REMOVAL OF SELECTED PHARMACEUTICAL RESIDUES FROM WASTEWATER USING ACTIVATED CARBON FROM RICE HUSKS AND THEIR BIODEGRADATION USING MICROORGANISMS

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Removal of Selected Pharmaceutical Residues from Wastewater using Activated Carbon from Rice Husks and their Biodegradation using Microorganisms

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DECLARATION

This thesis is my original work and has not been presented for the award of degree in any other University.

Signature …… Date……

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DEDICATION

I dedicate this work to my family. My wife Peris, my son Chris and my two daughters Monica and Shiroh. I also dedicate it to my mother Wahu wa Kahara who despite being very old has waited for me to go through the terrain of getting my third degree.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
EPA	Environmental Protection Agency
FDA	Food and Drugs Administration
FID	Flame Ionization Detector
FT-IR	Fourier Transform Infra-Red
GC-MS	Gas Chromatography-Mass Spectroscopy
HPLC	High Performance Liquid Chromatography
JKUAT	Jomo Kenyatta of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
LC	Liquid Chromatography
LC-MS/MS	Liquid Chromatography Tandem Mass Spectroscopy
LOD	Limit of Detection
LOQ	Limit of Quantification
MMSM	Minimum Mineral Salt Medium
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometer
NEV	Nevirapine
PARA	Paracetamol
PPB	Pharmacy and Poisons Board
RP	Reversed Phase
SEM	Scanning Electron Microscopy
SPE	Solid Phase Micro Extraction
TMP	Trimethoprim
WWTP	Waste Water Treatment Plant
XRD	X-ray Diffraction

ABSTRACT

Presence of pharmaceutical residues in wastewater discharges into rivers disrupts aquatic ecosystems and human health. The risk of exposure to these pharmaceutical residues becomes greater since most of them are not degraded during sewage treatment. The aim of this study was to develop a method of removing paracetamol, trimethoprim and nevirapine residues from wastewater using activated carbon made from rice husks and subject them to biodegradation by use of selected microorganisms. Analysis of pharmaceutical residues was done using liquid chromatography~~y~~ tandem mass spectrometry. Powdered rice husks were carbonated at different temperatures in the range of 300℃ - 600 ℃ then activated using phosphoric acid. Biochar carbonated at 500 ℃ was found to have the best adsorption properties. Characterization of the biochar was done using scanning electron microscopy, Fourier transform infrared spectroscopy and X-ray diffraction and their efficacy as adsorbents for the three pharmaceutical drugs evaluated. Adsorption of the three pharmaceutical drugs was found to increase with increase in contact time and adsorbent dosage, decrease with increase in initial drug concentration while pH did not have a significant effect on the adsorption rate. The optimum contact time for the three pharmaceutical drugs was 30 minutes while the optimum adsorbent dosage found to be 0.10 grams. Adsorption isotherms for the three pharmaceutical drugs fitted well in both Langmuir and Freundlich models. The Langmuir isotherms R^2 values were 0.9996, 0.9994 and 0.9831 while the Freundlich isotherm R^2 values were 0.977, 0.994 and 0.977 for paracetamol, trimethoprim and nevirapine, respectively. Kinetic studies were found to fit well in the pseudo-second order model with R^2 values approaching unity. This indicated that chemisorption was favoured over physisorption. Biodegradation of the three pharmaceutical drugs by *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureas* bacteria was also studied. Biodegradation was found to decrease with an increase in concentration from 0.5 ppm to 1.5 ppm for paracetamol and nevirapine but increased with an increase in pharmaceutical concentration for *P. aeroginosa.* Biodegradation by the bacteria was highest for trimethoprim (82%) followed by paracetamol (75%) and nevirapine the least (51%). The study gave a reliable assessment of the risks associated with the pharmaceuticals to living organisms and the remedial measures through possible biodegradation. According to the investigations, chemically activated rice husk biochar was shown to be an effective, low-cost adsorbent for use in removal of pharmaceutical residues from wastewater. Biodegradation of the three pharmaceuticals drugs using the selected microorganisms has been shown to be an effective method for their removal hence it should be tested in the field.

Key words: Pharmaceutical residues; Biodegradation; Wastewater; Activated carbon

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

A pharmaceutical is defined as any substance or a mixture of substances used in the diagnosis, treatment or prevention of a disease, disorder or abnormal physical state, or its symptoms in human beings or animals (Erick and Moore, 2007). A number of studies have detected the presence of pharmaceutical residues as pollutants of emerging concern in aqueous systems (Jones *et al*.,2004). Accumulation of pharmaceutical residues in aquatic systems are a source of concern due to their effects in the environment (Cecilia *et al*., 2020; Kairigo *et al*., 2020; K'oreje *et al*., 2016; Ngumba *et al*., 2016).

The increased presence of pharmaceutical residues in aqueous systems can be attributed to the high consumption of pharmaceutical drugs resulting from the ever-growing global population which leads to increase in volume of pharmaceuticals being consumed combined with their uncontrollable use. In addition, there is competition by pharmaceutical companies to produce new and more efficient products. Pharmaceuticals ingested into the body are excreted largely as unchanged form, conjugated form or metabolites in urine and feces. According to Gauthier *et al.*, (2010), up to 50%, of the original compound may be excreted unchanged.

The parent pharmaceutical compounds when released to the environment undergo different structural changes by a variety of biotic and non-biotic processes resulting in production of different transformation products some of which could be worse pollutants than the parent molecule (Jones, *et al.*, 2004). Although the concentration of pharmaceutical residues in wastewater have been found in nanograms per litre range, they have detrimental effects on aquatic flora and fauna. Pharmaceuticals, even at trace levels in aquatic systems, have potential adverse impact on human and ecological health (Kim, *et al*., 2007). When organisms such as fish are exposed to pharmaceuticals

residues, they cause endocrine malfunctions. For example, studies have shown that when male gold fish are exposed to oestrogens they develop gene mutations, low sperm count and finally develop female features (Kim *et al.*, 2007; Hook *et al*., 2007). Research on direct adverse effects on human health is scanty hence more studies are required. (Fawell and Nieuwenhuijsen, 2003). Pharmaceutical residues are known to cause antibiotic resistance. Antibiotic resistance is an issue of concern especially in developing countries where there are currently only few studies which have been documented to assess the extent of this problem (Neil, 2016).

Pharmaceuticals find their way into the environment through various mechanisms including direct disposal into the environment, excretion of human and animal waste, direct release from pharmaceutical industries, veterinary and agricultural practices and leachate from dumpsites, broken sewer pipes and blocked manholes (Mathenge, 2013). Another dominant pathway for pharmaceutical release in terrestrial and aquatic environment is through application of animal manure and biosolids containing excreted drugs residues to agricultural land as fertilizer which finally leach to the ground and surface water (Kemper, 2008). They can also be introduced to agricultural land through irrigation using wastewater (Gulkowska *et al*., 2008).

Globally the biggest source of pharmaceutical residue in the environment is the discharge of untreated sewage into water bodies. In Canada, for example an average of 3.25 billion litres of untreated sewage is discharged into surface waters (Daughton and Ternes, 1999). While developed countries have advanced wastewater treatment technologies for removing these pharmaceuticals from wastewater, this is not the case in developing countries such as those in Africa. In Kenya, studies have shown that wastewater treatment plants serve a small section of urban residents. A large proportion of wastewater from many informal settlements such as Kibra and Mathare are discharged directly into surface waters leading to large-scale contamination of the local water bodies (Ngumba *et al*., 2016). The problem of pharmaceutical residues in wastewater will worsen with the growing use of the pharmaceuticals from projected

population growth, ageing populations, and increased animal husbandry. Different methods for removal of pharmaceuticals in wastewater have been investigated such as Ozonation, UV treatment, use of activated carbon, fly ash resins and biosorbents (Isoda, et al., 2014; Chang *et al.,* 2004; Kilic, 2011). However, some of these methods are expensive and inaccessible. Hence there is need to develop cheaper and reliable methods of removing pharmaceuticals residues in wastewater. Biodegradation of pharmaceutical residues by microorganisms is an area that can be investigated as a viable cheaper option for removal of pharmaceuticals from wastewater. In biodegradation experiments microorganisms compete for the primary carbon source, which is usually the compound that is present at the highest concentration among the more easily degradable carbon sources. In this case the pharmaceutical is made to be the sole source of carbon and energy (Gauthier *et al*., 2010).

The pharmaceuticals selected for this study have been repeatedly found in the environment and are micro pollutants. These includes one antibiotic, trimethoprim (TMP) one pain killer, paracetamol (PARA) and one antiretroviral drug, nevirapine (NEV). The choice of pharmaceuticals drugs was based on their high annual therapeutic usage, solubility in water, stability in the environment and concern over their possible effect on human and aquatic organisms

The objective of this study was to evaluate the efficacy of rice husks carbonated at temperatures ranging from 300℃-600℃ in the removal of selected pharmaceutical residues from wastewater. The efficacy of selected microorganisms namely, *Pseudomonas aeroginosa, Escherichia coli, Bacillus subtilis and Staphylococcus aureas* in microbial degradation of the selected pharmaceutical drug residues using model solutions was investigated. Removal of the pharmaceutical residues from real wastewater was carried out in two study sites namely Jomo Kenyatta wastewater treatment plant. (JKUAT WWTP) and Nairobi River water.

1.2 Problem Statement

Pollution due to pharmaceutical residues has become a concern and especially in third world countries such as Kenya. Presence of pharmaceuticals residues in the environment has serious repercussions to both aquatic organisms and human health. Pollution from pharmaceutical wastes lead to challenges in getting fresh water and increase the cost of purifying it. The long-term effects of the pharmaceutical residues to aquatic organisms and on human health may be considerable. The fact that their ultimate fate is unknown creates uncertainty which adds to the risks associated with their presence in the environment.

In Kenya only a few studies have been reported on removal of pharmaceutical residues. (Cecilia *et al*., 2020; Kairigo *et al*., 2020; Koreje *et al*., 2016). These studies only assessed removal of pharmaceuticals by various wastewater treatment plants, hence the need for this study. One reason for scant research on removal of pharmaceutical residues is that, over the last two decades, it was difficult to conduct analyses at trace levels due to lack of selective and sensitive instruments. But since the late 1990's there has been emergence of numerous analytical methods that has enabled detection of these compounds in wastewater. One such technique is liquid chromatography tandem mass spectrometry. This method can measure pharmaceutical concentrations in nanograms per litre range (Ouma *et al*., 2021).

In third world countries, wastewater treatment plants are not well equipped to handle emerging pollutants such as pharmaceutical residues. Different methods have been developed to transform the pharmaceutical residues into less toxic forms and remove them from the environment (Smýkalová *et al.*, 2019). Conventionally, WWTPs use activated sludge processes and biological treatment (bio filtration) to remove waste but these methods have not been feasible in the removal of pharmaceutical residues (Mukoko *et al*.,2015). Passage through WWTPs removes a small percentage of pharmaceutical waste. (Petrovic *et al*., 2005). WWTPs are built to separate suspended solids and to remove easily degradable organic matter (Mulder *et al.*, 2015).

Therefore, there is a need to develop a novel adsorbent that can be used to remove pharmaceutical residues from wastewater, transform them to less toxic and more biodegradable forms for easier removal from the environment. One way to achieve this is to modify agricultural wastes such as rice husks and convert them to adsorbents that can be used in removal of pharmaceutical wastes. The second way is to subject the pharmaceutical residues to selected microorganisms and evaluate their possible biodegradation.

In this project, activated carbon prepared from rice (*Oryza glaberrima*) husks harvested from Mwea Kirinyaga county, was carbonated at temperatures of 300℃, 400℃ 500℃ and 600℃ and used in the removal of paracetamol, trimethoprim and nevirapine from wastewater. Biodegradation of the three pharmaceuticals using *Pseudomonas aeroginosa, Escherichia coli, Bacillus subtilis and Staphylococcus aureus* bacteria was also studied using model solutions that represented wastewater.

1.3 Justification

Several studies have indicated that there is high occurrence of pharmaceutical residues in water bodies (Ngumba *et al.,* 2016; Koreje *et al.,* 2016). Studies have shown the inefficiency of wastewater treatment plants in removal of pharmaceuticals residues. (Kolpin, *et al.*, 2002; Kairigo *et al*., 2020). Hence numerous methods have been developed to convert them into less toxic forms and remove them from the environment (Smkalová *et al*., 2019; Petrovic *et al*.,2005). However, their physical and chemical characteristics affect how well they are removed.

Conventionally WWTPs uses biological treatment (bio filtration) and activated sludge methods but biofiltration produce metabolites that are toxic to live microorganisms and more importantly, humans. Hence there is a risk in using them in removal of these organic pollutants. WWTPs are built to separate suspended solids and to reduce degradable dissolved organic matter, nitrogen and phosphorus, but not for removal of pharmaceuticals. (Bhamare and Kulkarni, 2019; Leal *et al*., 2010).

Various methods involving Oxidation Processes have been proposed to be efficient for the removal of harmful organic compounds in aquatic environments; which include activated carbon adsorption, membrane filtration, advanced oxidation processes which involves Fenton and photo Fenton oxidation, ozonation, photolysis, sonolysis and heterogeneous photo catalysis (Ngumba *et al.,* 2016). But these methods are expensive hence inaccessible in third world countries.

Hence there is a need to remove these pollutants from aquatic systems, preferably using a non-hazardous, cost-effective adsorbent with high adsorption capacity. It is also necessary to subject the pharmaceutical residues to biodegradation using microorganisms and assess whether their biodegradability and to what extent.

1.4 Hypothesis

i. Activated carbon made from rice husks carbonated at different temperatures cannot remove pharmaceutical residues from wastewater.
ii. *Pseudomonas aeroginosa, Escherichia coli, Bacillus subtilis and Staphylococcus aureus bacteria* do not biodegrade pharmaceutical residues in wastewater.

1.5 Objectives

1.5.1 General Objective

To evaluate the efficacy of activated carbon from rice husks in removal of pharmaceutical residues from wastewater and their biodegradation using microorganisms.

1.5.2 Specific Objectives

i. To thermally produce activated carbons from rice husks at different temperatures and characterize the carbon biochar using FT-IR, SEM and XRD

ii. To optimize sorption parameters such as pH, contact time, adsorbent dose and initial drug concentration for removal of paracetamol, trimethoprim and nevirapine residues from wastewater

iii. To determine the adsorption capacity of the activated rice husks biochar in removal of paracetamol, trimethoprim and nevirapine from wastewater.

iv. To investigate the efficacy of *P. aerogininosa, E. coli B.subtilis and S. aureas* bacteria in biodegradation of paracetamol, trimethoprim and nevirapine in aqueous media.

1.6 Scope of Study

The study dealt on pyrolytic synthesis and characterization of biochar derived from rice husk for removal of pharmaceutical residues from aqueous phase, its efficiency, adsorptive capacity and adsorption mechanisms. It also evaluated biodegradation of the selected pharmaceuticals using selected microorganisms.

1.7 Limitation of Study

The present research only dealt with removal of three pharmaceuticals. The biochar for adsorption of pharmaceuticals was derived only from rice husk biomass not any other biomasses. Regeneration study of the formulated rice husk biochar was not done. It dealt with biodegradation using four bacteria and not a range of microorganisms. Biodegradation experiments used model solutions instead of wastewater.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pharmaceutical Wastes and their Effects in the Environment

2.1.1 Problems Related to Pharmaceutical Wastes in the Environment

A pharmaceutical includes any substance or mixture of substances for use in diagnosis, treatment, mitigation or prevention of a disease, disorder or abnormal physical state, or its symptoms in human beings or animals (Erick and Moore, 2007). Pharmaceuticals are compounds which contain active ingredients that cure human and animal diseases. Due to rapid increase in population, coupled with emergence of new diseases, large quantities of various pharmaceutically active substances are being manufactured (Jones *et al*., 2004).

Paracetamol is a widely used drug for relieving pain and reducing fever. It is sold as over the counter (OTC) drug worldwide. It is one of the most often detected pharmaceutical products in sewage treatment plant effluents, surface water, and drinking water. Detection of this compound is greater in highly populated areas such as urban centers where drug usage is expected to reach elevated proportions. The Frequent occurrence of paracetamol in aquatic environments and drinking water has raised a concern about their potential effects on the environment and human health (De Luna *et al*.,2012; Wu *et al*., 2012).

The presence of antibiotics, such as trimethoprim, in natural water affects the environment in several ways. First, the presence of these kinds of compounds alters the natural flora and may lead to bacterial resistance. In this context, the development of economical and efficient advanced water treatment technologies is necessary (Ahmad *et al*., 2022; Lee *et al*., 2021).

Nevirapine is an antiviral drug which is in a class of pharmaceuticals designed to treat viral infections, such as influenza, herpes, hepatitis, and acquired immunodeficiency

syndrome (AIDs) (Peng *et al.,* 2014). It has been reported that up to 60% of administered dose of antiviral drugs are excreted by patients (Ncube *et al*., 2018). Just like other pharmaceutical drugs, the antiviral drugs are eventually released into receiving aquatic environments via effluent discharge of wastewater treatment plants. WWTPs removes only a small percentage of antiviral drugs. In some studies it was reported that the removal rates of nevirapine, lopinavir, efavirenz, and zidovudine after passage through WWTP were lower than 68% (Abafe *et al*., 2018; Prasse *et al.,* 2010). WWTPs have been identified as the primary sources for the discharge of antiviral drugs into the aquatic environment (Muriuki *et al*., 2020).

There are three types of pharmaceutical waste; Hazardous, non-hazardous and chemo pharmaceutical waste. Hazardous waste includes the Environmental Protection Agency (EPA) coded pharmaceutical waste or pharmaceuticals that are toxic, reactive, corrosive, and ignitable. This waste has detrimental effects on the environment and human health. There are two hazardous waste categories: listed and characteristic waste. Listed wastes contain pharmaceutical products for commercial use, while characteristic waste is controlled as they exhibit toxicity, reactivity, corrosivity, and ignitability. Non-Hazardous waste includes expired medicines, manufacturers sample, loose pills, damaged or contaminated patient medication, including packaging. Chemo wasteis obtained from a wide range of drugs used to disrupt the formation of cancer cells. (Nyaga *et al.,* 2020).

Pharmaceuticals are among a wide range of emerging toxicants such as cosmetics, personal care products which are used by modern society (Thomaidis *et al*., 2012). Over the last decade traces of pharmaceutical residues, though in concentrations ranging below 1000-1500 nanograms per litre range, have been reported in surface waters, wastewater, ground water and to a lesser extent in drinking water (WHO, 2011).

In many developed countries, there are more sensitive instruments which enable advanced analytical methods for analysis of pharmaceutical residues in aqueous systems. This leads to increased knowledge and interest in the effects of pharmaceutical products

once released in the environment. However, these advanced methods are not accessible in developing countries leading to lack of interest in the study of pharmaceutical residues since they occur in trace concentrations (Zuccato *et al*., 2006; Fawel and Niuewenhuijsen, 2003).

It has been reported that there is a link between water bound traces of pharmaceutical such as hormonally effective substances like estradiol which is used as a contraceptive pill that bring about shift in the sex ratio of some fish species. The study revealed that some males become more feminized in that they produce less sperm and instead start to produce eggs. Such effects may result in a reduction in population sizes of the fish which may have corresponding implications for the entire food chain (Hook *et al*., 2007). Analgesics such as ibuprofen have shown anti-bacterial and antimycotic properties. Some antidepressants such as Prozac (fluoxetine) and Zoloft (sertraline) have been reported to affect the spawning of shellfish (Jones, *et al*., 2004).

2.1.2 Pathways of Pharmaceutical Residues in the Environment

The major source of pharmaceutical residues is hospital wastewater. Significant quantities of pharmaceutical residues were detected in hospital effluent in Taipei, Taiwan (Katherine *et al*., 2011). Another source of pharmaceutical waste to the environment are the municipal wastewater treatment plants. According to Katherine *et al*. (2011), effluent from such plants increase the concentration of pharmaceuticals entering aquatic environment. Biosolids and dry sludge from these water treatment plants are also applied as fertilizers.

Leachates from municipal dumpsites where expired drugs and other medical waste are dumped is another source of pharmaceutical residues. Shala and Foster, (2010) analyzed pharmaceuticals in Anacostia River in United States and since the river didn't receive any wastewater discharge, they concluded that landfills or septic leachate could be the source of contamination. Jones *et al*. (2004) also associated leaching from landfill sites directly to the groundwater as a major source of pharmaceuticals in the environment.

Human and animal fecal waste is another contributor of pharmaceuticals in the environment. According to Khan, (2004), fifty percent of the drugs original compounds may be excreted unchanged. Some of these wastes are excreted as metabolites or conjugates which revert to original compounds. Pharmaceutical factories are also known to release medical waste in the environment. Some waste is disposed during the manufacturing process in the water system. A study in the USA found a thousand times higher levels of pharmaceutical ingredients in a wastewater treatment plant receiving water from such factories than from plants that received no such waste (Kessler, 2010). Figure 2.1 shows disposal route of pharmaceuticals in the environment.

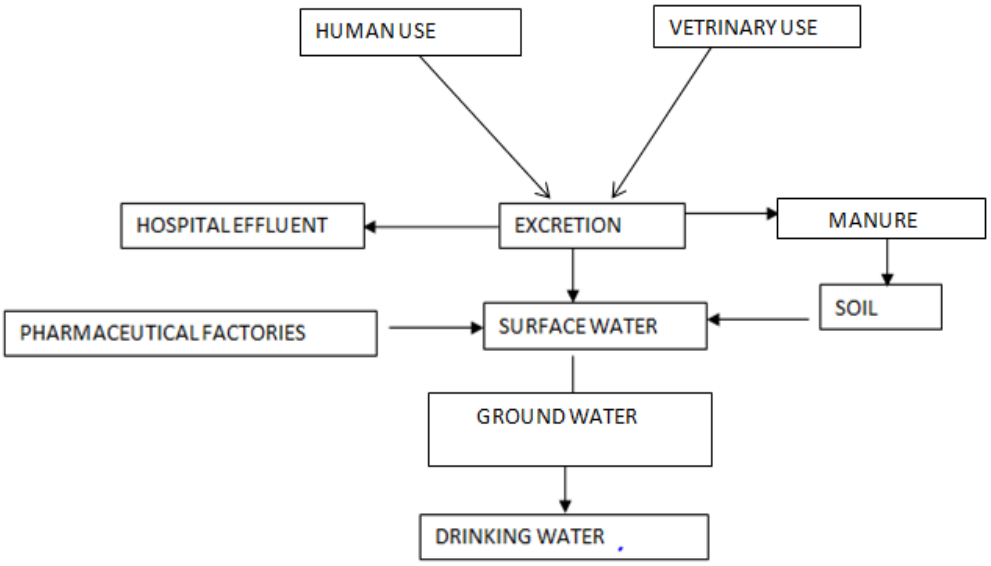

Figure 2.1: Disposal of pharmaceuticals in the environment (Kessler, 2010)

2.1.3 Regulations Governing Disposal of Pharmaceuticals

The Pharmacy and Poisons Board (PPB) defines pharmaceutical waste as waste containing expired medicines, unused or no longer needed drugs including items containing or contaminated by pharmaceuticals such as bottles, boxes, vials, ampules, gloves, and masks. Poor pharmaceutical waste management (PWM) may be deleterious to both human health and the environment, hence, the need for proper regulations (Sungpyo and Aga, 2007). Pharmaceutical waste management (PWM) is defined as all activities, both administrative and operational, for handling pharmaceutical waste. The procedure used to determine the guidelines for water quality gives rise to a vigorous debate among regulators, environmentalists and industries, each one defending their own point of view. The stakes are crucial on each side; protection of the environment including human and animal health on one side and billions of dollars in the market on the other. One of the major systems of regulation in the world is held in the United Stated under the jurisdiction of Food and Drug Administration (Sungpyo and Aga, 2007).

The Food and Drugs Administration (FDA) is the main Drug Regulatory Authority responsible for review, approval, and post approval compliance of all the drugs being marketed in the USA. Post approval changes to any drug product need to be notified to and approved by FDA before implementation. The environmental assessment procedure for a new drug is divided into two steps. Firstly, the manufacturer is asked to make an estimation of the expected introductory concentration (EIC) entering the environment based on five years of production (Sungpyo and Aga, 2007).

In Kenya the Pharmacy and Poisons Board (PPB), established under Chapter 244 of the Pharmacy and Poisons Act (2002), is the body responsible for the registration of pharmaceuticals and medical devices, whereas for testing and regulatory purposes, it is the National Quality Control Laboratory. However, it is believed to test less than 20% of samples. Most of the legal requirements are expected to be met by the importers. They provide drug samples to Kenya Bureau of Standards for quality checks and registration

and for national policy regulations adopted by the Ministry of Health. This includes an essential drugs list, using WHO guidelines, whose objective is to promote the availability of quality pharmaceutical products at affordable prices. (Chemwolo *et al*., 2010)

2.1.4 Fate of Pharmaceutical Residues in the Environment

The fate of pharmaceuticals in the environment is not well known since some drugs may be completely degraded in wastewater treatment plants, others remain completely unaffected (Jones *et al*., 2004). Adsorption by use of activated carbon and biodegradation by micro-organisms are the most common methods used to remove these pharmaceuticals (Ternes and Joss, 2006).

This research focused on three pharmaceuticals, each of which has been found in certain concentrations in the environment. (Ngumba *et al*.,2016a; Kairigo *et al*.,2020).

The main selection parameters of the drugs were their therapeutic use, stability, water solubility and the lacking knowledge of their occurrence, fate and behavior in the environment (Feng *et al*., 2009). The drugs included an antibiotic (Trimethoprim), a pain killer (Paracetamol) and an antiretroviral (Nevirapine).

2.1.4.1 Paracetamol

It is one of the most common pharmaceutical residues found in the environment. Although it is found in trace concentrations. Stackelberg, (2007), while working on efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds found that most ground waters in USA had paracetamol levels of 120 nanograms per litre.It has been detected in approximately 75% of natural waters such as rivers and lakes. It is commonly used as an analgesic for headaches and other pains. It is a white compound which is soluble in water at room temperature. Its structure consists of a benzene ring with one hydroxyl group substitution and an amide group in the para position. Figure 2.2 is the structure of paracetamol (N-(2,3,5,6-tetradeuterio-4-hydroxyphenyl) acetamide)

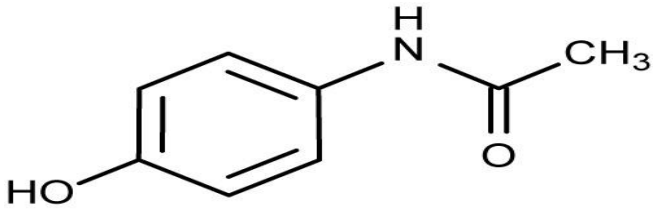

Figure 2.2: Structure of paracetamol (N-(2,3,5,6-tetradeuterio-4-hydroxyphenyl)acetamide)

2.1.4.2 Trimethoprim

This is a synthetic broad-spectrum antibiotic that inhibits the dihydrofolic acid synthesis in bacteria, hence killing the bacteria. It is used to treat many bacterial infections such as urinary tract infections, respiratory infections and middle ear infections (Ryan *et al*., 2011). The combination of sulfamethoxazole and trimethoprim obstructs two consecutive steps in the folic acid metabolism, hence interrupts the micro-organisms synthesis of RNA and DNA (Pérez *et al.,* 2005). In a study carried out by Ryan *et al.* (2011), the rate of loss of trimethoprim was found to be enhanced in wastewater effluent due to indirect photolysis reactions, specifically reactions with hydroxyl radicals and triplet excited states.

Trimethoprim was also found to be susceptible to indirect photolysis in wastewater effluents, with hydroxyl radical and triplet excited effluent organic matter being the responsible species (Ryan *et al.*, 2011). Research findings by Kathryn (2011), showed a concentration range of 2900-5000 ng/L of trimethoprim in untreated hospital wastewater. These concentrations are too high considering that WHO guidelines report that allowable limits of pharmaceuticals in surface waters, groundwater and partially treated water should be less than 100 ng/L. The concentrations in treated water should be below 50 ng/L (WHO, 2011). Similar studies done earlier by Kolpin (2002) reported a lower concentration range of 180-590 ng /L of trimethoprim in untreated wastewater although it was still above allowable limits. Figure 2.3 shows chemical structure of Trimethoprim (5-[(3, 4, 5-trimethoxyphenyl) methyl] pyrimidine-2,4-diamine).

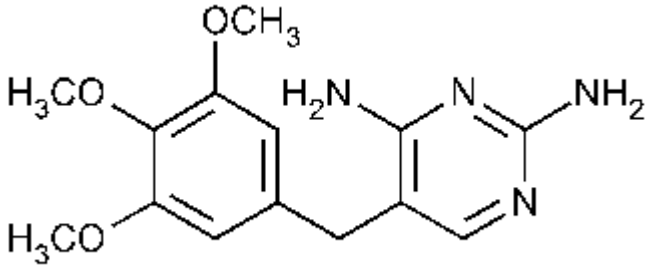

Figure 2.3: Structure of trimethoprim (5-[(3, 4, 5-trimethoxyphenyl) methyl] pyrimidine-2,4-diamine)

2.1.4.3 Nevirapine

Nevirapine is a white crystalline water-soluble drug which falls in the non-nucleoside reverse transcriptase inhibitor (NNRTI) class of antiretrovirals. The enzyme reverse transcriptase converts single-stranded viral RNA into DNA. Drugs in the NNRTI class stop HIV from replicating within cells by binding near reverse transcriptase's active site and inhibiting polymerase activity. Nevirapine (Viramune) is approved for the treatment of HIV infection in adults and children as part of a combination therapy (Samsodien *et al*., 2017). Nevirapine is practically insoluble in water with an aqueous solubility of 0.1 mg/ml (pH 7, Temp. 37°C). According to the Biopharmaceutical and classification Index, nevirapine is a Class II drug i.e., it has a high permeability and a low solubility (Caira, *et al*., 2011). The low rate of dissolution of Nevirapine is assumed to be the rate-limiting step for absorption of the drug (Sarkar *et al*., 2008). Figure 2.4 shows structure of Nevirapine 5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e] [1,4] diazepine molar mass 266.298 g/mol.

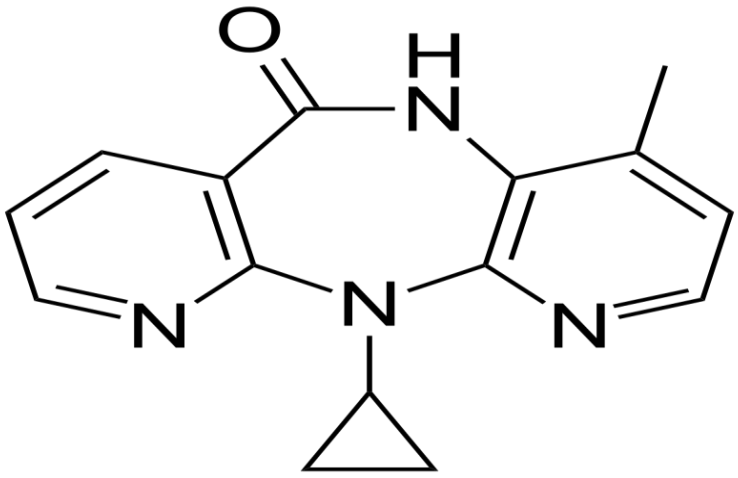

Figure 2.4: Structure of nevirapine 5,11-dihydro-6H-dipyrido[3,2-b:2',3'-e][1,4] diazepine

2.2 Removal of Pharmaceutical Residues from Wastewater

Different methods of removing pharmaceutical residues have been used all over the world. Most developed countries do monitor the presence of pharmaceutical wastes or personal care products in wastewater and open water. This practice is uncommon in third world countries including Kenya due to non-existence of locally suitable technology and the high economic investment required (Jones, 2003).

2.2.1 Activated Sludge Process

Activated sludge processes are employed in most conventional WWTPs. This is because they produce a secondary effluent that mostly complies with global and national quality standards for discharge into surface water bodies (Verlichi *et al*., 2012). The removal of pharmaceuticals in activated sludge processes includes two main mechanisms, biotransformation and sorption. Moreover, other mechanisms such as hydrogen bonding, ion exchange and surface complexation may intervene in the sorption process (Tolls, 2001). For the estimation of the removal via sorption to suspended solids and biomass solid–water distribution coefficients (K_d) have been introduced, which are defined as the ratio between the concentrations of a substance in the solid and in the aqueous phase at

equilibrium conditions WWTPs are specifically designed to remove a wide range of substances including particulate matter, nutrients, pathogens, carbonaceous biological matter and other macropollutants that can significantly raise the biochemical oxygen demand (BOD) or chemical oxygen demand (COD) of the effluent (Clara *et al*., 2005).

