I: Tables 1 and 2). Similar observations were made by Li *et al*. (2018) while applying used tea leaves pyrolyzed at 350, 400, 450, 500 and 550°C. Biochar pyrolysis temperature influences the pore structure and functional groups in the resultant biochar, which in turn affects adsorption. Lower pyrolysis temperatures tend to result in fewer functional groups and pore structures in biochars. The reverse (pore widening as well as coalescence of neighboring pores) predominates at higher temperatures, though too high temperatures might destroy them as well (Li *et al*., 2018).

The study indicated that on average, modified biochars were more effective than the unmodified biochars in the removal of CIP. These results suggested that modification using potassium hydroxide significantly improved the adsorption efficiency of the adsorbents (biochars). This could have been due to their higher specific surface area and improved hydrophobicity (Tang *et al*., 2022).

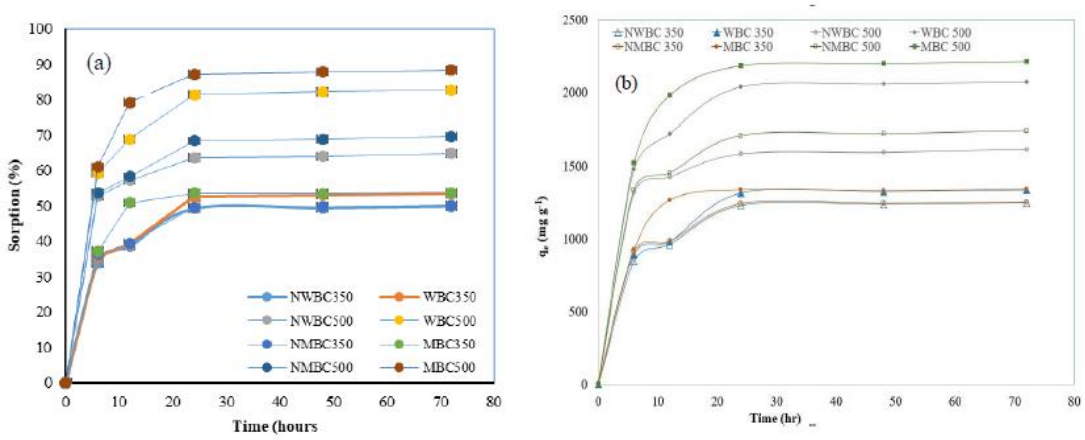


Figure 4.9: Percentage adsorption efficiency (a) and adsorbed amount (b) of CIP as a function of time.

4.3.1.2 Effect of pH on CIP adsorption

The pH of the batch reactor is an important factor to be considered in adsorption as it can alter the adsorption capacity of the adsorbents (biochars in this case) by affecting the amount of surface charges of the adsorbent and the ionization degree of the antibiotics (Ahmed, 2017). In this study, CIP adsorption on both modified and unmodified biochars as a function of pH (ranging from 2 to 12) was investigated. As shown in Figure 4 10, increase in the pH value of the solution from 2 to 8 exhibited an increase in the removal of CIP. For pH values above 8, the removal efficiency started decreasing (or remained almost constant for some biochars). The best removal percentage was 83.8 (at pH 6) by modified millet biochar produced at 500 °C (MBC500), followed by modified water hyacinth biochar (WBC500) at 83.0% at the same pH. For the other biochars, maximum removal efficiencies were obtained at pH 8, with the least removal (53.6%) being by unmodified water hyacinth biochar produced at 350 °C (Table 8, Appendix II).

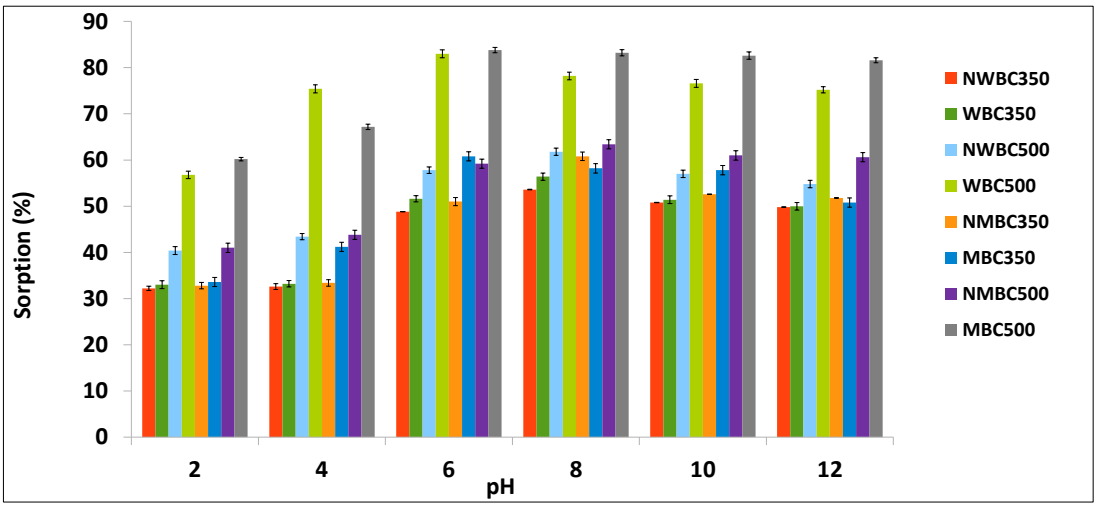


Figure 4.10: Effect of pH on CIP adsorption by the biochars.

It was previously illustrated that pH, which results in the modification of physical properties (such as charge) of compounds with ionizable chemical groups, contributes immensely to their adsorption and degradation (De Bel, Dewulf, Witte, Van Langenhove, & Janssen, 2009). Anhydrous CIP exists in two forms: as a zwitterion and the unionized form (Harrower, 2020; Mesallati, Mugheirbi, & Tajber, 2016). It is zwitterionic in the neutral pH range (De Bel *et al*., 2009; Zhu *et al*., 2021), which makes it better suited for cation exchange or bridging (Carrasquillo *et al*., 2008). That is, CIP exists as CIP^{+} at pH < 5.9 ± 0.15, $CIP^{\pm}$ and CIP^{0} at pH of 6.1 to 8.7, and CIP^{-} at pH > 8.89 ± 0.11 (Peng *et al*., 2015). For solution pH between 6 and 8, the biochar surfaces were strongly electronegative, and CIP thus existed primarily as a cationic species. This resulted in the observed strong CIP adsorption capacity on the biochars.

Thus, the relatively low adsorption of CIP at lower pH values can be ascribed to the electrostatic repulsions between CIP and the positively charged surface of the biochars, and vice versa (Tang *et al*., 2022). For pH values beyond 8, the adsorption decreased again due to electrostatic repulsion. Hence, the maximum adsorption capacity at equilibrium was obtained when the pH was around 8. This is in agreement with results of CIP adsorption onto biochar by previous authors (Jie *et al*., 2020; J. Li *et al*., 2018; Lili, Xin, Zhiping, & Sen, 2019).

The biochars had high adsorption efficiency at pH range of 6 to 8. This indicated that adsorption of CIP by biochar were pH dependent (Ahmed, 2017). As indicated by FTIR data, the biochars had functional groups such as –COOH and –OH which are known to vary pH values. At acidic pH, most of these functional groups are positively charged (protonated). At alkaline pH, the carboxylic acids and hydroxyl groups are

negatively charged (deprotonated). Decrease in adsorption ability at more acidic pH (2 to 4) could be due to electrostatic repulsion between the cationic antibiotics and the protonated functional groups of the biochar (Tang *et al*., 2022). Thus, electrostatic attraction was the main mechanism between CIP and the biochar surfaces (Min, 2020).

4.3.1.3 Effect of adsorbent dose on CIP adsorption

The effect of adsorbent dosage was studied for 24 hours at pH 8 which was the optimal condition for adsorption for the modified biochars. Modified biochars showed stronger adsorption efficiency (67.4% to 86.4%) compared to non-modified biochars (56.8% to 71.0%) as shown in Figure 4 11 and Table 14 of Appendix III. This could be due to additional functional groups observed on the biochar after modification. High- temperature biochar (BC500) exhibited better adsorption affinity compared to low- temperature biochars (BC350). Generally, the antibiotic adsorption rate increased with increasing biochar dosage and then started decreasing (or unchanged). The initial increase is because of an increase in the adsorption sites of specific surfaces for CIP to adsorb, which subsequently leads to increased adsorption rates (Wen, Chen, & Zhang, 2020). At higher doses, however, all the CIP in the solution was adsorbed and thus the adsorption rate started decreasing or remained unchanged. It is thus plausible that in the presence of limited adsorbate, an increase in the biochar dosage could only result in a surplus of biochar, which decreases the amount of CIP adsorbed per unit mass of the biochars (Wen *et al*., 2020).

For this study, a biochar dosage of 0.4 g L^{-1} was seen to be the optimum adsorbent dose for all biochars, after which the increase in the rate of adsorption started decreasing (or remained unchanged). This is similar to the observation by Arun and

Maharathi (2019) in which rice husk biochar produced at 200°C, 250°C and 300°C had maximum removal efficiency of 52%, 75% and 82% respectively, at optimum adsorption dosage of 0.6 g. Recently, Wen *et al*. (2020) reported a similar CIP removal potential by rabbit manure biochar produced at 400 ℃, 500 ℃, 600 ℃, and 700 ℃.

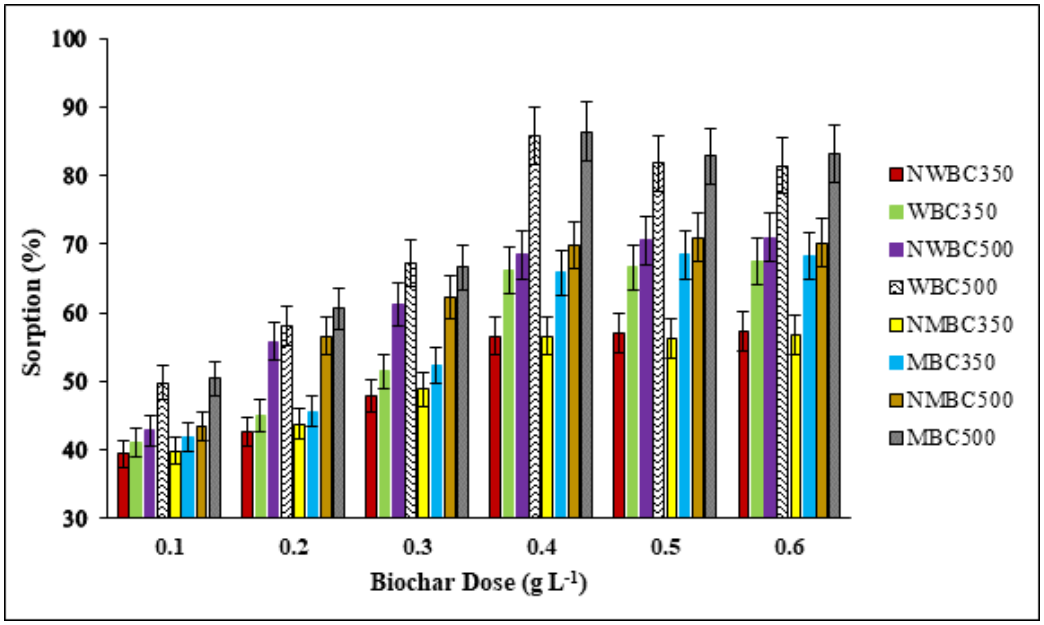


Figure 4.11: Effect of initial concentration on CIP adsorption

The effect of initial CIP concentration on its adsorption by the biochars was experimented for 24 hours at pH 8 and an adsorbent dosage of 0.4 g L^{-1} obtained in the preceding experiments. The study showed that as the concentration of CIP increases, the removal efficiency decreases (Figure 4 12) i.e., the removal efficiency was high at low antibiotic concentration and decreased as the concentration of the antibiotic increased. The highest adsorption was registered at an antibiotic concentration 5 mg L^{-1}. This could be due to limited number of effective adsorption pores at higher antibiotic concentrations (Ahsan *et al*., 2018; Li *et al*., 2018; Marzbali & Esmaieli, 2017). This is contrary to the observation by Arun and Maharathi (2019) in which the reverse was reported.

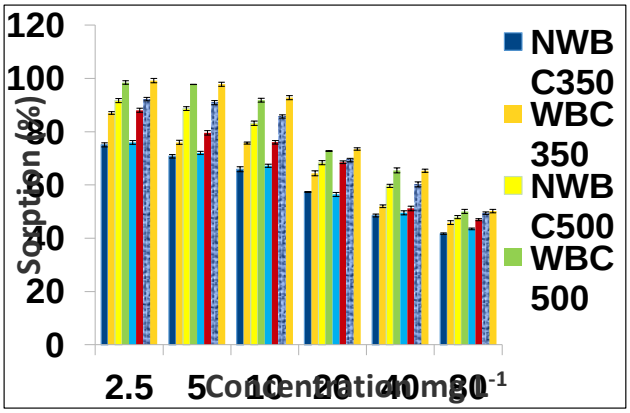


Figure 4.12: Effect of initial concentration on CIP adsorption by the biochars

4.3.2 Adsorption of Penicillin G

4.3.2.1 Effect of contact time on PNG adsorption

The removal efficiencies of Penicillin G (PNG) by the eight biochars under varying times of contact are depicted in Figure 4 13 (Table 3 of Appendix I). The highest adsorption was achieved after 24 hours, with the modified millet husk biochar and water hyacinth produced at 500 °C (MBC500 and WBC500) having the best removal efficiency of 88.0% and 82.4%, respectively. This was followed by unmodified millet husk biochar (NMBC500) and water hyacinth biochar (NWBC500) with adsorptions of 70.2% and 65.2%, respectively. The lowest percentage removal was 49.4% by the unmodified water hyacinth biochar from pyrolysis at 350 °C. Similar to CIP, modified biochars were more effective than the unmodified biochars in the removal of PNG. This indicates that chemical modification significantly improved the adsorption efficiency of the biochars (Tang *et al*., 2022).

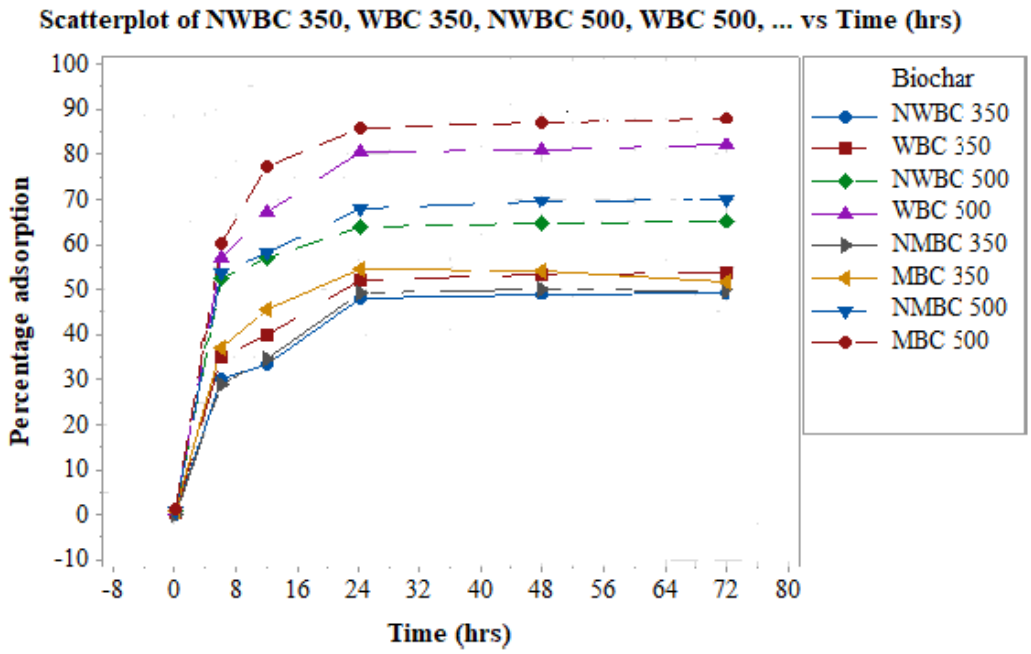


Figure 4. 13: Effect of contact time on PNG adsorption by the biochars

4.3.2.2 Effect of initial solution pH on PNG adsorption

The initial solution pH is critical for adsorption. As shown in Figure 4 14, increase in the pH value of the solution from 2 to 8 registered an increase in the removal of PNG when investigated in 24 hours. For pH values exceeding 8, the removal efficiency started decreasing. The highest adsorption was 84.8% (at pH 6) by modified water hyacinth biochar produced at 500 °C (WBC500), followed by modified millet husk biochar (MBC500) at 82.6% at the same pH. For the other biochars, maximum removal efficiencies were obtained at pH 8, with the least removal (61.4%) by unmodified millet husk biochar produced at 350 °C (Table 10 of Appendix II).