2.2.2 Wastewater Stabilization Ponds

Waste stabilization ponds (WSPs) are a very appropriate method of wastewater treatment in third world countries, where the climate is most favorable for this application. Their lower implementation costs and operational simplicity are commonly regarded as their main advantages. WSPs utilizes the natural attenuation processes for wastewater treatment (Mahmood *et al.* 2013). They use shallow basins that use natural factors such as biodegradation, sunlight, temperature, sedimentation, predation, and adsorption to treat wastewater. They are preferred to conventional WWTPs in developing countries and small communities due to the low operation and maintenance cost with minimum electrical energy and technical operation requirements. In addition, high wastewater treatment efficiency can be achieved in tropical and subtropical countries where the intensity of sun is high and minimum temperature variations (Zhang *et al*. 2012). However, limitations such as the need for large land area, regular removal of the sludge from the ponds, inefficiency in cold climates and oduor slow down the usage of the process especially in highly populated areas.

2.2.3 Activated Carbon

Several cheaper materials, including industrial and agricultural wastes, have been used to remove different pollutants from industrial effluents for their safe disposal into the biosphere. There are different types of adsorbents used in wastewater treatment. They include use of activated carbon, fly ash, resins and biosorbents. Activated carbon can be made from different waste products such as coffee residues or shells (Isoda *et al*., 2014). Activated carbon is used as an absorbent as well as a catalyst support. It has exceptional absorption kinetics (Tongpoothorn *et al*., 2011). Powdered activated carbon (PAC) is used due to its great surface area and ability to adsorb compounds. It has been used in

several studies for removal of organic matter and other contaminants in wastewater by adsorption (Negara, *et al*., 2019; Ndekei *et al*., 2021). According to a study by Chang *et al*. (2004), PAC can efficiently remove estrogens from the aqueous phase with up to 95% efficiency.

Activated carbons are made up of small hydrophobic graphite layers with disordered, irregular and heterogeneous surfaces bearing hydrophilic functional groups (Soto *et al.*, 2011). Synthesis of activated carbon is done through physical and chemical activation process (Hameed *et al.*, 2008; Moussavi *et al.*, 2012; Isoda *et al.*, 2014). Activation is responsible for enhancing chemical attack on the matrix of the precursor as well as fragmenting cellulose, hemicellulose and lignin.

Activated carbon is carbon produced from carbonaceous source materials such as nutshells, peat, wood, coir, lignite, coal, petroleum pitch, rice husks and maize cob. It can be produced by either physical or chemical activation. During activation the raw material is reacted with an acid, strong base or a salt. Chemical activation is always better than physical activation because it is done at lower temperatures, takes less time and it is cheaper (Aloko and Adebayo, 2007).

Activated carbon has been proposed to be an adsorbent for the removal of pharmaceuticals from water due to its unique physical chemical properties such as porosity and large specific surface area (Yoon, *et al*., 2003). Generally, activated carbon is applied at the polishing step for the removal of refractory compounds and precursors of disinfection by products in water treatment (Mahmoud *et al*., 2012; Kulkami., *et al*., 2010). Activated carbon has good adsorption capacity (Jones *et al*., 2003).

2.2.4 Rice Husks as Adsorbents

Rice husks are categorized as agricultural residues, produced as by-product of rice milling industries. They are exploited as low value energy resources or simply burnt at the field as they are considered a waste. This greatly affects the air quality, hence unfavourable to the environment. Rice husks contains organic materials such as lignin, hemicellulose, and cellulose, which make them to be easily turned into raw carbon materials (Wang *et al.,* 2010; Guo *et al*.,2003). The processing and transformation of rice husks into adsorbents with good adsorption properties has been found to alleviate problems of disposal and management of these waste by-products (Zhang, *et al* 2011).

Adachi *et al.* (2001), reported rice bran to be a potent sorbent for the removal of organochlorine compounds and benzene from industrial wastewaters. The rice husk is an abundant lignocellulosic biomass and this makes it suitable for biochar production. Lignocellulosic biomasses include those from agricultural residues (corn stover, crop straws and bagasse), from herbaceous crops, woody plants, forestry residue, wastepaper and other municipal green wastes that are mainly composed of cellulose, hemicellulose and lignin (Mohan *et al*., 2006).

Rice husks are modified through carbonization process which is the conversion of organic matter into porous carbon skeleton. This is done through heat or chemical treatment. Heat treatment produces a more porous carbon. Diverse structure and morphology of carbon materials derived from waste sources can be obtained, depending on the parameters such as heat or temperature-controlled carbonization process (Wang *et al.*, 2015; Saito and Arima, (2007).

Carbon materials with high lignin content generally produce the highest biochar yields when pyrolyzed at moderate temperatures (Demirbas *et al.,* 2008). The porosity of rice husks biochar can increase with time when the ash is dissolved and removed from the pores. According to Ahiduzzaman and Sadrui, (2016), chemical activation of biochar by use of phosphoric acid assists in developing more pores on the surface area of the

biochar which improves its adsorption capacity and porosity qualitatively and quantitatively.

Carbon materials for biochar production such as rice husks are readily available and at low-cost hence suitable for third world countries (Jindo *et al.,* 2014). Rice husk contains 20% silica, which is present in its hydrated amorphous form. During the pyrolysis procedure, the silica is converted to cristobalite, which is its crystalline form. The study involved adsorption of pharmaceuticals using rice husks biochar and biodegradation by use of microorganisms. A synergy between sorption and biodegradation was found to improve the removal of trimethoprim. using a highly efficient trimethoprim degrading bacteria strain, *Bacillus subtilis*, which was isolated from column reactors. In the removal process, this bacterium degrades trimethoprim to NH^{+}_{4}, and then further converted NH^{+}_{4} to NO_3^- in a continuous process (Liu, *et al.,* 2018).

2.2.5 Biodegradation of Pharmaceuticals Using Microorganisms

Biodegradation is defined as the transformation of organic molecules by bacteria, fungi or yeast to simple molecules through biological activity. Microorganisms can be used to biodegrade pharmaceuticals. The microorganism partially converts the drug and uses it as a primary carbon source or sometimes as the source of Nitrogen (Jones *et al*., 2007). Water and carbon dioxide are the final products of the degradation pathway (Murphy and Morrison, 2002). The first step in biodegradation is to expose the microorganisms to the target chemical compound which is to be degraded for a period of time. The microorganisms will have time to develop enzymes that will degrade the chemical which is usually not a readily used carbon or nitrogen source for that microorganism.

Microorganisms compete for the primary carbon source, which is usually the compound that is present at the highest concentration among the more easily degradable carbon sources. The dominant compounds in the influent are likely to be lipids or proteins while the pharmaceuticals are present in low concentrations. Biodegradation is therefore likely achieved by microorganisms with oligotrophic metabolism (Jones, *et al.*, 2007).

Biodegradation of pharmaceutical compounds is being considered as an environmentally friendly and low-cost option. It has been demonstrated to have the potential to eliminate pharmaceuticals by degrading them into innocuous end products such as CO_2 and H_2O. Microorganisms are the

main drivers of emerging antibiotic contaminant degradation in the environment (Liang, *et al*.,2019). The main fate of pharmaceuticals in environmental processes is sorption and biodegradation. Photodegradation and hydrolysis are also significant. Depending on the toxicity of the compound towards microorganisms, stability in the presence of air or light, pharmaceuticals can persist for years in the surroundings (Kümmerer, 2004).

A number of research studies on the fate of pharmaceuticals in the developed world use the available detailed knowledge of consumption patterns and amounts of pharmaceuticals consumed (Winker, 2008, Lindqvist, 2005). The comparison of quantity consumed and excreted via urine or feces to sewage system to the quantity detected in the WWTP influent/effluent gives needed knowledge on the removal efficiency during the WWTP processes. Most of the researchers focus on summarizing the studies on pharmaceutical biodegradation in the following aspects: the degrading bacteria, and the proposed metabolic/biodegrading pathways in microorganisms, enzymes, and possible intermediates. Pedrouzo *et al.* (2011) recorded the ability of isolated Penicillium species to transform paracetamol to 4-aminophenol and acetate. With the use of aryl acylamidase. 4-aminophenol was found to be a dead-end metabolite.

Another study by Li, *et al*. (2014) proposed and described the degradation pathway of paracetamol in soil microorganisms. It was shown that in the first step, the aromatic ring of paracetamol is hydroxylated to 3-hydroxyparacetamol, oxygenated to N-acetyl-p-benzoquinone imine, or methylated to p-acetanisidide. N-acetyl-p-benzoquinone imine is then metabolized to 1,4-benzoquinone which is more stable and critical toxic metabolite. p-acetanisidide is transformed to 4-methoxyphenol and in the next step to the 1,4-dimethoxybenzene. The presence of 2-hexenoic acid in the soil extract suggests the cleavage of the aromatic ring of paracetamol.

Microorganisms with a high natural ability to metabolize a wide range of various substances can be used as natural biocatalysts in sewage treatment processes. Unique features and great versatility of such strains increase the effectiveness of wastewater treatment and often decompose pollutants poorly susceptible to chemical degradation (Dasgupta *et al.,* 2013; Sipma *et al.,* 2010). Pharmaceuticals not detected in wastewater are completely degraded and metabolized in the organism or during transport of sewage (Jones *et al*., 2003). Biodegradation by micro-organisms and adsorption by use of activated carbon are the most common methods used to remove pharmaceuticals. (Ternes and Joss, 2006).

Pharmaceuticals in the environment affect most organisms, especially aquatic organisms which are vulnerable to them. This is because of the high solubility of most pharmaceutical drugs. Estrogen from birth control pills eventually enters water sources. Increasing levels of estrogen in the environment, via pharmaceuticals for the purposes such as menopause symptom relief and birth control pills, could be causing adverse effects on humans, such as reduced male sperm counts and sperm motility and young ages of puberty in girls (Roth, 2003).

2.2.5.1 Test Microorganisms

2.2.5.1.1 *Pseudomonas aeruginosa*

The *Pseudomonas* species are found in soils and water. They have the ability to proliferate even when the amount of nutrients is very low. These microorganisms have been recognized to degrade xenobiotic compounds. *Pseudomonas putida* has been used to biodegrade several pesticides such as desmedipham and promecarb and also acridine, a derivative of carbamazepine (Pia Arentsen *et al.,* 2005). In biodegradation of N-heterocycles, a microorganism that is closely related to *Pseudomonas fluorescens* has been found to breakdown carbamezole (Pia Arentsen *et al.,* 2005). It is a common gram-negative rod-shaped bacterium that causes disease in animals and plants. It thrives in low

oxygen environments. It is able to decompose hydrocarbons and has been used in breakdown of oil spills (Silva *et al*., 2014).

Pseudomonas aeruginosa strain HJ1012 was isolated on paracetamol as a sole carbon and energy source. Following paracetamol consumption, a carbon dioxide yield rate up to 71.4% confirmed that the loss of paracetamol was mainly via mineralization. Haldane's equation (2.1) adequately describes the relationship between the specific growth rate and substrate concentration.

$$v = \frac{V_{max}[S]}{K_M + [S]} \quad \text{.....(2.1)}$$

Where, V_{max} represents the maximum velocity achieved by the system, at maximum (saturating) substrate concentrations. K_M is the Michaelis constant; which is the substrate concentration at which the reaction velocity is 50% of the V_{max}. [*S*] is the concentration of the substrate *S*.

During paracetamol catabolism a total of 8 metabolic intermediates were identified and classified into aromatic compounds, carboxylic acids, and inorganic species (nitrite and nitrate ions). P-aminophenol and hydroquinone are the two key metabolites of the initial steps in the paracetamol catabolic pathway. Paracetamol is degraded predominantly via p-aminophenol to hydroquinone with subsequent ring fission, suggesting partially new pathways for paracetamol-degrading bacteria (Zhang *et al*., 2012). Figure 2.5 shows paracetamol catabolic pathway.

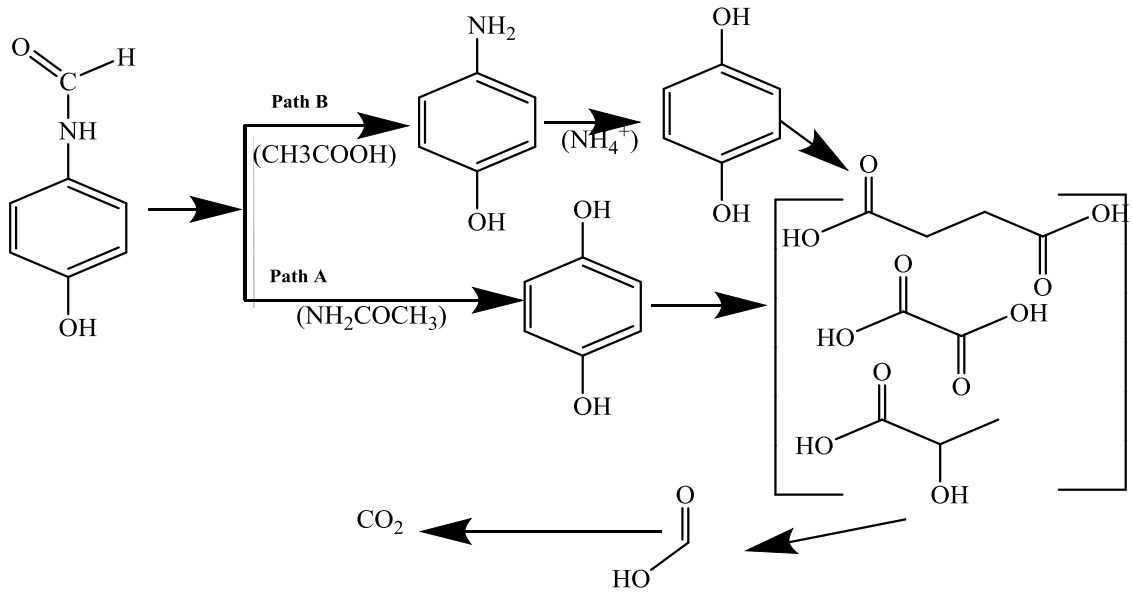

Figure 2.5: Paracetamol catabolic pathway (Zhang et al., 2013)

2.2.5.1.2 *Escherichia coli*

It is a Gram negative facultatively anaerobic bacteria commonly found in lower intestines of warm-blooded animals. *E. coli* bacteria has an advantage in fending off certain drugs. *Escherichia coli* DH5-á was chosen as a non-environmental strain, which is already a model bacterium for studying metabolism and adaptation. The results showed that this bacterium was able to tolerate high doses of the herbicide and completely degraded mesotrione after 3 hours of exposure, as determined by a high-performance liquid chromatography (Jaureguy, *et al*., 2008)

The recent progress in the understanding of the fundamentals that govern the degradation of aromatic compounds by *E. coli* makes this bacterium a very useful model system to decipher biochemical, genetic, evolutionary, and ecological aspects of the catabolism of such compounds (Diaz *et al*., 2001). Although *Escherichia coli* has long been recognized as the best-understood living organism, little was known about its abilities to use aromatic compounds as sole carbon and energy sources. It is involved in the catabolism of such compounds, like several aromatic acids (phenyl acetic acid, 3 and 4-hydroxyphenylacetic acid, phenyl propionic acid, 3-hydroxyphenylpropionic acid, and

3-hydroxycinnamic acid) and amines (phenyl ethylamine, tyramine, and dopamine) (Feng *et al*., 2009). Figure 2.6 shows trimethoprim biodegradation pathway.

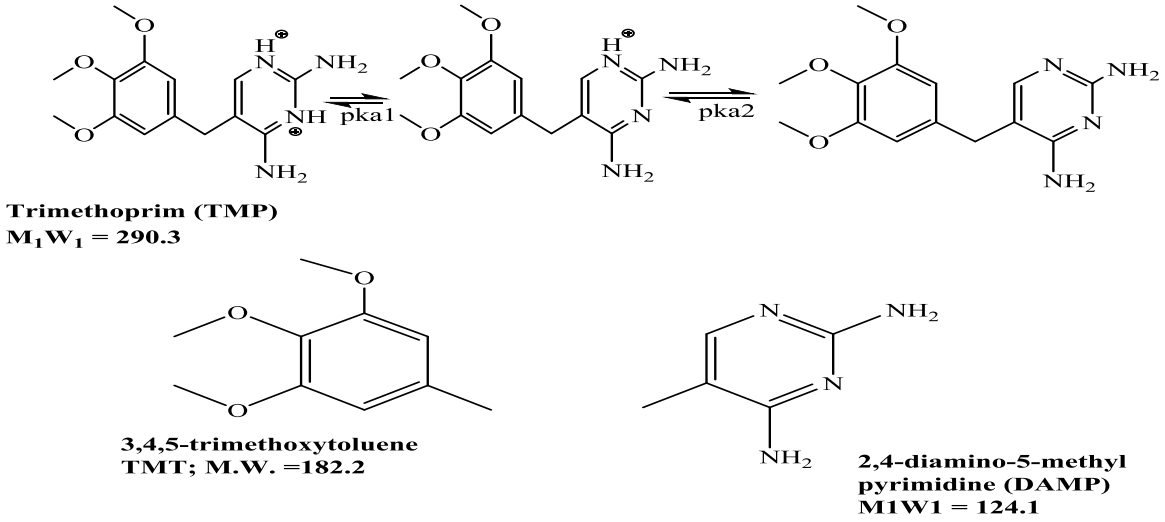

Figure 2.6: Trimethoprim biodegradation pathway (Feng et al., 2009).

2.2.5.1.3 *Bacillus subtilis*

It is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. It is rod-shaped, and can form a tough, protective endospore, allowing it to tolerate extreme environmental conditions. It has been found to biodegrade cephalexin in the treated sewage effluents more than other microorganisms (Barros, *et al*., 2013). A strain of *B. subtilis* 1556WTNC has the ability to survive in unfavourable environments also, it is safe and non-pathogenic, and does not produce toxic by-products. *Bacillus* spp. have developed strategies for survival in unfavourable environments. For instance, *Bacillus subtilis*, isolated from column reactors, degrades sulfamethoxazole and trimethoprim into NH_4^+, and then into NO_3^- in a continuous process (Liu, *et al*., 2018). After a four-day incubation period at 30°C, *B. subtilis* was able to transform approximately 40% of pyrene and 50% of benzo[a]pyrene, initially added at 20 $\mu g \cdot mL^{-1}$ (Hunter, *et al*., 2005). Figure 2.7 Shows nevirapine biodegradation pathway.

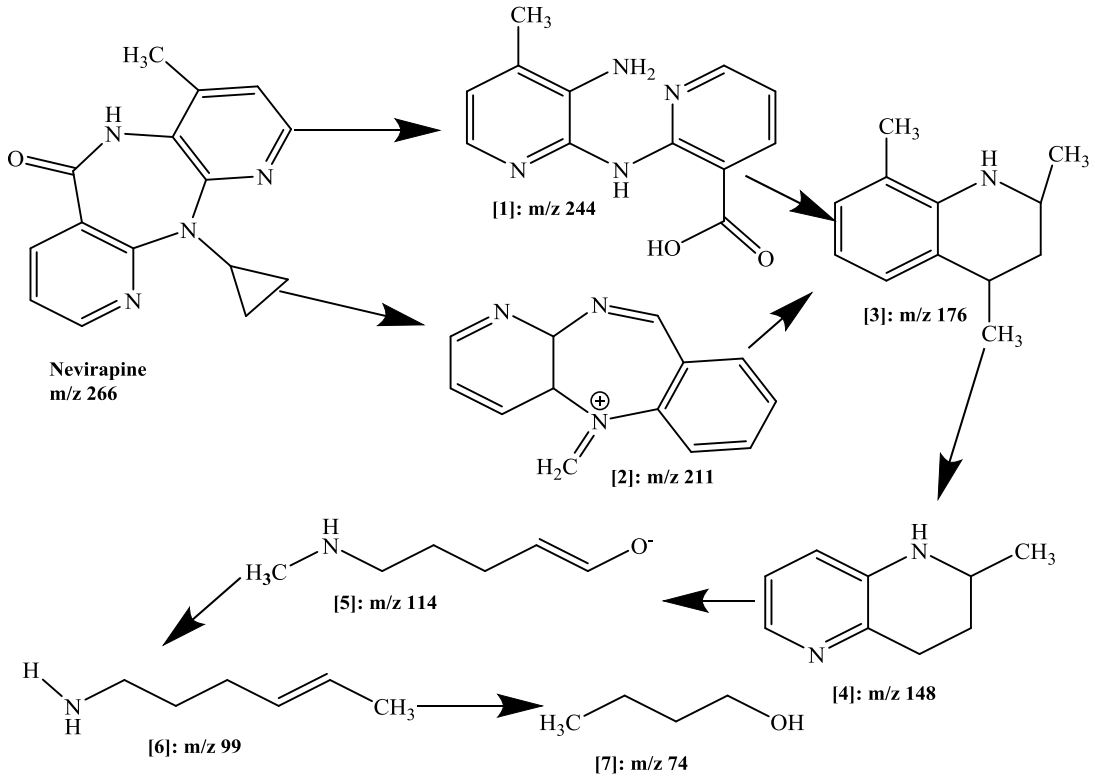

Figure 2.7: Nevirapine biodegradation pathway (Bhembe, et al., 2020)

2.2.5.1.4 *Staphylococcus aureus*

It is a Gram-positive, round-shaped bacterium, a member of the Firmicutes. It is often positive for catalase and nitrate reduction is a facultative anaerobe that can grow without the need for oxygen. Staphylococcus sp. strain 502A is promising enough to grow in minimum mineral salt media (MMSM) with only an insecticide acetamiprid as the nutrient source and is able to remove 61.68% of the toxic compound in 24 h from the effluent; to form a non- toxic clinically significant compound, Benzothiazole, with a significant metabolic pathway of degradation. Staphylococcus sp. strain 502A is potent enough to degrade a toxic insecticide like acetamiprid (61.68%) in comparatively high concentration of 50 mg L−1. The strain can be effectively utilized for further bioremediation (Tiyasha, *et al.*, 2015).

2.3 Analytical Techniques for Determination of Concentration of Pharmaceuticals

2.3.1 UV-Visible Spectroscopy

The Principle of UV-Visible Spectroscopy is based on the absorption of ultraviolet light or visible light by chemical compounds, which results in the production of distinct spectra. UV-visible spectroscopy is a fast analytical technique that measures absorbance (Barbosa *et al*., 2007). Its functions are guided by the Beer–Lambert law which states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. This method has been used to make predictions to evaluate food safety risks, growth rates and lag times of bacterial cultures while investigating the effect of pH values on growth parameters. Information about μ_{max} and λ is needed to make a prediction when studying growth kinetics, and this method has been preferred since it provides both parameters (Bidlas *et al*.,2008; Browne and Dowds, 2002).. Figure 2.8 shows the diagram of UV-Spectrophotometer.

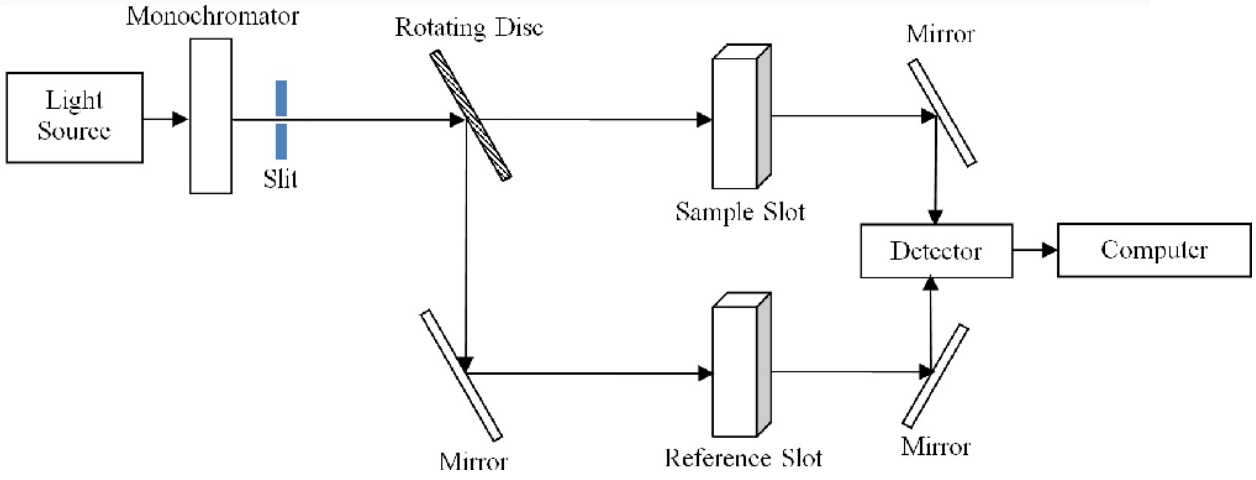

Figure 2.8: Diagrammatic representation of UV spectrophotometer (Skoog *et al*., 2017)

2.3.2 Liquid Chromatography-Mass Spectrometry

Liquid chromatography-mass spectrometry (LC-MS) is the combination of two selective techniques that allows the analyte(s) of interest in highly complex mixtures to be isolated

and measured. LC differentiates compounds by their Physico-chemical properties and MS differentiates compounds by mass (specifically their mass-to-charge ratio). It is this dual selectivity that makes LC-MS such a powerful analytical tool. LC-MS/MS is one of the analytical techniques used for the determination of pharmaceuticals and their metabolites because it is highly selective, specific and versatile. Liquid chromatography-mass spectroscopy is mostly used in the analysis of pharmaceuticals and their metabolites in a number of matrices. It has been revealed as a powerful analytical technique for rapid screening of ketoprofen, naproxen, diclofenac and ibuprofen (Olives. *et al*., 2012).

Many categories of activated pharmaceutical ingredients have been successfully identified in environmental samples using liquid chromatography with mass spectrometric detection. Advanced analytical methods like liquid chromatography (LC) are widely used in the fields of organic chemistry and biological sciences. It is used to separate mixtures into their individual chemical constituents so that these components may be subjected to robust analysis. Most chemists will have some degree of familiarity with a version of the methodology, whether it is routine high-performance liquid. One major benefit of an MS/MS detection system is that complete chromatographic resolution is not necessary and hence, multi-residue detection and quantification of closely eluting compounds is possible in a single rapid run (Petrovic *et al.,* 2005).

The use of high-performance liquid chromatography combined with mass spectrometry (HPLC–MS) or tandem mass spectrometer (HPLC–MS–MS) has proven to be the analytical technique of choice for most assays used in various stages of new drug discovery (Jenkins *et al*., 2004; Ackermann, *et al*., 2002; Hopf Gartner, *et al*., 2002). It is a powerful analytical technique that combines the separating power of liquid chromatography with the highly sensitive and selective mass analysis. Compared to the "traditional" technique of gas chromatography-mass spectrometry (GC-MS), LC-MS/MS is easier to use and is applicable for a substantially larger number of relevant analytes. With the development of LC-MS/MS, the widespread application of the proven principle

of isotope dilution mass spectrometry is now feasible not only in research but also for routine applications (Jenkins *et al*., 2004).

The resulting selectivity allows a particular analyte or analytes to be isolated from the mixture and gives confidence that the correct component is being measured. Since analytes are separated by their mass-to-charge ratio (*m/z*) the technique allows for the use of isotopically labeled internal standards, which may not separate by LC but can be separated by their mass difference. The use of stable isotopically labelled (SIL) internal standards can help control variability in a quantitative assay (Stokvis, *et al*., 2005). Figure 2.9 shows diagram of LC-MS/MS

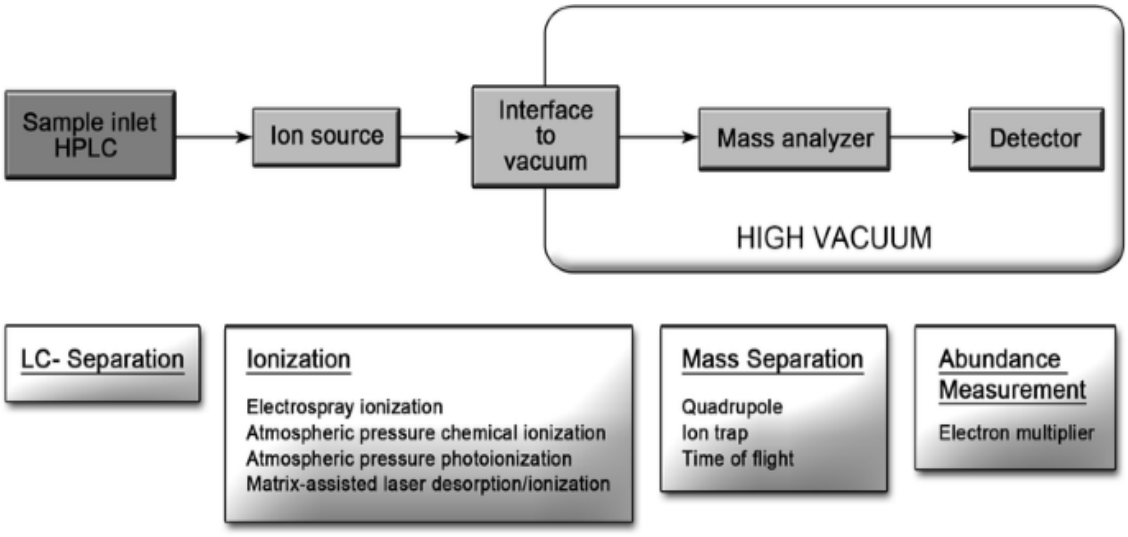

Figure 2.9: Diagram of LC-MS/MS (Leung and Fong, 2014)

2.3.2.1 Ionization Methods

In modern times the most extensively applied LC-MS interfaces are based on atmospheric pressure ionization (API) strategies like electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photoionization (APPI). Today, electrospray ionization (ESI) and atmospheric pressure

chemical ionization (APCI) are the most common API techniques in routine use for quantification of small molecules by LC-MS (Nxumalo, 2016).

Atmospheric pressure photoionization (APPI) was developed to increase ionization efficiencies of non-polar compounds such as polyaromatic hydrocarbons and steroids. The choice of the most appropriate ionization technique, as well as detection polarity, is based upon analyte polarity and LC operating conditions but many classes of compounds perform well using either technique or sometimes in both ion modes. Interfaces tend to be selected based upon individual preference derived from experience and available techniques as well as the magnitude of any matrix effects. These interfaces became available in the 1990s after a two-decade long research and development process (Takino *et al*., 2003).

Matrix effect occurs because the ionization mechanism is prone to disturbance from some components of the sample, especially with biological samples. If these components co-elute with the analyte of interest, then data can become biased or have poor precision. Typically, the ionization mechanism is suppressed, meaning that a lower response than expected is observed (Ngumba *et al*., 2016). There are three approaches to reducing this effect, one is chromatographic. This involves physical separation of the analytes and interference on column, so they elute at different times. The second one is extraction. This involves selective removal of the interferences during the sample preparation stage. The third one is compensation. This is where stable isotopically labelled (SIL) internal standard or matrix matched standards are used so that any effect is consistent (Stokvis, *et al*., 2005).

The elution time of a SIL internal standard will be virtually identical to that of its unlabeled counterpart and will therefore (in theory) undergo the same amount of matrix effect. Using response ratio to determine concentrations will therefore compensate between different matrices. Matrix matching is the process of ensuring that all standards, quality control (QC) samples and test samples are in an identical matrix so that any ion suppression is constant (Li, *et al*., 2019).

2.3.2.1.1 Electrospray Ionization (ESI)

There have been considerable efforts directed at understanding the mechanisms involved in ion production for electrospray because understanding how ions are generated from the mobile phase into the gas-phase is invaluable in diagnosing problems such as loss of sensitivity and matrix effects (Kang, *et al*., 2008). There are a number of differing theories, but ionization takes place in the liquid phase and involves an interactive process where the LC eluent flows through a metal capillary contained within the probe. Droplets are formed by nebulization of the LC flow into a spray as it leaves the electrospray capillary. A charge is transferred onto the droplets by applying a large (2-5 kV) potential difference between the electrospray capillary and counter electrode. The droplet size is reduced by evaporating the mobile phase by the use of a heated drying gas. This desolation increases charge density on the surface of the smaller droplets. Electric repulsion due to the charge density results in droplet fission.

When this exceeds the surface tension of the droplet it results in coulombic fission. Gas-phase ions are formed as the droplet "explodes" and are sampled typically through some form of orifice. The main advantage of the use of ESI for quantitative LC-MS is the formation of protonated or de-protonated molecules with little fragmentation, ideal for selection of precursor ions and for maximizing sensitivity (Santos *et al*., 2004). The major limitation is ion suppression or enhancement effects due to presence of co-eluting analytes or co-eluting matrix components. This "matrix effect" is recognized as one of the major sources of uncertainty in LC-MS and LC-MS/MS). In addition, response can be non-linear at high concentrations and optimum pH for ESI response can conflict with choices made to control LC selectivity.

2.3.2.1.2 Atmospheric Pressure Chemical Ionization (APCI)

APCI is a soft ionization technique, unlike ESI, and it frequently results in some degree of fragmentation that is useful for structural characterization. The investigation of relatively non-polar compounds that fall within the scope of LC separations is better suited for APCI. It is a method of gas-phase ionization that includes the following

coordinated processes: the LC eluent flows through a silica capillary contained within the probe. Nebulizing the LC flow into a spray result in the formation of droplets. A heater inside the probe is used to evaporate the solvent, resulting in gas-phase molecules. Since APCI uses gas-phase ionization, it is less susceptible to ion suppression compared to ESI. To avoid problems with the analyte signal being suppressed due to preferred ionization of solvents or additives with relatively higher ionization energies, great care must be taken while choosing the kind of solvent and additives used for the mobile phase (Byrdwell, 2001).

2.3.2.1.3 Atmospheric Pressure Photoionization (APPI)

This is a soft ionization method for liquid chromatography-mass spectrometry (LC-MS) known as atmospheric pressure photoionization ionization (APPI) uses photochemical activity to ionize materials in the gas phase. he APPI facilitates the analytical detection of weakly polar and non-polar compounds by mass spectrometry. Molecules are ionized using a vacuum ultraviolet light source operating at atmospheric pressure (105 Pa), either by direct absorption followed by electron ejection or through ionization of a dopant molecule that leads to chemical ionization of target molecules. The sample is typically a solvent spray that is heated and nebulized before being vaporized. The benefit of APPI is that it ionizes molecules across a broad range of polarity and is particularly useful for ionization of low polarity molecules for which other popular ionization methods such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) are less suitable (Himmelsbach *et al*., 2009; Hanold *et al.,* 2004).

2.3.2.2 The Mass Analyzer

The traditional view of the mass spectrometer is of a large, floor standing instrument utilizing a large electromagnet to provide a high-resolution mass spectrum. In practice, this type of instrument has seen only limited application for LC-MS. As mentioned above, most LC-MS instruments use an analyzer comprising several components in tandem, with the most popular for quantitative LC-MS being the triple quadrupole (QqQ) spectrometers..

2.3.2.2.1 Tandem Mass Spectrometry

Despite the availability of a wide range of mass analyzers each with different performance characteristics, QqQ instruments have been the preferred choice for most routine targeted quantification assays as they offer the best performance characteristics for quantitation. The basic principle of QdQ is the selection of a precursor ion, fragmentation of this ion, usually by collision-induced dissociation (CID) and measurement of the mass-to-charge ratio (*m/z*) of the product ions formed. The traditional approach to mass spectrometry involves scanning a wide mass range to obtain a mass spectrum.

However, for LC-MS applications QqQ instruments are typically operated in selected reaction monitoring (SRM) mode which is also called multiple reaction monitoring (MRM) by some suppliers. This involves continuously monitoring a small number of selected transitions (*i.e.,* mass numbers) for each analyte, typically, one precursor ion to a couple of product ions. This approach provides a significant gain in sensitivity compared with acquiring full spectral data. Further gains have been achieved by steady innovation in instrumentation. Modern electronic technology and faster data acquisition interfaces, together with better designs of collision cell to allow faster clearing of ions from the cell, significantly shorten the minimum dwell times that can be used for each precursor/product ion pair monitored, without significantly reducing signal-to-noise ratios (S/N) or introducing crosstalk. The latter is the term used to describe the phenomenon when the fragment ions from one SRM transition are scanned out during another transition. The tandem mass spectroscopy has been successfully employed for the determination of non-steroidal anti-inflammatory drugs in bovine plasma using (Dowling & Malone, 2011).

2.3.2.3 Detectors

Liquid chromatographs are equipped with a means to continuously monitor the column effluent and to recognize the presence of solute. Only small sample sizes are used with most HPLC columns, so a detector must have high sensitivity (Tautenhahn, *et al*., 2008).

The type of detector that has the most universal application is the differential refractometer. This device continuously monitors the refractive index difference between the mobile phase (pure solvent) and the mobile phase containing sample (column effluent). The most widely used HPLC detectors are the photometric detectors. These detectors measure the extent of absorption of ultraviolet or visible radiation by the sample. A third type of detector that has only limited use is the fluorescence detector. This type of detector is extremely sensitive; its use is limited to samples

2.3.2.4 Direct Infusion Sample Introduction

Sometimes, especially with thermally labile compounds, it is possible to introduce samples directly to the spectrometer in the liquid phase. This method is called direct infusion. In this case, ionization takes place in the condensed phase, and a syringe pump is necessary to continuously deliver the sample into the spectrometer ion source. Other techniques are used for direct infusion, but syringe pumps are the most common and reliable and they are good for tuning the instrument (Garofolo, 2004). Syringe pumps are also commonly used for delivery calibration solution and matrix addition in MS.

2.3.2.5 Analysis of LC-MS/MS Data

Most LC instruments are online with an integrator and a computer for data handling. For quantitative analysis of LC data, operating parameters such as rate of solvent flow must be controlled. In modern instruments, the whole system including the pump, injector, detector and data system is under control of a computer.

2.3.2.6 Stationary Phases in LC

The adsorbents in LC are typically small diameter porous materials. In the early days of HPLC, solid supports were coated with a liquid stationary phase. Columns with these packings had short lifetimes as a gradual decrease in resolution because there was continuous loss of the liquid stationary phase with use of the column. This problem was remedied by the discovery of methods for chemically bonded stationary phases. The major advantage of a bonded stationary phase is stability. Since it is chemically bonded,

there is very little loss of stationary phase with column use. The use of non-polar chemically bonded stationary phases with a polar mobile phase is referred to as reverse phase LC. This technique separates sample components according to hydrophobicity. It is widely used for the separation of all types of biomolecules. Typical solvent systems are water, methanol, water acetonitrile, and water-tetrahydrofuran mixtures

2.3.2.7 Solvents for LC Operation

The eluting power of a solvent is related to its polarity. Chromatographic solvents have been organized into a list according to their ability to displace adsorbed solutes (eluting power, ε°). The ε° increases with an increase in polarity. Using the eluotropic series makes solvent choice less a matter of trial and error. Occasionally, a single solvent does not provide suitable resolution of solutes. Solvent binary mixtures can be prepared with eluent strengths intermediate between the ε° values for the individual solvents (Kluters *et al*., 2015).

2.3.2.7.1 Gradient Elution in LC

The common difficulty encountered in the analysis of multi-component samples is referred to as the general elution problem. It is due to the fact that the components have a wide range of *K*D values, and no single solvent system is equally effective in displacing all components from the column. The general elution problem is solved by the use of gradient elution. This is achieved by varying the composition of the mobile phase during elution. In practice, gradient elution is performed by beginning with a weakly eluting solvent (low ε°) and gradually increasing the concentration of a more strongly eluting solvent (higher ε°). The weaker solvent is able to improve resolution of components having low *K*D value. In addition, the gradual increase in ε° of the solvent mixture decreases the line broadening and provides a more effective separation. The choice of solvents for gradient elution is still somewhat empirical, but data from eluotropic series may give some guidelines. Modern HPLC equipment is equipped with solvent

programming units that control gradient elution in a stepwise or continuous manner (Kluters *et al*., 2015).

2.3.2.7.2 Isocratic Mode Elution

If the composition of the mobile phase remains constant throughout the HPLC separation, the separation is deemed an isocratic elution. Chromatographers are cautioned to avoid gradient elution when isocratic elution will do (Schellinger and Carr, 2006). Here, the concentration of the mobile phase is constant throughout the chromatographic process. In this process, we can observe the peak width increasing with retention time linearly in the chromatogram. However, this leads to a disadvantage – the late-eluting peaks for late elution get very flat and broad.

Therefore, these broad peaks become difficult to be recognized as peaks. Moreover, in isocratic elution, the selectivity does not change according to the column dimensions. This means selectivity does not depend on the changes in column dimensions. Here, the length and diameter are considered as column dimensions. Therefore, the peaks elute in the same order (Ehlert *et al*., 2010).

2.3.3 Fourier Transform Infrared Spectrometer (FT-IR)

The FTIR instrument sends infrared radiation of about 10,000 to 100 cm^{-1} through a sample, with some radiation absorbed and some passed through. FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. The absorbed radiation is converted into rotational and/or vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000 cm^{-1} to 400cm^{-1}, representing a molecular fingerprint of the sample. Each molecule or chemical structure will produce a unique spectral fingerprint, making FTIR analysis a great tool for chemical identification. This technique is useful for analyzing the chemical composition of smaller particles, typically 10 -50 microns, as well as larger areas on the surface.

2.3.3.1 Working of FT-IR

The interferometer consists of a beam splitter, a fixed mirror, and a mirror that translates back and forth, very precisely. The beam splitter is made of a special material that transmits half of the radiation striking it and reflects the other half. Radiation from the source strikes the beam splitter and separates it into two beams. One beam is transmitted through the beam splitter to the fixed mirror and the second is reflected off the beam splitter to the moving mirror. The fixed and moving mirrors reflect the radiation back to the beam splitter. Again, half of this reflected radiation is transmitted, and half is reflected at the beam splitter, resulting in one beam passing to the detector and the second back to the source. It uses the standard KBr method, with spectral resolution set at 4 cm^{-1} and the scanning range from 400 to 4000 cm^{-1} (Ponce *et al*., 2013). Figure 2.10 shows block diagram of FT-IR.

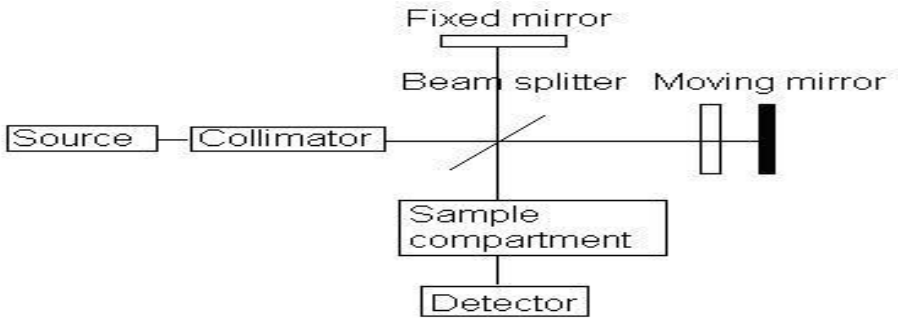

Figure 2.10: Block diagram of FT-IR (Griffiths and de Haseth, 2007)

2.3.4 X-ray Diffractometer

X-ray scattering techniques are a family of non-destructive analytical techniques which reveal information about the crystal structure, chemical composition, and physical properties of materials and thin films. These techniques are based on observing the scattered intensity of an X-ray beam hitting a sample as a function of incident and scattered angle, polarization, and wavelength or energy. (Griffiths and de Haseth, 2007).

2.3.4.1 Working of XRD

X-ray diffractometers consist of three basic elements: An X-ray tube, a sample holder, and an X-ray detector. X-rays are generated in a cathode ray tube by heating a filament to produce electrons, accelerating the electrons toward a target by applying a voltage, and bombarding the target material with electrons. When electrons have sufficient energy to dislodge inner shell electrons of the target material, characteristic X-ray spectra are produced. Figure 2.11 shows the diagram of XRD.

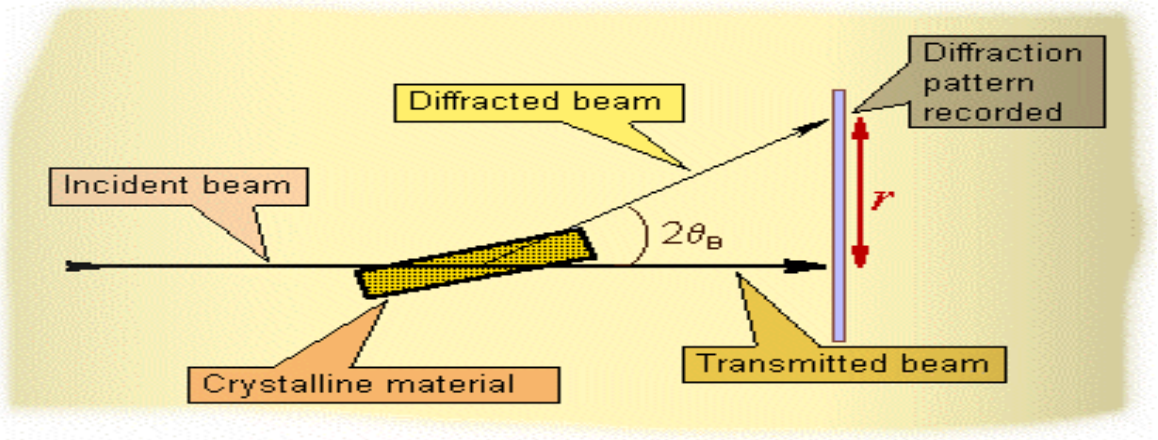

Figure 2.11: Diagram of XRD (Al Masud and Chowdhury, 2020)

2.3.5 Scanning Electron Microscope (SEM)

The Scanning Electron Microscope (SEM) is used for observation of specimen surfaces. When the specimen is irradiated with a fine electron beam (called an electron probe), secondary electrons are emitted from the specimen surface (Yamada *et al.*, 2004). Topography of the surface can be observed by two-dimensional scanning of the electron probe over the surface and acquisition of an image from the detected secondary electrons SEMs provide a 3D image of the surface of the sample. The number of secondary electrons that can be detected, and thus the signal intensity, depends, among other things, on specimen topography (Klein *et al.*, 2012; Butterfield *et al.*, 2017).

The SEM uses Energy-Dispersive X-Ray Spectroscopy (EDS) in the production of elemental maps, which accurately represent the distribution of elements within samples. The most typical use is elemental analysis, mineral orientation, morphology and contrasts (Debbie, 2008). Morphology indicates the shape and size, while topography indicates the surface features of an object or "how it looks", its texture, smoothness or roughness. Likewise, composition means elements and compounds that constitute the material, while crystallography means the arrangement of atoms in the materials (Khan and Lo, 2018). Due to the very narrow electron beam, SEM micrographs have a large depth of field yielding a characteristic three-dimensional appearance useful for understanding the surface structure of a sample (Echlin *et al*., 2013). Figure 2.12 is the diagram of Scanning Electron Microscope

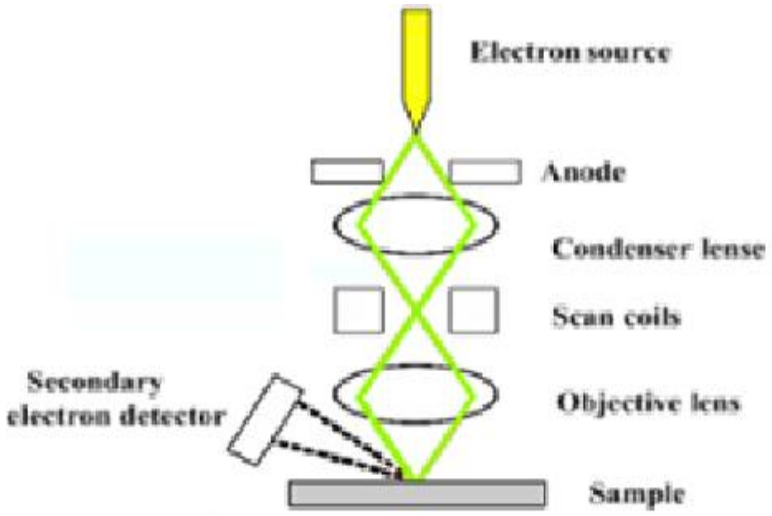

Figure 2.12: Diagram of scanning electron microscope (Debbie, 2008)

2.4 Adsorption Isotherms

Several studies have adopted the use of mathematical modeling and representation to represent adsorption (Mahmoud *et al*., 2012; Kilic *et al*.,2011; Soto *et al*., 2011). Langmuir and Freundlich isotherms are the most commonly used isotherms used to

describe adsorption. They form the basis of feasibility study on any potential adsorbent (Alok *et al*., 2007).

2.4.1 Langmuir Isotherm

Langmuir isotherm is more widely used compared to freundlich. It assumes uniform adsorption energies along the homogeneous adsorbent surface (Soto *et al*., 2011) and equal affinity in all the adsorption sites (Kilic *et al*., 2011). It predicts no interaction among adsorbed molecules (Alterior *et al*., 2008) as well as single adsorption mechanism (Hameed *et al*., 2008). It is also based on the assumption that molecules form a monolayer on the free surface (Mahmoud *et al*., 2012). Equation for the Langmuir adsorption isotherm is used in the estimation of the maximum adsorption capacity (qmax). Where "qmax" is the maximum quantity of adsorbate adsorbed per gram of activated carbon. The equation is represented as:

$$qe = \frac{qm\ bCe}{1+bCe} \quad \text{..... (2.2)}$$

Where q_m is the monolayer adsorption capacity (mg/g), b is the Langmuir constant (L/mg), *Ce*is the equilibrium constant (mg/L) and *qe* is the amount adsorbed on adsorbent at equilibrium (mg/L) (Soto *et al*., 2011). A plot of $\frac{Ce}{qe}$ against Ce gives a straight line.

The above equation can be rearranged to give the linearized equation (2.3)

$$\frac{C_e}{q_e} = \frac{1}{q_m^b} + \frac{C_e}{q_m} \quad \text{..... (2.3)}$$

2.4.2 Freundlich Isotherm

Freundlich isotherm is an empirical equation suited for non-ideal systems with highly heterogeneous surfaces (Alterior *et al*., 2008; Hameed *et al*., 2008). It gives good interpretation of data over a restricted concentration range. The equation is represented as:

$$qe = K_F Ce^{1/n} \quad \text{..... (2.4)}$$

Where *KF* is the capacity of adsorption (L/mg) and *n* is the intensity of adsorption.

A plot of Log qe against Ce gives a straight line where the constants $\frac{1}{n}$ and log K_F can be determined from the intercept and the slope respectively. The above equation (2.4) can be represented in linear form to give equation (2.5)

$$\text{Log} \quad q_e = Log\ K_f + \left(\frac{1}{n}\right) LogCe \quad \text{.....(2.5)}$$

2.5 Kinetics Studies

Evaluation of adsorption kinetics is carried out using pseudo-first - order, pseudo – second order and intra-particle diffusion models (Kilic *et al*., 2011).

2.5.1 Pseudo-First Order Kinetic Model

Lagergren pseudo-first order is expressed as:

The Pseudo-first order model is based on the assumption that the adsorption rate is proportional to the number of available sites (Lin and Wang, 2009). The model of Pseudo first–order is given by equation 2.6.

$$\ln(q_e - q_t) = \ln q_e - K_1 t \quad \text{..... (2.6)}$$

Where q_e (mg/g) and $q_t(mg/g)$ are the adsorption capacity at equilibrium and at time t (min) respectively and qe is the concentration of the pharmaceutical adsorbed

K_1 (min^{-1}) is the Pseudo first-order rate constant of adsorption. The value of K_1 and q_e are calculated from the slope and intercept respectively of the linear plot of $\ln(q_e - q_t)$ versus $\ln q_e$.

2.5.2 Pseudo Second Order Kinetic Model

The equation for the pseudo-second-order is expressed as:

$$\frac{t}{q_t} = \frac{1}{K^2 q_e^2} + \frac{t}{q_e} \quad \text{.....(2.7)}$$

Where $q_e\left(\frac{mg}{g}\right)$ and $q_t\left(\frac{mg}{g}\right)$ are the adsorption capacities at equilibrium and at time $t\ (min)$spectively and q_e^2 is the concentration of pharmaceutical adsorbed. K_2 and q_e^2 are obtained from a plot of $\frac{t}{q_t}$ versus t.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Areas

3.1.1 Study Area 1: Nairobi River

Nairobi river basin (1.1997° S, 37.1571° E) is made up of three main rivers; Mathare, Ngong and Nairobi. It flows through many formal settlements including Kawangware, Majengo and Dandora. All these settlements are densely populated hence prone to poor disposal of pharmaceuticals. Ngong River which drains to Nairobi River passes through Africa's largest slum, the Kibra slums (Erulkar and Mathaka, 2007; Ngumba *et al*., 2016). Figure 3.1 shows the Nairobi River basin.

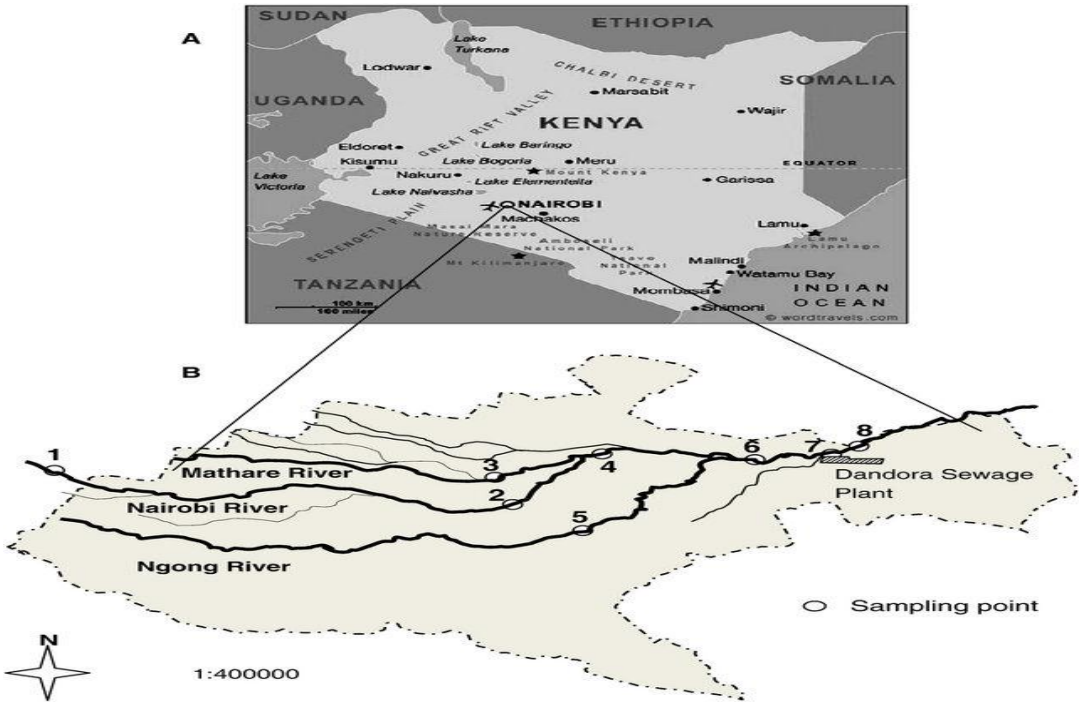

Figure 3.1: Nairobi River water basin map (K'oreje *et al*., 2012)

3.1.2 Study Area 2: JKUAT Wastewater Treatment Plant

The Wastewater Treatment Plant (WWTP) in Jomo Kenyatta University of Agriculture and Technology (JKUAT) is situated 32 kilometers from Nairobi central district (1°5'33"S 37°1'6"E) and it serves a population of over 20,000 residents (Ngumba *et al*., 2016). Jomo Kenyatta WWTP is located within Jomo Kenyatta University. The university covers an area of 200 acres. It serves a student population of about 10,000. This WWTP uses stabilization ponds which include anaerobic, facultative and maturation ponds with retention time of 60-90 days and it employs primary mechanical processing followed by biological treatment (Blánquez, *et al.*, 2020). Figure 3.2 shows location of JKUAT WWTP.

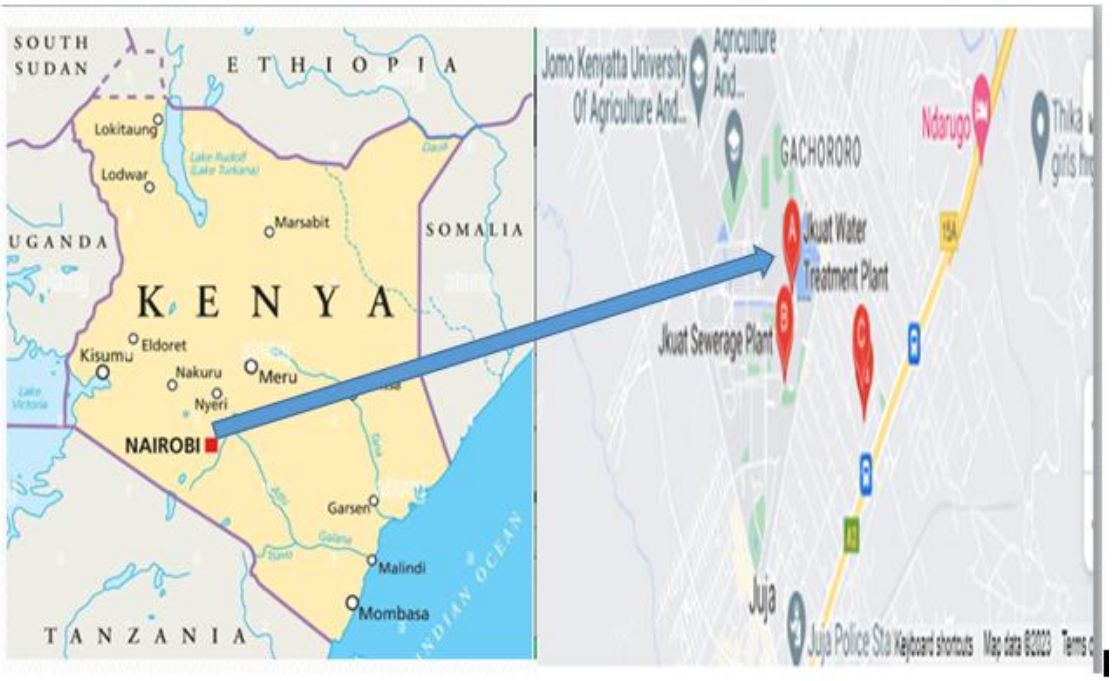

Figure 3.2: Location of JKUAT waste water treatment plant

3.2 Sample Collection

The rice (*Oryza glaberrima)* husks were collected from Nice Rice Millers Ltd located at 0`40`0`S and 37’220`E. It is one of the biggest millers in Mwea Kirinyaga county and is

allocated in Wang`uru town. The rice in Nice Millers Ltd is obtained from farmers in all sub- county schemes that is Tembere, Nguka (Mwea), Thiba, Wamumu and Karaba. The rice husks were put in the sacks and then transported to Jomo Kenyatta University of Agriculture and Technology analytical chemistry laboratory, where preparation of samples, pyrolysis and adsorption analysis were carried out. Bacteria for biodegradation experiments were obtained from JKUAT botany laboratories while some were cultured.

3.2.1 Wastewater Sampling

Wastewater samples were collected from two sampling points. One was Nairobi River at the river road junction while the one was JKUAT WWTP. In JKUAT WWTP the samples were collected before and after treatment, two times a day and four times a month. Sampling was done in August 2018, which is usually a dry month preceding short rains. It was done for two consecutive months. From each of the effluent and influent sampling points, 200cm^3 of wastewater were collected by grab method (n=8) over a period of 8 hours and pooled together into a 1 L composite sample. They were then taken to Jomo Kenyatta University of Agriculture and Technology analytical chemistry laboratory where they were stored at 4°C and analyzed within 24 hours. Batch experiments were done using model solutions made by spiking ethanol-water mixture 50/50 with the three drugs: paracetamol, trimethoprim, and nevirapine. Later experiments were done using wastewater obtained from Jomo Kenyatta University WWTP and Nairobi River water.

3.2.2 Rice Husks Sample Preparation

The rice husk samples were prepared according to the method by Wong *et al.,* (2003). The rice husks were initially washed thoroughly with tap water to remove mud, any other adhering particles and other water-soluble impurities. They were then dried at 110°C for 8 hours, ground to fine powder and sieved to obtain a powder with a particle size of 1 - 2 mm.

3.3 Chemicals and Reagents

Methanol, acetonitrile and formic acid were HPLC-grade and were purchased from Sigma Andrich (Germany) while paracetamol, trimethoprim and nevirapine standards were obtained from Universal Corporation Ltd, Kenya. Ultrapure water which was used throughout the study was obtained from Kenyatta university chemistry laboratory. Unless otherwise stated all the other reagents used were of analytical grade or above.

3.3.1 Preparation of Stock and Calibration Standard Solutions

Individual Stock solutions of each pharmaceutical were prepared by dissolving 0.001mg in 1 ml of methanol/ water mixture (50/50) equivalent to 1000ppm per litre and stored at -4℃ in the dark. Working solutions were prepared by appropriate dilutions of the stock solution (Schaefer *et al.,* 2019).

3.4 Instrumentation

All pharmaceutical concentrations were analyzed using LC-MS/MS (Agilent HP 1100 LC) coupled to a Waters Quattro-Ultima mass spectrometer. Characterization of activated rice husks was done using scanning electron microscopy (SEM), Fourier transform infrared (FTIR) and X-ray diffractometer (XRD). Optical density was measured using UV spectrophotometer. A Millipore filtration unit equipped with 0.45um cellulose acetate filter membrane was used for wastewater filtration. A digital pH meter (211 HANNA, UK) was used for pH measurement and optimization (Vera *et al.,* 2020). Oasis hydrophilic –lipophilic HLB 6cc. (200 mg) solid phase extraction cartridges purchased from waters (Milford, USA) (Tan *et al.,* 2015). PDF micro filters and Whatman filter papers were obtained commercially.

3.5 Carbonization and Chemical Activation of Rice Husks

About100 g of rice husks were carbonated at temperatures of 300℃, 400℃, 500℃ and 600℃. in a muffle furnace (Advatec-KL-420 Japan) for a duration of 2 hours. The samples were then crushed and sieved to a size of about 800 micrometers and then kept

in an oven at 100℃ for 24 hours (Zhang *et al.*, 2017). The rice husks biochar was then impregnated with 1 dm^3 of 3 M H_3PO_4, at 80℃ for 3 hours. It was then cleaned with distilled water until a pH of 7 was attained and dried in an oven at 105°C for 24 hours. The dried samples were finally stored in a desiccator.

3.6 Determination of Physical Properties of Rice Husks Activated Carbon

3.6.1 Determination of Moisture Content

The method proposed by Zhang *et al*. (2012) was adopted. Moisture content was determined by oven drying 5.0 g of rice husks at 110℃ for 24 hours.

The experiment was done in triplicate. The moisture content was calculated using equation 3.1.

$$\%\ \text{Moisture Content} = \frac{\text{Initial mass} - \text{Mass of dried rice husks}}{\text{Initial mass}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.1)$$

3.6.2 Determination of Ash Content

The ash content was determined using a method adopted from Otaru *et al*. (2013). 10.0 g of rice husks samples carbonated at temperatures of 300℃, 400℃, 500℃ and 600℃, were oven dried separately at 80℃ for 24 hours. The dried samples were then heated in a muffle furnace at 800℃ for 3 hours in open crucibles. After heating, the crucibles and their contents were cooled in a desiccator and then weighed. The experiment was done in triplicate. The ash content was calculated using equation 3.2.

$$\%\ Ash\ content = \frac{\text{Remaining solid weight}}{\text{Original material weight}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.2)$$

3.6.3 Bulk Density

The bulk density was determined using a method adopted from Sugumaran *et al.*, (2012). A glass cylinder (25 cm^3) was filled with finely ground activated carbon powder and dried overnight in an oven at 80℃. The cylinder was cooled and then tapped for 1 - 2 minutes for the carbon to compact and the bulk density calculated using equation 3.3.

$$Bulk\ density = \frac{\text{Weight of dry material(g)}}{\text{Volume of packed dry material(cm3)}} \quad \text{.....} \quad (3.3)$$

The experiments were carried out in triplicate and the average value recorded

3.6.4 Determination of Iodine Number

The method was adopted from Birbas *et al*. (2011). Activated carbon of mass 0.1 g was put into the conical flask containing 25 cm^3 of iodine solution. The sample was mixed with iodine solution for about a minute by swirling. The activated carbon – iodine mixture was then filtered and 10 cm^3 of the filtered solution transfered into another flask using a volumetric pipette. The filtered solution was then titrated with 0.04 N sodium thiosulphate solution until it becomes colourless. The experiment was done in triplicate. Iodine number was calculated as shown in equation (3.4)

$$\text{Id. No.} = \frac{(Vb-Vs).N.1.5M1}{m} \quad \text{.....} \quad (3.4)$$

Where:(Vb-Vs) is the difference between results of test without biosorbent and the test with biosorbent (ml of sodium thiosulfate 0,1N); N, normality of sodium thiosulfate solution (mol/L); MI, Molar mass of iodine (126.9 g/mol); m, the mass of biosorbent (g).

3.6.5 Determination of Percentage Yield

This was done using a method adopted from Gaskin *et al*. (2008). Rice husks samples (10.0 g) were put in a muffle furnace and heated at 400℃ for one hour, cooled and washed several times. This was done in triplicate. The final sample was then dried to a constant mass in an oven at 110℃. Thereafter, the percentage yield was calculated as

$$\text{Percentage yield} = \frac{Mass\ of\ biochar}{Mass\ of\ oven\ dried\ rice\ husks} \times 100 \quad \text{.....} \quad (3.5)$$

3.7 Characterization of Rice Husks Biochar Carbonated at Different Temperatures

Functional groups on the surface of biochar, surface morphology and degree of crystallinity are parameters that affect the efficiency of the rice husks activated carbon. They were analysed using FTIR, SEM and XRD respectively.

3.7.1 Fourier Transform Infra-Red Spectroscopy (FT-IR) Characterization

The functional groups were determined using a Shimadzu 8400 Fourier Transform Infrared spectrophotometer Shimadzu, Japan operated between 400 and 4000 cm^{-1}. A 0.02 g sample of rice husk activated carbon was ground into a powder and then mixed with 0.3 g of anhydrous KBr. The pellets were obtained by pressing the mixture in a vacuum before analysis to obtain respective IR spectra (Wanakai *et al.,* 2019).