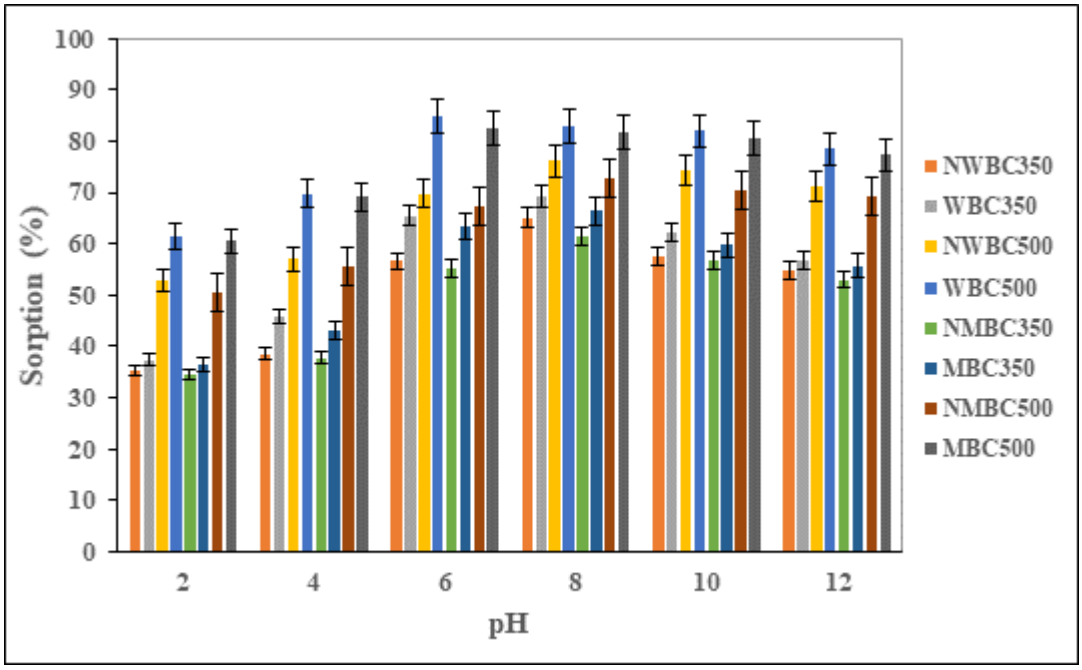


Figure 4 14: Effect of initial solution pH on PNG adsorption by the biochars

4.3.2.3 Effect of adsorbent dosage on PNG adsorption

The effect of adsorbent dosage was studied for 24 hours at pH 8 which was the highest adsorption for the modified biochars. Modified biochars exhibited the highest adsorption efficiency vis-à-vis non-modified biochars as shown in Figure 4 15 (Table 16 of Appendix III). This higher adsorption by the modified biochars could be attributed to the additional functional groups that resulted from biochar modification using potassium hydroxide. High temperature biochar (BC500) had higher adsorption affinities when compared to low temperature biochars (BC350). Following a similar trend to that of CIP, adsorption of PNG increased with increasing biochar dosage and began decreasing with adsorbent dosage of 0.5 g L^{-1} (or higher) for NMBC500 and MBC500. The initial increment is plausibly due to the accompanying increase in the adsorption sites of PNG which is known to facilitate adsorption (Wen *et al.*, 2020). At higher adsorbent doses however, all the PNG molecules in solution was adsorbed and hence the adsorption rate started decreasing (Wen *et al.*, 2020). It was interesting to note that modified and unmodified millet husk

biochars (NMBC500 and MBC500) had maximum adsorption of 78.2% and 85.0%, respectively, at adsorbent dosage of 0.4 g L^{-1} unlike the corresponding biochars of water hyacinth biochar (NWBC500 and WBC500) which had maximum adsorptions of 87.6% and 80.8% at adsorbent dosage of 0.6 g L^{-1}.

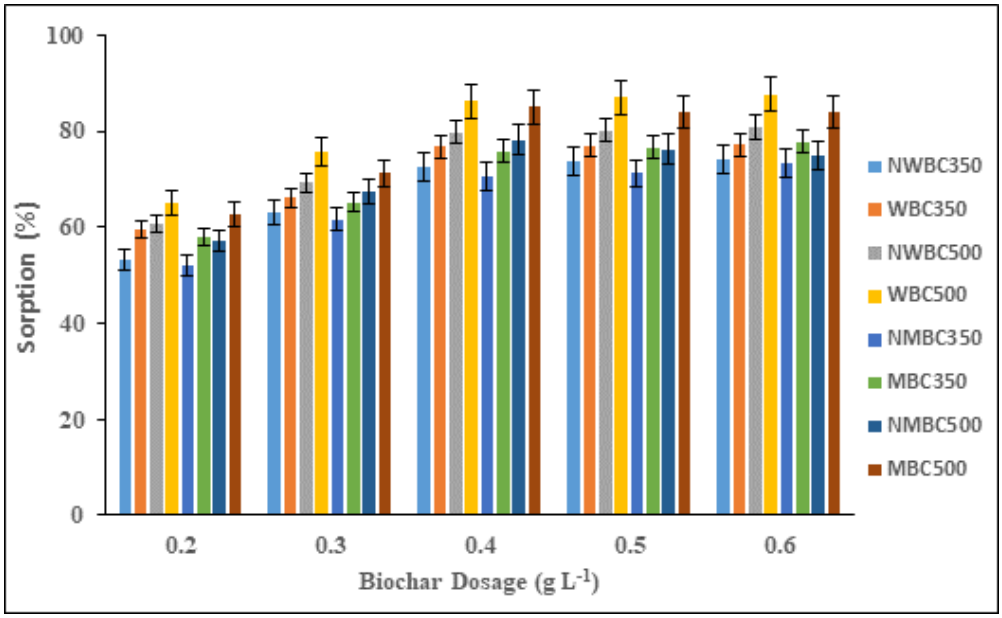


Figure 4.15: Effect of adsorbent dosage on the removal of PNG by the biochars

4.3.2.4 Effect of initial concentration on PNG adsorption

The influence of initial PNG concentration on its adsorption by the biochars was experimented for 24 hours at pH 8 and adsorbent dosage of 0.4 g L^{-1} obtained in the preceding experiments. The study illustrated that initial increase in PNG concentration led to increased antibiotic removal (Figure 4.16; Table 22 of Appendix IV) for some biochars. However, PNG removal decreased as the concentration of the antibiotic increased. The highest adsorption for the biochars were recorded at PNG concentration of 5.0 mg L^{-1} for the modified biochar (MBC350) while the modified biochars had maximum adsorptions at 2.5 mg L^{-1}. This could be due to reduced number of effective adsorption pores at higher antibiotic concentrations (Ahsan *et al.*, 2018; Li *et al.*, 2018; Marzbali and Esmaieli, 2017).

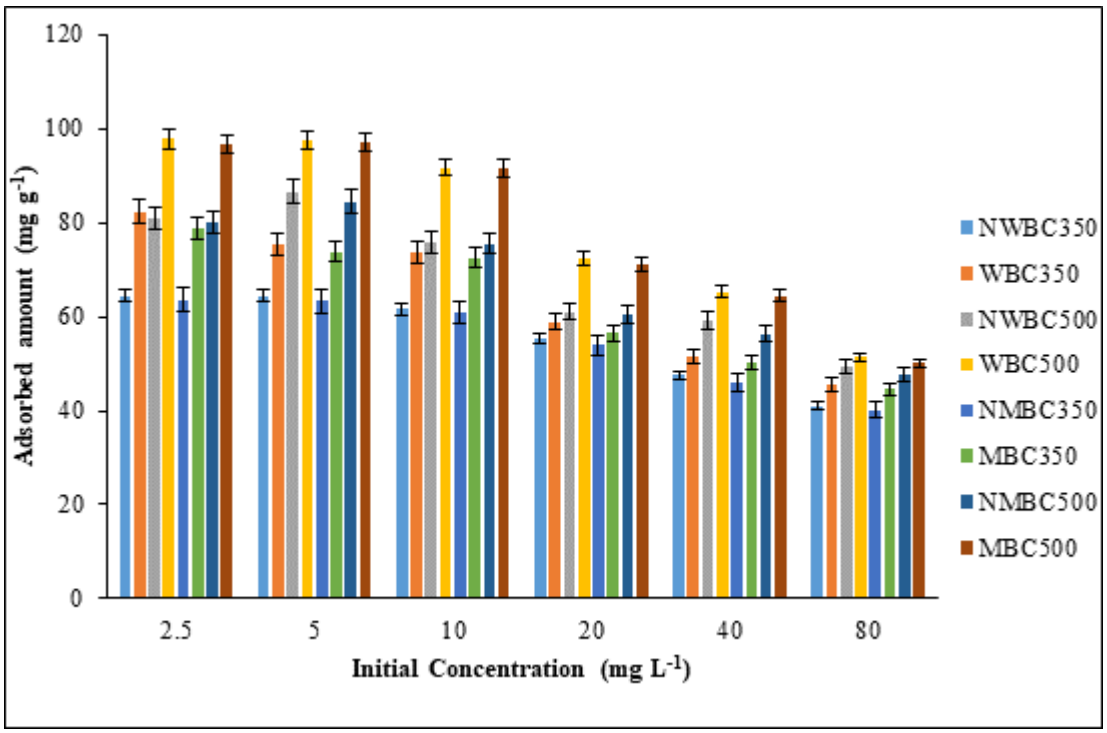


Figure 4.16: Effect of initial concentration on PNG adsorption by the biochars

4.3.3 Adsorption of Sulfamethoxazole (SMX)

4.3.3.1 Effect of contact time on SMX adsorption

The removal efficiencies of Sulfamethoxazole (SMX) by the eight biochars under varying times of contact are depicted in Figure 4 17 (Table 6 of Appendix I). The highest adsorption was achieved after 72 hours, with the modified millet husk biochar produced at 500 ℃ (MBC500) and water hyacinth produced at 500℃ (WBC500) having the best removal of 80.2% and 88.2%, respectively. This was followed by modified water hyacinth biochar (WBC350) and millet husk biochar (MBC350) with adsorptions of 70.4% and 65.0%, respectively. The lowest percentage removal was 45.4% by the unmodified millet husk biochar (MBC350). Similar to CIP and PNG, modified biochars were more effective than the unmodified biochars in the removal of SMX.

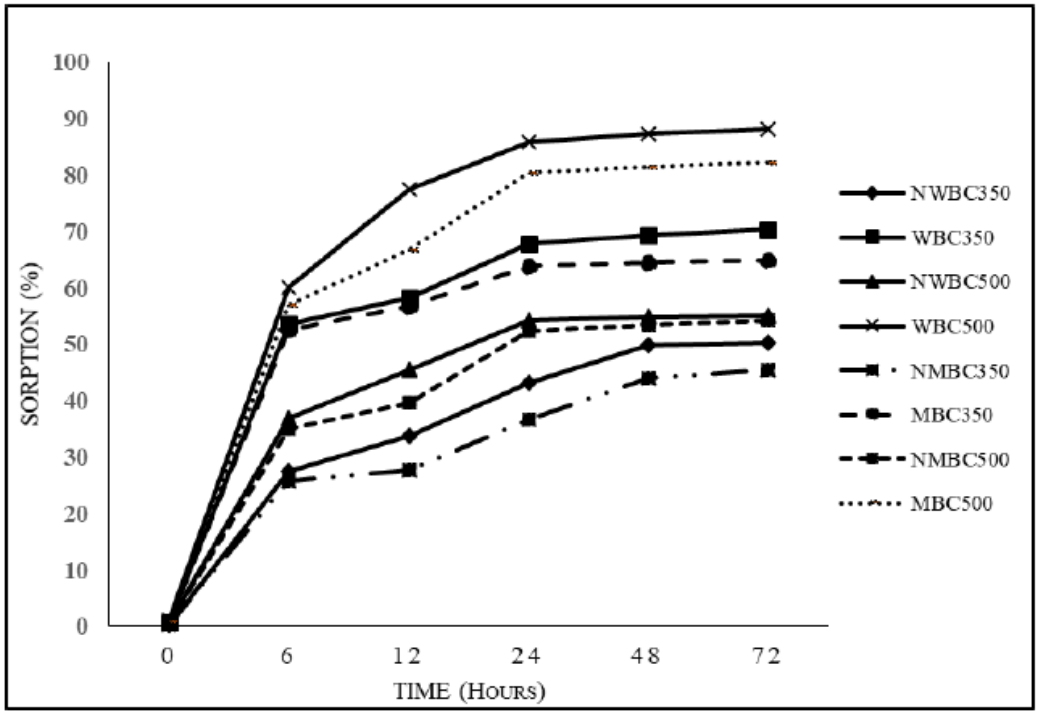


Figure 4.17: Effect of contact time on the adsorption of SMX by the biochars

4.3.3.2 Effect of initial solution pH on SMX adsorption

The effect of initial pH of the solution on the removal of SMX from aqueous solution was also evaluated. Increase in the pH of the solution led to increase in the removal of SMX when investigated in 24 hours (Figure 4 18, Table 12 of Appendix II). For pH above 8, the removal efficiency of the adsorbent started reducing. The highest adsorptions were 85.2% and 82.6% by modified water hyacinth biochar and millet husk biochar produced at 500 °C (WBC500 and MBC500) at pH 6. The other biochars had maximum removal efficiencies at pH 8, with the least removal (60.4%) by unmodified water hyacinth biochar produced at 350 °C.

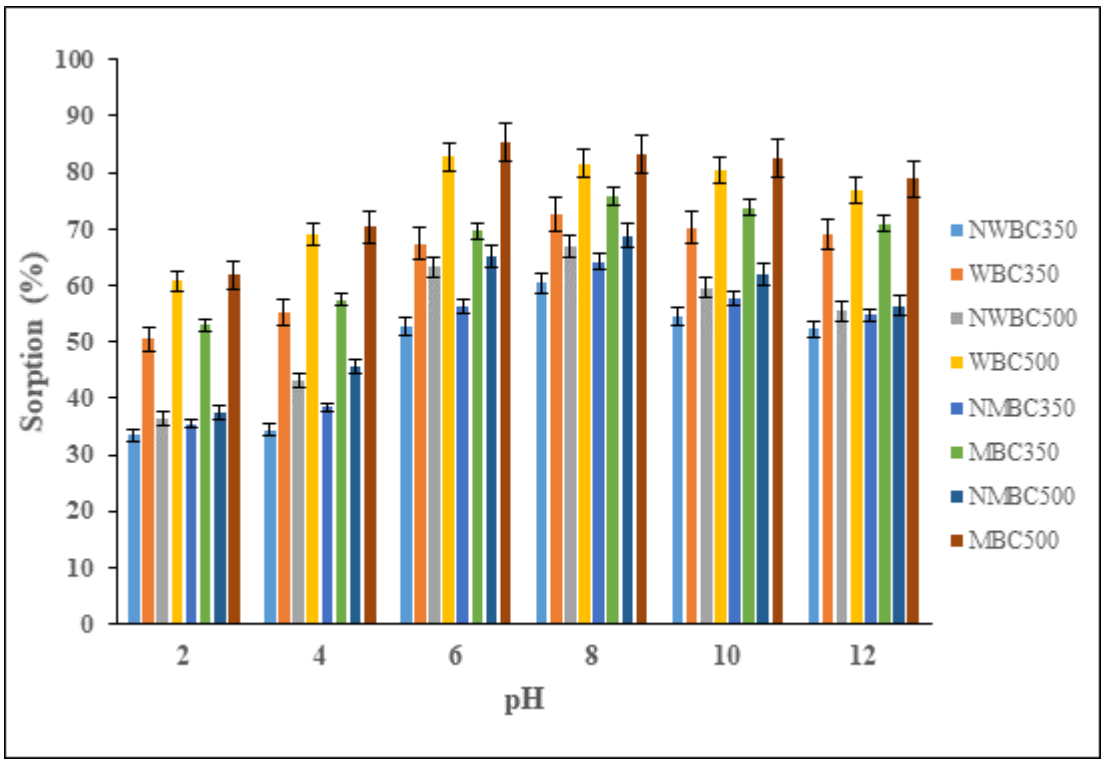


Figure 4.18: Effect of initial solution pH on SMX adsorption by the biochars

4.3.3.3 Effect of adsorbent dosage on SMX adsorption

The effect of adsorbent dose on SMX adsorption was studied for 24 hours at pH 8 which was the most suitable adsorption conditions for the modified biochars. Modified biochars elicited the best adsorption capacity as depicted in Figure 4 19 (Table 18 of Appendix III). This higher adsorption by the modified biochars could be attributed to the increased adsorption sites that resulted from biochar modification using potassium hydroxide. Akin to CIP and PNG, high temperature-pyrolyzed biochars (BC500) had higher adsorption affinities than the low temperature-pyrolyzed biochars (BC350). Similar to PNG and CIP, adsorption of SMX increased with increasing biochar dosage and began levelling afterwards. The initial increment is explained by the increase in the adsorption sites for SMX following increase in adsorbent dosage, which is known to facilitate adsorption (Wen *et al.*, 2020). At higher adsorbent doses, all the SMX molecules in the solution were all adsorbed and

hence the adsorption rate started decreasing or remained unchanged (Wen *et al*., 2020). The modified millet husk and water hyacinth biochars (MBC500 and WBC500) had maximum adsorption of 87.6% and 85.6%, respectively, at adsorbent dosage of 0.4 g L^{-1}. All the other biochars, unlike with CIP and PNG, had maximum adsorptions at adsorbent dosage of 0.4 g L^{-1}. Attempts to experiment beyond this dosage, however, led to a decrease in the adsorption of SMX with adsorbent dosage of 0.6 g L^{-1}.