3.7.2 Determination of Surface Morphology

This was analyzed using scanning electron microscopy technique (SEM, Quanta FEG 250) at 5.00KV. Everhart – Thornley detector (ETD) was used as a detector and a working distance (WD) of 10.1 mm was used for high resolution imaging. The activated carbon sample was ground to a powder and mounted on the standard specimen stubs using adhesive tape and then coated with gold layer to prevent the sample from charging (Negara *et al.,* 2019).

3.7.3 Determination of Degree of Crystallinity of the Biochar

The degree of crystallinity or amorphous nature of rice husks activated carbon determined at the department of geology and mining in Nairobi Kenya using a Riguka Miniflex II X-ray diffractometer Riguka, Japan, fitted with a nickel filtered Cu-Kα radiation source. Rice husks activated carbon were dried and finely ground and tested at 40 kV and 40 mA, using argon filled proportional counter detector. A scan mode $2\Theta = 5 – 90°$ and a scan rate of 3◦ / min was used to collect x-ray diffraction patterns (Madivoli *et al.,* 2020).

3.8 LC-MS/MS Determination of Concentration of Pharmaceuticals

Paracetamol, trimethoprim and nevirapine concentrations were determined using an Agilent 100 series (USA) HPLC systems consisting of a binary pump, a vacuum degasser an auto-sampler and a column oven. It was fitted with a reversed phase C18 column Kinetex EVO (100mm x 3.0mm, 5mm particle size 100 A). For trimethoprim and nevirapine which were not very soluble in water, the mobile phase constituted of 70% deionized water and 30% acetonitrile. For paracetamol the mobile phase was 80% water and 20% acetonitrile. The de-ionized water and acetonitrile were both spiked with 0.1% formic acid as a buffer and to help in generation of protonated molecular ions. Isocratic elution was used with a total run time of 5 minutes while the retention time was between 2 and 2.5 minutes. The flowrate was 0.45 ml/min while column temperature was maintained at 40℃. The residual concentrations were determined using LC-MS/MS while the percentage removal was calculated using the equation:

$$\% \text{ Removal} = \frac{Co - Ct}{Co} \text{x } 100 \quad \ldots\ldots \quad (3.6)$$

Where Co is the initial concentration (mg/L) Ct is the final concentration after time t (mg/L). The amount of pharmaceutical adsorbed at equilibrium qe (mg g^{-1}) was calculated according to the equation

$$Qe = \left(\frac{ci - ce}{m}\right)V \quad \ldots\ldots \quad (3.7)$$

Where:

Qe: the equilibrium drug concentration on adsorbent (mg/g).

Ci: the initial concentration (mg/L).

Ce: the equilibrium concentration (mg/L).

V: the volume of drug solution (L).

m: the weight of adsorbent (g).

A Quattro ultima micromass triple quadruple mass spectrometer (Quattro mass UK) was used as both the detector and mass analyzer. Nitrogen gas generated by a nitrogen generator (Peak Scientific UK) was used as a desolvation and cone gas. Desolvation temperature was 350℃ while source temperature was 150℃. Argon gas was used as a collision gas at a collision pressure of 2.8×10^{-4} M bars. Analysis was done in electro spray ionization positive mode (ESI +) operating in a multiple reaction monitoring (MRM) with a dwell time of 200 ms. Two productions were generated from the precursor ion with one ion used for quantification while the other was used for confirmation (Martos *et al.,* 2010).

3.9 Method Performance Characteristics

The LC-MS/MS analytical method performance characteristics of linearity, selectivity and precision were evaluated.

3.9.1 Linearity

The linear regression equations and correlation co-efficient (R^2) for each of the three pharmaceutical drugs were used to determine linearity (Saadati *et al.,* 2013).

3.9.2 Limit of Detection (L.O.D)

The limit of detection was determined using calibration standards. It was evaluated as L.O.D = 3.3 σ/s where s is the slope of the calibration curve and σ is the standard deviation of the four replicate measurements of blank solution (Evard *et al.,* 2016).

3.9.3 Limit of Quantification (L.O.Q)

The limit of quantitation was determined using calibration standards using the formula; L.O.Q = 10 σ/s where s is the slope of the calibration curve and σ is the standard deviation of the response (Ikonen *et al.,* 2020).

3.9.4 Recovery Studies

For recovery studies, duplicate sets of 200 ml of a blank made of water that did not contain detectable quantities of pharmaceuticals and spiked with paracetamol, trimethoprim and nevirapine were taken through the extraction process with oasis HLB (6 c.c 200 mg) SPE cartridges. The recoveries were calculated by comparing the response of the analyte in the standard solution to that of the post-extract blank spiked with the analyte at the same concentration, equation 3.7 (Biselli *et al.*, 2013).

$$\% \text{ Recovery} = \frac{\text{Sample peak area}}{\text{Standard peak area}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.8)$$

3.10 Determination of Optimum Carbonation Temperature of the Biochar

In this study, rice husks biochar was carbonated at temperatures of 300, 400, 500 and 600℃ for a constant duration of 2 hours. The temperature was varied with an aim of obtaining the optimum carbonation temperature that would obtain a biochar with the best adsorption capacity for paracetamol, trimethoprim and nevirapine from wastewater systems (Fu *et al.,* 2019). The biochar carbonated at the optimum temperature was used to perform batch equilibrium studies.

3.11 Batch Equilibrium Experiments

Adsorption experiments were carried out using activated carbon carbonated at temperatures described in (3.11) to explore the effect of different parameters which may affect adsorption of the pharmaceutical drugs. This was done using a method adopted from Li *et al*. (2007). After the activation, rice husks biochar was applied in adsorption of paracetamol, trimethoprim and nevirapine. The parameters optimized included pH, contact time, adsorbent dosage and initial drug concentration.

3.11.1 Determination of Optimum Contact Time

Stock solutions containing 1 ppm of paracetamol, trimethoprim, and Nevirapine were prepared in 25 ml volumetric flasks. A series of five (5) mililitres of the 1 ppm drug

solution was placed in 100 ml conical flasks and 0.1 g of activated carbon of rice husks was added to each flask. The contents were placed on rotator shaker at different time (min) intervals of 15, 30, 60, 90 and 120 min. After completion of the pre-set intervals, the solutions were filtered and kept at 4℃. (Nassar *et al.,* 2019).

3.11.2 Determination of Optimum Adsorbent Dosage

Five mililitres (5ml) of each drug solution was placed in six different flasks containing 0.0125, 0.05, 0.1, 0.2, 0.25.and 0.5 g of activated carbon of rice husks. The flasks were shaken at 150 rpm for 60 minutes and kept to be analyzed after filtration using LC-MS/MS (Yu *et al.,* 2020).

3.11.3 Determination of Optimum pH

Five millilitres (5 ml) of the drug solutions were placed in conical flasks and the pH of the solutions adjusted using 0.1 M NaOH and 0.1 M HCl. 0.1 g of activated carbon of rice husks were then added to the flasks containing the different drug solutions at pH of 3, 5, 7, 9 and 11. The contents were placed on a rotator shaker, shaken at 135 rpm, filtered and the filtrate kept to be analyzed using an LC-MS/MS (Yu *et al.,* 2020).

3.11.4 Determination of Optimum Initial Drug Concentration

Five milliliters (5 ml) of aqueous solution of each drug were placed in the conical flasks containing 0.1 g activated carbon of rice husks containing initial drug concentrations ranging from 0.1 to 1.5 ppm. The contents were then shaken at 150 rpm and filtered.

3.12 Wastewater Analysis for Pharmaceutical Residues

3.12.1 Extraction of Pharmaceutical Residues from Wastewater

Paracetamol, trimethoprim and nevirapine residues were extracted from wastewater using solid phase extraction which is a four-step process that include conditioning the cartridge, loading, washing, and analyte elution The solid phase extraction process used Oasis hydrophilic-lipophilic (HLB) 6 c.c /200 mg cartridges. They were conditioned

using 3 ml methanol HPLC grade followed by 3 ml of deionized water. Sample loading was done at a flow rate of 10 ml per minute using SPE vacuum manifold the cartridges were then dried in a vacuum for 10 minutes The analytes were then eluted with 3 ml methanol and evaporated in a stream of nitrogen and then reconstituted with 1 ml of water: methanol mixture 80/20 (v/v) (Dasenaki and Thomaidis, 2015).

3.13 Biodegradation by use of Microorganisms

3.13.1 Microbial Assay Preparation

The assay was prepared using a method by Sharma *et al.* (2020). Microorganisms all from American Type Culture Collections (ATCC), included *Bacillus subtilis* (11778), *Escherichia coli* (25922), *Staphylococcus aureus* (25923) and *Pseudomonas aeroginosa* (27853). They were all obtained from botany laboratory at JKUAT. Before initiating biodegradation studies, the microorganisms were cultured in an optimal nutrient medium in order to grow a healthy breed of microorganisms that can withstand toxicity of the pharmaceuticals. Culturing was performed in a laminar flow hood to ensure a sterile environment (Rechab *et al.,* 2018; Bernal *et al.,* 2017). The healthy isolates were then transferred onto other plates and stored in a freezer. The medium used was HIMEDIA M002-5 Nutrient broth which was prepared by dissolving 13.0 grams in 1000 ml de-ionized water and autoclaved at 121°C. Minimum mineral salt medium (MMSM) prepared in de-ionized water contained the following compounds; KH_2PO_4, NH_4Cl, $Na_2HPO_4.2H_2O$, $CaCl_2.2H_2O$, $FeCl_3.6H_2O$, $MgSO_4.7H_2O$ and K_2HPO_4. The medium's pH was thereafter adjusted to 7.0 and then autoclaved for 20 min at 121°C to kill the existing microorganisms.

3.13.2 Acclimatization of Microorganisms to Pharmaceuticals

Acclimatization is a process where microorganisms are exposed to pharmaceuticals they are supposed to biodegrade so that they can develop appropriate enzymes and get used to the pharmaceuticals. Frozen microorganisms meant for future studies were transferred into conical flasks containing minimum mineral salt medium together with some amount

of glucose to alleviate the microorganisms from starvation. The pharmaceuticals were added in each flask in concentrations to be used for biodegradation experiments. The microorganisms were allowed to grow in presence of 0.5, 1.0 and 1.5 mg/ml of the respective pharmaceuticals for several days, removed and stored at a temperature of 4℃ (da Silva *et al.*, 2021).

3.13.3 Evaluation of Microorganisms Tolerance to Pharmaceuticals.

Effects of pharmaceuticals concentration on growth of the microorganisms was investigated by inoculating acclimatized microorganisms into flasks containing a small amount of glucose and MMSM. This was preceded with separate addition of nevirapine and trimethoprim drugs into these flasks in a range of 0.5 - 10 µg/L upon which the solutions optical densities were measured at 600 nm using a Shimadzu model UV – 1601 PC, Japan.

3.14 Biodegradation Experiments

A volume of 98 ml of minimum mineral salt medium (MMSM) was put into 500 ml conical flasks. They were each spiked with the pharmaceuticals to obtain concentrations of 0.5 µg / ml, 1.0 µg/ ml and 1.5 µg / ml respectively (Plate 3.1). Two mililitres of acclimatized bacterial isolate were then placed in each flask to make up a volume of 100 ml. To prevent possible photodegradation, the flasks were covered with aluminium foil. The temperature was set at 25°C with a rotating speed of 150 rpm. Plate 3.1 shows the biodegradation flasks containing the spiked minimum mineral salt media and the microorganisms.

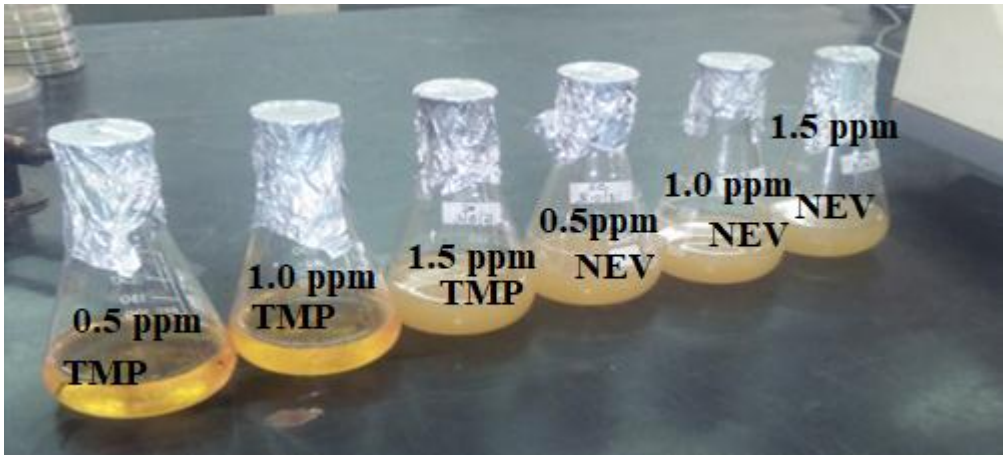

Plate 3.1: Biodegradation experimental flasks containing different concentrations of pharmaceuticals inoculated with bacteria

3.14.1 Control Experiments

Three control experiments were set up. The first one without the microorganisms to account for the drug's abiotic degradation, the second with dead biomass to account for sorption to the biomass and the third one to determine the effect of autoclaving at temperatures of 121°C meant to kill other microorganisms present (Al-Gheethi *et al*., 2019; Gauthier *et al.*, 2010).

3.14.2 Autoclaved and Non-Autoclaved Pharmaceuticals

Two model solutions were prepared in triplicate flasks containing 50 % deionized water and 50 % methanol at concentration of 0.5 ppm of each pharmaceutical. One flask was autoclaved while another was not autoclaved. These flasks were taken through the same conditions as those used in biodegradation experiments. After the experiment, one milliliter of each solution was filtered with a PVDF filter and put in an HPLC vial and its concentration determined using LC-MS/MS.

3.14.3 Abiotic Control

The abiotic control was also prepared in de-ionized water- methanol mixture, with the pharmaceutical present in the media but without any microbe. The abiotic control was

used to determine the degradation of the pharmaceuticals in the conditions used during the experiments (Gauthier *et al.,* 2010).

3.14.4 Sorption by Dead Biomass

The dead biomass was prepared by autoclaving the HIMEDIA M002-5 broth containing each of the selected microorganisms at 121°C for 20 minutes. The broth containing the non-living cells was then lyophilized overnight in Labconco freeze drier (JAPAN). The product was then ground into a fine powder and was used as dead biomass. Control with dead biomass was used to measure the extent to which the pharmaceuticals adsorbed on to the autoclaved biomass (Lucas *et al.,* 2018; Vasiliadou *et al.,* 2013).

3.14.5 Monitoring Bacterial Growth

Assessment of bacterial growth was made possible by diluting 1 ml of biodegradation contents in the flasks with 2 ml of deionized water and measuring the absorbance using a UV-vis spectrophotometer (Shimadzu model UV – 1601 PC) at a wavelength of 600 nm (Emeka *et al.,* 2014). This was done after a period of 4 hours until the optical density started to decrease. To estimate drug concentration, 2 ml of the culture medium was taken and centrifuged at (6000 rpm) for 10 min and the supernatant filtered through a Millipore filter (0.45 μm) and stored at -4℃ in the dark until analysis.

3.15 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) was used. Experiments for statistical data analysis were carried out in triplicates and statistical tools such as mean, standard deviation, sum of square error (SSE), chi – square test and correlation coefficient (r) used to process experimental data. Adsorption data was fitted to the Langmuir, Freundlich, pseudo-first order and pseudo-second-order models. Coefficient of determination (R^2) and chi – square tests were used to determine the best fit for various models.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Physical Properties of Rice Husks Carbonated at Different Temperatures

The physical properties, ash content, bulk density, iodine number, and percentage yield that affect the adsorption capacity of a given adsorbent were determined at 300 ℃, 400 ℃, 500 ℃, and 600 ℃ and results presented in in Table 4.1.

Table 4.1: Physical Properties of Rice Husks Carbonated at Different Temperatures (n=3)

Pyrolysis Temperature (℃)	Ash Content (%) Mean± SD	Bulk Density (g/cm^3) Mean± SD	Iodine Number (g/100g) Mean± SD	Percentage Yield (%) Mean± SD
300	40.07 ± 0.02	6.20± 0.01	805.00 ± 0.02	80.60 ± 0.01
400	38.34 ± 0.03	8.30 ± 0.01	956.00 ± 0.02	50.50 ± 0.02
500	32.00 ± 0.01	9.42± 0.01	1010.00 ± 0.03	30.60 ± 0.02
600	25.00 ± 0.02	12.00± 0.01	1206.00± 0.01	18.40 ± 0.01

4.1.1 Ash Content

From Table 4.1, it was observed that the ash content decreased from 40.07 ± 0.02 to 25.00 ± 0.02 with an increase in pyrolytic temperature of between 300 to 600 ℃. The findings are in concurrence with a study conducted by Ndekei *et al.,* (2021), where it was found that the ash content of biochar decreases with an increase in pyrolytic temperature because higher temperatures volatilize the inorganic materials, such as

minerals and salts, which make up the ash. This leaves behind a more carbon-rich biochar with lower ash content. However, it is important to note that the ash content of biochar can also be affected by the feedstock used, the particle size, and the pyrolysis conditions. According to Abdullah *et al.,* (2001), while preparing and characterizing activated carbon from Gelam wood bark, they recorded the same trend. The lower the ash content the higher the surface area. Thus, it is preferable to use activated carbon from rice husks with lower ash content because ash blocks the pores on the carbon surface, reducing mechanical strength as well as adsorptive capacity.

4.1.2 Bulk Density

The bulk density is an important parameter that determines the interparticle spaces between activated carbon of rice husks (Sharma *et al*., 2004). From Table 4.1 bulk density increases with an increase in temperature, that is 620± 0.01 at a temperature of 300 ℃ to 1200± 0.01 at 600 ℃. Iheanacho *et al.* (2021), while working on adsorption of phenol onto corn cob activated carbon concluded that bulk density is principally influenced by the particle size as well as the structure of cellulose-based fiber which is also dependent on carbonation temperature. It is ideal to use activated carbon from rice husks with a higher bulk density since it avails more interparticle spaces for adsorbate molecules to lodge (Sharma *et al*., 2004). A higher bulk density implies that the particles are packed more tightly together, which leaves less space between them. This affects the adsorption capacity of the activated carbon, as the molecules to be adsorbed need to have space to move around and interact with the surface of the carbon. Therefore, it is ideal to use activated carbon from rice husks with a higher bulk density since it avails more interparticle spaces for adsorbate molecules to lodge.

4.1.3 Iodine Number

Iodine Number is accepted as the most fundamental parameter used to characterize activated carbon performance (Du *et al.,* 2021). It is the amount of iodine adsorbed per 100 g of the material (Hilp, 2002). One important application of iodine adsorption number is that it elicits the surface area of the material as it indicates the macrostructure

of adsorbents which reflects the reaction and adsorption abilities. From Table 4.1, iodine number increased with an increase in pyrolytic temperature. The highest iodine value of 1206± 0.01mg/100g was obtained when the carbonizing temperature was 600 ℃. During the studies, it was noted that increase in the pyrolytic temperature is directly proportional to the surface area, implying that rice husks activated at 600 ℃ had the greatest surface area. The surface area value is much smaller when carbonizing under low temperature due to the low porosity resulting from incomplete carbonization. According to Martin *et al.,* (2003), materials with a high iodine adsorption number have a large surface area, which means that they have a high capacity for adsorbing other molecules. This makes them useful for different applications, such as adsorbing toxins and pollutants, removing impurities from liquids and gases, and catalyzing chemical reactions.

4.1.4 Percentage Yield

The percentage yield is the difference in weight of the respective samples before and after carbonization. The percentage yield decreased with an increase in temperature, that is from 80.6 ± 0.01 - 18.4 ± 0.01 %. As the temperature increases, the equilibrium shifts to the direction of the endothermic reaction, in the process, releasing volatiles through pore opening and gasification at high temperatures. Yahya *et al. (*2018), while reviewing photocatalyst adsorbents for wastewater treatment found that rice husks carbonated at high temperatures (400-900 ℃), leads to decomposition of polymeric structures and production of most of the non-carbon elements, primarily hydrogen, oxygen, and nitrogen, in the form of tars and gases. The remnants form a rigid carbon skeleton; aromatic sheets and strips. Aromatic sheets are made up of benzene rings, which are six-membered rings that are each bonded to three other carbon atoms. Further, aromatic strips are made up of multiple aromatic sheets that are bonded together.

4.2 Characterization Results

The rice husk's biochar was characterized in terms of functional groups, surface morphology, and crystalline nature. These factors highly affect the adsorbate-adsorbent interactions.

4.2.1 FT-IR Spectra of Raw Rice Husks and Activated Biochar

Figure 4.1 indicates the Infrared spectra of raw rice husks and activated carbon obtained at different temperatures between 300 – 600 ℃.

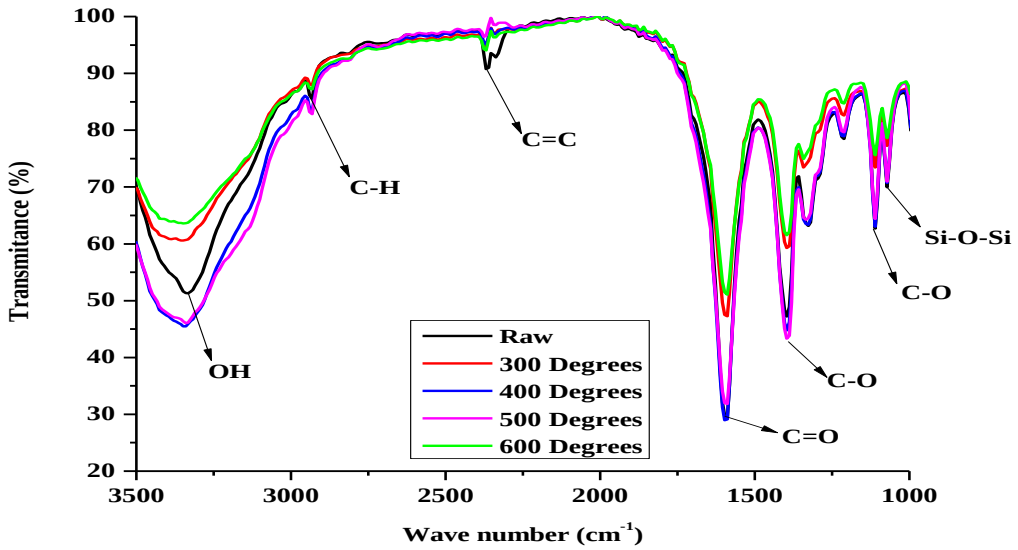

Figure 4.1: Infrared spectra of variation of temperature of rice husks biochar on the surface functional groups.

The Infrared spectra (Figure 4.1), indicates variation of temperature on the biochar's surface functional groups. The raw rice husk IR spectra depicted four major peaks at 1392 cm^{-1} (-C-O stretching vibrations), 3402 cm^{-1} (O-H stretching vibrations), 2368 cm^{-1} (-C=C stretching vibration), and 1113 cm^{-1} (C-O group). The peak ranging between 1600~1800 cm^{-1} is due to the (-C=O stretching vibrations), and 1074 cm^{-1} (Si-O-Si group) (Liu *et al.*, 2018). The surface functional groups of activated rice husks are

affected by the temperature at which they are carbonated. At low temperatures, the main functional groups present are hydroxyl (-OH) groups and alkene (C=C) groups and as the temperature is increased, these groups are decomposed. The prominent band 1074 cm^{-1} in the rice husk ash coupled with narrow peaks are attributed to Si–O–Si stretching (Kiran and Prasad, 2019). The regions (1000-1200 cm^{-1}) contain different bands related to aromaticity, out-of-plane C–H bending, and their varying degrees of substitution. With increased temperature from 300 °C to 600 °C (charring), there was a gradual loss in peaks at 2930 cm^{-1} and 2368 cm^{-1}.which can be attributed to loss of aromatic hemicelluloses leaving behind a porous carbon skeleton.

Optimal pyrolytic temperature for ash removal of pharmaceuticals may vary depending on the specific pharmaceutical and the wastewater matrix. Therefore, it is important to conduct experiments to optimize the pyrolytic temperature for each specific application. Further, the functional groups on the surface of the ash affect its adsorption capacity. Some functional groups, such as hydroxyl groups, are more polar than others and can form hydrogen bonds with the pharmaceutical molecules.

4.2.2 Surface Morphology of Rice Husks Carbonated at Different Temperatures

SEM images of biochar carbonated at different temperatures were taken to compare the morphological changes in the pore structure. The SEM image of biochar carbonated at 300 ℃ (Plate 4.1) shows the presence of a plain surface without well-distinguished pores, whereas at 400 ℃ regular pores start to form. There is also evidence of several button-like structures interspaced with small pores, which were lacking in the rice husks carbonated at 300 ℃. The button-like structures and pores for rice husks activated at 400 °C are attributed to volatiles escaping from the surface because of rapid thermal degradation (Ahmad *et al*., 2012). When rice husks are heated to 400 °C, the volatile components of the husk, such as water, hemicellulose, and cellulose, begin to vaporize. This vaporization creates pressure inside the husk, which causes the husk to expand and form pores. The volatiles also escape from the surface of the husk, leaving behind button-like structures.

Rice husks carbonated at 500 °C had regular pores, button-like structures as well as a larger surface area. At 500 °C the structures of the biochar became more ordered and the pores became wider. The findings are in concurrence with a study conducted by Mopoung *et al.*, (2020) who observed that with a temperature of 500 °C, there was complete degradation of volatile matter and hence destruction of adjacent walls of micro-pores leading to formation of wider pores and bigger cracks hence increasing the surface area. Due to the outlined properties biochar carbonated at 500°C was selected as the optimum. The small valleys and craters observed were the regions from which the volatiles had escaped. Pyrolysis of the cellulosic material selectively consumes the matrix leaving the silica fibers. When the carbonation temperature is increased to 600 ℃ the number of pores reduced and the surface was covered with a lot of ash. The reduction in porosity can be attributed to the realignment or destruction of pore structure at higher temperatures. Angin, (2013) while working on safflower seed cake found that at 600 ℃, biochar had lower surface area owing to the narrowing or closing of the pores. Plates 4.1 shows the SEM micrographs of rice husks carbon pyrolyzed at temperatures between 300 - 600 ℃.

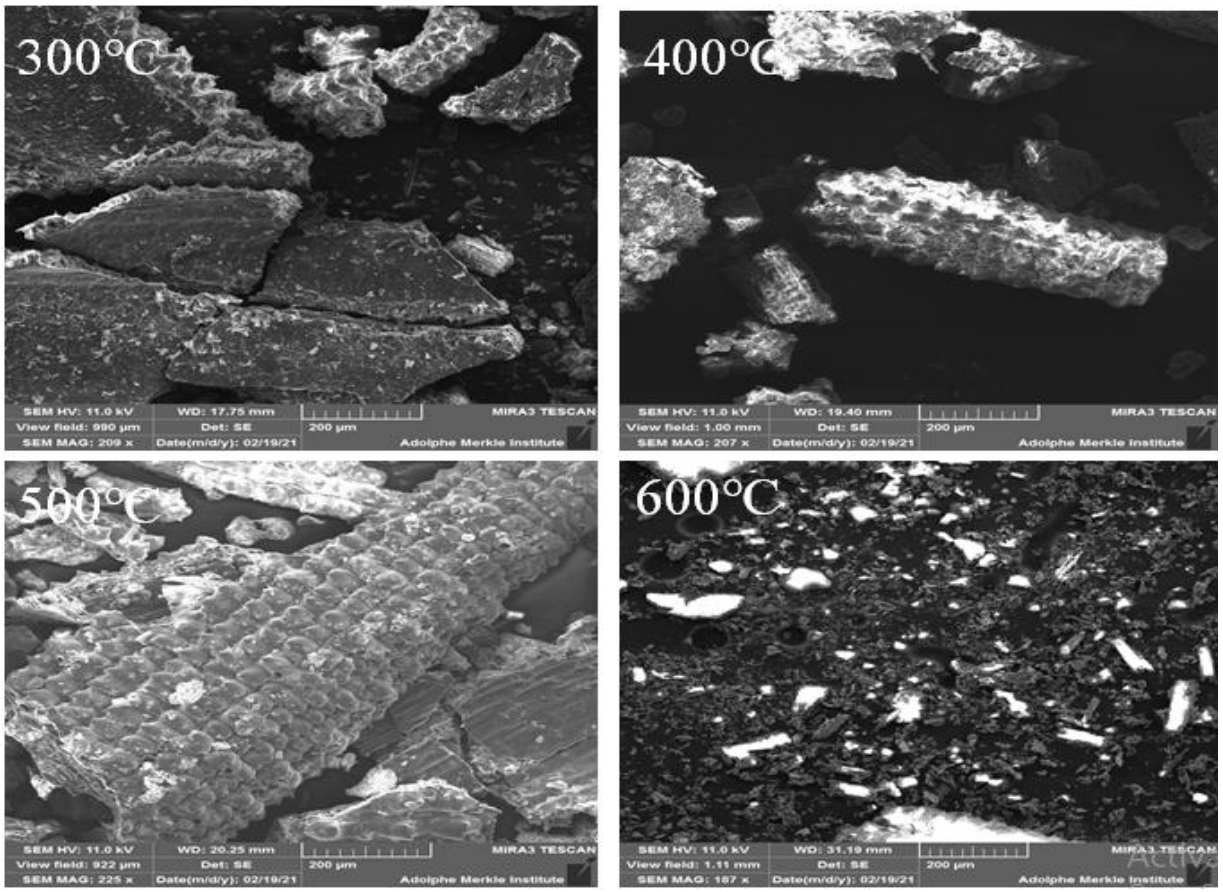

Plate 4.1: SEM Micrographs of rice husks pyrolyzed at temperatures ranging from 300 - 600 ℃

4.2.3 Degree of Crystallinity of Rice Husks Biochar

From Figure 4.2, it was observed that there were intense broad peaks around 2Θ values of 20 ℃ indicating that pyrolytic temperature did not significantly affect the disordered carbon particles. A broad single peak indicates that the activated carbon from rice husks is amorphous. Materials that are amorphous as depicted in the study do not have a regular crystalline structure, so they do not produce sharp peaks in an XRD spectrum. Instead, they produce broad peaks.

The broader the peak, the more disordered the material is. A study conducted by Bidayatul *et al. (*2018) and Zhu *et al*. (2014), found that the crystalline nature of

activated carbon affects its adsorption capacity for pharmaceutical residues. Figure 4.2 shows the effect of varying pyrolysis temperature on X-ray diffraction of rice husks carbonated at temperatures between 300 – 600 ℃ and the raw rice husks.

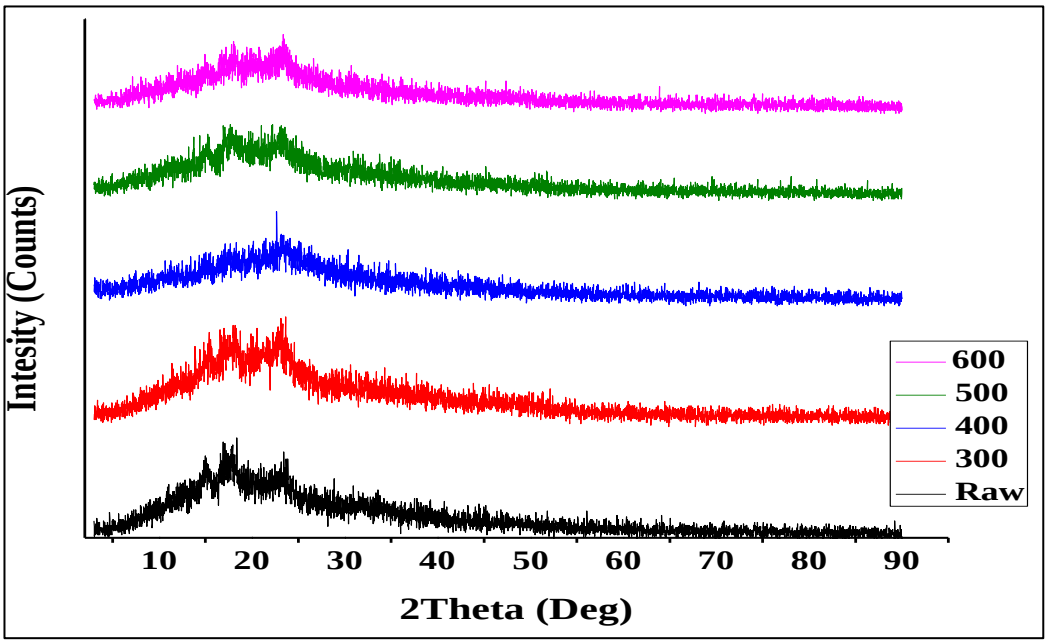

Figure 4.2: X-Ray diffraction of rice husks activated carbon carbonated at different temperatures

4.3 Method Performance Characteristics for LC-MS analysis of pharmaceuticals

Method performance characteristics of linearity, recovery and limits of detection were evaluated.

4.3.1 Linearity

For all the analytes, linear calibration curves were obtained with a range of between 5-1000µg/L. The coefficients of determination R^2 values were 0.9944, 0.9933 and 0.9901 for paracetamol, trimethoprim and nevirapine respectively while the linear regression equations were y=13891x +1175, y=134789x +3556 and y=13066x +16. Where y is the

peak area while x is the pharmaceutical concentration. Some representative calibration curves are given in Appendix XIII-XV.

4.3.2 Recovery

Recovery was evaluated by comparing the peak areas of pre-spiked samples with those of standards of similar concentrations. 200 cm^3 of de-ionised water samples were spiked with pharmaceutical drugs at concentrations of 0.25 µg/L, 1.0 µg/L and 2.5 µg/L which translates into a range of 50 µg/L, 200 µg/L and 500 µg/L respectively in the final pharmaceutical samples. The volumes were taken through the SPE process and later reconstituted. The percentage mean recoveries were paracetamol (65), trimethoprim (78) and nevirapine (92) as shown in Table 4.2.

4.3.3 Detection Limits

The limit of detection (LOD) and the limit of quantification (LOQ) were determined based on the definition that LOD is the lowest analyte concentration that gives a signal response with a signal to noise ratio of 3 while LOQ has a signal to noise ratio of 10. Mathematically they were calculated as

$LOD=3.3\ \sigma/s$……...4.1

$LOQ=10\ \sigma/s$……...4.2

Where "σ" is the standard deviation of the blank while "s" is the slope of the regression curve but since the response signal of the blank is zero it is impossible to obtain a peak area for the blank. The standard deviation of the y intercept was used to approximate the standard deviation of the response of the blank (Appendix XXVI). Table 4.2: shows the summary of method performance characteristics for paracetamol, trimethoprim and nevirapine where linear calibration curves were obtained at concentrations ranging from 5-1000µg/L.

Compound	Linearity (R^2)	LOD(µg/L)	LOQ(µg/L)	Recovery(%)
Paracetamol	0.9944	2.03	5.01	65
Trimethoprim	0.9933	1.55	8.50	78
Nevirapine	0.9901	1.86	4.69	92

Table 4.2: Method Performance Characteristics for Paracetamol, Trimethoprim and Nevirapine

Results in Table 4.2 show that calibration curves exhibited good linearity with correlation coefficient r^2= 0.99 in almost all the batches. The analytes' LOQ varied between the three pharmaceuticals, with an overall concentration range of 5.01 µg/L to 8.50 µg/L. The average recoveries for both spiked JKUAT WWTP and Nairobi River water were paracetamol (65%), trimethoprim (78%) and nevirapine (92%) (Table 4.2). MRM parameters were also optimized to ensure reproducible and reliable results and are summarized in Table 4.3.

Table 4.3: Optimized MRM Parameters for Analysis of Pharmaceutical Residues

Compound	Retention time (min)	Isotopic mass (DA)	parent ion(MZ)	Cone voltage(V)	Product ion(MZ)	Collision energy(CE)
PARA	2.40	151.1	152	30	110, 93	25, 30
TMP	2.89	290.1	291	35	230, 123	30, 35
NEV	2.20	266.1	267	40	230, 202	20, 25

From Table 4.3 the following information for each compound is revealed. Retention time (min) is the time it takes for the compound to elute from the mass spectrometer. Isotopic mass is the mass of the compound, including its isotopes; Parent ion (MZ) is

the mass-to-charge ratio of the molecular ion of the compound; Cone voltage (V) is the voltage applied to the mass spectrometer cone, which affects the fragmentation of the compound; Product ion (MZ) is the mass-to-charge ratio of one of the daughter fragment ions of the compound; Collision energy (CE) is the energy of the collision between the compound and the gas in the mass spectrometer, which also affects the fragmentation of the compound.

4.4 Optimum Carbonation Temperature of the Biochar

To find the biochar to be used in the subsequent experiments the adsorption capacity for the biochar carbonated at different temperatures were tested according to a method by Fu *et al.* (2019). The biochars carbonated at different temperatures, were used to adsorb paracetamol, trimethoprim and nevirapine before optimization process to ascertain the optimum biochar that will be used for subsequent experiments. Rice husks biochar carbonated at 500 ℃ was found to have a removal efficiency of 99% for trimethoprim, 85% paracetamol and 80% (Figure 4.3). It was hence used for subsequent sorption studies. It was used to optimize parameters such as contact time, pH, adsorbent dosage, and initial drug concentration. It had also been confirmed to be the optimum biochar during analysis of surface morphology (Plate 4.1). Figure 4.3 shows the effect of varying pyrolysis temperature on adsorption capacity of paracetamol, trimethoprim, and nevirapine.

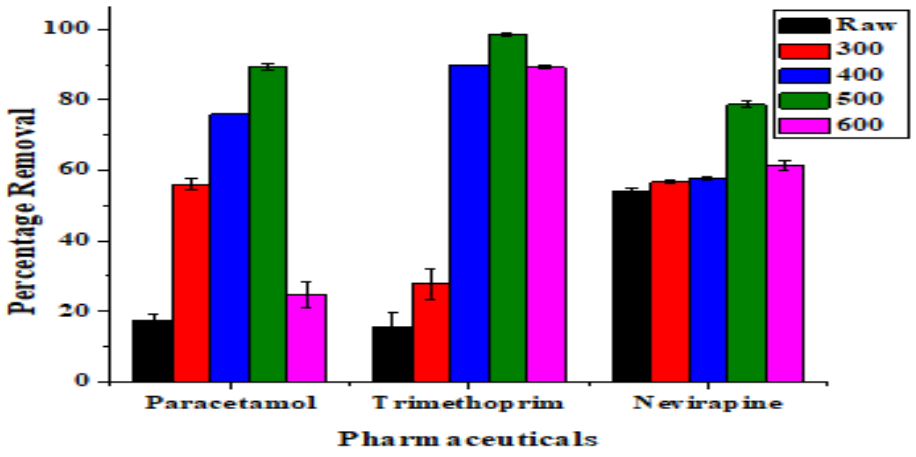

Figure 4.3: Variation of pyrolysis temperature on percentage removal of paracetamol, trimethoprim and nevirapine

4.5 Optimization of Adsorption Parameters for Paracetamol, Trimethoprim and Nevirapine

This was carried using the biochar carbonated at 500 ℃ since it had been confirmed to have the highest percentage removal (Figure 4.3, Plate 4.1).

4.5.1 Optimization of pH

The pH of a solution is known to affect the surface charge of the adsorbent which would in turn affect the adsorbent–adsorbate interactions (Bernal *et al.,* 2017). The electrical interactions taking place on the solid surface play an important role in adsorption kinetics (Vergil and Barlas, 2009). This implies that the type of charge on the surface, the formation of hydrogen bonds, and the diffusion rate are all affected by the electrical interactions, as well as influence the rate of adsorption. For instance, a positively charged surface will attract negatively charged adsorbate molecules, and this will increase the rate of adsorption.

In the present study, the influence of pH on adsorption of the three pharmaceuticals on to activated carbon of rice husks was done in the pH range of 3-10. This range was based

on studies by (Mukoko *et al*., 2015) who found that the pH of a solution affects the charge of the pharmaceutical molecules and the activated carbon surface. While trimethoprim and paracetamol had high removal rates of over 80% (Figure 4.4), Nevirapine had the lowest percentage removal rate. This was attributed to its physical chemical properties. Jain, *et al* (2014) stated that the pH, adsorbent dose and temperature lead to low removal of acyclovir which is a retroviral drug. Babas, *et al* (2021) gave the same reason for low removal of sofosbuvir.

This study revealed that pH did not have a significant effect on adsorption of trimethoprim, paracetamol and nevirapine (Figure 4.4). Statistical analysis revealed there is no significant difference ($P \geq 0.05$). Analysis of variance for the three pharmaceutical drugs revealed that the value of F calculated was less than F critical (Appendices I-III). This therefore revealed that the means of the different pH values were not significantly different. It was therefore decided that a pH of 7 be taken to be optimum for the rest of the experiments since drugs are found in water which has a pH value close to 7. Figure 4.4 shows the effect of pH for removal of trimethoprim (TMP), paracetamol (PARA), and nevirapine (NEV).

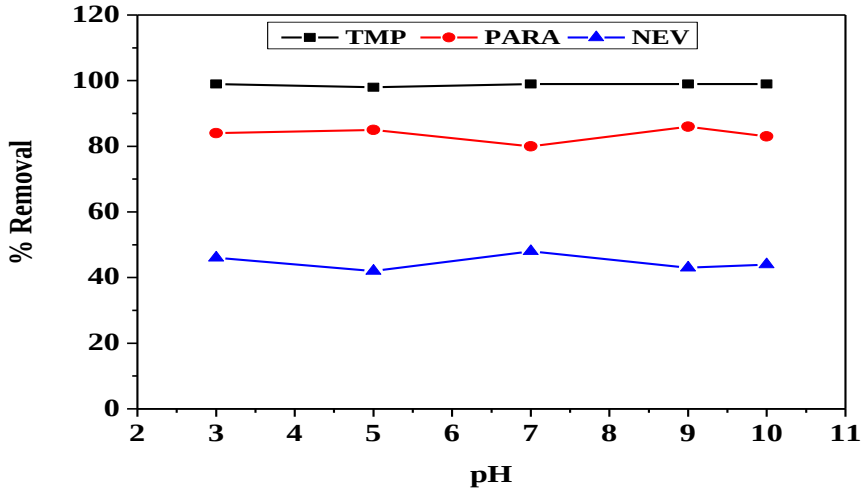

Figure 4.4: Optimization of pH on removal of trimethoprim, paracetamol and nevirapine

4.5.2 Optimization of Contact Time

The effect of contact time on the adsorption of the three pharmaceutical drugs was monitored for a period of up to 120 minutes. It was observed that there was an increase in the percentage removal of the three pharmaceuticals with time. At equilibrium, the rate of pharmaceutical molecules being adsorbed is equal to the rate at which the molecules are being desorbed (Mukoko *et al.*, 2015). The three pharmaceutical drugs required an average optimum time of 30 minutes for maximum adsorption to take place. The amounts of pharmaceuticals removed within this optimum time were 99%, 65% and 50% for trimethoprim, paracetamol and nevirapine respectively (Figure 4.5). The initial increase in the adsorption of all three drugs was due to the availability of a large number of adsorption sites on the adsorbent but adsorption became constant when the pores became saturated. Also in the first stage, the sorbate molecules are being adsorbed onto a surface where there are no other such molecules already attached, and consequently the adsorbent-adsorbate interactions are negligible (Nche *et al.*, 2017). From the results obtained trimethoprim reached maximum adsorption in 25 minutes, paracetamol in 30 minutes while nevirapine took 35 minutes to reach equilibrium (Figure 4.5). Statistical analysis of the data revealed that there is a significant difference ($P \leq 0.05$) in the amount

of pharmaceutical molecules adsorbed with increase in contact time. ANOVA tests for the three molecules reveal that the value of F calculated is greater than F critical implying that there is a significant difference between the means at different time intervals (Appendices IV-VI). Figure 4.5 shows the effect of contact time on the percentage removal of trimethoprim, paracetamol, and nevirapine.

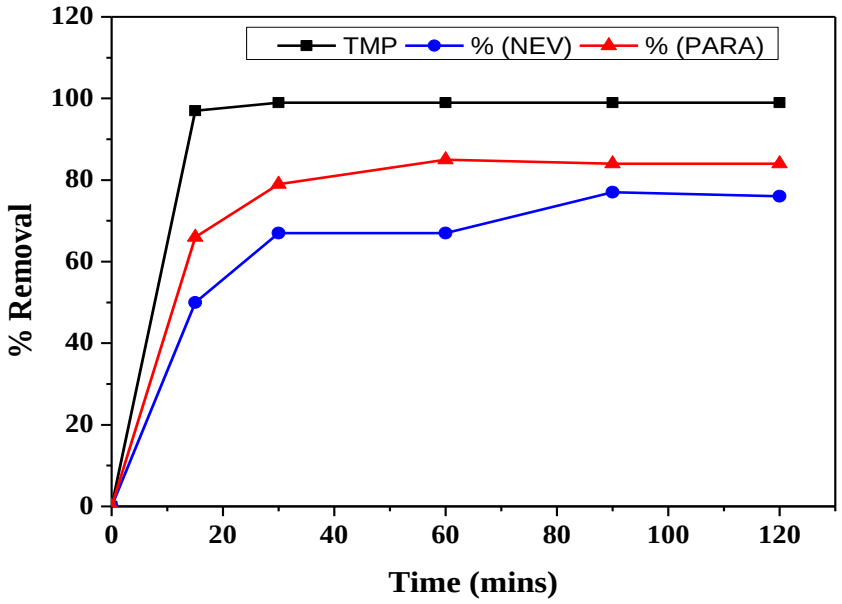

Figure 4.5: Optimization of contact time on percentage removal of trimethoprim, paracetamol and nevirapine

4.5.3 Optimization of Adsorbent Dosage

The study revealed that an increase in adsorbent dose increased the amount of pharmaceutical removed from the solutions. Increasing the adsorbent dose from 0.01125 g to 0.2 g had a subsequent increase in the percent removal of the pharmaceutical in water. Different masses of activated carbon showed varied adsorption abilities for trimethoprim, paracetamol, and nevirapine. The trend was that, for the three drugs, percentage removal increased with an increase in the mass of adsorbent until equilibrium was attained (Figure 4.6). The optimum adsorbent dosage was found to be 0.1 g with %

removals of 99, 58, and 54 for trimethoprim, paracetamol and nevirapine respectively (Figure 4.6). Kumar *et al* (2010), also reported that the percentage removal initially increased sharply with an increase in adsorbent dosage, but beyond a value of 25 g/L, the percentage removal reached an almost constant value. This is attributed to an increase in the number of adsorption sites due to an increase in surface area as the mass increases (Babel and Kurniawan, 2003). Further increase in adsorbent dose does not alter the percentage removal. This is due to the binding of almost all pharmaceutical molecules to the adsorbent surface and the establishment of equilibrium between the molecules on the adsorbent and in the solution (Garg *et al.,* 2003).

The decrease in removal before equilibrium is attributed to aggregation and overlapping of the activated carbon resulting in a decrease in effective surface area for the adsorbent (Kilic *et al*., 2011). It was also noted that the loading capacity gradually decreases due to over-saturation of the pores. Statistical analysis of the data reveals that there is a significant difference ($P \leq 0.05$) of the pharmaceuticals at different adsorbent dosages. ANOVA tests for the three molecules reveal that the value of F calculated is greater than F critical implying that there is a significant difference between the means at different values of the sorbent masses (Appendices VII-IX). Figure 4.6 shows the effect of varying adsorbent dose on the percentage removal of trimethoprim, paracetamol, and nevirapine.

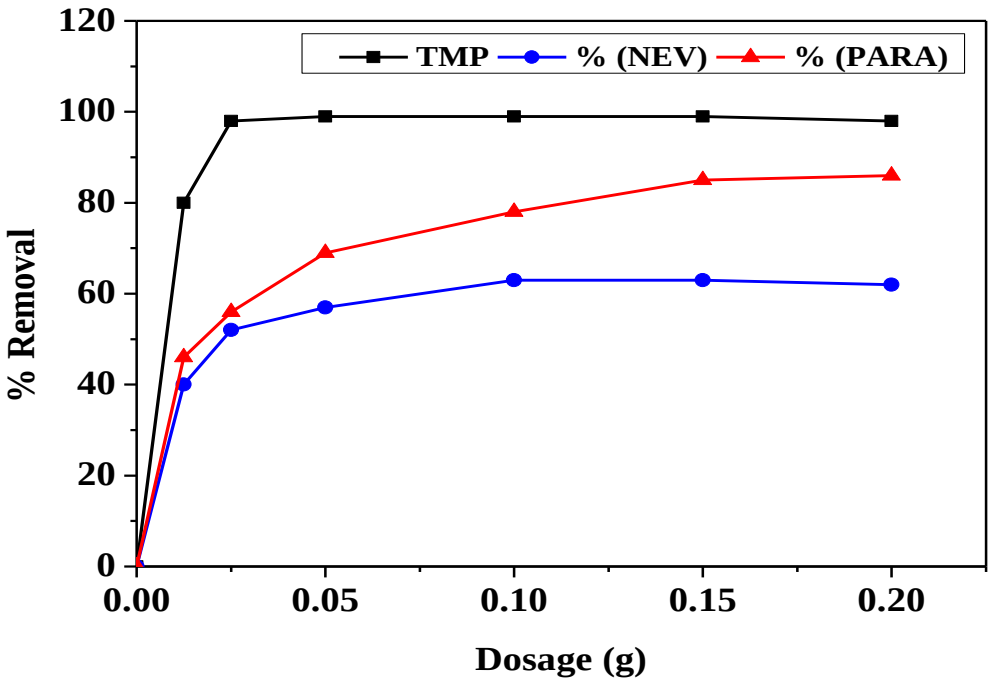

Figure 4.6: Optimization of adsorbent dose on percentage removal of trimethoprim, paracetamol and nevirapine

4.5.4 Optimization of Initial Drug Concentration on Percentage Removal

It was observed that adsorption of the three pharmaceuticals decreases with an increase in initial concentrations of the three drugs (Figure 4.7). The higher initial adsorption can be attributed to the availability of more sites on the activated carbon than the solute molecules in solution (Pakhre and Srivastava, 2012). At higher concentrations, the pharmaceutical molecules are more than the sites available. The percentage removal of the three drugs increased with an increase in initial drug concentration up to a maximum of 88% trimethoprim, 82 % paracetamol, and 75% nevirapine (Figure 4.7). Thereafter, there is a decrease in removal as the concentration increases.

Statistical analysis of the data reveals that there is a significant difference ($P \leq 0.05$) of the pharmaceuticals at different initial drug concentrations. ANOVA tests for the three

molecules reveal that the value of F calculated is greater than F critical implying that there is a significant difference between the means at different values of initial drug concentrations (Appendices X-XII). Figure 4.7 shows the effect of varying initial drug concentrations on the percentage removal of trimethoprim, paracetamol and nevirapine.

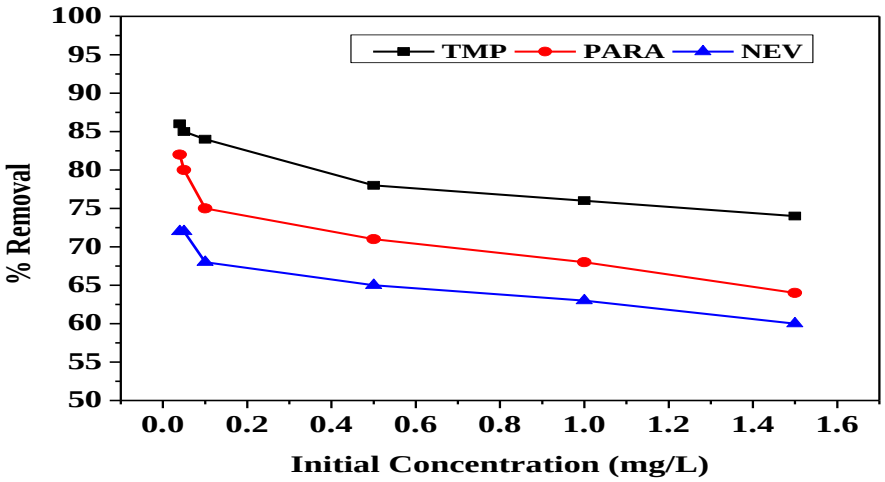

Figure 4.7: Optimization of initial drug concentration on percentage removal of trimethoprim, paracetamol and nevirapine

4.6 Adsorption Isotherms

The distributions of chemical components between the liquid and solid phase of the adsorbent are explained using Langmuir and Freundlich models. The R^2 values for the Freundlich model were 0.977, 0.994, and 0.977 while the Langmuir adsorption model was 0.9996, 0.9994, and 0.9831 respectively as summarized in Table 4.4. Both the Freundlich and Langmuir adsorption plots gave a good fit which meant that the adsorption of the pharmaceuticals by the rice husks charcoal fitted both the Freundlich and Langmuir models (Foo and Hameed, 2010). This implies that the adsorption was monolayer, occurring at finite number of localized sites and multilayer where non uniform distribution over a heterogenous surface also occurred. Figures 4.8-4.13 show

the plots for Freundlich and Langmuir adsorption isotherms of paracetamol, nevir**a**pine, and trimethoprim on rice husks activated carbon.

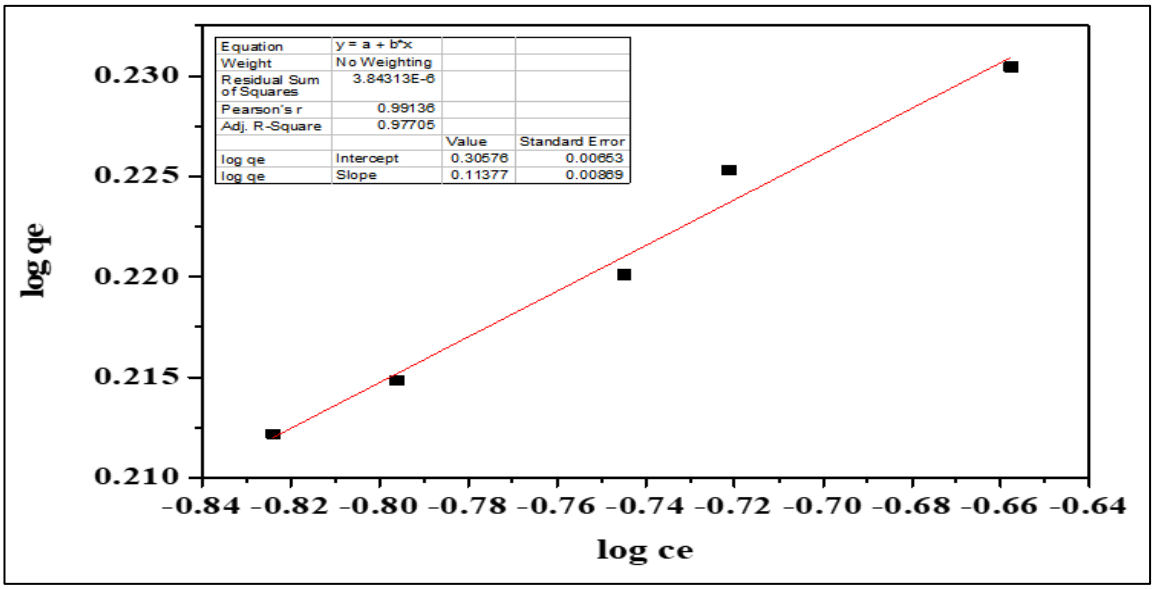

Figure 4.8: Freundlich adsorption isotherm for adsorption of paracetamol

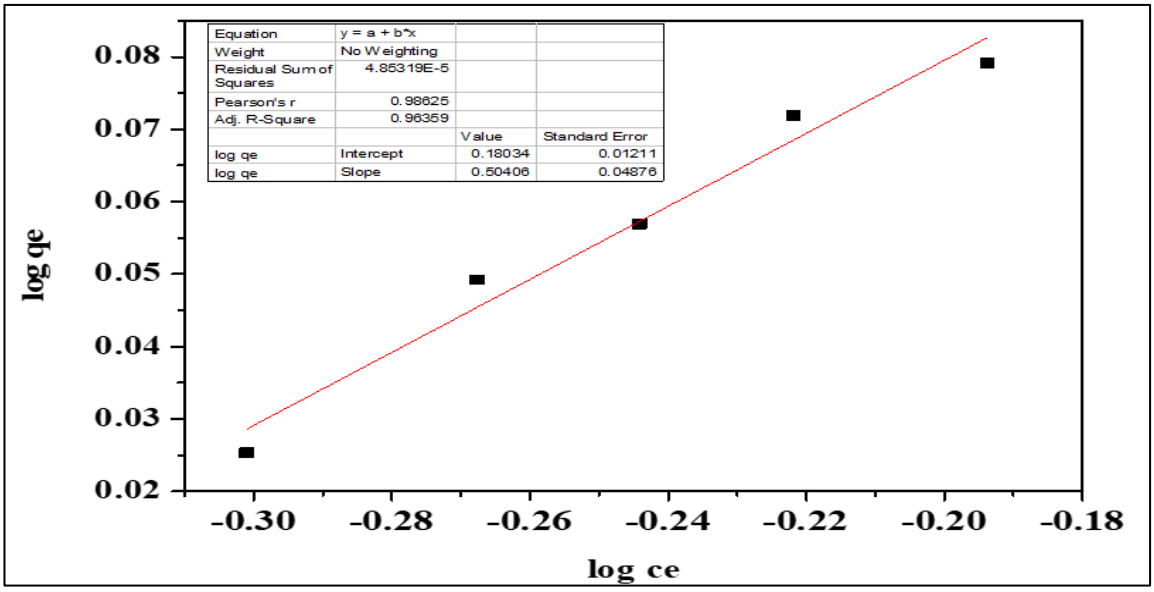

Figure 4.9: Freundlich adsorption isotherm for adsorption of nevirapine

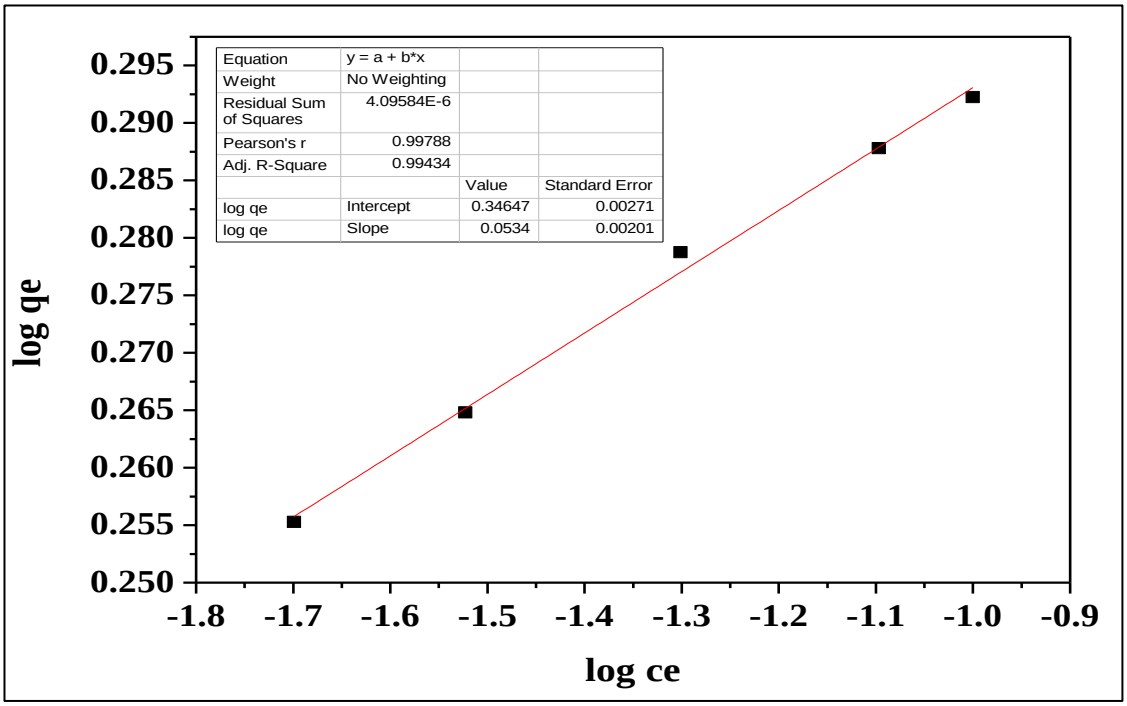

Figure 4.10: Freundlich adsorption isotherm for adsorption of trimethoprim

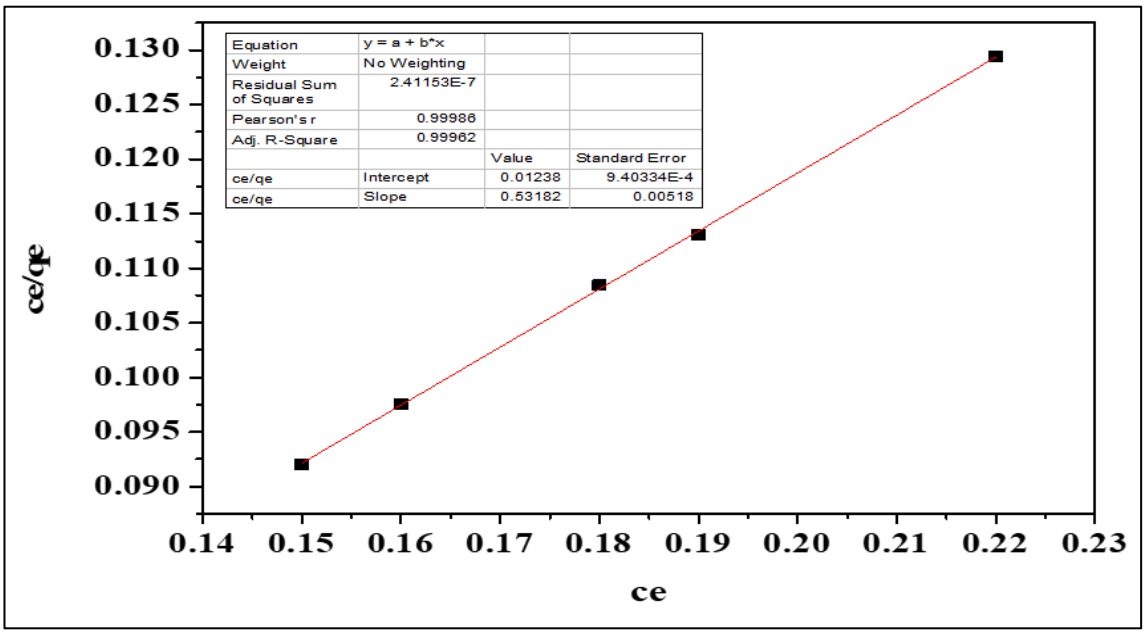

Figure 4.11: Langmuir adsorption isotherm for adsorption of paracetamol

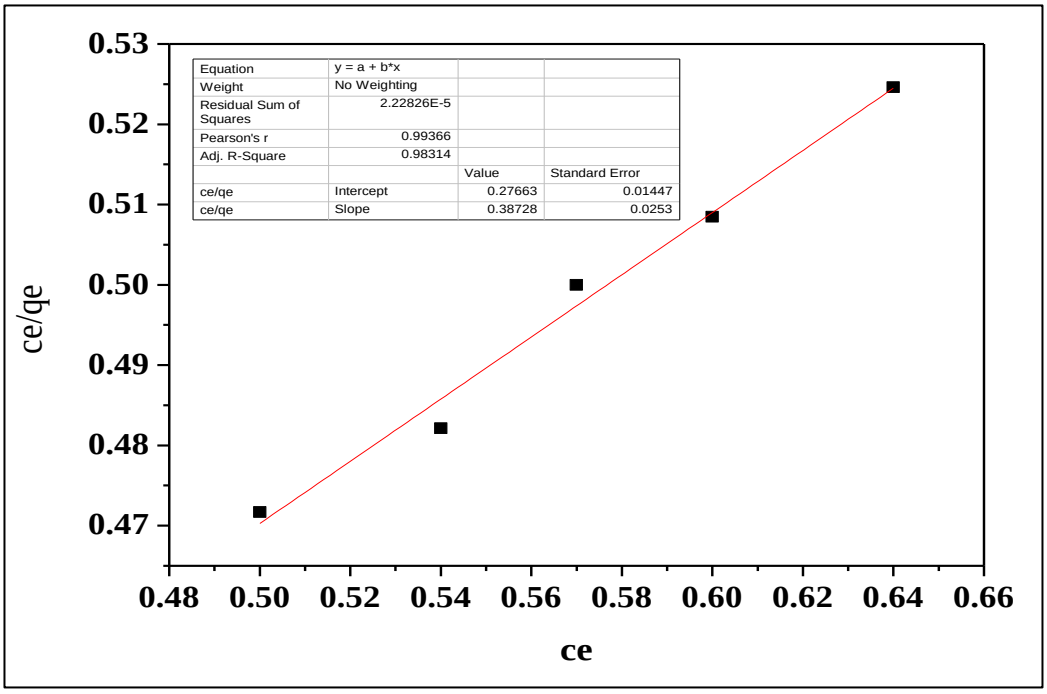

Figure 4.12: Langmuir adsorption isotherm for adsorption of nevirapine

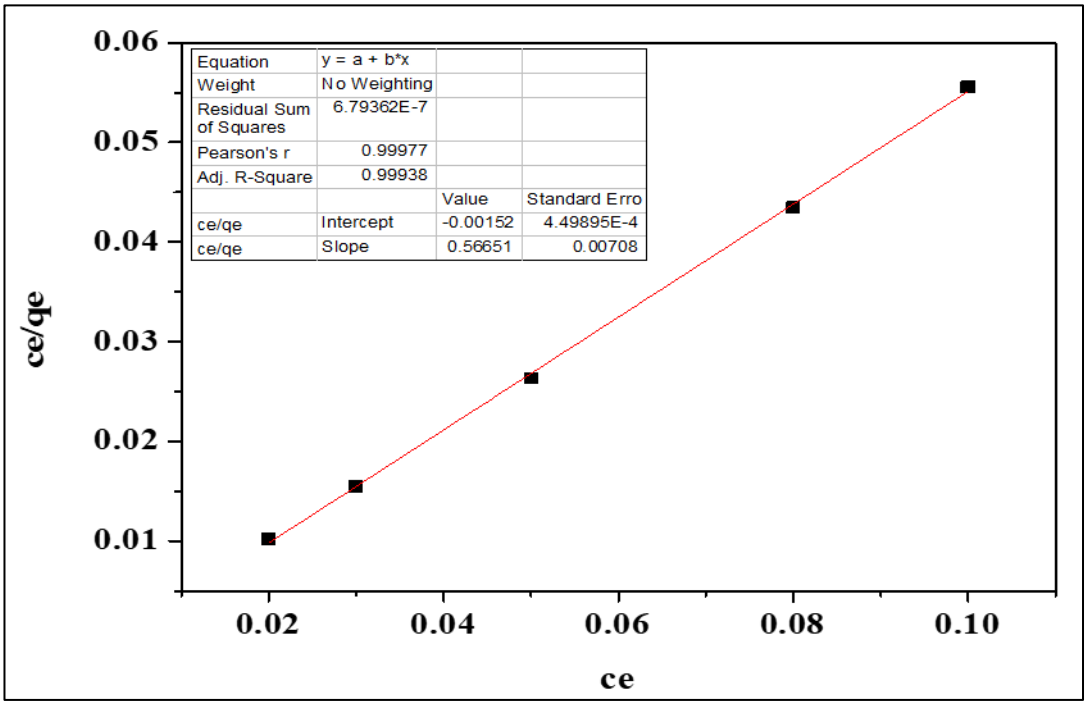

Figure 4.13: Langmuir adsorption isotherm for adsorption of trimethoprim

Table 4.4 shows a summary of Langmuir and Freundlich isortherm parameters

Table 4.4: Langmuir and Freundlich Isotherm Parameters for Adsorption of Paracetamol, Trimethoprim and Nevirapine

	Langmuir			Feundlich			
	b	q_m	R^2	K_f	1/n	n	R^2
Para	42.958	1.880335	0.99962	2.0219	0.11377	8.789663	0.97705
TMP	-372.704	1.765194	0.99938	2.2206	0.0634	15.77287	0.99434
Nev	1.399993	2.582111	0.98314	1.5147	0.50406	1.983891	0.97705

From Table 4.4 The R^2 values for the Freundlich model were 0.977, 0.994, and 0.977 while for the Langmuir adsorption model were 0.9996, 0.9994, and 0.9831 respectively. When these values approach unity, it illustrates that both the Freundlich and Langmuir adsorption plots gave a good fit which meant that the adsorption of the pharmaceuticals by the rice husks charcoal obeyed both the Freundlich and Langmuir models

4.7 Adsorption Kinetic Studies

Evaluation of the adsorption kinetics of pharmaceuticals, dyes, and metal ions in water has been done by application of various kinetic models. In this study, the mechanism of adsorption was investigated using characteristic constants of adsorption determined from Lagergren's pseudo-first order, and pseudo-second-order kinetic rate equation. Figures 4.14 to 4.19 shows the kinetic studies of the adsorption process for the pseudo-first order and second order plots for degradation of trimethoprim, paracetamol, and nevirapine respectively.