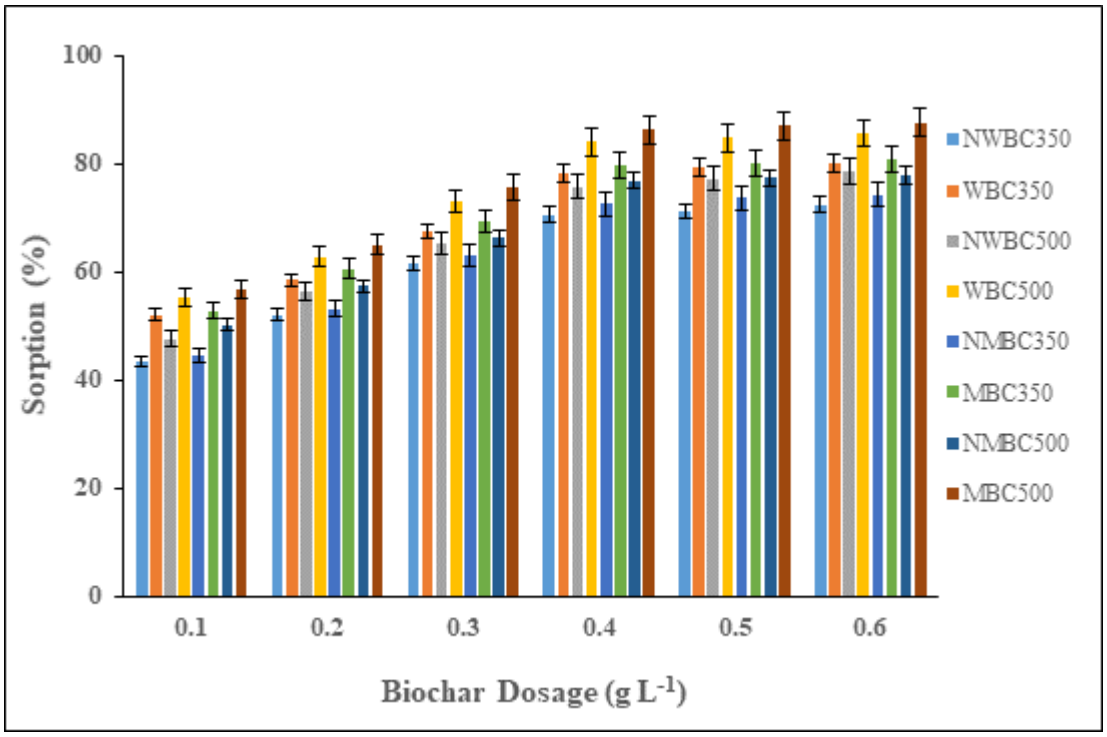


Figure 4.19: Effect of adsorbent dosage on SMX adsorption by biochars.

4.3.3.4 Effect of initial concentration on SMX adsorption

The influence of initial SMX concentration on its adsorption by the biochars was experimented for 24 hours at pH 8 and adsorbent dose of 0.4 g L^{-1} obtained in the previous experiments. The results indicated that initial increase in SMX concentration led to increased antibiotic removal (Figure 4 20). However, SMX adsorption decreased as its concentration increased. The highest adsorptions were recorded at a

concentration of 2.5 mg L^{-1} for unmodified biochars (NWBC500, NMBC350, NMBC500) and the modified biochar (MBC500). Distinct cases were modified and unmodified biochars (NWBC350, WBC350, WBC500 and MBC350) which had maximum adsorptions at 5.0 mg L^{-1}. However, these maximum absorptions were, however, insignificantly higher than those obtained at 2.5 mg L^{-1}.

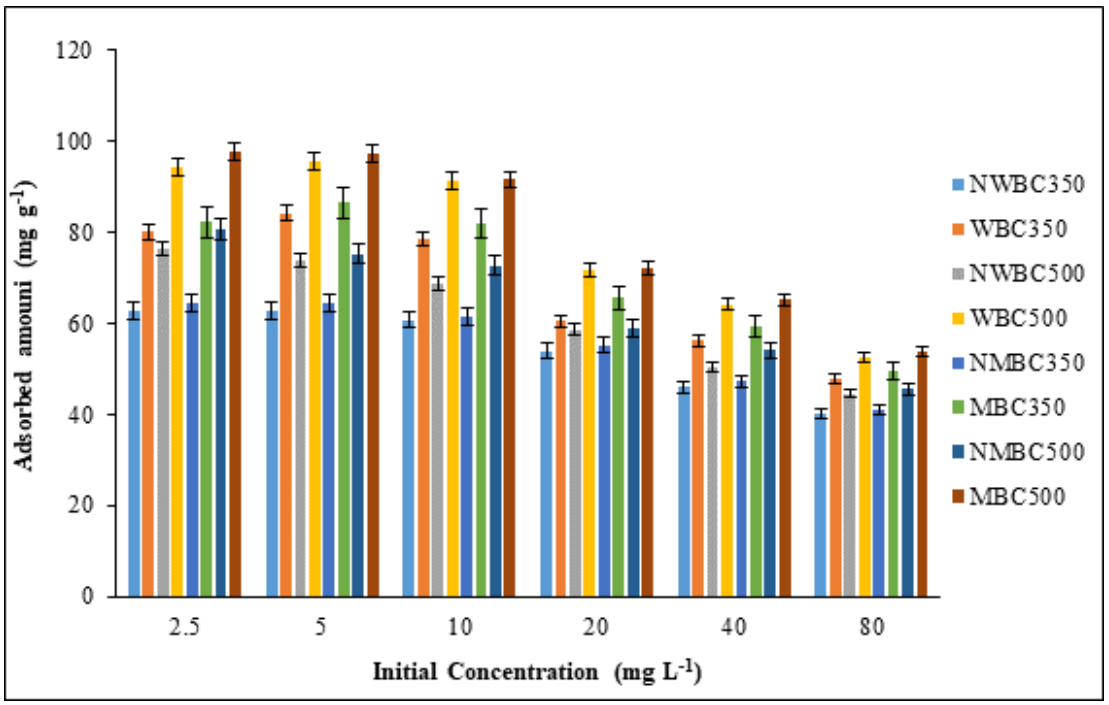


Figure 4 20: Effect of initial concentration on SMX adsorption by the biochars.

4.3.4 Modelling of sorption Kinetics

4.3.4.1 Modelling of sorption kinetics for ciprofloxacin

The concentration of CIP in batch solutions (5 mg L^{-1}) was measured at different sampling times to determine its adsorption kinetics by the different biochars during the adsorption process. The parameters for three adsorption kinetics models are shown in Table 4: 9. The corresponding pseudo-first order and pseudo-second order kinetic model fitting are presented in Figure 4 *21* and Figure 4 *22*, respectively, and the intra-particle kinetic model fitting in Figure 4.23. The values of R^2 from the pseudo-second-order (PSO) model fitting for all 8 treatments were above 0.99, which was

much higher than that of the pseudo-first-order model whose range was 0.66-0.700 (Figure 4 *21*, Table 4: *9*). Based on linearity, CIP adsorption onto biochar best fitted into the PSO kinetic model, indicating that CIP adsorption by the biochars was a chemical adsorption process, and that the number of available active sites on the surface of biochar was significantly related to its adsorption capacity (Li *et al*., 2020). The rate constants (K_2) of the 8 biochars, based on the PSO model, ranged from 0.0009 to 0.0017, indicating a slow CIP adsorption rate onto biochar (Fierro *et al*., 2008). Increase in temperature from 350 ^oC to 500 ^oC had no effect on the rate constant (K_2) of NMBC & WBC, but caused an increase for NWBC (0.0009 to 0.0016) and a decrease for MBC (0.0017 to 0.0009). Increase in temperature increased the initial adsorption rate (h) in all the treatments except NMBC. Activation of biochar caused a decrease in rate constant (K_2) for WHB (350 ^oC and 500 ^oC) and MB (500 ^oC), but an increase for MB (350 ^oC). Biochar activation resulted in decreased h for WHB (350 ^oC & 500 ^oC) and increased h for MB (350 ^oC & 500 ^oC). Therefore, activation is necessary only for millet biochar, especially the one prepared at 500 ^oC. WHB should be used without activation. Generally, for all treatments, h>>>>>>K_2, meaning that differences in adsorption among the treatments was mainly due to the initial adsorption (h), given the relatively low values of the adsorption rate (K_2). For WBC, improved adsorption can be achieved by using higher pyrolysis temperatures, which result in higher h values.

Table 4 9: Adsorption kinetic model parameters for the 8 biochar treatments

Biochar	Pseudo-first order		Pseudo-second order		
	K_1 (min^{-1})	R^2	K_2 (µg mg^{-1} min^{-1})	H (µg g^{-1} min^{-1})	R^2
NWBC350	0.081	0.7001	0.0009	104.2	0.9974
WBC350	0.083	0.6997	0.0008	98.0	0.9963
NWBC500	0.079	0.6877	0.0016	270.3	0.9995
WBC500	0.087	0.6996	0.0008	222.2	0.9986
NMBC350	0.080	0.6981	0.0012	125.0	0.9974
MBC350	0.074	0.6657	0.0017	204.1	0.9988
NMBC500	0.083	0.696	0.0012	125.0	0.9989
MBC500	0.087	0.6955	0.0009	277.8	0.9988

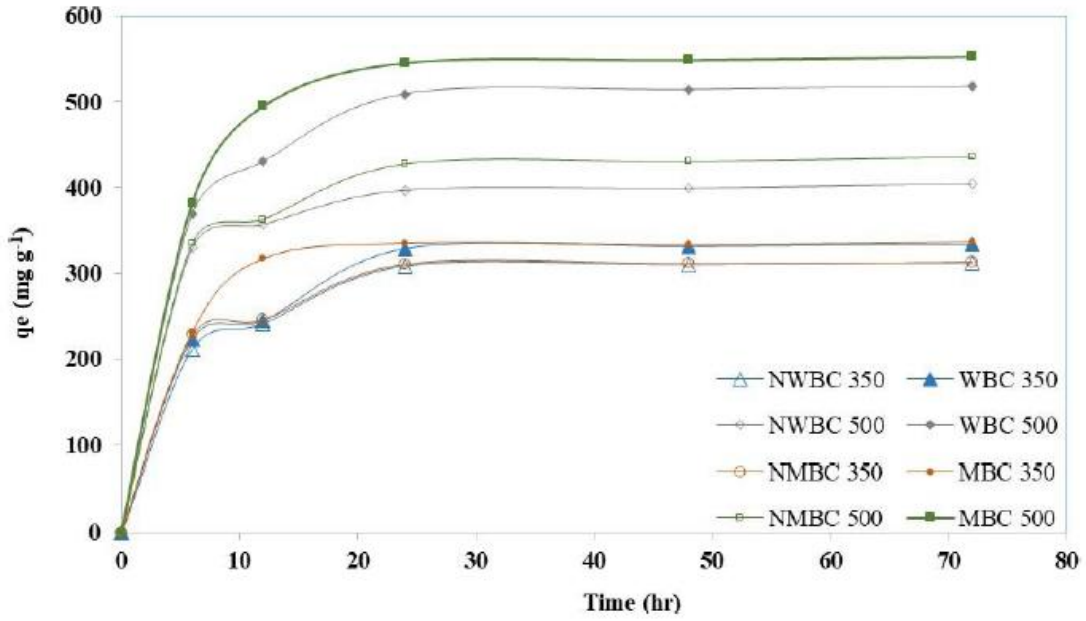


Figure 4 21: Pseudo-first order plot for the adsorption of ciprofloxacin

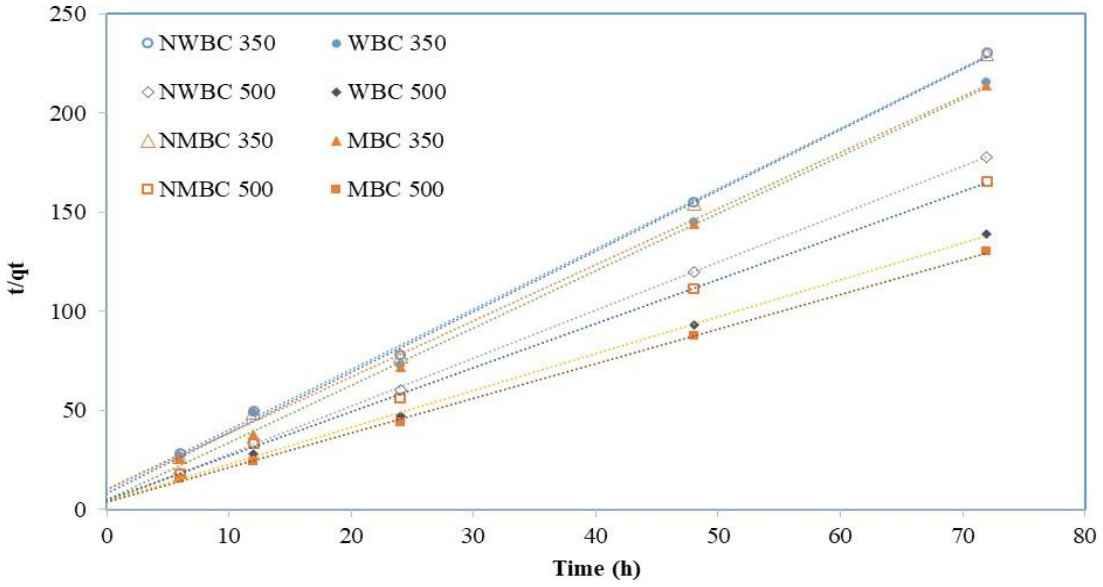


Figure 4.22: Pseudo-second-order plot for CIP adsorption by different biochars

Table 4 10: Model parameters for the Intra-particle kinetic model

	NWBC 350	WBC 350	NWBC 500	WBC 500	NMBC 350	MBC 350	NMBC 500	MBC 500
K_{id1}	0.0331	0.0385	0.0217	0.0438	0.0299	0.0231	0.0319	0.0420
C_1	0.1549	0.1519	0.2952	0.3042	0.1735	0.2223	0.2795	0.3439
R_1^2	0.9924	0.9894	0.9956	0.9961	0.9898	0.9987	0.9924	1.0000
K_{id2}	0.0286	0.0323	0.0195	0.0395	0.0252	0.0244	0.0276	0.0417
C_2	0.1594	0.1581	0.2975	0.3085	0.1783	0.221	0.2839	0.3442
R_2^2	0.9118	0.8642	0.9573	0.9645	0.8674	0.9451	0.9138	0.9933
K_{id3}	0.0012	0.0014	0.0021	0.0025	0.0006	0.0003	0.0022	0.002
C_3	0.302	0.3215	0.386	0.4967	0.3079	0.3329	0.416	0.5354
R_3^2	0.964	0.9943	0.9309	1	0.8416	0.1277	0.9495	0.988

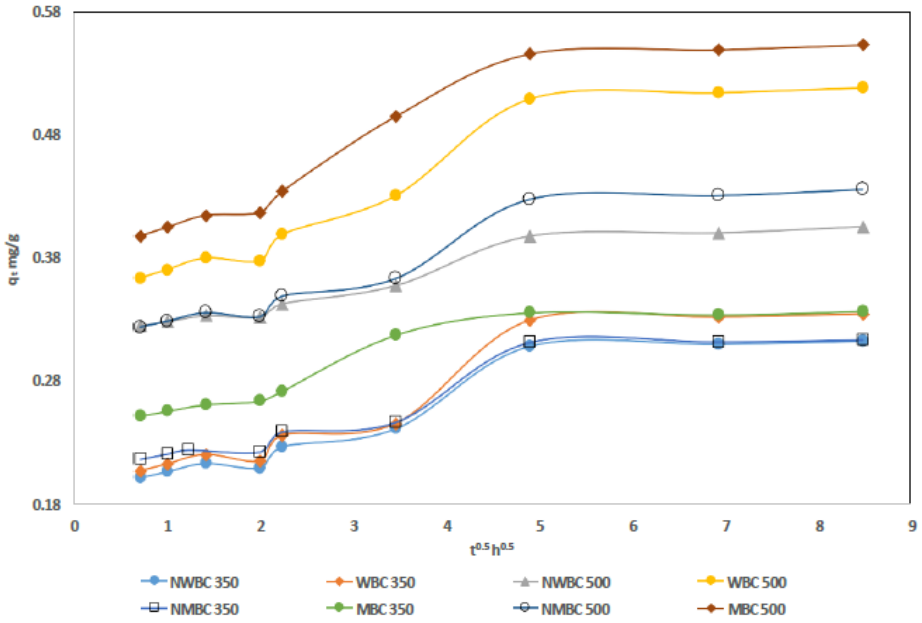


Figure 4.23: Intra-particle diffusion model for the sorption of ciprofloxacin

From Figure 4.23, the data exhibited multi-linear (three) plots, and the first line does not pass through the origin (0, 0). It is generally accepted that whenever the model line passes through the origin, adsorption is dominated by intra-particle diffusion and, if not, it is controlled by the multiple processes. This implies that two or more steps influenced the sorption process. It has been shown that adsorption kinetics includes 3 mass transfer processes: (i) the external (or film) diffusion which entails the transfer of adsorbate in the liquid film around the adsorbent; (ii) the internal (or intraparticle) diffusion which entails transfer of adsorbate in the pores of the adsorbent; and (iii) the adsorption onto the active sites (Wang and Guo, 2022).