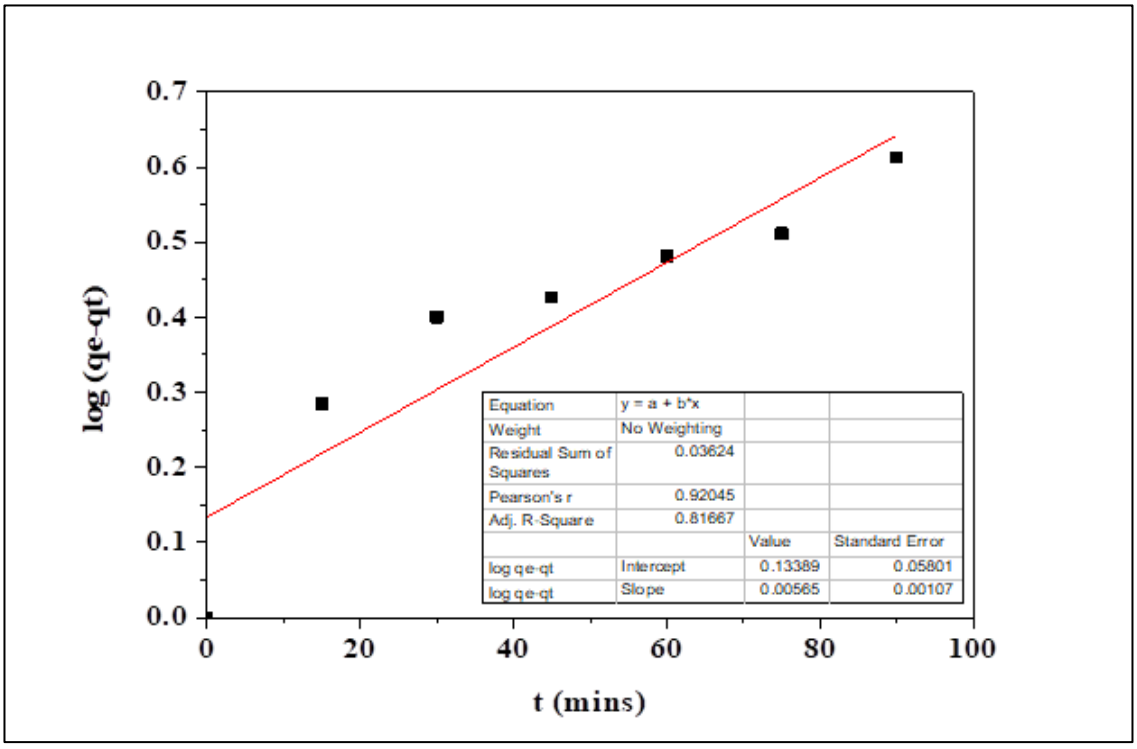

Figure 4.14: Pseudo first-order plot for adsorption of trimethoprim

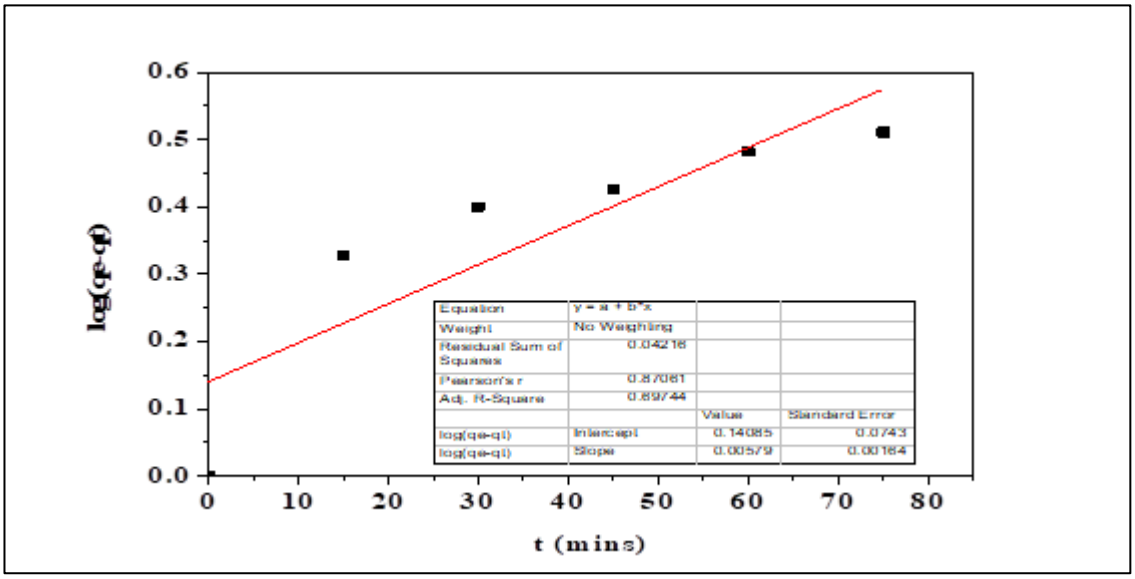

Figure 4.15: Pseudo first-order plot for adsorption of paracetamol

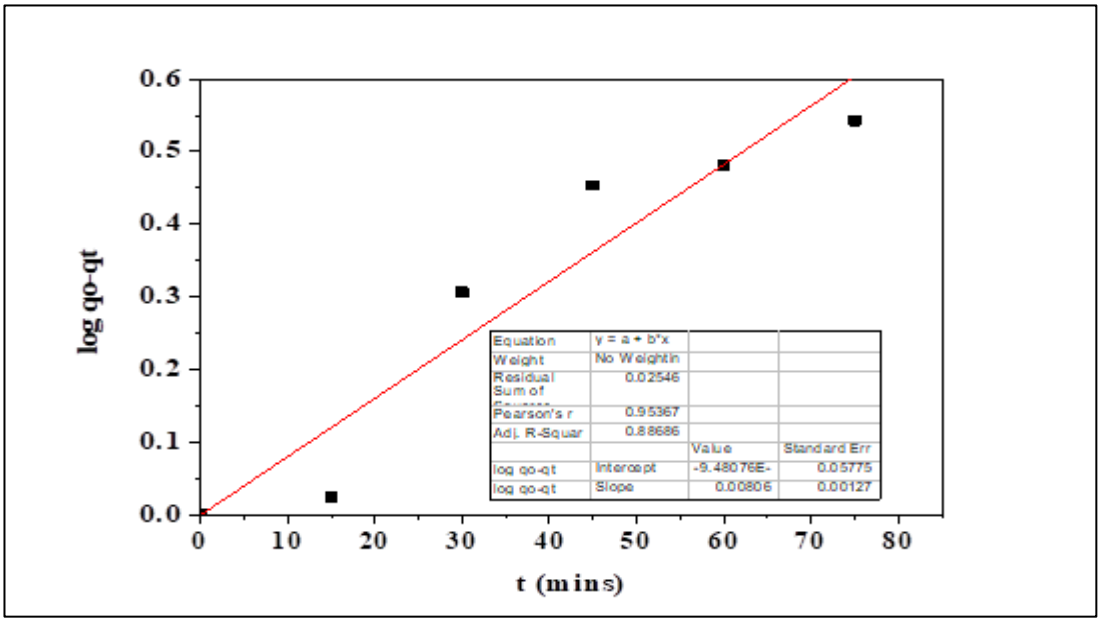

Figure 4.16: Pseudo first-order plot for adsorption of nevirapine

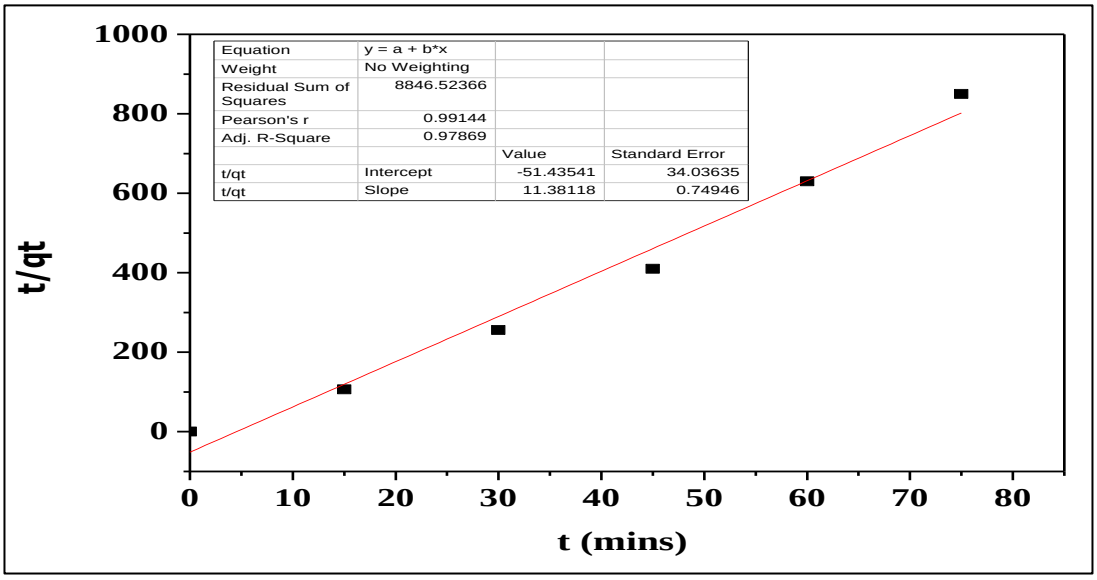

Figure 4.17: Pseudo second order plot for adsorption of paracetamol

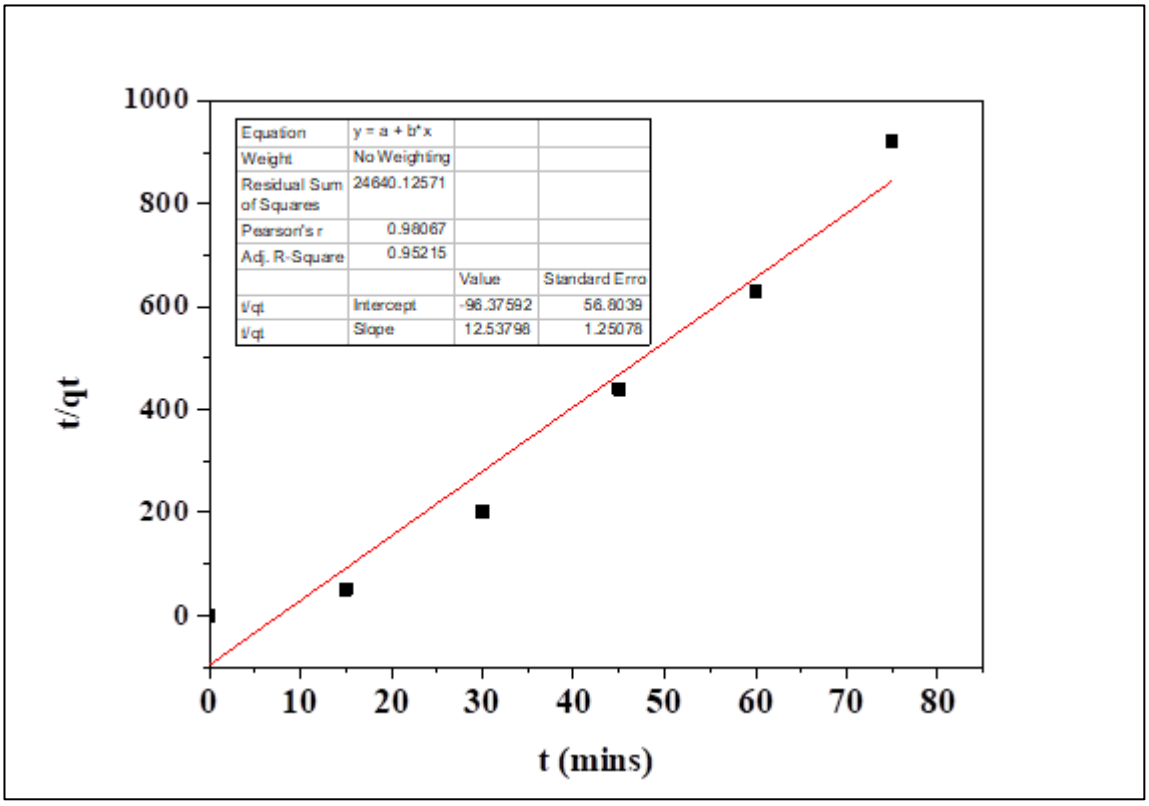

Figure 4.18: Pseudo second order plot for adsorption of nevirapine

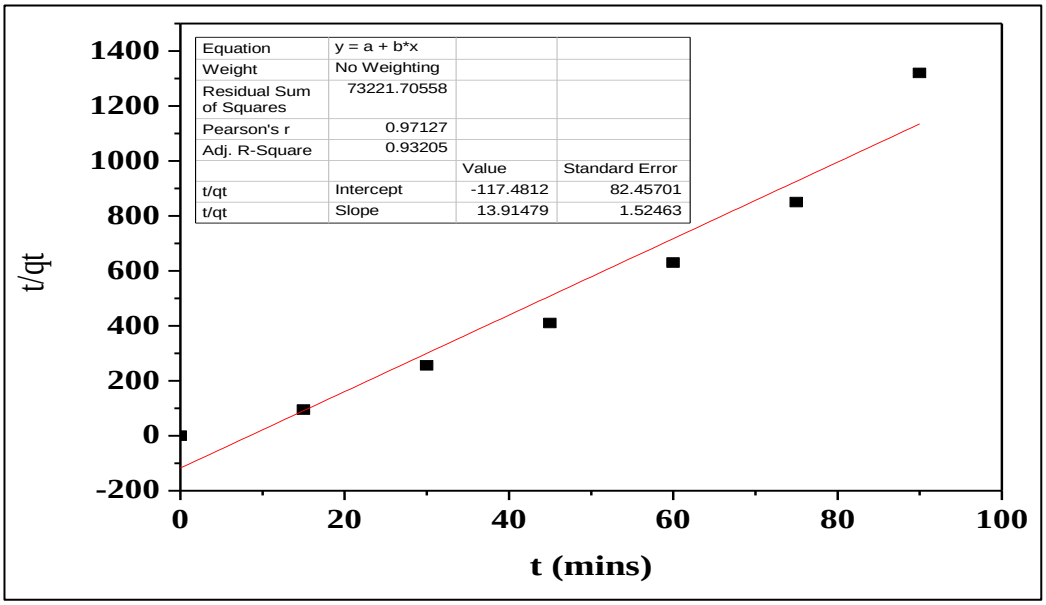

Figure 4.19:Pseudo second order plot for adsorption of trimethoprim

4.7.1 Kinetic Parameters for the Removal of Trimethoprim, Paracetamol and Nevirapine

The kinetic parameters for the removal of trimethoprim, paracetamol, and nevirapine using activated carbon of rice husks are summarized in Table 4.5.

Table 4.5: Kinetic Parameters for the Removal of Trimethoprim, Paracetamol and Nevirapine

	pseudo first order			pseudo second order		
	k_1	R^2	qe	k_2	R^2	qe
tmp	$5.65x10^{-3}$	0.8166	1.3611	-1.64811	0.93205	$7.1866x10^{-2}$
para	$66.997x10^{-1}$	0.69744	1.3831	-2.51833	0.9769	$8.786x10^{-2}$
nev	$8.00066x10^{-3}$	0.88686	3.2302	-1.63112	0.95215	$7.9758x10^{-2}$

From the data, the Pseudo- second order model recorded R^2 values which were closer to unity compared to a pseudo-first-order model which was unfavorable, hence the adsorption of the three pharmaceuticals used pseudo-second-order kinetics meaning that chemisorption was favoured more than physisorption.This implies that adsorbate molecules formed chemical bonds with the adsorbent.

4.8 Pharmaceuticals Removal from Wastewater using Activated Carbon

4.8.1 Levels of Pharmaceuticals Residues at Jomo Kenyatta University Wastewater Treatment Plant and Nairobi River Water

After the levels of the three pharmaceuticals were established, they were extracted through solid phase extraction. The major purpose of SPE is to clean up the sample, concentrate analytes of interest and removal of matrix that hinder or enhance ionization (Semreen *et al.,* 2019). Figure 4.20 shows the concentration of paracetamol, nevirapine, and trimethoprim in JKUAT WWTP effluent and influent and in Nairobi River water.

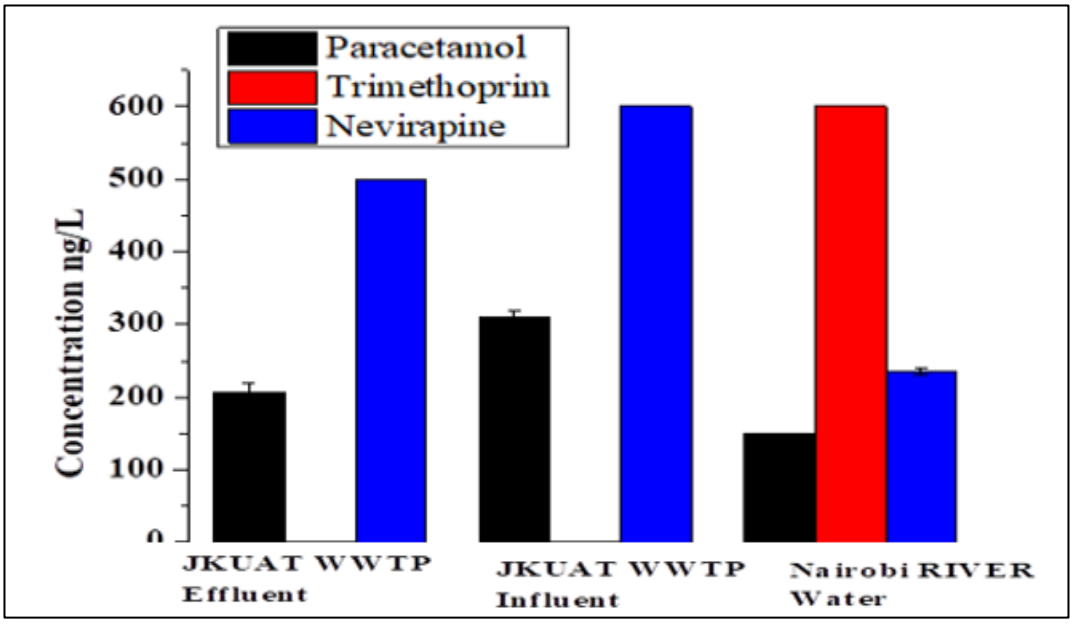

Figure 4.20: Concentration of paracetamol, nevirapine and trimethoprim in JKUAT WWTP effluent, influent and Nairobi river water

The occurrence of the selected pharmaceuticals in JKUAT WWTP and Nairobi River water was investigated (Figure 4.20). In the JKUAT WWTP effluent, Nevirapine had the highest concentration (500±0.02 ng/L), followed by paracetamol (207±0.01 ng/L) while trimethoprim was below the detection limit. In the influent, a slightly higher concentration of nevirapine (600 ± 0.01ng/L) and paracetamol (310±0.01 ng/L) was recorded while trimethoprim was below the detection limit. The concentrations of the three pharmaceuticals were highest in Nairobi River wastewater samples. Trimethoprim had the highest concentration, at 600 ng/L, followed by nevirapine at 235 ng/L, and paracetamol at 150 ng/L. This agrees with a study conducted by Ngumba *et al*, (2016) whose finding was 769 ng/L and 769 ng/L for nevirapine and trimethoprim respectively in the Nairobi water basin. Kairigo *et al*. (2020), found higher concentrations of trimethoprim of 5600 ng/L and 900 ng/L at Mutheu river and Gatei WWTP respectively. For the influent and effluent samples, there was a decrease in paracetamol concentration from 310-210 ng/L while nevirapine decreased from 600-235 ng/L. This represents the removal of between 20-50% by the WWTP. The variation in percentage removal of the

pharmaceuticals is attributed to a number of factors, including the type of WWTP, the treatment processes, as well as the concentration of the pollutants in the influent and effluent water. However, the results of this study suggest that the WWTP in question is not effective at removing these pollutants from wastewater. Such decreasing concentrations were also reported by other workers (Cecilia *et al*., 2020; Koreje *et al*., 2016).

The occurrence of the selected pharmaceuticals in JKUAT WWTP and Nairobi River water was investigated (Figure 4.20). In the JKUAT WWTP effluent, Nevirapine had the highest concentration (500±0.02 ng/L), followed by paracetamol (207±0.01 ng/L) while trimethoprim was below the detection limit. In the influent, a slightly higher concentration of nevirapine (600 ± 0.01ng/L) and paracetamol (310±0.01 ng/L) was recorded while trimethoprim was below the detection limit. Nairobi river wastewater samples had higher concentrations of the three pharmaceuticals. Trimethoprim had the highest concentration (600±0.02 ng/L) followed by nevirapine (235±0.03 ng/L) while paracetamol had the lowest concentration at 150±0.02 ng/L. This agrees with a study done by Ngumba *et al*, (2016), though they found higher concentrations of nevirapine (769 ng/L) and trimethoprim (769 ng/L) in the Nairobi water basin. Kairigo *et al*. (2020), found higher concentrations of trimethoprim of 5600 ng/L and 900 ng/L at Mutheu river and Gatei WWTP respectively. For the influent and effluent samples, there was a decrease in paracetamol concentration from 310-210 ng/L while NVP decreased from 600-235 ng/L. This represents the removal of between 20-50% by the WWTP. Such decreasing concentrations were also reported by other workers (Cecilia *et al*., 2020; Koreje *et al*., 2016).

4.8.2 Results of Adsorption of Pharmaceutical Residues from JKUAT WWTP and Nairobi River Water using Activated Carbon Biochar.

The application of rice husk activated carbon to remove paracetamol, trimethoprim and nevirapine from wastewater obtained from the two sampling sites was evaluated and the results are depicted in Table 4.6 and 4.7. It should be noted that trimethoprim levels in

JKUAT WWTP were below the detection limit hence its removal using the adsorbent was not possible.

Table 4.6: Adsorption of Pharmaceutical Residues Using Activated Carbon of Rice Husks in JKUAT WWTP

	JKUAT WWTP Before Adsorption (ng/L)			JKUAT WWTP After Adsorption (ng/L)		
Pharmaceutical	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 1**	**Sample 2**	**Sample 3**
Paracetamol	320	310	300	BDL	BDL	BDL
Nevirapine	600	600	600	BDL	BDL	BDL

BDL- Below Detection Limit

Table 4.7: Adsorption of Pharmaceutical Residues using Activated Carbon of Rice Husks in Nairobi River water

	Nairobi River Water Before Adsorption(ng/L)			Nairobi River Water After Adsorption(ng/L)		
Pharmaceutical	**Sample 1**	**Sample 2**	**Sample 3**	**Sample 1**	**Sample 2**	**Sample 3**
Paracetamol	150	150	150	BDL	BDL	BDL
Trimethoprim	600	600	600	BDL	BDL	BDL
Nevirapine	230	235	230	BDL	BDL	BDL

BDL- Below Detection Limit

After subjecting the water samples to the adsorbent at the optimized conditions it was found to remove 99% of the pharmaceuticals (Table 4.6 and 4.7). Since the pharmaceuticals were in small concentrations the high percentage removals agrees with the observation made during optimization on the effect of initial drug concentration

where removal increased with a decrease in initial drug concentration. This can be attributed to the fact that the adsorbent contains many adsorption sites where the drug molecules could attach before the sites became saturated (Mukoko *et al*., 2015).

4.9 Microbial Biodegradation

The use of indigenous microorganisms as well as optimizing their biodegradation parameters such as concentration of microorganisms, temperature, pH, and time has a great influence on biodegradation efficiency. Furthermore, they enhance the quality of the degradation process without necessarily polluting the environment (Al-Gheethi *et al.*, 2019). To determine the levels of pharmaceuticals that could be tolerated by the microorganisms, each strain of microorganism was subjected to various concentrations of the pharmaceuticals, and their growth was monitored (Figures 4.21-4.23).

In effect, low concentrations of the pharmaceuticals were utilized resulting in a reduction in toxicity. However, under the conditions used (1ppm of each drug) in the biodegradation experiments, the microorganisms were only minimally affected leading to a conclusion that lower concentrations should be used in the biodegradation experiments (Akhtar *et al.,* 2016). The MIC used ranged from 0.5-1.5 ppm. Figure 4.21, 4.22 and 4.23 shows the effect of varying pharmaceutical drugs concentration on the growth of microorganisms.

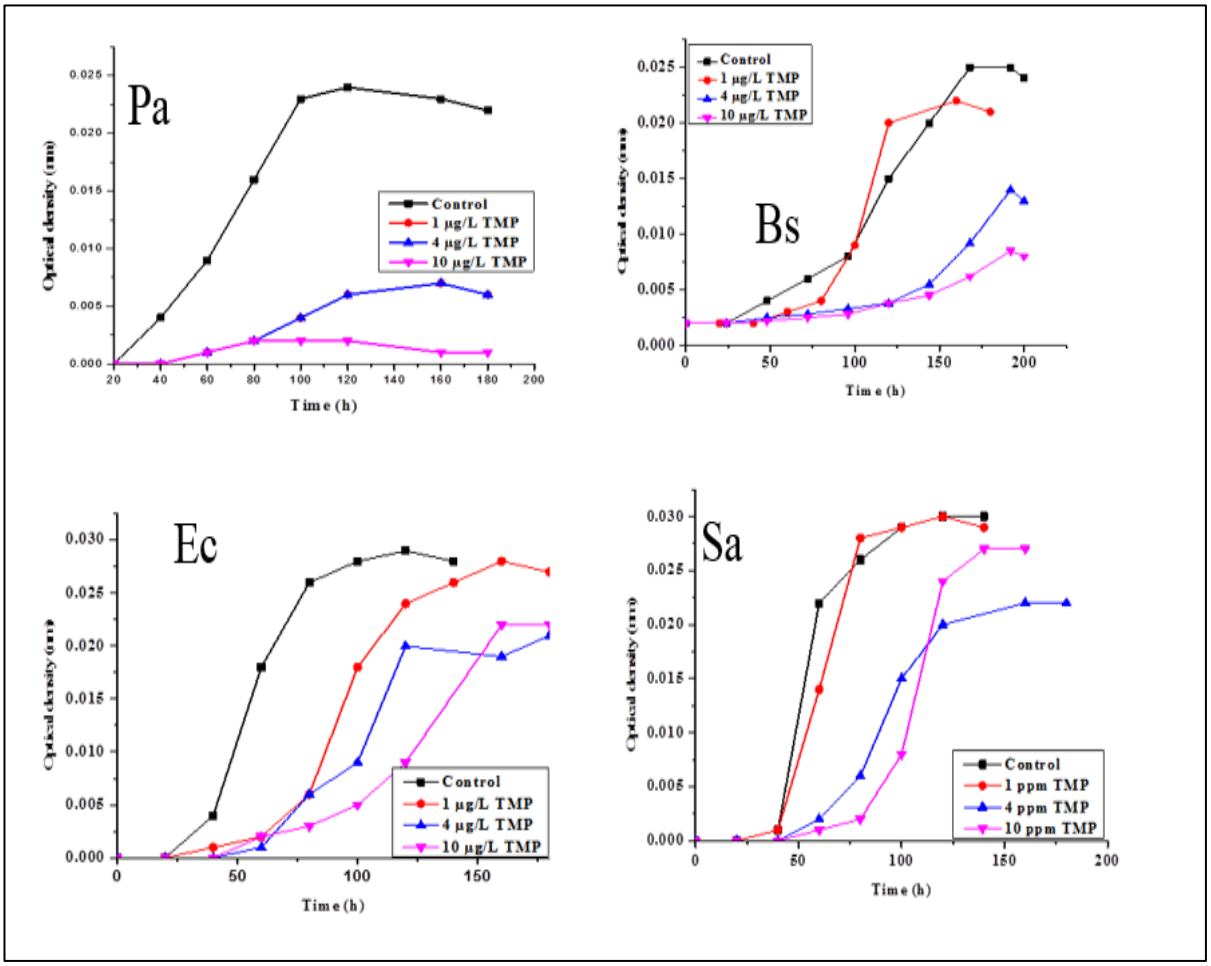

Figure 4.21: Effect of trimethoprim concentration on the growth of *P. aeroginosa*, *B. subtilis*, *E. coli*, and *S. aureus*

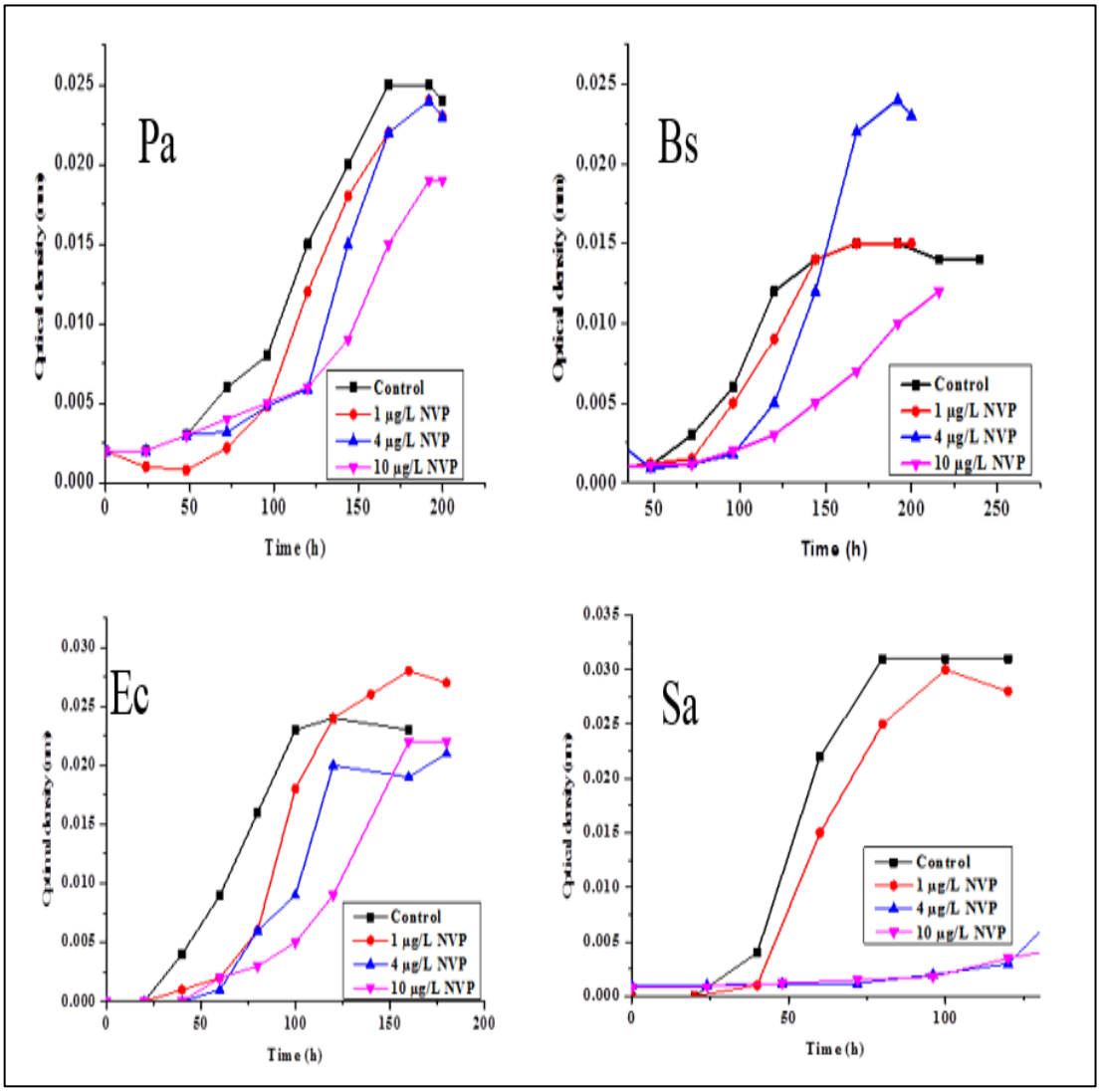

Figure 4.22: Effect of nevirapine concentration on the growth of *P. aeroginosa*, *B. subtilis*, *E. coli*, and *S. aureus*

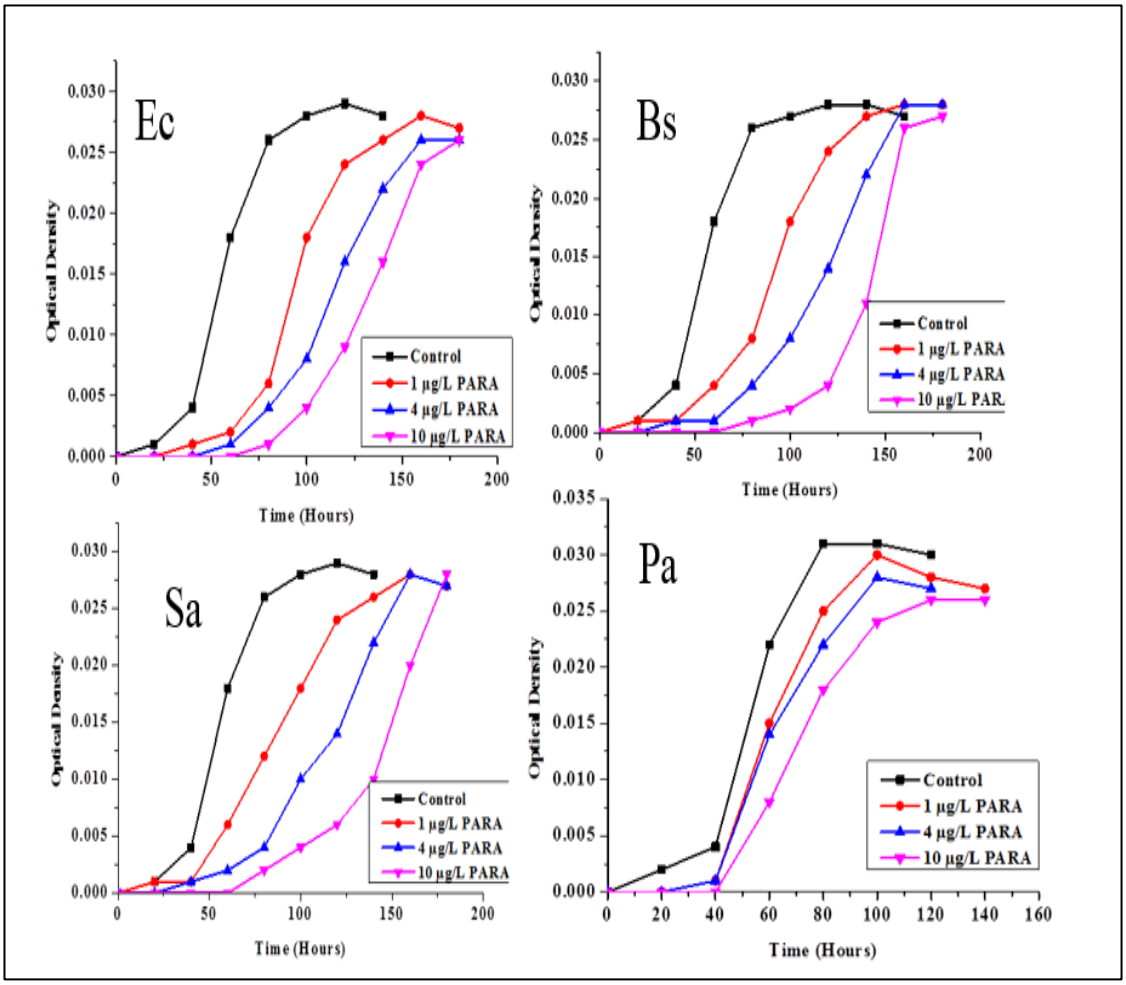

Figure 4.23: Effect of paracetamol concentration on the growth of *P. aeroginosa, B. subtilis, E. coli*, and *S. aureus*

4.9.1 Biodegradation Studies

Figures 4.24-4.26 show representative chromatographic peaks for biodegradation of selected pharmaceutical drugs by selected microorganisms. Biodegradation is implied by reduction in peak area of the sample compared with that of standard because concentration is directly proportional to peak area.

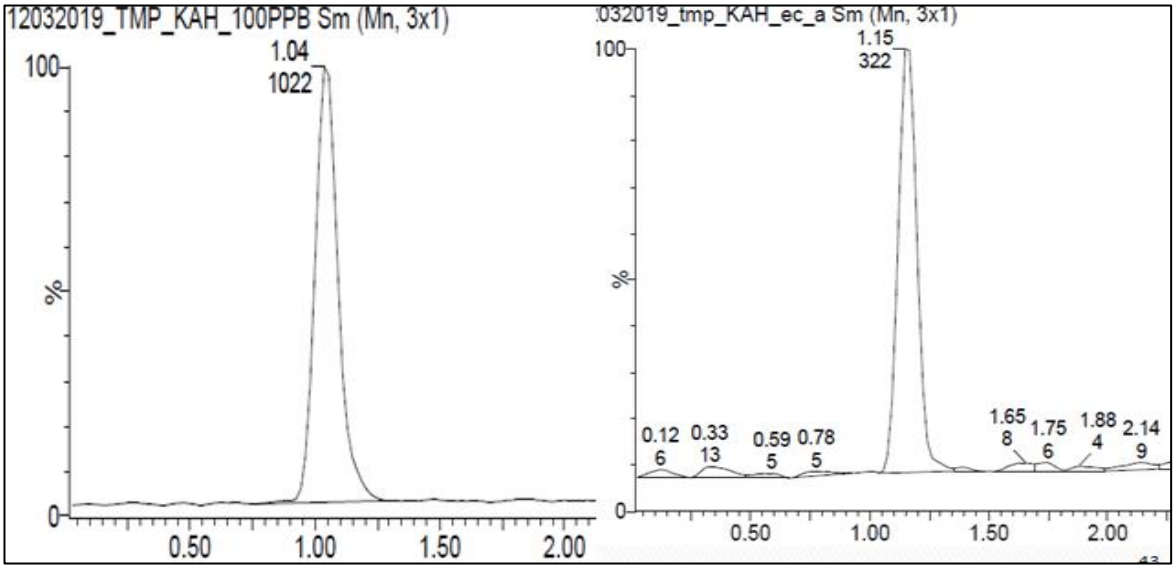

Figure 4.24: Chromatogram showing biodegradation of trimethoprim by *Escherichia coli* bacteria depicted by reduction in peak area.

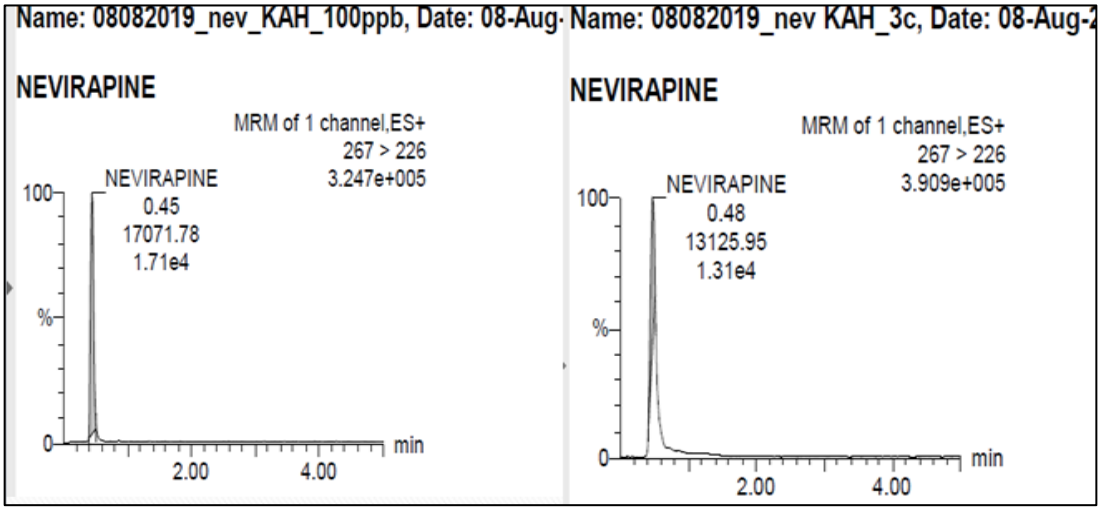

Figure 4.25: Chromatogram for Biodegradation of nevirapine by *Escherichia coli* bacteria depicted by reduction in peak area

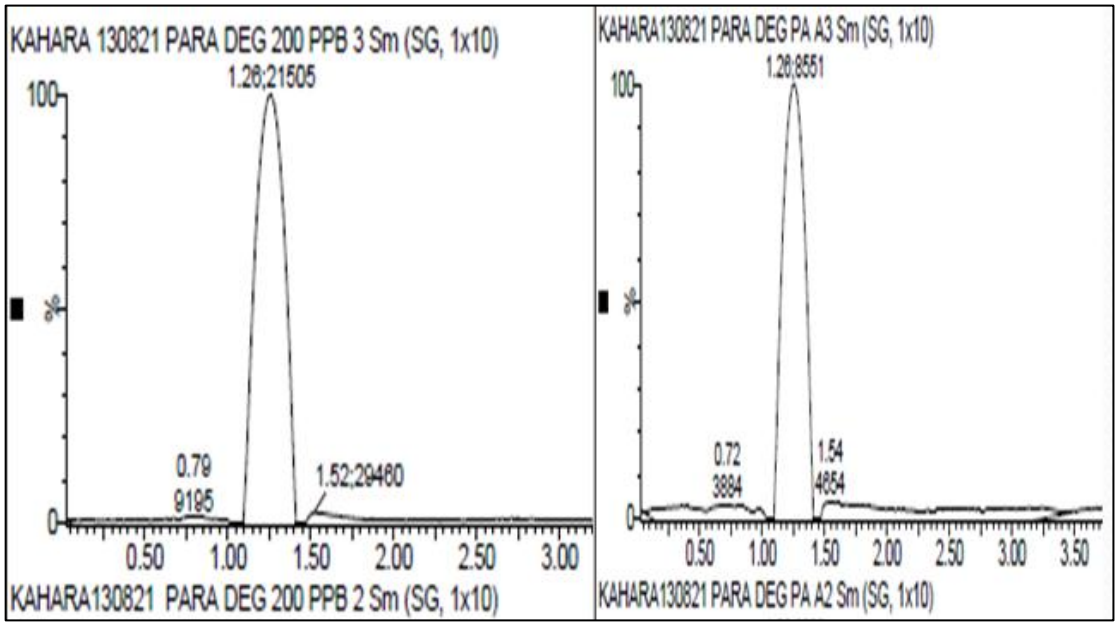

Figure 4.26: Chromatogram for Biodegradation of paracetamol by *Escherichia coli* bacteria depicted by reduction in peak area

4.9.1.1 Effect of Autoclaving on the Pharmaceuticals Biodegradation

The effect of whether the pharmaceuticals were affected by heating at 121℃ (autoclaving) was evaluated and the the results presented in Table 4.8.

Table 4.8: Effect of Heat on Biodegradation

	Concentration (ppm) n=3		
	Trimethoprim	**Nevirapine**	**Paracetamol**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**
Autoclaved	0.44±0.02	0.45±0.01	0.44±0.01
Not Autoclaved	0.42±0.01	0.43 ±0.01	0.43±0.02

It was revealed that the change in the concentration of the pharmaceuticals due to autoclaving was negligible showing that autoclaving did not affect the biodegradation of the pharmaceuticals except for trimethoprim which was affected minimally (Table 4.8). Statistical analysis of the data revealed that there was no significant difference ($P \geq 0.05$) of the concentrations of the three pharmaceuticals. ANOVA tests for the concentrations of the three molecules revealed that the value of F calculated was less than F critical implying that there was no significant difference between the means of the different values of autoclaved and non-autoclaved pharmaceuticals. (Appendices XVI-XVIII).

4.9.1.2 Microbial Degradation Results

The percentage biodegradation for the three pharmaceutical concentrations of 0.5, 1.0, and 1.5 ppm was averaged and the results are summarized in Table 4.9.

Table 4.9: Average Percentage Biodegradation by Microorganisms

Percentage Microbial Biodegradation(n=3)				
	EC	BS	PA	SA
Microorganisms	MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD
TMP	82±0.01	79±0.01	85±0.02	80±0.02
PARA	74±0.01	64±0.03	92±0.01	60±0.01
NEV	59±0.01	60±0.02	34±0.01	51±0.03

In the biodegradation process, the pharmaceuticals were aerobically degraded into water and carbon dioxide as well as used as the essential carbon source for microbial growth (Chen *et al*., 2010; Hasan *et al*., 2011; Jun *et al*., 2013). When the percentage degradation for the four microorganisms was averaged for each drug, (Table 4.9) it was revealed that trimethoprim was the most biodegradable (82%) followed by paracetamol (73%) while nevirapine was the least at (51%). Analysis of variance revealed that there is a significant difference in biodegradation of the three pharmaceutical molecules by the microorganisms since F calculated was higher than F critical (Appendix XIX-XXI). Between the different microorganisms there is no significant difference since the F calculated is less than F critical (Appendix XXII-XXV). Equation 4.3 was used to tabulate the percentage biodegradation of the selected pharmaceuticals.

$$\text{Biodegradation \%} = \left(\frac{\text{Initial Concentration} - \text{Final Concentration}}{\text{Initial Concentration}}\right) X100 \quad \text{.....(4.3)}$$

4.10 Microbial Degradation of Different Concentrations of Nevirapine, Trimethoprim, and Paracetamol by *E. coli, B. subtilis, S. aureus,* and *P. aeruginosa* Bacteria

An increase in concentration from 0.5ppm-1.5ppm, for nevirapine (Figures 4.27) and paracetamol (Figures 4.29), leads to a decrease in percentage biodegradation by all bacterial strains. For trimethoprim (Figure 4.28), microorganisms *E. coli, B. subtilis*, and *S. aureus* showed increased degradation with increasing concentration, whereas P. aeruginosa showed decreased degradation with increasing concentration. The disparity in the rate of biodegradation is related to the difference in pharmaceutical tolerance of each bacterial strain and the medium used in each setup (Al-Gheethi *et al*., 2019). The findings are consistent with previous study (Jun *et al.,* 2013), where he reported paracetamol to have biodegradation of more than 70% by *P. aeroginosa*. Wadhah *et al.* (2018), reported a higher percentage of 80% and at a lesser time of 120 hours. Figures 4.27-4.29 show percentage degradation of varied concentrations of nevirapine, trimethoprim, and paracetamol using *E. coli, B. subtilis, S. aureus*, and *P. aeruginosa*.

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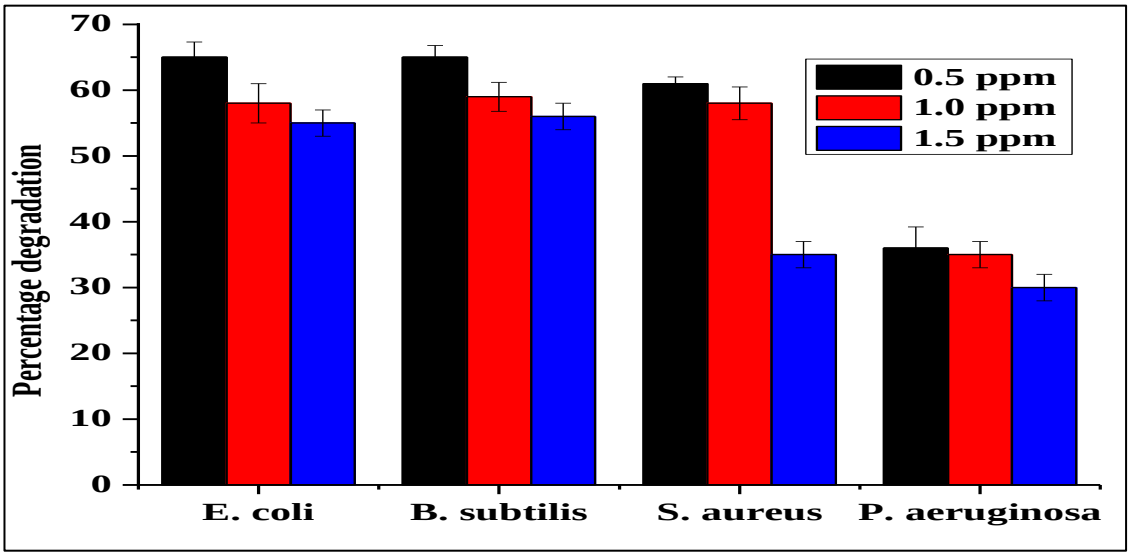


Figure 4.27:Percentage degradation of different concentrations of nevirapine by selected microorganisms

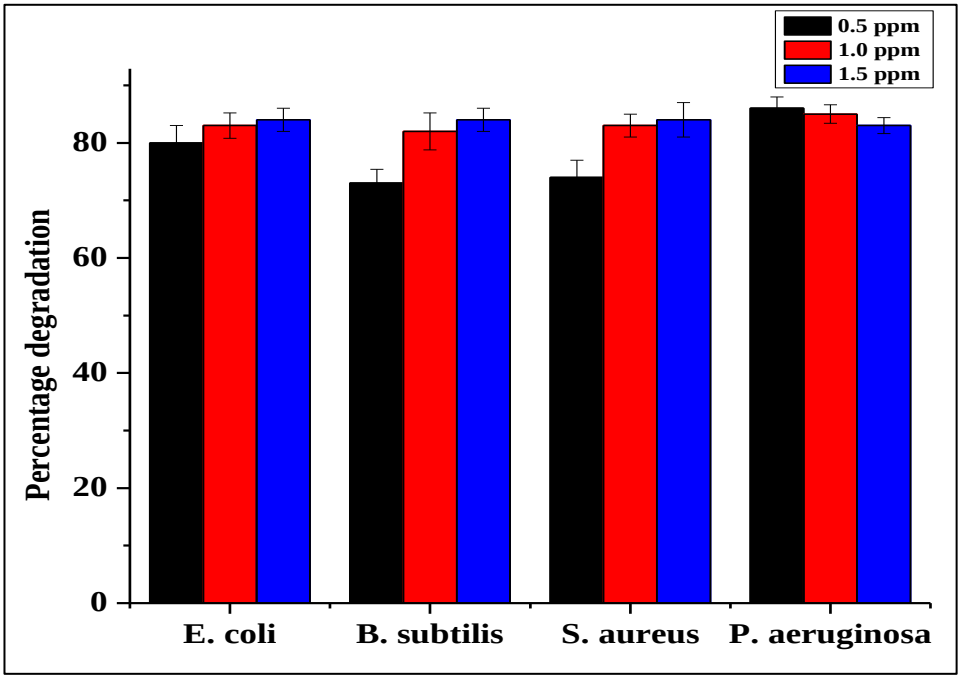


Figure 4.28: Percentage degradation of different concentrations of trimethoprim by selected microorganisms

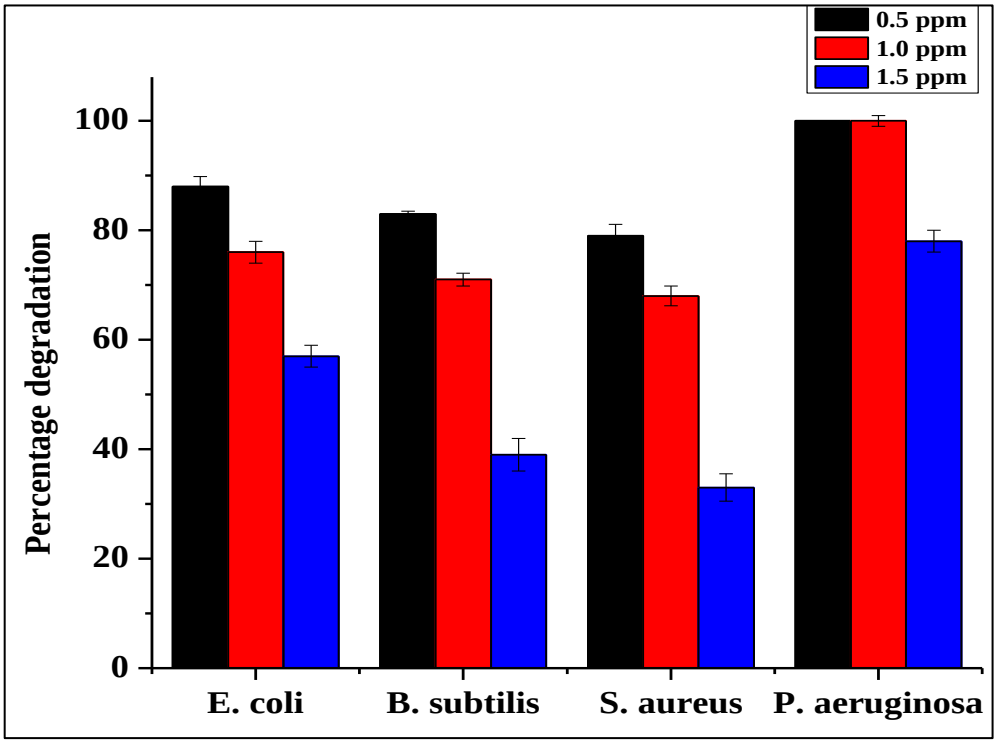


Figure 4.29: Percentage degradation of different concentrations of paracetamol by selected microorganisms

Adsorption and biodegradation are two of the most promising methods for removing pharmaceutical residues from wastewater. Adsorption is the process of attracting and retaining molecules on the surface of a solid. Biodegradation is the process of breaking down organic molecules by living organisms. Biochar derived from activated carbon of rice husks is a good adsorbent for pharmaceutical residues. It has a high specific surface area and a large pore volume, which provides a lot of surface area for the molecules to attach to. Additionally, biochar has a negative charge, which can attract positively charged pharmaceutical residues. Biodegradation can also be used to remove pharmaceutical residues from wastewater. There are many different microorganisms that can degrade pharmaceutical residues. The most effective microorganisms for biodegradation will depend on the specific pharmaceutical residues present in the wastewater.

Combining adsorption and biodegradation can be a very effective way to remove pharmaceutical residues from wastewater. Adsorption can remove the majority of the residues, and then biodegradation can be used to break down the remaining residues. This combination of methods can achieve a high level of removal efficiency.

CHAPTER FIVE

CONCLUSIONS AND RECOMMEDATIONS

5.1 Conclusions

From the study the following conclusions were made:

1. Activated carbon biochar derived from rice husks can remove paracetamol, trimethoprim and nevirapine from wastewater and model solutions. The biochar carbonated 500℃ exhibited the best porosity which enabled it to remove over 90% of the three pharmaceutical drugs.
2. FTIR characterization of the rice husks biochar indicates that it was dominated by a number of functional groups - OH, CH, C=C, C-O, C=O, N-O and Si-O-Si which are key in the adsorption process while SEM analysis revealed that biochar carbonated at 500℃ was the optimal. It contained regular shaped pores which acted as active sites for adsorption. Characterization by XRD revealed a broad single peak around 2Ø which indicated that the activated biochar was amorphous
3. Adsorption parameters results revealed that removal of the pharmaceuticals under study increases with an increase in contact time, adsorbent dose, whereas for initial drug concentration it decreases while pH it did not have a significant effect on the adsorption.
4. Experimental data was fitted to two isotherm models, Langmuir and Freundlich and the equilibrium fitted well to both isotherms. The adsorption data was fitted into two kinetic models pseudo first order and pseudo second order and it was found to fit pseudo second order since the R^2 values were closer to unity compared to pseudo first order.
5. On microbial degradation the torelance of *P. aeroginosa B. subtilis, E. coli* and *S. aureas* to phamaceuticals was tested. It was found that increase in

concentration of the pharmaceuticals leads to a decrease in growth of the microorganism hence low concentrations of the pharmaceuticals were used.

6. The four microorganisms *Pseudomonas aeroginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* showed good efficacy to biodegrade paracetamol, trimethoprim and nevirapine.

5.2 Recommendations

The following recommedations can be made from this study and for future work.

5.2.1 Recommendations from this Work

1. More studies should be carried out on recyclability of rice husks adsorbent and the best mode of its disposal after use.
2. Cheaper environmentally friendly adsorbents such as activated carbon of rice husks should be used extensively in water treatment to remove pharmaceuticals residues.
3. Relevant authorities should provide consumer education on proper disposal of expired or unused drugs.
4. The efficiency of the rice husk biochar in the removal of other types of contaminants like organic, inorganic pollutants, pesticides waste effluents should also be investigated.
5. Authorities should consider separating waste treatment, if possible, at the source of wastewater prone to high concentrations e.g., hospital wastewater.
6. Pharmaceutical companies should be tasked to assess how their products are discarded and where necessary make sure that they do not reach the water ways.
7. The results should aid scientists and engineers in planning and designing of WWTPs

5.2.2 Recommendations for Further Works

1. Future researchers should dwell on comparative studies on effectiveness, efficiency and efficacy of rice husk, rice straw and other biomasses.

2. On microbial degradation, further studies are needed to identify metabolites associated with the selected pharmaceuticals.
3. Further studies are required on how pharmaceuticals might affect a selected microbial community in terms of genomics and metabolomics and at what levels.
4. Further studies should be done on how presence of hormonal pharmaceuticals such as female hormones might affect human males who drink water containing these hormones for a long period
5. Further studies on how to subject the selected microorganisms in removal of the selected pharmaceuticals from real wastewater instead of from model solutions.

REFERENCES

Abdel–Gawad, S. A. and Abd El–Aziz, H. M. (2019). Removal of Pharmaceuticals from Aqueous Medium Using Entrapped Activated Carbon in Alginate. *Air, Soil and Water Research*, *12*, 1-7.

Abdullah, A. H., Kassim, A., Zainal, Z., Hussein, M. Z., Kuang, D., Ahmad, F. and Wooi, O. S, (2001). Preparation and Characterization of Activated Carbon from Gelam Wood Bark.*Malaysian Journal of Analytical Sciences*, *7*(1) 65-68.

Ackermann, B. L., Berna, M. J. and Murphy, A. T. (2002). Recent Advances in Use of LC/MS/MS for Quantitative High-Throughput Bioanalytical Support of Drug Discovery. *Current Topics in Medicinal Chemistry*, *2*(1), 53-66.

Adachi, A., Ikeda, C., Takagi, S., Fukao, N., Yoshie, E., and Okano, T. (2001). Efficiency of Rice Bran for Removal of Organochlorine Compounds and Benzene from Industrial Wastewater. *Journal of Agricultural and Food Chemistry*, *49*(3), 1309-1314.

Ahiduzzaman, M. and Sadrul Islam, A. K. M. (2016). Preparation of Porous Bio-Char and Activated Carbon From Rice Husk By Leaching Ash and Chemical Activation. *Springerplus*, *5*(1), 1-14.

Ahmad, H. A., Ahmad, S., Cui, Q., Wang, Z., Wei, H., Chen, X. and Tawfik, A. (2022). The Environmental Distribution and Removal of Emerging Pollutants, Highlighting the Importance of Using Microbes as a Potential Degrader: A Review. *Science of the Total Environment*, *809*, 1-14.

Ahmad M, Lee SS, Dou X. (2012). Effects of Pyrolysis Temperature on Soybean Stover and Peanut Shell-Derived Biochar Properties and TCE Adsorption in Water. *Bioresource Technology 118*, 536–544.

Akhtar, M. F., Hanif, M. and Ranjha, N. M. (2016). Methods of Synthesis of Hydrogels. A Review. *Saudi Pharmaceutical Journal*, *24*(5), 554-559.

Al-Gheethi, A., Noman, E., Radin Mohamed, R. M. S., Ismail, N., Bin Abdullah, A. H. and Mohd Kassim, A. H. (2019). Optimizing of Pharmaceutical Active Compounds Biodegradability in Secondary Effluents by B-Lactamase from

Bacillus Subtilis Using Central Composite Design. *Journal of Hazardous Materials*, *365*, 883–894.

Al Masud, M. A. and Chowdhury, M. A. H. (2020). Analyzing the Effect of Annealing Temperature on the Crystalline Parameters of ZNO Nanoparticles. 99, 104

Alok, M., Lisha, K. and Jyoti M. (2007). Freundlich and Langmuir Adsorption Isotherms and Kinetics for the Removal of Tartrazine from Aqueous Solutions Using Hen Feathers. *Journal of Hazardous Materials , 146*, 243-248.

Aloko, D.F. and Adebayo G.A. (2007). Production and Characterization of Activated Carbon from Agricultural Waste (Rice- Husk and Corn-Cob). *Journal of Engineering and Applied* Sciences, *2*(2), 440–480.

Alterior, K. R., Carene, B., Emmanuel, E., Lambert, J., Ehrhardt. J. and Gaspard, S., (2008). Adsorption Studies of Methylene Blue and Phenol onto Vetiver Roots Activated CarbonPrepared by Chemical Activation. *Journal of Hazardous Materials*, *10*, 1-11.

Alzaydien, A. S. (2016). Physical, Chemical and Adsorptive Characteristics Of Local Oak Sawdust Based Activated Carbons. *Asian Journal of Scientific Research*, *9*(2), 45-56.

Angın, D. (2013). Effect of Pyrolysis Temperature and Heating Rate on Biochar Obtained from Pyrolysis of Safflower Seed Press Cake. *Bioresource Technology*, *128*, 593-597.

Anthérieu, S., Le Guillou, D., Coulouarn, C., Begriche, K., Trak-Smayra, V., Martinais, S. and Fromenty, B. (2014). Chronic Exposure to Low Doses of Pharmaceuticals Disturbs the Hepatic Expression of Circadian Genes in Lean and Obese Mice. *Toxicology and Applied Pharmacology*, *276*(1), 63-72.

Babas, H., Kaichouh, G., Khachani, M., Karbane, M. E., Chakir, A., Guenbour, A. and Zarrouk, A. (2021). Equilibrium and Kinetic Studies for Removal of Antiviral Sofosbuvir from Aqueous Solution by Adsorption on Expanded Perlite: Experimental, Modelling and Optimization. *Surfaces and Interfaces*, *23*(100962), 1-10

Babel, S. Kurniawan, T.A. (2003). Low-Cost Adsorbents for Heavy Metals Uptake from Contaminated Water: A Review. *Journal of Hazardous Materials. 97*, 219–243.

Baçaoui, A., Yaacoubi, A., Dahbi, A., Bennouna, C., Luu, R. P. T., Maldonado-Hodar, F. J. and Moreno-Castilla, C. (2001). Optimization of Conditions for The Preparation of Activated Carbons from Olive-Waste Cakes. *Carbon Journal* 39(3), 425-432.

Barbosa-García, O., Ramos-Ortiz, G., Maldonado, J. L., Pichardo-Molina, J. L., Meneses-Nava, M. A., Landgrave, J. E. A. and Cervantes-Martínez, J. (2007). UV–Vis Absorption Spectroscopy and Multivariate Analysis as a Method to Discriminate Tequila. *Spectrochimica Acta Part A: Molecular And Biomolecular Spectroscopy*, 66(1), 129-134.