The first stage (represented by the first four data points) is assumed to be due to external resistance to mass transfer surrounding the particles and is significant only in the early stages of adsorption. The deviation of the lines from the origin (Figure 4.23) may be due to differences in the rate of mass transfer in the initial stages of adsorption. Furthermore, such deviation of straight line from the origin indicates that

the pore diffusion is not the sole rate-controlling step. Whereas this adsorption rate is usually attributed to boundary layer diffusion effects or external mass transfer effects (Li *et al*., 2020), this may not apply in this case because there seems to be no correlation between K_{id1} and C_1 (Table 4: *10*). For instance, NMBC500 and MBC500 have C_1 values of 0.2795 and 0.3439, and K_{id1} values of 0.0319 and 0.0420, respectively. Therefore, boundary layer thickness ($C_{1)}$ effects had no significant influence on differences in initial mass transfer among the adsorbents. The range of K_{id1} values was 0.0217 - 0.0420.

The second linear portion (data point 4 to 8) is the gradual adsorption stage with controlling intra-particle diffusion. This stage has been attributed to macropore and mesopore diffusion. The relationship between K_{id2} and C_2 in the 8 biochars is the same as that noted for K_{id1} and C_1 above. Generally, values of K_{id2} were the same or lower than the corresponding K_{id1} values for each treatment. The range of K_{id2} values was 0.0195 - 0.0417. The third stage is attributed to micropore diffusion (Ferro *et al*., 2008; Li *et al*., 2020). The relationship between K_{id3} and C_3 in the 8 biochars is the same as that noted for K_{id1}/ K_{id2} and C_1/ C_2 above. The range of K_{id3} values was 0.0003 - 0.0025. Therefore, for every treatment, $K_{id3} <<< K_{id2} \leq K_{id1}$. The three-stage diffusion has been reported by other authors, although most have reported that the second stage, where intra-particle diffusion is rate controlling, is the rate determining step (Wang and Guo, 2022). Given that the slowest step is usually the rate limiting step, the third stage could be rate limiting for sorption of CIP to the adsorbents.

The high K_{id1}/ K_{id2} values relative to K_{id3} means that fast adsorption occurred at the initial stages, with the 3rd stage being very slow. This is consistent with the results of the PSO model fitting, which showed that adsorption was mainly influenced by the

initial adsorption (h), with the adsorption rate (K_2) being very slow. This lends further credence to the choice of the PSO over the PFO kinetic model. The PFO model assumes that the rate of change of solute uptake with time is directly proportional to the difference in saturation concentration and the amount of solid uptake with time, while PSO kinetics model assumes that the rate-limiting step is chemisorption and the adsorption rate is dependent on the adsorption capacity rather than on concentration of adsorbate (Fierro *et al*., 2008).

4.3.4.2 Modelling of sorption kinetics for Penicillin G

Batch sorption equilibrium experiments (5 mg L^{-1}) were carried out to determine the sorption kinetics of Penicllin G (PEG) by the various biochars. The concentration of PEG was measured at various sampling times during the adsorption process. The parameters for pseudo-first order and pseudo-second order kinetic models are shown in Table 4:9 and that for intra-particle kinetic model in Table 4:*10*. The corresponding pseudo-first order and pseudo-second order models fitting are presented in Figure 4.21 and Figure 4.22, respectively, and in Figure 4.23 for the intra-particle kinetic model. Similar to CIP, R^2 values for the pseudo-second-order (PSO) kinetic model fitting for all 8 treatments were above 0.99, which was much higher than that of the pseudo-first-order model (PFO), with a range of 0.69-0.700 for seven biochars (Figure 4 *23*, Table 4: *9*). Data for MBC350 fitted poorly into PFO kinetic model with an R^2 value of 0.2972. Therefore, PEG adsorption onto biochar best fitted into the PSO kinetic model. This suggest that the biochars' adsorption of PEG was a chemical process and that the amount of active sites that were available on the surface of the biochar had a major impact on the material's ability to adsorb PEG (Li *et al*., 2020). Based on the PSO model, the rate constants (K_2) of the 8 biochars, ranged from 0.0008 to 0.0016, which was quite similar to that of CIP, also indicating a slow PEG

adsorption rate on to biochar (Fierro *et al*., 2008). Increase in temperature from 350 ^{o}C to 500 ^{o}C had no effect on the rate constant (K_2) of WBC, but caused an increase for NWBC (0.0009 to 0.0016) and a decrease for MBC (0.0018 to 0.0009) and NMBC (0.0011 to 0.0006). Increase in temperature increased the initial adsorption rate (h) in all the treatments except NMBC that was the same for NMBC350 and NMBC500. Activation of biochar resulted in an increased rate constant (K_2) for millet husk biochar, MBC (350 ^{o}C and 500 ^{o}C) and a decrease in K_2 for water hyacinth biochar WBC (350 ^{o}C and 500 ^{o}C). In a similar trend, biochar activation resulted in decreased h for WHB (350 ^{o}C and 500 ^{o}C) and increased h for MBC (350 ^{o}C and 500 ^{o}C). Therefore, activation is necessary only for millet biochar (MBC) and WHB should be used without activation. Generally, for all treatments, h>>>>>>K_2, meaning that differences in adsorption among the treatments was mainly due to the initial adsorption (h), given the relatively low values of the adsorption rate (K_2). For WBC, improved adsorption can be achieved by using higher pyrolysis temperatures, which result in higher h values.

Table 4. 11: Adsorption kinetic model parameters for PEG for the 8 biochar treatments

Biochar	Pseudo-first order		Pseudo-second order		
	K_1 (min^{-1})	R^2	K_2 (µg mg-1 min^{-1})	h (µg g^{-1} min^{-1})	R^2
NWBC350	0.0831	0.7000	0.0009	104.2	0.996
WBC350	0.0827	0.7003	0.0008	98.0	0.9984
NWBC500	0.0799	0.6906	0.0016	270.3	0.9998
WBC500	0.0877	0.7005	0.0008	222.2	0.9993
NMBC350	0.0838	0.7010	0.0011	125.0	0.9956
MBC350	0.0438	0.2972	0.0018	204.1	0.9969
NMBC500	0.0824	0.6951	0.0006	125.0	0.9997
MBC500	0.0868	0.6963	0.0009	277.8	0.9995

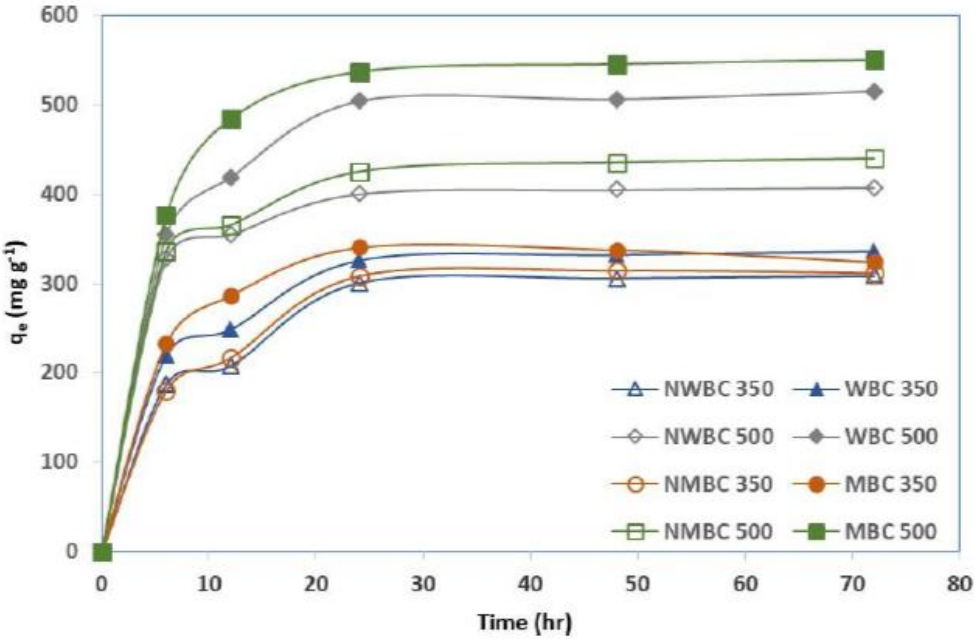


Figure 4.24: Pseudo-first order plot for the adsorption of PEG

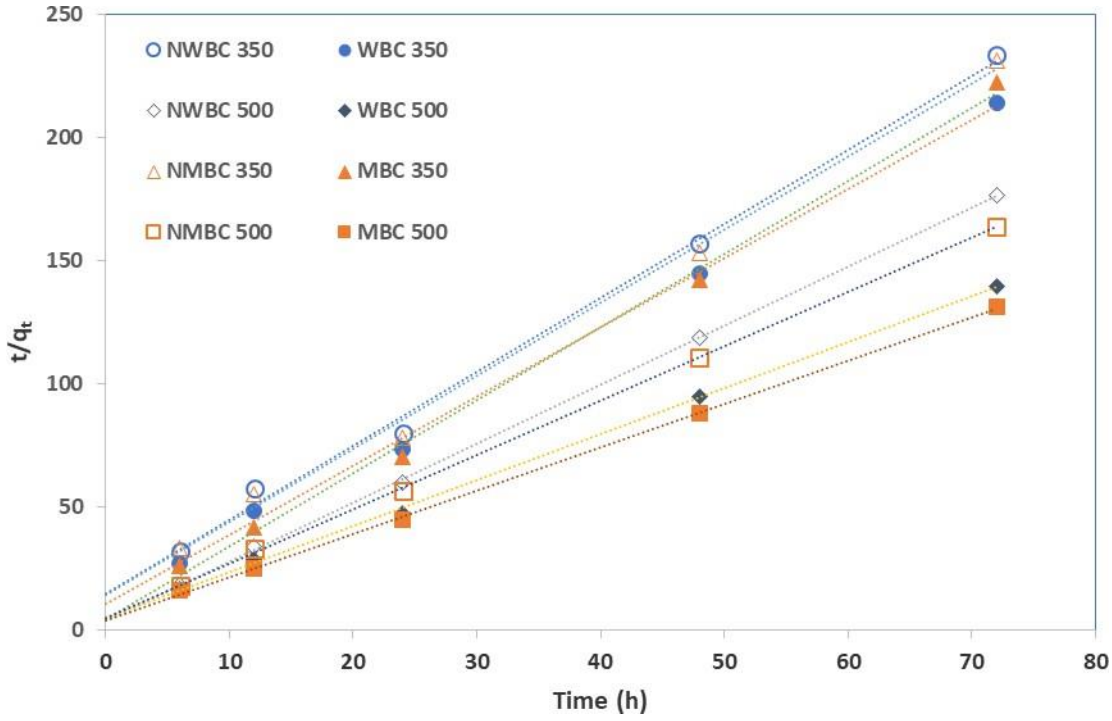


Figure 4.25: Pseudo-second-order plot for PEG adsorption by different biochars

The intra-particle kinetic model of PEG (Figure 4.*26*) exhibited three multi-linear plots, where the first line does not pass through the origin. Therefore, multiple processes (two or more steps) influenced the sorption process and was not

dominated by intra-particle diffusion. The adsorption kinetics of PEG included three mass transfer processes (similar to CIP): (i) external (or film) diffusion, which is the process by which adsorbate is transferred into the liquid film surrounding the adsorbent; (ii) the internal (or intraparticle) diffusion which entails transfer of adsorbate in the pores of the adsorbent; and (iii) the adsorption onto the active sites (Wang and Guo, 2022).

The first stage, as described for CIP, is only important in the early stages of adsorption and is thought to be caused by external resistance to mass transfer surrounding the particles. The deviation of straight line (Figure 4.*25*) from the origin indicates that the pore diffusion is not the sole rate-controlling step. Adsorption rate at this stage is typically attributed to boundary layer diffusion effects or external mass transfer effects (Li *et al*., 2020). However, it does not apply in this case for PEG as there was weak correlation between K_{id1} to and C_1 (r = 0.048); no meaningful correlation was established for K_{id1} to and C_1 of individual treatments. For example, the C_1 values of NMBC 500 and MBC 500 were 0.3103 and 0.3694, respectively, and the Kid_1 values are 0.017 and 0.0246, respectively. K_{id1} values ranged from 0.017 to 0.0249, which was slightly higher than values for CIP. From the results, it can therefore be concluded that boundary layer thickness ($C_{1)}$ effects had no significant influence on differences in initial mass transfer among the adsorbents.

The second stage showing gradual adsorption is attributed to macropore and mesopore diffusion. The relationship between K_{id2} and C_2 for PEG adsorption by the 8 biochars was the same as that noted for the first stage, showing a weak and negative correlation (r = - 0.186). The range of K_{id2} values was 0.0227- 0.0452. Generally, values of K_{id2} were higher than the corresponding K_{id1} values for each treatment,

contrary to the observation for CIP, which were the same or slightly lower for K_{id2}. For the third stage, which is attributed to micropore diffusion (Ferro *et al*., 2008; Li *et al*., 2020), the range of K_{id3} values was 0.002 - 0.0041 for seven biochars. K_{id3} value of MBC350 was -0.0044. The relationship between K_{id3} and C_3 in the 8 biochars was the same as that noted for K_{id1}/ K_{id2} and C_1/ C_2 above, having a weak correlation (r = 0.321). Therefore, for every treatment, $K_{id3} <<< K_{id1} < K_{id2}$. The third stage may be rate limiting step for the sorption of PEG to the adsorbents, since the slowest step is typically the one that limits the rate.

The high K_{id2} followed by K_{id1} values relative to K_{id3} means that fast adsorption occurred during the initial stages (second and first stages), with the 3rd stage being very slow. This supports the results of the PSO model fitting, which showed that adsorption was mainly influenced by the initial adsorption (h), with the adsorption rate (K_2) being very slow.

Table 4.123: Model parameters for the Intra-particle kinetic model for PEG

	NWBC 350	WBC 350	NWBC 500	WBC 500	NMBC 350	MBC 350	NMBC 500	MBC 500
K_{id1}	0.0212	0.0196	0.0129	0.0249	0.0222	0.0139	0.0170	0.0246
C_1	0.1510	0.1899	0.3118	0.3290	0.1496	0.2314	0.3103	0.3694
R_1^2	1	1	1	1	1	1	1	1
K_{id2}	0.0392	0.0353	0.0227	0.0452	0.0415	0.0295	0.0290	0.0420
C_2	0.0973	0.1449	0.2850	0.276	0.0948	0.1922	0.2769	0.3333
R_2^2	0.8527	0.8962	0.9376	0.9611	0.8945	0.9707	0.9266	0.9939
K_{id3}	0.0022	0.0028	0.0020	0.0030	0.001	-0.0044	0.0041	0.0037
C_3	0.2903	0.3124	0.3908	0.4881	0.304	0.3638	0.4052	0.5187
R_3^2	0.9904	0.9985	0.987	0.8278	0.3793	0.816	0.9781	0.9927

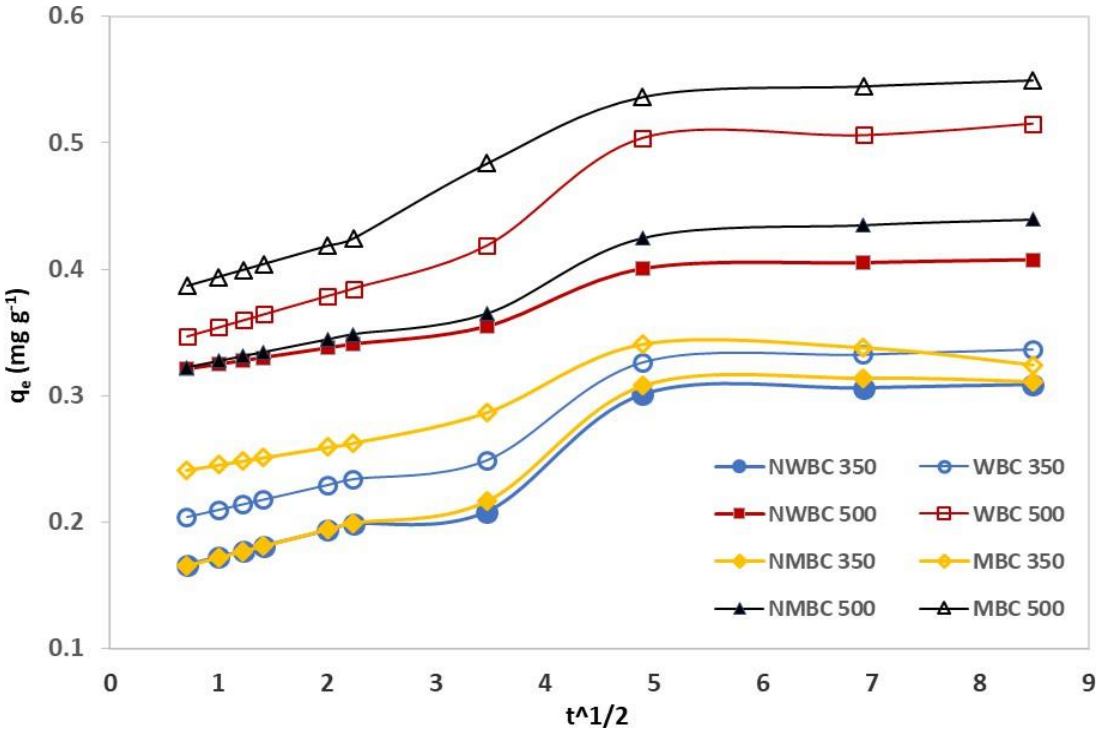


Figure 4.26: Intra-particle diffusion model for the sorption of penicillin G

4.3.4.3 Modelling of sorption kinetics for Sulfamethoxazole

Similar to CIP and PEG, the concentration SMX was measured at various sampling times during the adsorption process in batch sorption equilibrium experiments (5 mg L^{-1}) and the data was modelled into three different kinetic models. The parameters for pseudo-first order and pseudo-second order kinetic models are shown in Table 4:4 and that for intra-particle kinetic model in Table 4:5, and the corresponding pseudo-first order and pseudo-second order models fitting are presented in Figure 4.27and Figure 4.*28*, respectively, and the in Figure 4.6*9* for the intra-particle kinetic model. R^2 values for the pseudo-first-order model (PFO) ranged from 0.6657-0.7001, whereas that of pseudo- second-order (PSO) kinetic model fitting for all 8 treatments were above 0.996 (Table 4:13, Figure 4.28); a similar observation as for the other two studied antibiotics. The PSO kinetic model thus provides the best fit for SMX adsorption onto biochar, which indicate that adsorption by the biochars was a

chemical adsorption process. The rate constants (K_2) of the 8 biochars, ranged from 0.0004 to 0.0031 for the PSO kinetic model, indicating a slow PEG adsorption rate onto the biochar. The effects of temperature and biochar activation on rate constants and initial adsorption rate (h) for PSO were probed. Increase in temperature from 350 ^oC to 500 ^oC had no effect on the rate constant (K_2) of WBC, but caused an increase for NWBC (0.0004 to 0.0022) and a decrease for millet husk biochars. A similar trend was observed for h vales, whereby an increase in temperature from 350 ^oC to 500 ^oC had no effect on the initial adsorption rate (h) for WBC, increased h the for NWBC (52.1 to 454.5), and a decrease for millet husk biochars. Activation of biochar resulted in an increase in rate constant (K_2) for WBC350 only, while it had no effect for MBC500 and caused a decrease for WBC 500 and MBC 350. Biochar activation showed varied effects on h values; it caused an increase of h values for WBC350 and MBC500, a decrease for WBC500 and MBC350 (Table 4:14). Therefore, activation is necessary for millet biochar (MBC) prepared at 500 ^oC and WBC prepared at 350 ^oC. Generally, for all treatments, h>>>>>>K_2; therefore, the differences in adsorption among the treatments was mainly due to the initial adsorption (h).

Table 4:13 Adsorption kinetics model parameters for SMX for the 8 biochar treatments

Biochar	Pseudo-first order		Pseudo-second order		
	K_1 (min^{-1})	R^2	K_2 (µg mg-1 min^{-1})	h (µg g^{-1} min^{-1})	R^2
NWBC350	0.0797	0.7001	0.0004	52.1	0.9988
WBC350	0.0824	0.6997	0.0009	196.1	0.9997
NWBC500	0.0824	0.6877	0.0022	454.5	0.9997
WBC500	0.0815	0.6996	0.0009	196.1	0.9997
NMBC350	0.0765	0.6981	0.0032	312.5	0.9964
MBC350	0.0795	0.6657	0.0015	263.2	0.9998
NMBC500	0.0829	0.6960	0.0007	90.9	0.9983
MBC500	0.0877	0.6955	0.0007	196.1	0.9993

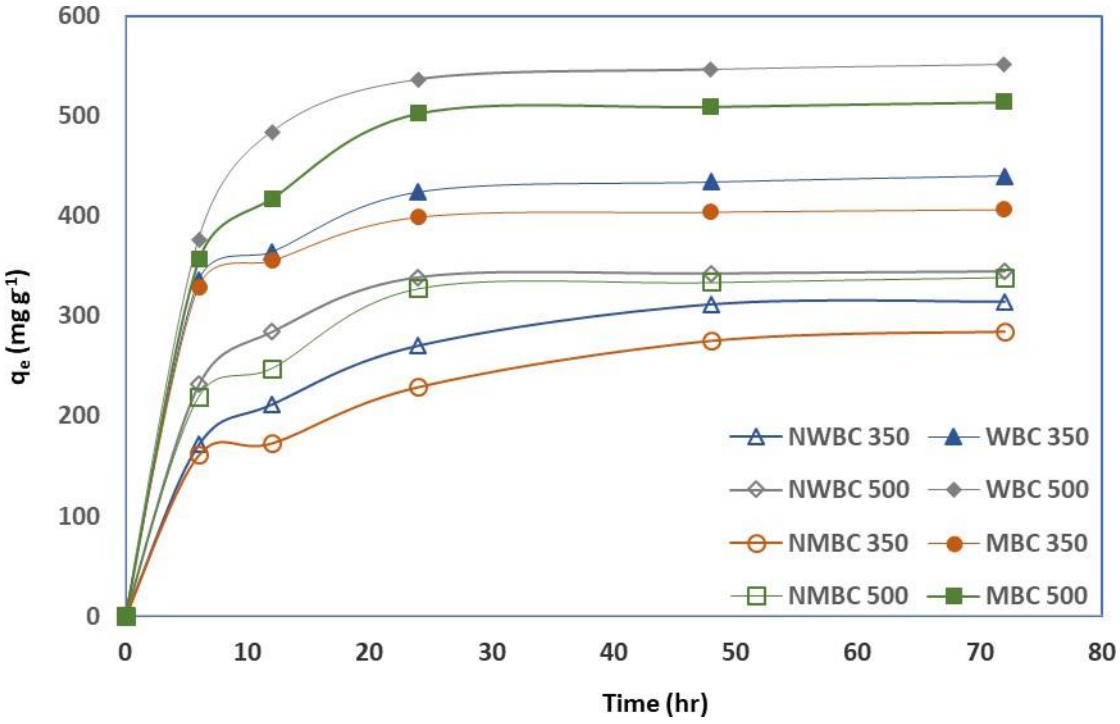


Figure 4.27: Pseudo-first order plot for the adsorption of SMX

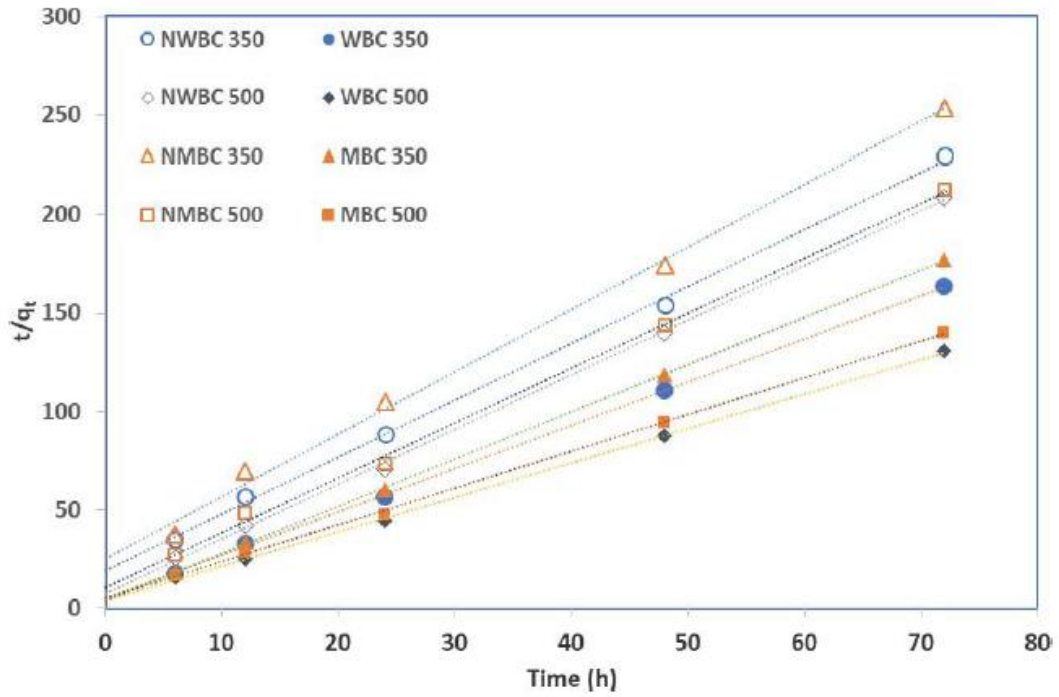


Figure 4.287: Pseudo-second-order plot for SMX adsorption by different biochars

Similar to the aforementioned results of intra-particle kinetic model of CIP and PEG, SMX data followed the same trend exhibiting three multi-linear plots (Figure 4.29),

where the lines do not pass through the origin (0, 0) (Wang and Guo, 2022). Therefore, the preceding explanations given for CIP and PEG hold for SMX too.

The rate constant for the first stage, K_{id1}, values ranged from 0.0125 to 0.0249, whereas C_1 ranged from 0.1068 to 0.3689. There was a weak and negative correlation between K_{id1} to and C_1 (r = -0.137). No specific correlation could be established between individual K_{id1} and C_1 for individual treatments. For example, WBC 500 and MBC 500 had C_1 values of 0.3689 and 0.3294, and K_{id1} values of 0.0248 and 0.0249, respectively. From this observation, pore diffusion was not the sole rate-controlling step (plot deviated from the origin) and boundary layer thickness (C_1) effects had no significant influence on differences in initial mass transfer among the adsorbents (Li *et al*., 2020).

The relationship between K_{id2} and C_2 for SMX adsorption by the 8 biochars was also weak (similar to that of first stage) correlation (r = 0.265). The range of K_{id2} values was 0.022 - 0.0445. The values of K_{id2} were generally higher than the corresponding K_{id1} values for each treatment. The second stage showing gradual adsorption is normally attributed to macropore and mesopore diffusion. The third stage of intra-particle model gave K_{id3} values of 0.0016-0.0158. Adsorption in this stage is attributed to micropore diffusion (Ferro *et al*., 2008; Li *et al*., 2020). Unlike CIP and PEG, there was a moderately strong but negative relationship between K_{id3} and C_3 in the 8 biochars weak correlation (r = -0.765) for SMX. However, for individual treatments, no correlation could be established relationship between K_{id3} and C_3. The relationship was similar to that noted for K_{id1}/ K_{id2} and C_1/ C_2 and $K_{id3} << K_{id1} < K_{id2}$; third stage was considered as the rate limiting step for sorption of SMX to the adsorbents.

Similar to PEG, the high K_{id2} followed by K_{id1} values relative to K_{id3} indicate that fast adsorption occurred at the initial stages (second and first stages), with the 3rd stage being very slow; hence supporting results of the PSO model fitting, which showed that adsorption was mainly influenced by the initial adsorption (h), with the adsorption rate (K_2) being very slow.

Table 4.14: Model parameters for the Intra-particle kinetic model for SMX

	NWBC 350	WBC 350	NWBC 500	WBC 500	NMBC 350	MBC 350	NMBC 500	MBC 500
K_{id1}	0.0242	0.0174	0.0173	0.0248	0.0224	0.0125	0.0202	0.0249
C_1	0.1285	0.3081	0.2175	0.3689	0.1068	0.3128	0.1873	0.3294
R_1^2	1	1	1	1	1	1	1	1
K_{id2}	0.033	0.0292	0.0312	0.0419	0.0273	0.022	0.0362	0.0445
C_2	0.1045	0.2751	0.1828	0.3337	0.0895	0.287	0.1411	0.2777
R_2^2	0.9772	0.929	0.9808	0.9928	0.9285	0.9431	0.8943	0.9573
K_{id3}	0.0126	0.0046	0.0016	0.0042	0.0158	0.0021	0.0031	0.0031
C_3	0.213	0.4017	0.3309	0.516	0.1556	0.3886	0.3121	0.4872
R_3^2	0.8506	0.9968	0.9923	0.987	0.9167	0.987	0.9999	0.9991

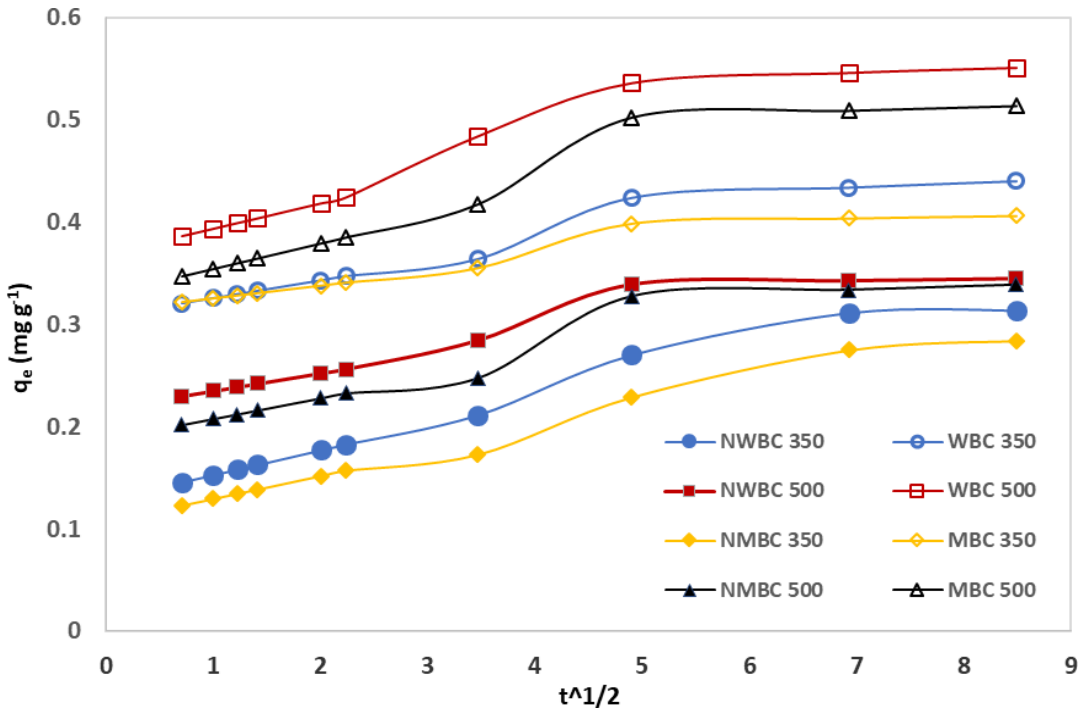


Figure 4.29: Intra-particle diffusion model for the sorption of Sulfamethoxazole

4.3.5 Modelling of sorption capacity of the antibiotics using adsorption isotherms

4.3.5.1 Adsorption isotherms of Ciproloxacin

The adsorption process of ciprofloxacin by the biochars at different initial concentrations was evaluated using three commonly used sorption isotherm models. The models fitting parameters including linear regression coefficients (R^2), and other constants of the sorption isotherm models are given in Table 4: 6 and the corresponding linear plots in Figure 4.30 for the Freundlich isotherm. The sorption process could be described by both Freundlich and Langmuir isotherm models. The Freundlich model gave the best fit with all $R^2 > 0.97$, and that of Langmuir isotherm had $R^2 > 0.90$. Specifically, for Langmuir isotherm, six biochars had $R^2 > 0.96$, whereas WBC500 and MBC 500 had R^2 of 0.90 and 0.91, respectively.

From the Freundlich isotherm, it can be concluded that sorption of CIP was through the multilayer and heterogenous surfaces of the biochars (Jie *et al*., 2020). High K_f values (>700) were obtained for biochars prepared at 500 ^oC where among these, the activated biochars showed the highest K_f values (Table 4:15). This confirmed the increased surface functionalities and porosity for biochar prepared at higher temperatures and further enhancement of sorption through activation of biochar. Further, the 1/n values were >0.1 indicating increased heterogeneity and strong adsorption. Adsorption is considered satisfactory when the Freundlich constant n takes values within the range 1– 10. According to Table 4:16, CIP adsorption is thus satisfactory for the 8 biochars, given that the range for n values is 1.50 to 2.95. The n value is also taken to be an indicator of mesopore volumes (Fierro *et al*., 2008), which are responsible for strong sorption: the higher the n value, the higher the mesopore volumes. Increase of temperature from 350 ^oC to 500 ^oC increased K_f and n (decreases 1/n) for all the 4 biochars. Activation also increased K_f and n or all the

4 biochars. This means that preparation of biochar at higher pyrolysis temperatures improves the sorption capacity of the adsorbents by increasing the mesopore volumes, while activation etches the biochar surface thereby broadening it into an array of mesopores or macropores - from the initial micropore structures - resulting in a heterogenous surface that increases the available active adsorption sites (Zhang *et al*., 2020; Zhong *et al*., 2021). The Freundlich adsorption isotherm assumes that adsorption occurs on a heterogeneous surface through a multilayer adsorption mechanism, and that the adsorbed amount increases with concentration of the adsorbate (Fierro *et al*., 2008).

The Langmuir isotherm reveals that the sorption was favourable since the separation factor R_L is greater than zero and less than 1 ($0<R_L<1$) (Da¸browski, 2001). Increase of temperature from 350 ^oC to 500 ^oC increased K_L but decreased R_L for all the 4 biochars. Activation had the same effect. The Langmuir adsorption model (Fierro *et al*., 2008) is based on the assumption that a maximum limiting uptake exists, corresponding to a saturated monolayer of adsorbate molecules at the adsorbent surface. This means that preparation of biochar at higher pyrolysis temperatures and activation increased the maximum adsorption limit (K_L) of the adsorbents, while at the same time lowering the separation factor (R_L), hence resulting in improved sorption of CIP.

The R^2 values for the Temkin isotherm were significant except for MBC500 (R^2 = 0.006), but lower than those for the Freundlich and Langmuir isotherm models. The variation of adsorption energy, b_T, was positive for all biochars (with significant R^2) indicating exothermic adsorption reaction. This means that the sorption of CIP to the biochars was spontaneous. Increase of temperature (350 ^oC to 500 ^oC) and

activation increased b_T and K_T in all the biochars, implying increase in the spontaneity of the sorption process.

Table 4: 15: Different Adsorption Isotherms for Ciprofloxacin with Biochar

Biochar	Freundlich Isotherm			Langmuir Isotherm			Temkin Isotherm		
	K_f	1/n	R^2	K_L	R_L	R^2	b_T	K_T	R^2
NWBC350	337.13	0.661	0.9978	0.160	0.9398	0.9918	1559.20	124.40	0.7978
WBC350	502.57	0.579	0.9915	0.455	0.8460	0.9915	3347.95	29238.02	0.6852
NWBC500	712.36	0.514	0.9950	0.667	0.7894	0.9791	5124.70	8978443	0.7564
WBC500	1277.03	0.339	0.9865	15.000	0.1429	0.9024	17617.76	1.9E+24	0.6331
NMBC350	434.56	0.668	0.9960	0.120	0.9542	0.9949	1538.68	119.42	0.8061
MBC350	532.97	0.575	0.9926	0.455	0.8460	0.9627	3255.18	23832.18	0.7309
NMBC500	798.73	0.486	0.9942	1.250	0.6667	0.9608	7580.49	1.741E+10	0.7152
MBC500	1160.38	0.385	0.9653	2.000	0.5556	0.9137	-71230.40	0.841631	0.0060

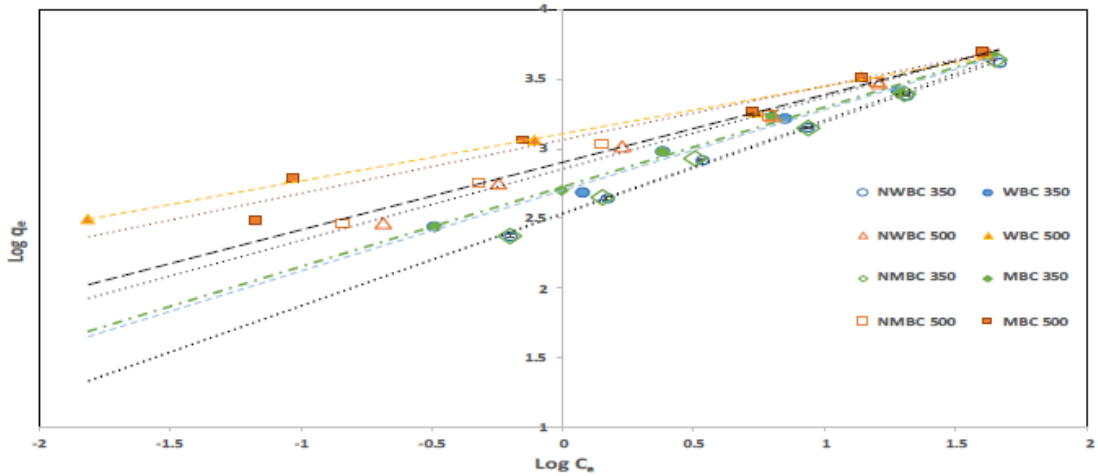


Figure 4:30 Freundlich model fitting for the sorption of CIP by the biochars

4.3.5.2 Adsorption isotherms of Penicillin G

Sorption data of PEG was evaluated using Freundlich, Langmuir and Temkin sorption isotherm models. The models fitting parameters including linear regression coefficients (R^2), and other constants are given in Table 4:16 and the corresponding linear plots in Figure 4.30 for the Freundlich isotherm. The Freundlich model gave the best fit with $R^2 > 0.97$ for seven biochars, while NMBC500 and MBC500 had $R^2 > 0.82$. Langmuir isotherm had $R^2 > 0.93$ for seven biochars, but the model failed for NMBC500 and MBC500 ($R^2 = 0.24$ and 0.02, respectively).

Sorption of PEG occurred through the multilayer and heterogenous surfaces of the biochars (Jie *et al*., 2020), as supported by observed results from Freundlich isotherm. K_f values followed the order NWBC350 < NMBC350 < WBC350 < MBC350 < NWBC500 = WBC500 < NMBC500 < MBC500. High K_f values (> 1000) were obtained for biochars prepared at 500 ^oC where among these, the activated biochars showed the highest Kf values (Table 4:16). Observed Kf values therefore confirmed the increased porosity and surface functionalities for biochar prepared at higher temperatures and further enhancement of sorption through activation of biochar. All the 1/n values were >0.1 and ranged from 0.52 – 0.75, indicating increased heterogeneity and strong adsorption. The n value that is also taken to be an indicator of mesopore volumes (Fierro *et al*., 2008), confirmed high mesopore volumes, hence strong adsorption of PEG by the biochars. Increase of temperature from 350 ^oC to 500 ^oC increased K_f and n (decreases 1/n) for all biochars except WBC350. Similarly, activation also increased K_f and n for all biochars except NWBC500. Therefore, the preparation of biochar at higher pyrolysis temperatures improved the sorption capacity of the adsorbents (except for activated water hyacinth biochar prepared at 350 ^oC) by increasing the mesopore volumes (Zhang *et al*., 2020; Zhong *et al*., 2021).

The Langmuir isotherm indicated that the sorption was favourable for all biochars whose R^2 values were > 0.93 as the separation factor R_L was greater than zero and less than 1 ($0<R_L<1$) (Da¸browski, 2001). It should be noted that Langmuir isotherm was not consistent in observed trends for the changes in K_L and R_L upon increase of pyrolysis temperature and activation of biochars. As earlier noted, the model failed for NMBC500 and MBC 500.

The R^2 values for the Temkin isotherm were significant except for MMBC500 (R^2 = 0.3211) and MBC500 (R^2 = 0.1046), but lower than those for the Freundlich and Langmuir isotherm models. The variation of adsorption energy, b_T, was positive for all biochars (with significant R^2) indicating exothermic adsorption reaction, which indicated that the sorption of PEG to these particular biochars was spontaneous.

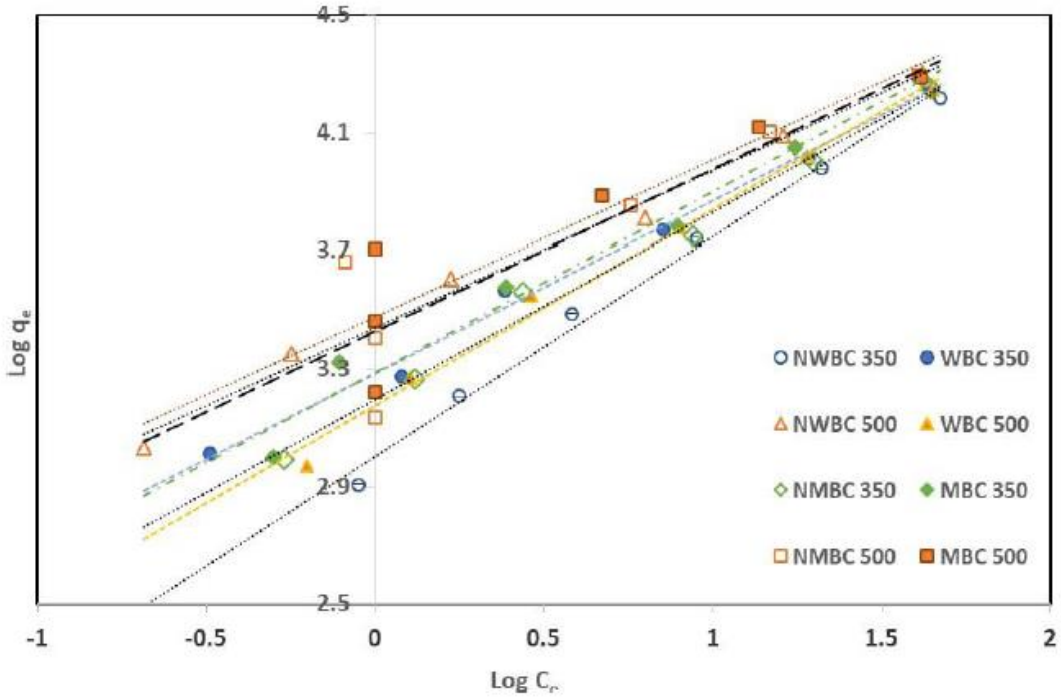


Figure 4 31: Freundlich model fitting for the sorption of PEG by the biochars

Table 4 16: Adsorption Isotherms parameters of Penicillin G with different biochars

Biochar	Freundlich Isotherm			Langmuir Isotherm			Temkin Isotherm		
	K_f	1/n	R^2	K_L	R_L	R^2	b_T	K_T	R^2
NWBC350	1003.0	0.7450	0.9927	0.0456	0.9910	0.9991	8260.0	694663.6	0.9899
WBC350	1922.2	0.5857	0.9929	0.2650	0.9497	0.9734	2307.8	7.3897	0.7506
NWBC500	2761.2	0.5298	0.9917	0.2702	0.9487	0.9333	3082.7	19.2492	0.8439
WBC500	1495.9	0.6643	0.9917	0.0978	0.9808	0.9979	973.3	2.6278	0.8501
NMBC350	1568.9	0.635	0.9904	0.1775	0.9657	0.9884	1850.3	5.077614	0.7888
MBC350	1941.3	0.6109	0.9764	0.1463	0.9716	0.9567	2013.8	6.697256	0.8541
NMBC500	2674.9	0.5475	0.8213	-10.0580	-0.9885	0.2442	-27002.4	4.45E-10	0.3211
MBC500	3000.5	0.5306	0.8458	-16.4122	-0.4381	0.0236	-20202.3	1.11E-07	0.1046

4.3.5.3 Adsorption isotherms for Sulfamethoxazole

The adsorption data for SMX was fitted to three sorption isotherm models, whose fitting parameters including R^2, and other constants are given in Table 4:17 and the corresponding linear plots in Figure 4.*32* for the Freundlich isotherm. The sorption process could be described by both Freundlich and Langmuir isotherm models. The Freundlich model gave the best fit with all $R^2 > 0.99$ for six biochars and $R^2 > 0.82$ for NMBC500 and MBC500. For Langmuir isotherm model $R^2 > 0.95$ for six biochars, except NMBC500 and MBC500 whose data could not fit this model. In general, sorption data could be explained using both Freundlich and Langmuir isotherm models.

Sorption was therefore through the multilayer and heterogenous surfaces of the biochars (Jie *et al*., 2020), as supported by R^2 values of the Freundlich model and the high K_f values (> 950) (Table 4.17). Activation increased K_f values for WBC350 and MBC350, but resulted to a decrease in K_f for the other biochars. 1/n values were >0.1 indicating increased heterogeneity and strong adsorption. Therefore, adsorption of SMX was satisfactory for the 8 biochars, given that the range for n values is 1.34 to 2.39, and considering that n value is taken to be an indicator of mesopore volumes (Fierro *et al*., 2008), which are responsible for strong sorption (the higher the n value, the higher the mesopore volumes). Increase of temperature from 350 ^oC to 500 ^oC increased K_f and n (decreased 1/n) for 3 biochars (decreased for WBC350). Therefore, preparation of biochar at higher pyrolysis temperatures improved the sorption capacity of the three adsorbents by increasing the mesopore volumes. Also, activation modifies the biochar surface thereby widening it into an array of mesopores or macropores - from the initial micropore structures - resulting in a heterogenous surface that increases the available active adsorption sites (Zhang *et al*., 2020;

Zhong *et al*., 2021). This explains the observed sorption characteristics of the biochars that occurred on a heterogeneous surface through a multilayer adsorption mechanism.

Similar to Freundlich isotherm model, the Langmuir isotherm revealed that the sorption was favourable since the separation factor R_L was greater than zero and less than 1 ($0<R_L<1$) for six biochars (Da¸browski, 2001). The R^2 values for the Temkin isotherm were significant except for MBC500 (R^2 = 0.3842) and MBC500 (R^2 = 0.2353). However, they were lower than those for the Freundlich and Langmuir isotherm models. The variation of adsorption energy, b_T, was positive for all biochars (with significant R^2) indicating exothermic adsorption reaction, indicating that the sorption of SMX to these biochars was spontaneous.

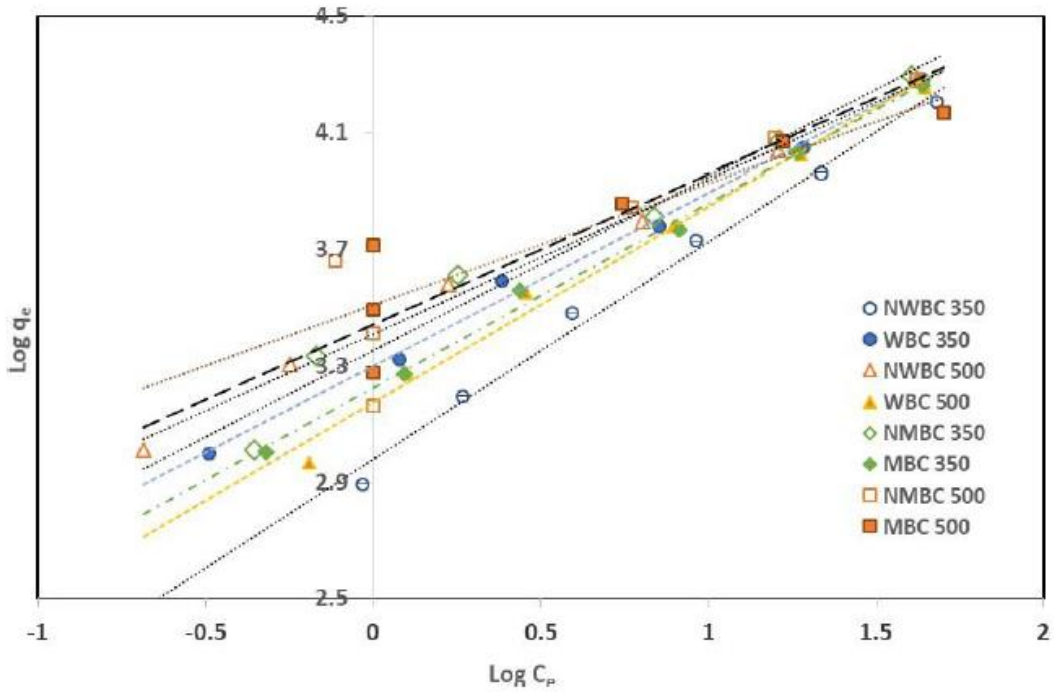


Figure 4.32: Freundlich model fitting for the sorption of SMX by the biochars

Table 4.17: Adsorption isotherms parameters of Sulfamethoxazole with different

Biochar	Freundlich Isotherm			Langmuir Isotherm			Temkin Isotherm		
	K_f	1/n	R^2	K_L	R_L	R^2	b_T	K_T	R^2
NWBC350	953.0	0.7487	0.9919	0.0410	0.9919	0.9990	7950.0	709117.7	0.9871
WBC350	1997.6	0.5949	0.9948	0.1277	0.9751	0.9584	1988.3	6.6911	0.8715
NWBC500	2580.5	0.5295	0.9944	0.1960	0.9623	0.9961	2239.2	7.8084	0.8196
WBC500	1489.4	0.6746	0.9886	0.0845	0.9834	0.9968	969.7	2.6382	0.8512
NMBC350	2266.2	0.5952	0.9667	0.1286	0.9749	0.9531	2212.6	8.8026	0.8876
MBC350	1677.3	0.6352	0.9953	0.2151	0.9587	0.9834	2073.5	6.2315	0.7703
NMBC500				-3.0214			-9611.6		
	2775.2	0.5171	0.8181		2.5271	0.3941		0.0005	0.3842
MBC500				-2.6266			-5827.0		
	3230.0	0.418	0.8196		2.1067	0.1237		0.0089	0.2353

4.4 Possible interaction mechanisms of biochar with antibiotics

The possible interaction mechanisms of biochar with the antibiotics during the adsorption process were evaluated using CIP as the representative compound.

For all the biochars, there appears to be more aggregation of particles after adsorption (Figures 4.32). This indicates that other than getting sorbed onto the biochar, CIP could have resulted in binding of particles through complexation rections. The presence of functional groups in biochars, including C=O, N-H, C-N and hydroxyl groups (Figure 4.21 to Figure 4.23), played a significant role in the sorption of CIP. CIP is a fluoroquinolone antibiotic compound containing multiple functional groups, including carboxylic acid (C=O), amino (N-H), and aromatic (C-N) moieties (Fig 1). One potential sorption mechanism is through electrostatic interactions between the positively charged amino groups of CIP and negatively charged functional groups, such as carboxylate groups present on the biochar surface (Lili *et al*., 2019), and these interactions are multidimensional (Jia at al., 2013). These interactions can involve ion exchange processes, where the negatively charged functional groups of biochar attract and retain the positively charged CIP molecules (Jie *et al*., 2020).

In addition, hydrogen bonding can occur between hydroxyl groups present on biochar

surface and CIP functional groups such as carboxylic acids and amino groups (Li *et al*., 2020). Hydrogen bonding can enhance CIP sorption and stability on biochar surface. Furthermore, π-π interactions may occur between CIP aromatic rings and biochar aromatic functional groups such as the Ar-C bonds mentioned in the FT-IR spectra (Mesallati *et al*., 2016). These interactions can contribute to adsorption of CIP through π-stacking, where the aromatic rings of CIP align and interact with the aromatic structures of biochar (Peng *et al*., 2016). Furthermore, the introduction of hydroxyl groups through biochar activation can further enhance CIP sorption. This is because hydroxyl groups provide additional active sites for hydrogen bonding and electrostatic interactions with CIP, thus increasing the overall sorption capacity and affinity of the activated biochar for CIP molecules (Li *et al*., 2020). Similar interaction mechanisms can be inferred for PEG and SMX due to the presence of the determined functional groups in the biochar materials (before adsorption) and the chemical structures of the two antibiotics.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

River Sosiani had twenty-eight detectable antibiotics, twenty-two of which had a 100% detection frequency and the remaining 4 showed detection frequencies ranging from 5 to 47%. Detectable concentrations of the pharmaceuticals in water ranged between 0.1 and 247 ng L^{-1} and 0.01 and 974 µg kg^{-1} in the sediments. The sulfonamide, sulfamethoxazole, had the highest concentration in water (247 ng L^{-1}), whereas penicillin G showed the highest concentrations in sediments (414 – 974 µg kg^{-1}).

Risk quotients (RQ_w) calculated indicated that sulfamethoxazole and ciprofloxacin were of high ecological risk in surface water (RQw values of 1.11 and 3.24, respectively), whereas penicillin V, ampicillin, penicillin G, norfloxacin, enrofloxacin, erythromycin, tylosin, and lincomycin were of medium ecological risk in surface water of River Sosiani.

The percentage yield of water hyacinth biochar (WBC) and millet husk biochar (MBC) biochars prepared at 350°C and 500 °C were WBC350 (48.78%) > WBC500 (42.28%) > MBC350 (38.25%) > MBC500 (36.13%). Generally, modified biochar had higher adsorption capacities compared to unmodified biochar.

Biochar derived from water hyacinth and millet husks can be used as adsorbents for ciprofloxacin, penicillin G and sulfamethoxazole removal in contaminated water. Sorption kinetics for the three tested antibiotics (Ciprofloxacin, Penicillin G and Sulfamethoxazole) was best described by pseudo-second-order kinetics (R^2 > 0.99, and therefore the adsorption was a chemical adsorption process. A three multi-layer

diffusion characteristics of the antibiotics was displayed, consistent with the intra-particle diffusion kinetic model.

Pore diffusion was not the sole rate-controlling step and boundary layer thickness effects had no significant influence on differences in initial mass transfer among the adsorbents. Diffusion was attributed to macropore, mesopore and micropore diffusion in the microporous structures of the biochars. The sorption process could be best described by the Freundlich isotherm ($R^2 > 0.97$) for all biochars, hence occurring through the multilayer and heterogenous surfaces of the biochars.

The FTIR results showed that the major effect of sorption was shifting and enhancement of existing peaks. Though direct complexation of antibiotics with biochar surfaces may not have occurred, SEM micrographs indicated aggregation of particles after adsorption, which indicated that the compounds could have resulted in the binding of particles through complexation reactions. The interaction mechanisms of biochar with the antibiotics were electrostatic interactions between oppositely charged functional groups in biochar and the compounds, π-π interactions between aromatic rings of the compounds and biochar aromatic functional groups, and hydrogen bonding. From the observations of this study, the SEM micrographs showed that biochar derived from water hyacinth had large pores on the surfaces, and modification with KOH caused the development of more porosity, which resulted in better adsorption of contaminants.

5.2 Recommendations

From this study, the following are recommended:

i. Further studies should investigate the efficacy of the biochars in the removal of the other antibiotics identified in River Sosiani water and sediment samples when compared with commercial adsorbents such as activated carbon.

ii. More studies on monitoring and assessing the risks of antibiotics in water and sediments of the Sosiani River should be conducted to guarantee its safety for different uses.

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APPENDICES

Appendix I: Effect of contact time on adsorption of Ciprofloxacin, Penicillin G and Sulfamethoxazole by water hyacinth and millet husk biochars

1. CIPROFLOXACIN

Table 1. Parameters of the effect of contact time on adsorption of ciprofloxacin by water hyacinth and millet husk biochars

Time (hours)	**C_i**	**NWBC 350 (C_e)**	**WBC 350 (C_e)**	**NWBC 500 (C_e)**	**WBC 500 (C_e)**	**NMBC 350 (C_e)**	**MBC 350 (C_e)**	**NMBC 500 (C_e)**	**MBC 500 (C_e)**
0	5	5	5	5	5	5	5	5	5
6	5	3.31	3.22	2.36	2.04	3.18	3.14	2.32	1.95
12	5	3.07	3.04	2.14	1.56	3.03	2.46	2.09	1.04
24	5	2.54	2.37	1.82	0.93	2.52	2.32	1.58	0.64
48	5	2.53	2.35	1.8	0.89	2.51	2.33	1.56	0.61
72	5	2.51	2.33	1.76	0.86	2.49	2.31	1.52	0.58

C_i is the initial concentration and C_e the final concentration of CIP in the solution

Table 2. Effect of contact time on percentage removal of ciprofloxacin by water hyacinth and millet husk biochars

Time (hours)	**NWBC 350**	**WBC 350**	**NWBC 500**	**WBC 500**	**NMBC 350**	**MBC 350**	**NMBC 500**	**MBC 500**
0	0	0	0	0	0	0	0	0
6	33.8	35.6	52.8	59.2	36.4	37.2	53.6	61
12	38.6	39.2	57.2	68.8	39.4	50.8	58.2	79.2
24	49.2	52.6	63.6	81.4	49.6	53.6	68.4	87.2
48	49.4	53.0	64	82.2	49.8	53.4	68.8	87.8
72	**49.8**	**53.4**	**64.8**	**82.8**	**50.2**	**53.8**	**69.6**	**88.4**

2. PENICILLIN G

Table 3. Parameters of the effect of contact time on adsorption of Penicillin G by water hyacinth and millet husk biochars

Time (hours)	**C_i**	**NWBC 350 (C_e)**	**WBC 350 (C_e)**	**NWBC 500 (C_e)**	**WBC 500 (C_e)**	**NMBC 350 (C_e)**	**MBC 350 (C_e)**	**NMBC 500 (C_e)**	**MBC 500 (C_e)**
0	5	4.99	4.97	4.96	4.95	4.99	4.96	4.96	4.94
6	5	3.50	3.24	2.37	2.16	3.56	3.14	2.31	1.99
12	5	3.33	3.01	2.16	1.65	3.27	2.71	2.08	1.13
24	5	2.59	2.39	1.80	0.97	2.54	2.28	1.60	0.71
48	5	2.55	2.34	1.76	0.95	2.49	2.30	1.52	0.64
72	5	2.53	2.31	1.74	0.88	2.51	2.41	1.49	0.60

Table 4. Effect of contact time on percentage removal of penicillin G by water hyacinth and millet husk biochars

Time (hours)	**NWBC 350**	**WBC 350**	**NWBC 500**	**WBC 500**	**NMBC 350**	**MBC 350**	**NMBC 500**	**MBC 500**
0	0.2	0.6	0.8	1.0	0.2	0.8	0.8	1.2
6	30.0	35.2	52.6	56.8	28.8	37.2	53.8	60.2
12	33.4	39.8	56.8	67.0	34.6	45.8	58.4	77.4
24	48.2	52.2	64.0	80.6	49.2	**54.4**	68.0	85.8
48	49.0	53.2	64.8	81.0	**50.2**	54.0	69.6	87.2
72	**49.4**	**53.8**	**65.2**	**82.4**	49.8	51.8	**70.2**	**88.0**

2. SULFAMETHOXAZOLE

Table 5. Parameters of the effect of contact time on adsorption of sulfamethoxazole by water hyacinth and millet husk biochars

Time (hours)	**C_i**	**NWBC 350 (C_e)**	**WBC 350 (C_e)**	**NWBC 500 (C_e)**	**WBC 500 (C_e)**	**NMBC 350 (C_e)**	**MBC 350 (C_e)**	**NMBC 500 (C_e)**	**MBC 500 (C_e)**
0	5	4.99	4.96	4.96	4.94	4.99	4.96	4.97	4.95
6	5	3.63	2.32	3.15	1.99	3.71	2.37	3.25	2.15
12	5	3.31	2.09	2.73	1.13	3.62	2.16	3.02	1.66
24	5	2.84	1.61	2.29	0.71	3.17	1.81	2.38	0.98
48	5	2.51	1.53	2.26	0.63	2.80	1.77	2.33	0.93
72	5	2.49	1.48	2.24	0.59	2.73	1.75	2.29	0.89

Table 6. Effect of contact time on the percentage removal of sulfamethoxazole by water hyacinth and millet husk biochars

Time (hours)	**NWBC 350**	**WBC 350**	**NWBC 500**	**WBC 500**	**NMBC 350**	**MBC 350**	**NMBC 500**	**MBC 500**
0	0.2	0.8	0.8	1.2	0.2	0.8	0.6	1.0
6	27.4	53.6	37.0	60.2	25.8	52.6	35.0	57.0
12	33.8	58.2	45.4	77.4	27.6	56.8	39.6	66.8
24	43.2	67.8	54.2	85.8	36.6	63.8	52.4	80.4
48	49.8	69.4	54.8	87.4	44.0	64.6	53.4	81.4
72	**50.2**	**70.4**	**55.2**	**88.2**	**45.4**	**65.0**	**54.2**	**82.2**

Appendix II: Effect of solution pH on adsorption of Ciprofloxacin, Penicillin G and Sulfamethoxazole by water hyacinth and millet husk biochars

1. CIPROFLOXACIN

Table 7. Parameters of the effect of solution pH on the adsorption of ciprofloxacin by water hyacinth and millet husk biochars

pH	C_i	NWBC 350 (C_e)	WBC 350 (C_e)	NWBC 500 (C_e)	WBC 500 (C_e)	NMBC 350 (C_e)	MBC 350 (C_e)	NMBC 500 (C_e)	MBC 500 (C_e)
2	5	3.39	3.35	2.98	2.16	3.36	3.32	2.95	1.99
4	5	3.37	3.34	2.83	1.23	3.33	2.94	2.81	1.64
6	5	2.56	2.42	2.11	0.85	2.45	1.96	2.04	0.81
8	5	2.32	2.18	1.91	1.09	1.96	2.09	1.83	0.84
10	5	2.46	2.43	2.15	1.17	2.37	2.11	1.95	0.87
12	5	2.51	2.5	2.26	1.24	2.41	2.46	1.97	0.92

Table 8. Effect of solution pH on the percentage removal of ciprofloxacin by water hyacinth and millet husk biochars

pH	NWBC 350	WBC 350	NWBC 500	WBC 500	NMBC 350	MBC 350	NMBC 500	MBC 500
2	32.2	33	40.4	56.8	32.8	33.6	41.0	60.2
4	32.6	33.2	43.4	75.4	33.4	41.2	43.8	67.2
6	48.8	51.6	57.8	**83.0**	51.0	**60.8**	59.2	**83.8**
8	**53.6**	**56.4**	**61.8**	78.2	**60.8**	58.2	**63.4**	83.2
10	50.8	51.4	57.0	76.6	52.6	57.8	61.0	82.6
12	49.8	50	54.8	75.2	51.8	50.8	60.6	81.6

2. PENICILLIN G

Table 9. Parameters of the effect of solution pH on the adsorption of Penicillin G by water hyacinth and millet husk biochars

Time (hours)	C_i	NWBC 350 (C_e)	WBC 350 (C_e)	NWBC 500 (C_e)	WBC 500 (C_e)	NMBC 350 (C_e)	MBC 350 (C_e)	NMBC 500 (C_e)	MBC 500 (C_e)
0	5	4.99	4.97	4.96	4.95	4.99	4.96	4.96	4.94
6	5	3.50	3.24	2.37	2.16	3.56	3.14	2.31	1.99
12	5	3.33	3.01	2.16	1.65	3.27	2.71	2.08	1.13
24	5	2.59	2.39	1.80	0.97	2.54	2.28	1.60	0.71
48	5	2.55	2.34	1.76	0.95	2.49	2.30	1.52	0.64
72	5	2.53	2.31	1.74	0.88	2.51	2.41	1.49	0.60

Table 10. Effect of solution pH on the percentage removal of Penicillin G by water hyacinth and millet husk biochars

pH	NWBC 350	WBC 350	NWBC 500	WBC 500	NMBC 350	MBC 350	NMBC 500	MBC 500
2	32.2	33	40.4	56.8	32.8	33.6	41.0	60.2
4	32.6	33.2	43.4	75.4	33.4	41.2	43.8	67.2
6	48.8	51.6	57.8	**83.0**	51.0	60.8	59.2	**83.8**
8	**53.6**	**56.4**	**61.8**	78.2	**60.8**	**58.2**	**63.4**	83.2
10	50.8	51.4	57	76.6	52.6	57.8	61	82.6
12	49.8	50	54.8	75.2	51.8	50.8	60.6	81.6

3. SULFAMETHOXAZOLE

Table 11. Parameters of the effect of solution pH on the adsorption of sulfamethoxazole by water hyacinth and millet husk biochars

pH	C_i	NWBC 350 (C_e)	WBC 350 (C_e)	NWBC 500 (C_e)	WBC 500 (C_e)	NMBC 350 (C_e)	MBC 350 (C_e)	NMBC 500 (C_e)	MBC 500 (C_e)
2	5	3.33	2.48	3.18	1.96	3.23	2.35	3.13	1.91
4	5	3.28	2.24	2.84	1.55	3.08	2.13	2.72	1.48
6	5	2.36	1.63	1.84	0.86	2.19	1.52	1.75	0.74
8	5	1.98	1.37	1.65	0.92	1.79	1.21	1.56	0.84
10	5	2.27	1.49	2.02	0.98	2.12	1.31	1.90	0.88
12	5	2.39	1.55	2.23	1.16	2.26	1.46	2.18	1.06

Table 12. Effect of solution pH on the percentage removal of sulfamethoxazole by water hyacinth and millet husk biochars

pH	NWBC 350	WBC 350	NWBC 500	WBC 500	NMBC 350	MBC 350	NMBC 500	MBC 500
2	33.4	50.4	36.4	60.8	35.4	53.0	37.4	61.8
4	34.4	55.2	43.2	69.0	38.4	57.4	45.6	70.4
6	52.8	67.4	63.2	**82.8**	56.2	69.6	65.0	**85.2**
8	**60.4**	**72.6**	**67.0**	81.6	**64.2**	**75.8**	**68.8**	83.2
10	54.6	70.2	59.6	80.4	57.6	73.8	62.0	82.4
12	52.2	69.0	55.4	76.8	54.8	70.8	56.4	78.8

Appendix III: Effect of adsorbent dosage on adsorption of Ciprofloxacin, Penicillin G and Sulfamethoxazole by water hyacinth and millet husk biochars

1. CIPROFLOXACIN

Table 13. Parameters of the effect of adsorbent dosage on the adsorption of Ciprofloxacin by water hyacinth and millet husk biochars

Dosage	C_i	NWBC 350 (C_e)	WBC 350 (C_e)	NWBC 500 (C_e)	WBC 500 (C_e)	NMBC 350 (C_e)	MBC 350 (C_e)	NMBC 500 (C_e)	MBC 500 (C_e)
0.1	5	3.03	2.95	2.86	2.51	3.01	2.91	2.83	2.48
0.2	5	2.87	2.75	2.21	2.1	2.81	2.72	2.17	1.97
0.3	5	2.61	2.43	1.94	1.64	2.56	2.39	1.89	1.67
0.4	5	2.17	1.69	1.58	0.71	2.17	1.71	1.51	0.68
0.5	5	2.15	1.67	1.47	0.91	2.19	1.58	1.45	0.86
0.6	5	2.14	1.63	1.45	0.93	2.16	1.59	1.49	0.84

Table 14. Effect of adsorbent dosage on the percentage removal of Ciprofloxacin by water hyacinth and millet husk biochars

Dosage	NWBC 350	WBC 350	NWBC 500	WBC 500	NMBC 350	MBC 350	NMBC 500	MBC 500
0.1	39.4	41	42.8	49.8	39.8	41.8	43.4	50.4
0.2	42.6	45	55.8	58	43.8	45.6	56.6	60.6
0.3	47.8	51.4	61.2	67.2	48.8	52.2	62.2	66.6
0.4	56.6	66.2	68.4	**85.8**	56.6	65.8	69.8	**86.4**
0.5	57.0	66.6	70.6	81.8	56.2	**68.4**	**71.0**	82.8
0.6	**57.2**	**67.4**	**71.0**	81.4	56.8	68.2	70.2	83.2

2. PENICILIIN G

Table 15. Parameters of the effect of adsorbent dosage on the adsorption of Penicillin G by water hyacinth and millet husk biochars

pH	C_i	NWBC 350 (C_e)	WBC 350 (C_e)	NWBC 500 (C_e)	WBC 500 (C_e)	NMBC 350 (C_e)	MBC 350 (C_e)	NMBC 500 (C_e)	MBC 500 (C_e)
2	5	3.24	3.13	2.36	1.93	3.28	3.18	2.47	1.97
4	5	3.07	2.71	2.15	1.51	3.11	2.84	2.22	1.54
6	5	2.17	1.73	1.51	0.76	2.24	1.82	1.64	0.87
8	5	1.75	1.54	1.19	0.85	1.93	1.68	1.36	0.91
10	5	2.12	1.89	1.28	0.90	2.16	2.01	1.48	0.97
12	5	2.26	2.16	1.44	1.07	2.35	2.21	1.53	1.13

Table 16. Effect of adsorbent dosage on the percentage removal of Penicillin G by water hyacinth and millet husk biochars

Adsorbent dosage	**NWBC 350**	**WBC 350**	**NWBC 500**	**WBC 500**	**NMBC 350**	**MBC 350**	**NMBC 500**	**MBC 500**
0.1	44.4	50.8	52.8	56.8	43.4	49.8	52.0	55.4
0.2	53.2	59.6	60.6	65.0	52.0	58.0	57.2	62.8
0.3	63.0	66.2	69.2	75.6	61.6	65.2	67.4	71.2
0.4	72.6	76.8	79.8	86.2	70.6	75.8	**78.2**	**85.0**
0.5	73.6	77.0	80.2	87.0	71.2	76.6	76.2	84.0
0.6	**74.2**	**77.2**	**80.8**	**87.6**	**73.4**	**77.8**	75.0	83.8

3. SULFAMETHOXAZOLE

Table 17. Parameters of the effect of adsorbent dosage on the adsorption of Sulfamethoxazole by water hyacinth and millet husk biochars

Absorbent Dosage	**C_i**	**NWBC 350 (C_e)**	**WBC 350 (C_e)**	**NWBC 500 (C_e)**	**WBC 500 (C_e)**	**NMBC 350 (C_e)**	**MBC 350 (C_e)**	**NMBC 500 (C_e)**	**MBC 500 (C_e)**
0.1	5	2.83	2.40	2.62	2.23	2.78	2.36	2.49	2.16
0.2	5	2.40	2.08	2.18	1.86	2.34	1.97	2.13	1.75
0.3	5	1.92	1.63	1.74	1.35	1.85	1.54	1.69	1.22
0.4	5	1.47	1.09	1.21	0.80	1.37	1.01	1.16	0.69
0.5	5	1.44	1.03	1.14	0.76	1.32	0.99	1.13	0.65
0.6	5	1.38	1.00	1.07	0.72	1.29	0.96	1.11	0.62

Table 18. Effect of adsorbent dosage on the percentage removal of Sulfamethoxazole by water hyacinth and millet husk biochars

Dosage	**NWBC 350**	**WBC 350**	**NWBC 500**	**WBC 500**	**NMBC 350**	**MBC 350**	**NMBC 500**	**MBC 500**
0.1	43.4	52.0	47.6	55.4	44.4	52.8	50.2	56.8
0.2	52.0	58.4	56.4	62.8	53.2	60.6	57.4	65.0
0.3	61.6	67.4	65.2	73.0	63.0	69.2	66.2	75.6
0.4	70.6	78.2	75.8	84.0	72.6	79.8	76.8	86.2
0.5	71.2	79.4	77.2	84.8	73.6	80.2	77.4	87.0
0.6	**72.4**	**80.0**	**78.6**	**85.6**	**74.2**	**80.8**	**77.8**	**87.6**

Appendix IV: Effect of initial concentration on adsorption of Ciprofloxacin, Penicillin G and Sulfamethoxazole by water hyacinth and millet husk biochars

1. CIPROFLOXACIN

Table 19. Parameters of the effect of initial concentration on the adsorption of Ciprofloxacin by water hyacinth and millet husk biochars

Initial Concentration	NWBC 350 (C_e)	WBC 350 (C_e)	NWBC 500 (C_e)	WBC 500 (C_e)	NMBC 350 (C_e)	MBC 350 (C_e)	NMBC 500 (C_e)	MBC 500 (C_e)
2.5	0.623	0.324	0.207	0.0387	0.601	0.299	0.195	0.0209
5.0	1.461	1.201	0.567	0.113	1.397	1.019	0.456	0.109
10.0	3.412	2.423	1.68	0.818	3.278	2.396	1.43	0.72
20.0	8.533	7.121	6.333	5.452	8.713	6.296	6.13	5.302
40.0	20.59	19.21	16.133	13.821	20.183	19.53	15.907	13.857
80.0	46.627	43.341	41.667	39.956	45.176	42.431	40.515	39.854

Table 20. Effect of initial concentration on percentage removal of Ciprofloxacin by water hyacinth and millet husk biochars

Initial Concentration	NWBC 350	WBC 350	NWBC 500	WBC 500	NMBC 350	MBC 350	NMBC 500	MBC 500
2.5	**75.080**	**87.040**	**91.720**	**98.452**	**75.960**	**88.040**	**92.200**	**99.164**
5.0	70.780	75.980	88.660	97.740	72.060	79.620	90.880	97.820
10.0	65.880	75.770	83.200	91.820	67.220	76.040	85.700	92.800
20.0	57.335	64.395	68.335	72.740	56.435	68.520	69.350	73.490
40.0	48.525	51.975	59.668	65.448	49.543	51.175	60.233	65.358
80.0	41.716	45.824	47.916	50.055	43.530	46.961	49.356	50.183

2. PENICILLIN G

Table 21. Parameters of the effect of initial concentration on the adsorption of Penicillin G by water hyacinth and millet husk biochars

Initial Concentration	NWBC 350 (C_e)	WBC 350 (C_e)	NWBC 500 (C_e)	WBC 500 (C_e)	NMBC 350 (C_e)	MBC 350 (C_e)	NMBC 500 (C_e)	MBC 500 (C_e)
2.5	0.892	0.443	0.481	0.0578	0.912	0.536	0.498	0.089
5.0	1.781	1.231	0.676	0.127	1.834	1.313	0.784	0.143
10.0	3.842	2.632	2.416	0.829	3.924	2.744	2.451	0.856
20.0	8.96	8.212	7.788	5.542	9.213	8.715	7.897	5.764
40.0	21.012	19.435	16.311	13.916	21.623	19.878	17.522	14.214
80.0	47.172	43.512	40.463	38.965	47.831	44.353	41.766	39.981

Table 22. Effect of initial concentration on the percentage removal of Penicillin G by water hyacinth and millet husk biochars

Initial Concentration	**NWBC 350**	**WBC 350**	**NWBC 500**	**WBC 500**	**NMBC 350**	**MBC 350**	**NMBC 500**	**MBC 500**
2.5	64.320	**82.280**	80.760	**97.688**	**63.520**	**78.560**	80.080	96.440
5.0	**64.380**	75.380	**86.480**	97.460	63.320	73.740	**84.320**	**97.140**
10.0	61.580	73.680	75.840	91.710	60.760	72.560	75.490	91.440
20.0	55.200	58.940	61.060	72.290	53.935	56.425	60.515	71.180
40.0	47.470	51.413	59.223	65.210	45.943	50.305	56.195	64.465
80.0	41.035	45.610	49.421	51.294	40.211	44.559	47.793	50.024

3. SULFAMETHOXAZOLE

Table 23. Parameters of the effect of initial concentration on the adsorption of sulfamethoxazole by water hyacinth and millet husk biochars

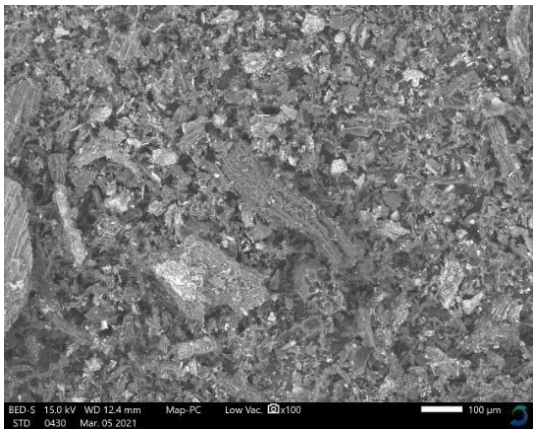
Initial Concentration	**NWBC 350 (C_e)**	**WBC 350 (C_e)**	**NWBC 500 (C_e)**	**WBC 500 (C_e)**	**NMBC 350 (C_e)**	**MBC 350 (C_e)**	**NMBC 500 (C_e)**	**MBC 500 (C_e)**
2.5	0.931	0.498	0.586	0.144	0.891	0.443	0.481	0.0582
5.0	1.854	0.784	1.314	0.209	1.783	0.676	1.233	0.128
10.0	3.924	2.145	3.122	0.854	3.844	1.789	2.732	0.834
20.0	9.213	7.897	8.273	5.674	8.962	6.877	8.216	5.539
40.0	21.623	17.524	19.878	14.322	21.102	16.313	18.354	13.909
80.0	47.831	41.765	44.355	37.819	47.173	40.462	43.521	36.956

Table 24. Effect of initial concentration on the percentage removal of sulfamethoxazole by water hyacinth and millet husk biochars

Initial Concentration	**NWBC 350**	**WBC 350**	**NWBC 500**	**WBC 500**	**NMBC 350**	**MBC 350**	**NMBC 500**	**MBC 500**
2.5	62.760	80.080	76.560	94.240	64.360	82.280	80.760	97.672
5.0	62.920	84.320	73.720	95.820	64.340	86.480	75.340	97.440
10.0	60.760	78.550	68.780	91.460	61.560	82.110	72.680	91.660
20.0	53.935	60.515	58.635	71.630	55.190	65.615	58.920	72.305
40.0	45.943	56.190	50.305	64.195	47.245	59.218	54.115	65.228
80.0	40.211	47.794	44.556	52.726	41.034	49.423	45.599	53.805

Appendix V: Scanning electron micrographs of selected of the water hyacinth and millet husk biochars before and after adsorption

NWB500-AB



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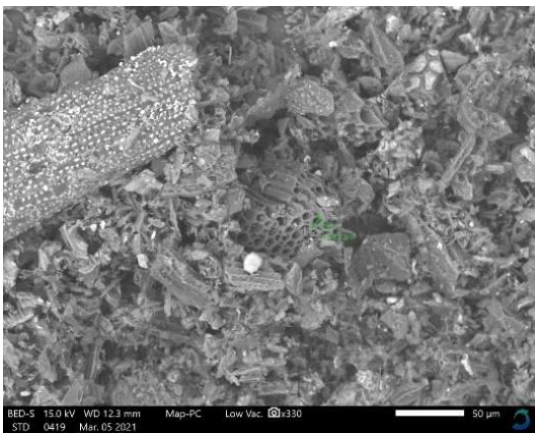
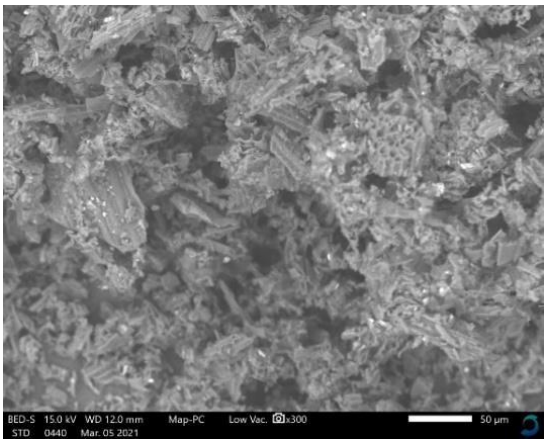


NMB350-AB



MB350-AB

Appendix VI: Published peer-reviewed articles from this thesis

1. **Chemtai, C.**, Kengara, F. O., & Ngigi, A. N. (2023). Levels and ecological risk of pharmaceuticals in River Sosiani, Kenya. Environmental monitoring and assessment, 195(3), 431. https://doi.org/10.1007/s10661-023-11022-1
2. **Chemtai, C**., Kengara, F. O., & Ngigi, A. N. (2023). Ciprofloxacin sorption by non-activated and activated biochar derived from millet husks and water hyacinth. *Sustainable Chemistry for the Environment* (under peer review).

Appendix VII: Plagiarism Awareness Certificate

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PLAGIARISM AWARENESS CERTIFICATE

This certificate is awarded to

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PHD/ACH/07/18

In recognition for passing the University's plagiarism

Awareness test for Thesis entitled: **OCCURRENCE OF ANTIBIOTICS IN SEDIMENTS AND SURFACE WATER OF RIVER SOSIANI, ELDORET, KENYA AND THEIR SORPTIVE REMOVAL USING BIOCHARS** With similarity index of 25% and striving to maintain academic integrity.

Word count: 50055

Awarded by

Prof. Anne Syomwene Kisilu
CERM-ESA Project Leader Date: 08/04/2024