Barros, F. F. C., Simiqueli, A. P. R., de Andrade, C. J. and Pastore, G. M. (2013). Production of Enzymes from Agroindustrial Wastes By Biosurfactant-Producing Strains of Bacillus Subtilis. *Biotechnology Research International*, 1-9.

Benzaoui, T., Djabali, D. and Selatnia, A. (2018). Adsorption of Copper (II) Ions from Aqueous Solution Using Bottom Ash of Expired Drugs Incineration. *Adsorption Science and Technology, 36*(1–2) 114–129.

Bernal, V., Erto, A., Giraldo, L.,and Moreno-Piraján, J. C. (2017). Effect of Solution pH on the Adsorption of Paracetamol on Chemically Modified Activated Carbons. *Molecules*, *22*(1032), 1-14.

Bhamare, V. S. and Kulkarni, R. M. (2019). Photocatalytic Degradation of Pharmaceutical Drug Zidovudine by Undoped and 5% Barium Doped Zinc Oxide Nanoparticles during Water Treatment: Synthesis and Characterisation. *International Journal of Applied Pharmaceutics*, *11*(1), 227–236.

Bhembe, Y. A., Lukhele, L. P., Hlekelele, L., Ray, S. S., Sharma, A., Vo, D. V. N. and Dlamini, L. N. (2020). Photocatalytic Degradation of Nevirapine with a Heterostructure of Few-Layer Black Phosphorus Coupled with Niobium (V) Oxide Nanoflowers (FL-BP@ Nb2O5). *Chemosphere*, 261, 128159.

Bidayatul, A, Atika, Z., Wahyu H. and Piarah, D.(2018). Analysis of Chemical and Physical Properties of Biochar from Rice Husk Biomass. *Journal of Physics: Conference Series* 979, 012038

Bidlas, E., Du, T. and Lambert, R. J. (2008). An Explanation for the Effect of Inoculum Size on MIC and the Growth/No Growth Interface. *International Journal of Food Microbiology*, *126*(1-2), 140-152.

Birbas, D. (2011). Preparation of Activated Carbon: Forest Residues Activated Carbon with Phosphoric Acid and Zinc Sulfate. Msc Thesis, Royal Institute of Technology, Sweden.

Biselli, S., Schwalb, U., Meyer, A. and Hartig, L. (2013). A Multi-Class, Multi-Analyte Method for Routine Analysis of 84 Veterinary Drugs In Chicken Muscle Using Simple Extraction and LC-MS/MS. *Food Additives and Contaminants: Part A*, *30*(6), 921-939.

Blánquez, P., Hom-Díaz, A., Vicent, T. and Guieysse, B. (2020). Microalgae-Based Processes for the Removal of Pharmaceuticals in Wastewater. *Removal and Degradation of Pharmaceutically Active Compounds in Wastewater Treatment* . 191, 222.

Brausch, J. M., Connors, K. A., Brooks, B. W. and R. and, G. M. (2012). Human Pharmaceuticals in the Aquatic Environment: A Review of Recent Toxicological Studies and Considerations for Toxicity Testing. *Reviews of Environmental Contamination and Toxicology Volume 218*, 1-99.

Browne, N. and Dowds, B. C. A. (2002). Acid Stress in the Food Pathogen Bacillus Cereus. *Journal of Applied Microbiology*, *92*(3), 404-414.

Butterfield, N., Rowe, P. M., Stewart, E., Roesel, D. and Neshyba, S. (2017). Quantitative Three-Dimensional Ice Roughness from Scanning Electron Microscopy. *Journal of Geophysical Research: Atmospheres*, *122*(5), 3023-3041.

Byrdwell, W. C. (2001). Atmospheric Pressure Chemical Ionization Mass Spectrometry for Analysis of Lipids. *Lipids*, *36*(4), 327-346.

Caira, M.R.,Bourne, S.A., Samsodien, H. Engel, E. and Liebenberg, W. (2011). Co-Crystals of the Antiretroviral Nevirapine: Crystal Structures, Thermal Analysis and Dissolution Behaviour. *Cryst Eng Comm 14*(7), 2335-2596.

Cecilia M., Kairigo, P., Patrick H.,Ngumba., Raude.,J., Gachanja, A. and Tuhkanen, T.(2020). Mass Loading, Distribution, and Removal of Antibiotics and Antiretroviral Drugs in Selected Wastewater Treatment Plants in Kenya. *Science of the Total Environment, 743*(140655), 1-56.

Celiz, M. D., Tso, J. and Aga, D. S. (2009). Pharmaceutical Metabolites in the Environment: Analytical Challenges and Ecological Risks. *Environmental Toxicology and Chemistry*, *28*(12), 2473-2484.

Chang, S., Waite, T. D., Ong, P. E., Schäfer, A. I. and Fane, A. G. (2004). Assessment of Trace Estrogenic Contaminants Removal by Coagulant Addition, Powdered Activated Carbon Adsorption and Powdered Activated Carbon/Microfiltration Processes. *Journal of Environmental Engineering*, *130*(7), 736-742.

Chemwolo, A. K., Ngoni, J. and Clark, B. (2010). Perceptions and Views Of Regulatory Pharmacists on the Registration System for Generic Drugs for Human use in Kenya. *East African Medical Journal*, *87*(3), 120-126.

Chen, C. Y., Chen, S. C., Fingas, M. and Kao, C. M. (2010). Biodegradation of Propionitrile by Klebsiella Oxytoca Immobilized in Alginate and Cellulose Triacetate Gel. *Journal of Hazardous Materials*, *177*(1-3), 856-863.

Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N. and Kroiss, H. (2005). Removal of Selected Pharmaceuticals, Fragrances and Endocrine Disrupting Compounds in a Membrane Bioreactor and Conventional Wastewater Treatment Plants. *Water Research*, *39*(19), 4797-4807.

Dasenaki, M. E. and Thomaidis, N. S. (2015). Multi-Residue Determination of 115 Veterinary Drugs and Pharmaceutical Residues in Milk Powder, Butter, Fish Tissue and Eggs using Liquid Chromatography–Tandem Mass Spectrometry. *Analytica Chimica Acta*, *880*, 103-121.

Dasgupta, D., Ghosh, R. and Sengupta, T. K. (2013). Biofilm-Mediated Enhanced Crude Oil Degradation by Newly Isolated Pseudomonas Species. *International Scholarly Research Notices*, (250749), 1-13.

da Silva, M. N., Tagliapietra, B. L., do Amaral Flores, V. and dos Santos Richards, N. S. P. (2021). In Vitro Test to Evaluate Survival in the Gastrointestinal Tract of Commercial Probiotics. *Current Research in Food Science*, *4*, 320-325.

Daughton, C. G. and Ternes, T. A. (1999). Pharmaceuticals and Personal Care Products in the Environment: Agents of Subtle Change? *Environmental Health Perspectives*, *107*(suppl 6), 907-938.

Debbie, S. (2008). Principles and Practice of Variable Pressure/Environmental Scanning Electron Microscopy (VP-ESEM). John Wiley and Sons.

de Luna, M. D. G., Veciana, M. L., Su, C. C. and Lu, M. C. (2012). Acetaminophen Degradation by Electro-Fenton and Photoelectro-Fenton using a Double Cathode Electrochemical Cell. *Journal of Hazardous Materials*, *217*, 200-207.

Demirbas, E., Kobya, M. and Konukman, A. E. S. (2008). Error Analysis of Equilibrium Studies for the Almond Shell Activated Carbon Adsorption of Cr(VI) from Aqueous Solutions. *Journal of Hazardous Materials*, *154*(1–3), 787–794.

Desbrow, C. E. J. R., Routledge, E. J., Brighty, G. C., Sumpter, J. P. and Waldock, M. (1998). Identification of Estrogenic Chemicals in STW Effluent. 1. Chemical Fractionation and In Vitro Biological Screening. *Environmental Science and Technology*, *32*(11), 1549-1558.

Díaz, E., Ferrández, A., Prieto, M. A. and García, J. L. (2001). Biodegradation of Aromatic Compounds by Escherichia coli. *Microbiology and Molecular Biology Reviews*, *65*(4), 523-569.

Dowling, G., Malone, E. (2011). Analytical Strategy for the Confirmatory Analysis of the Non-Steroidal Anti-Inflammatory Drugs Firocoxib, Propyphenazone, Ramifenazone and Piroxicam in Bovine Plasma By Liquid Chromatography Tandem Mass Spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *56*(2), 359-365.

Du, C., Liu, B., Hu, J. and Li, H. (2021). Determination of Iodine Number of Activated Carbon by the Method of Ultraviolet–Visible Spectroscopy. *Materials Letters*, *285*, 129137.

Duaa ,J., Ali R., Al-Bayati1, A. and Ramzie R. A. (2016). Adsorption – Desorption and Theoretical Study of Propranolol Hydrochloride Drug on Chitosan and Cellulose Acetate Surfaces. *British Journal of Pharmaceutical Research 10*(4), 1-8.

Echlin, P., Fiori, C. E., Goldstein, J., Joy, D. C. and Newbury, D. E. (2013). Advanced Scanning Electron Microscopy and X-Ray Microanalysis. Springer Science and Business Media. *629*, 1-454.

Ehlert, S., Trojer, L., Vollmer, M., van de Goor, T. and Tallarek, U. (2010). Performance of HPLC/MS Microchips in Isocratic and Gradient Elution Modes. *Journal of Mass Spectrometry*, *45*(3), 313-320.

Emeka, E. E., Ojiefoh, O. C., Aleruchi, C., Hassan, L. A., Christiana, O. M., Rebecca, M. and Temitope, A. E. (2014). Evaluation of Antibacterial Activities of Silver Nanoparticles Green-Synthesized using Pineapple Leaf (Ananas comosus). *Micron*, *57*, 1-5.

Erick, O. V. and Moore, M. M. (2007) Assessing the Assessments: Pharmaceutical in the Environment. *Environment Impact Assessment Review, 27*(6), 1244 – 1251.

Erulkar, A. and Matheka, J. K. (2007). Adolescence in the Kibera Slums of Nairobi, Kenya.

Evangelista, S., Cooper, D. G., and Yargeau, V. (2010). The Effect of Structure and a Secondary Carbon Source on the Microbial Degradation of Chlorophenoxy Acids. *Chemosphere, 79*(11), 1084-1088.

European Commission 2012. Directive of the European Parliament and of the Council Amending Directives 2000/60/EC and 2008/105/EC as Regards Priority Substances in the Field of Water Policy. European Commission, Brussels

Evard, H., Kruve, A. and Leito, I. (2016). Tutorial on Estimating the Limit of Detection using LC-MS Analysis, Part I: Theoretical Review. *Analytica Chimica Acta*, *942*, 23-39.

Fawell, J. and Nieuwenhuijsen, M. J. (2003). Contaminants in Drinking Water Environmental Pollution and Health. *British Medical Bulletin*, *68*(1), 199-208.

Feng, L., Guang- Guo, Y., Jiang – Liang, Z., Ji-Feng, Y. and Lan-Feng, Z. (2009). Effects of Six Selected Antibiotics on Plant Growth and Soil Microbial and Enzymatic Activities. *Journal of Environmental Pollution 157*(5), 1636 – 1642.

Foo, K. Y. and Hameed, B. H. (2010). Insights into the Modeling of Adsorption Isotherm Systems. *Chemical Engineering Journal*, *156*(1), 2–10.

Fu, Y., Shen, Y., Zhang, Z., Ge, X. and Chen, M. (2019). Activated Bio-Chars Derived from Rice Husk Via One-and Two-Step KOH-Catalyzed Pyrolysis for Phenol Adsorption. *Science of the Total Environment, 646*, 1567-1577.

Garg, V.K, Gupta, R. Yadav, A.B. and Kumar R. (2003). Dye Removal from Aqueous Solution by Adsorption on Treated Sawdust. *Bioresource Technology, 89*(2), 121–124.

Garofolo, F. (2004). *LC-MS Instrument Calibration* (p. 197e220). John Wiley and Sons, Inc., Hoboken, NJ, USA.

Gaskin, J. W., Steiner, C., Harris, K., Das, K. C. and Bibens, B. (2008). Effect of Low-Temperature Pyrolysis Conditions on Biochar for Agricultural Use. *Transactions of the ASABE*, *51*(6), 2061-2069.

Gaspari, M. and Cuda, G. (2011). Nano LC–MS/MS: A Robust Setup for Proteomic Analysis.

Gauthier, H., Yargeau, V. and Cooper, D. G. (2010). Biodegradation of Pharmaceuticals by *Rhodococcus rhodochrous* and *Aspergillus niger* by Co-metabolism. *Science of the Total Environment, 408*(7), 1701-1706.

Grasse, E. K., Torcasio, M. H. and Smith, A. W. (2016).Teaching UV–Vis Spectroscopy with a 3D-Printable Smartphone Spectrophotometer. *Journal of Chemical Education*, *93*(1), 146-151.

Griffiths, P. and de Hasseth, J. A. (2007). Fourier Transform Infrared Spectrometry (2nd edition.).

Gulkowska, A., Leung, H. W., So, M. K., Taniyasu, S., Yamashita, N., Yeung, L. W. and Lam, P. K. (2008). Removal of Antibiotics from Wastewater by Sewage Treatment Facilities in Hong Kong and Shenzhen, China. *Water Research*, *42*(1-2), 395-403.

Guo, J., Gui, B., Xiang, S. X., Bao, X. T., Zhang, H. J. and Lua, A. C. (2008). Preparation of Activated Carbons by Utilizing Solid Wastes from Palm Oil Processing Mills. *Journal of Porous Materials*, *15*(5), 535-540.

Guo, Y., Yu, K., Wang, Z. and Xu, H. (2003). Effects of Activation Conditions on Preparation of Porous Carbon from Rice Husk. *Carbon (New York, NY)*, *41*(8), 1645-1648.

Hameed, B. H., Tan, I. A. W. and Ahmad, A. L. (2008). 'Adsorption Isotherm, Kinetic Modeling and Mechanism of 2,4,6-Trichlorophenol on Coconut Husk-Based Activated Carbon.' *Chemical Engineering Journal*, *144*(2), 235 - 244.

Hanold, K. A., Fischer, S. M., Cormia, P. H., Miller, C. E. and Syage, J. A. (2004). Atmospheric Pressure Photoionization. 1. General properties for LC/MS. *Analytical Chemistry*, *76*(10), 2842-2851.

Hasan, S. A., Ferreira, M. I. M., Koetsier, M. J., Arif, M. I. and Janssen, D. B. (2011). Complete Biodegradation of 4-fluorocinnamic Acid by a Consortium Comprising Arthrobacter sp. Strain G1 and Ralstonia sp. Strain H1. *Applied and Environmental Microbiology*, *77*(2), 572-579.

Hilp M. (2002). "Determination of Iodine Values According to Hanuš Using 1,3-dibromo-5,5-dimethylhydantoin (DBH)." *Journal of Pharmaceutical and Biomedical Analysis*, *28* (1), 81–86.

Himmelsbach, M., Buchberger, W. and Reingruber, E. (2009). Determination of Polymer Additives by Liquid Chromatography Coupled with Mass Spectrometry. A Comparison of Atmospheric Pressure Photoionization (APPI), Atmospheric Pressure Chemical Ionization (APCI), and Electrospray Ionization (ESI). *Polymer Degradation and Stability*, *94*(8), 1213-1219.

Hook, S. E., Skillman, A. D., Small, J. A. and Schultz, I. R. (2007). Temporal Changes in Gene Expression in Rainbow Trout Exposed to Ethynyl Estradiol. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, *145*(1), 73-85.

Hopfgartner, G., Husser, C. and Zell, M. (2002). High-Throughput Quantification of Drugs and their Metabolites in Biosamples by LC-MS/MS and CE-MS/MS: Possibilities and Limitations. *Therapeutic Drug Monitoring*, *24*(1), 134-143.

Huhta, H. K., Rytkönen, J. and Sassi, J. (2007). Estimated Nutrient Load from Waste Waters Originating from Ships in the Baltic Sea Area. *VTT TIEDOTTEITA, 2370*.

Hunter, R. D., Ekunwe, S. I., Dodor, D. E., Hwang, H. M. and Ekunwe, L. (2005). *Bacillus Subtilis* as A Potential Degrader of Pyrene and Benzo [A] Pyrene. *International Journal of Environmental Research and Public Health*, *2*(2), 267-271.

Iheanacho, O. C., Nwabanne, J. T., Obi, C. C. and Onu, C. E. (2021). Packed Bed Column Adsorption of Phenol onto Corn Cob Activated Carbon: Linear and Nonlinear Kinetics Modeling. *South African Journal of Chemical Engineering*, *36*, 80-93.

Ikonen, K. E., Wehde, K. E., Khalida, H. and Kenttämaa, H. I. (2020). Bias, Limit of Detection and Limit of Quantitation for the ASTM D2425 Method Updated in 2019. *Journal of Chromatography A*, *1614*, 460705.

Isoda, M, Rodriques, R, Silvia, A, Gondalves, M, Mandeli, D, Fiqudinedo, F.C.A. and Carvalho, W.A (2014). Optimization of Preparation Conditions of Activated Carbon from Agricultural Waste Utilizing Factorial Design. *Powder Technology*, *256*, 175- 181.

Jagtoyen, M. and Derbyshire, F. (1998). Activated Carbons from Yellow Poplar and White Oak by H_3PO_4 Activation. *Carbon*, *36*(7-8), 1085-1097.

Jain, S., Vyas, R. K., Pandit, P. and Dalai, A. K. (2014). Adsorption of Antiviral Drug, Acyclovir from Aqueous Solution on Powdered Activated Charcoal: Kinetics,

Equilibrium, and Thermodynamic Studies. *Desalination and Water Treatment*, *52*(25-27), 4953-4968.

Jaseem, M., Kumar, P. and John, R. M. (2017). An Overview of Waste Management in Pharmaceutical Industry. *The Pharma Innovation*, *6*(3, Part C), 158.

Jaureguy, F., Landraud, L., Passet, V., Diancourt, L., Frapy, E., Guigon, G. and Brisse, S. (2008). Phylogenetic and genomic diversity of human bacteremic Escherichia coli strains. *BMC genomics*, *9*, 1-14.

Jenkins, K. M., Angeles, R., Quintos, M. T., Xu, R., Kassel, D. B. and Rourick, R. A. (2004). Automated High Throughput ADME Assays for Metabolic Stability and Cytochrome P450 Inhibition Profiling of Combinatorial Libraries. *Journal of Pharmaceutical and Biomedical Analysis*, *34*(5), 989-1004.

Jindo, K., Mizumoto, H., Sawada, Y., Sanchez-Monedero, M. A. and Sonoki, T. (2014). Physical and Chemical Characterization of Biochars Derived from Different Agricultural Residues. *Biogeosciences*, *11*(23), 6613–6621.

Jones, O. A., Voulvoulis, N. and Lester, J. N. (2003). Potential Impact of Pharmaceuticals on Environmental Health. *Bulletin of the World Health Organization*, *81*, 768-769.

Jones, O.A.H, Voulvoulos, N. and Lester, J.N (2004). Potential Ecological and Human Health Risks Associated with the Presence of Pharmaceutically Active Compounds in the Adequate Environment. *Critical Reviews in Toxicology, 34(*4), 335 – 350.

Jones, O.A.H, Voulvoulis, N. and Lester, J.N (2007). The Oddurence in a Sewage Treatment Works Utilizing Activated Sledge Treatment. *Environmental Pollution1 45*(3), 738 – 744.

Jun Hu, Li L. Zhang, Jian M. Chen. and Yu Liu (2013). Degradation of Paracetamol by Pseudomonas aeruginosa strain HJ1012. *Journal of Environmental Science and Health*, Part A: *Toxic/Hazardous Substances and Environmental Engineering*, *48*(7), 791-799.

Kairigo, P., Ngumba, E., Sundberg, L. R., Gachanja, A. and Tuhkanen, T. (2020). Occurrence of Antibiotics and Risk of Antibiotic Resistance Evolution in Selected Kenyan Wastewaters, Surface Waters and Sediments. *Science of the Total Environment*, *720*, 137580.

Kang, W. and Kim, E. Y. (2008). Simultaneous Determination of Aceclofenac and its Three Metabolites in Plasma using Liquid Chromatography–Tandem Mass Spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *46*(3), 587-591.

Kanjilal, T., Bhattacharjee, C. and Datta, S. (2016). Utilization of S. *Aureus* Strain 502A in Biodegradation of Insecticide Acetamiprid from Wetland Wastewater. *Desalination and Water Treatment*, *57*(28), 13190-13206.

Katherine, Y. B., Martha, J. M. W., Kathy, A., Traexler, M. P., Audra, M. and Jeff, B. (2011). Emerging Pollutants. *Water Environment Research, 83*(10), 1906-1984.

Kathryn D. (2011)."Pharmaceutically Active Compounds in Residential and Hospital Effluent, Municipal Wastewater, and the Rio Grande in Albuquerque, New Mexico."

Kemira, K. (2003). About Waste Water. Helsingborg.

Kemper, N (2008). Veterinary Antibiotics in the Aquatic and Terrestrial Environment. A Review. *Ecological Indicators 8*(1), 1-13.

Kessler, R. (2010). INDUSTRY ISSUES: Pharmaceutical Factories as a Source of Drugs in Water.

Khan, M. and Lo, I. M. (2018). A Holistic Review of Hydrogel Applications in the Adsorptive Removal of Aqueous Pollutants: Recent Progress, Challenges, and Perspectives. *Water Research*, *106*, 259-271.

Khan, S. J. and Ongerth, J. E. (2004). Modelling of Pharmaceutical Residues in Australian Sewage By Quantities of Use and Fugacity Calculations. *Chemosphere*, *54*(3), 355-367.

Kilic, M., Apaydin-Varol, E. and Putun, A. E., (2011). Adsorptive Removal of Phenol from Aqueous Solutions on Activated Carbon Prepared from Tobacco Residues:

Equilibrium, Kinetics and Thermodynamics. *Journal of Hazardous Materials, 189*, 397 - 403.

Kim, Y., Choi, K., Jung, J., Park, S., Kim, P. G. and Park, J. (2007). Aquatic Toxicity of Acetaminophen, Carbamazepine, Cimetidine, Diltiazem and Six Major Sulfonamides, and their Potential Ecological Risks in Korea. *Environment International*, *33*(3), 370-375.

Kiran, B. R. and Prasad, M. N. V. (2019). Biochar and Rice Husk Ash Assisted Phytoremediation Potentials of Ricinus Communis L. For Lead-Spiked Soils. *Ecotoxicology and Environmental Safety*, *183*, 109574.

Klein, T., Buhr, E. and Frase, C. G. (2012). TSEM: A Review of Scanning Electron Microscopy in Transmission Mode and Its Applications. *Advances in Imaging and Electron Physics*, *171*, 297-356.

Kluters, S., Hafner, M., Von Hirschheydt, T. and Frech, C. (2015). Solvent Modulated Linear pH Gradient Elution for the Purification of Conventional and Bispecific Antibodies: Modeling and Application. *Journal of Chromatography A*, *1418*, 119-129

Kolpin, D. W., Furlong, E. T., Meyer, M. T., Thurman, E. M., Zaugg, S. D., Barber, L. B. and Buxton, H. T. (2002). Pharmaceuticals, Hormones, and other Organic Wastewater Contaminants in US Streams, 1999−2000: A National Reconnaissance. *Environmental Science and Technology*, *36*(6), 1202-1211.

K'oreje, K. O., Demeestere, K., De Wispelaere, P., Vergeynst, L., Dewulf, J. and Van Langenhove, H. (2012). From multi-residue screening to target analysis of pharmaceuticals in water: development of a new approach based on magnetic sector mass spectrometry and application in the Nairobi River basin, Kenya. *Science of the Total Environment*, *437*, 153-164

K'oreje, K. O., Vergeynst, L., Ombaka, D. P. O., Wispelaere, P. De, Okoth, M. O., Langenhove, H. R. and Van Demeestere, K. (2016). Occurrence Patterns of Pharmaceutical Residues in Wastewater, Surface Water and Groundwater of Nairobi and Kisumu City, Kenya. *Chemosphere*, *149*, 238–244.

Kulkarni, P. and Chellam, S. (2010). Disinfection By-Product Formation following Chlorination of Drinking Water: Artificial Neural Network Models and Changes in Speciation with Treatment. *Science of the Total Environment, 408* (19), 4202–4210.

Kumar, P.S., Vincent, C., Kirthika, K. and Kumar, S.K. (2010). Kinetics and Equilibrium Studies of Pb^{2+} Ion Removal from Aqueous Solutions by use of Nano-Silversol-Coated Activated Carbon. *Brazilian Journal of Chemical Engineering, 27*(2), 339-346.

Kümmerer, K. (2004). Resistance in the Environment. *Journal of Antimicrobial Chemotherapy*, *54*(2), 311-320.

Leal, J. E., Thompson, A. N. and Brzezinski, W. A. (2010). Pharmaceuticals in Drinking Water: Local Analysis of the Problem and Finding a Solution through Awareness. *Journal of the American Pharmacists Association, 50*(5), 600–603.

Lee, B. C. Y., Lim, F. Y., Loh, W. H., Ong, S. L. and Hu, J. (2021). Emerging Contaminants: An Overview of Recent Trends for their Treatment and Management using Light-Driven Processes. *Water* 2021, 13, 2340.

Leung, K. S. Y. and Fong, B. M. W. (2014). LC–MS/MS in the Routine Clinical Laboratory: Has its Time Come? *Analytical and Bioanalytical Chemistry*, *406*, 2289-2301.

Li, D., Steimling, J. A., Konschnik, J. D., Grossman, S. L. and Kahler, T. W. (2019). Quantitation of Mycotoxins in Four Food Matrices Comparing Stable Isotope Dilution Assay (SIDA) with Matrix-Matched Calibration Methods by LC–MS/MS. *Journal of AOAC International*, *102*(6), 1673-1680.

Li, J., Ye, Q. and Gan, J. (2014). Degradation and Transformation Products of Acetaminophen in Soil. *Water Research, 49*, 44-52.

Li, M., Hou, Y. L. and Zhu, B. (2007). Phosphorus Sorption–Desorption by Purple Soils of China in Relation to their Properties. *Soil Research, 45*(3), 182-189.

Li, Y., Sun, S., Ma, M., Ouyang, Y. and Yan, W. (2007). The Effects of Activated Carbon Supports on the Structure and Properties of TiO_2 Nanoparticles Prepared by Sol-gel Method. *Applied Surface Science*, *253*(23), 9254 – 9258.

Liang, B., Kong, D., Qi, M., Yun, H., Li, Z., Shi, K. and Wang, A. (2019). Anaerobic Biodegradation of Trimethoprim with Sulfate as an Electron Acceptor. *Frontiers of Environmental Science and Engineering*, *13*(6), 1-10.

Lin, J. and Wang, L. (2009). Comparison between Linear and Non-Linear Forms of Pseudo-First Order and Pseudo-Second-Order Adsorption Kinetic Models for the Removal of Methylene Blue by Activated Carbon. *Frontiers of Environmental Science and Engineering in China*, *3*(3), 320-324.

Lindqvist, N., Tuhkanen, T. and Kronberg, L. 2005. Occurrence of Acidic Pharmaceuticals in Raw and Treated Sewages and in Receiving Waters. Tampere, Turku, Finland. *Water Research, 39*, 2219-2228.

Liu, M., Zhao, S., Wang, Z., Wang, Y., Liu, T., Li, S. and Tu, P. (2014). Identification of Metabolites of Deoxyschizandrin in Rats by UPLC–Q-TOF-MS/MS Based on Multiple Mass Defect Filter Data Acquisition and Multiple Data Processing Techniques. *Journal of Chromatography B*, *949*, 115-126.

Liu, Q., Li, M., Liu, X., Zhang, Q., Liu, R., Wang, Z. and Zhang, F. (2018). Removal of Sulfamethoxazole and Trimethoprim from Reclaimed Water and the Biodegradation Mechanism. *Frontiers of Environmental Science and Engineering*, *12*(6), 1-8.

Liu, Y., Chen, K., Dong, F., Peng, S., Cui, Y., Zhang, C. and Zhang, H. (2018). Effects of Hydrolysis of Precursor on the Structures and Properties of Polymer-Derived Sibn Ceramic Fibers. *Ceramics International*, *44*(9), 10199-10203.

Lucas, D., Castellet-Rovira, F., Villagrasa, M., Badia-Fabregat, M., Barceló, D., Vicent, T. and Rodríguez-Mozaz, S. (2018). The Role of Sorption Processes in the Removal of Pharmaceuticals by Fungal Treatment of Wastewater. *Science of the Total Environment*, *610*, 1147-1153.

Madivoli, E. S., Kareru, P. G., Makhanu, D. S., Wandera, K. S., Maina, E. G., Wanakai, S. I. and Kimani, P. K. (2020). Synthesis of Spherical Titanium Dioxide Microspheres and its Application to Degrade Rifampicin. *Environmental Nanotechnology, Monitoring and Management*, *14*, 100327.

Mahmood, Q., Pervez, A., Zeb, B. S., Zaffar, H., Yaqoob, H., Waseem, M. and Afsheen, S. (2013). Natural Treatment Systems as Sustainable Ecotechnologies for the Developing Countries. *Biomed Research International*, (796373), 1-19.

Mahmoud, D. K, MohdSalleh, M.A, AabdulKkarim, W.A. Idris, A. and Abidin, Z.Z. (2012). Batch Adsorption of Basic Dye using Acid treated Kenaffibre Char. Equilibrium, Kinetic and Thermodynamic Studies. *Chemical Engineering Journal, 181,* 449 – 457.

Martin, M. J., Artola, A., Balaguer, M. D. and Rigola, M. (2003). Activated Carbons Developed from Surplus Sewage Sludge for the Removal of Dyes from Dilute Aqueous Solutions. *Chemical Engineering Journal*, *94*(3), 231-239.

Martos, P., Thompson, W., and Diaz, G. (2010). Multiresidue Mycotoxin Analysis in Wheat, Barley, Oats, Rye and Maize Grain by High-Performance Liquid Chromatography-Tandem Mass Spectrometry. *World Mycotoxin Journal*, *3*(3), 205-223.

Mathenge, S. G. (2013). Assessment of Selected Antibiotics and Heavy Metals in Untreated Wastewater, Vegetables and Soils in Eastern Nairobi, Kenya. *Unpublished PhD Thesis Kenyatta University.*

Mohan, D., Pittman, C. U. and Steele, P. H. (2006). Pyrolysis of Wood/Biomass for Bio-oil: A Critical Review. *Energy and Fuels*, *20*(4), 848–889.

Moll, D. M. and Frick, E. A. (1999). Presence of Pharmaceutical in Treated Wastewater Effluent and Surface Water Supply System. Metropolitan, Antlanta, Georgia.

Mopoung, S., Udeye, V., Viruhpintu, S., Yimtragool, N. and Unhong, V. (2020). Water Treatment for Fish Aquaculture System by Biochar-Supplemented Planting Panel System. *The Scientific World Journal*, (7901362), 1-8.

Moussavi, G., Alahabadi, A., Yaghmaeian, K. and Eskandari, M. (2013). Preparation, Characterization and Adsorption Potential of NH_4Cl-Induced Activated Carbon for the Removal
of Amoxicillin Antibiotic. *Chemical Engineering Journal,* 217, 119 - 128.

Mukoko, T., Mupa, M. and Dziike, F. (2015). Preparation of Rice Hull Activated Carbon for the Removal of Selected Pharmaceutical Waste Compounds in Hospital Effluent. *Journal of Environmental and Analytical Toxicology, 10*, 4172/2161-0525.S7-008

Mulder, M., Antakyali, D.and Ante, S. (2015). Costs of Removal of Micropollutants from Effluents of Municipal Wastewater Treatment Plants-General Cost Estimates for the Netherlands Based on Implemented Full Scale Post Treatments of Effluents of Wastewater Treatment Plants in Germany and Switzerland. *STOWA and Waterboard the Dommel, the Netherlands*, 1-52

Murphy, B. L and Morrison, R.D. (2002). Introductions to Environmental Forensics London U.K Academic Press.

Nassar, M. Y., Ahmed, I. S. and Raya, M. A. (2019). A Facile and Tunable Approach for Synthesis of Pure Silica Nanostructures from Rice Husk for the Removal of Ciprofloxacin Drug from Polluted Aqueous Solutions. *Journal of Molecular Liquids*, *282*, 251-263.

Nche, N. A. G., Bopda, A., Tchuifon, D. R. T., Ngakou, C. S., Kuete, I. H. T. and Gabche, A. S. (2017). Removal of Paracetamol from Aqueous Solution by Adsorption onto Activated Carbon Prepared from Rice Husk. *Journal of Chemical and Pharmaceutical Research*, *9*(3), 56-68.

Ndekei, A., Muigai-Gitita, N. N. and Mbui, D. (2021). Synthesis and Characterization of Rice Husk Biochar and its Application in the Adsorption Studies of Lead and Copper. *International Research Journal of Pure and Applied Chemistry*, *22*(4), 36-50.

Negara, D. N. K. P., Nindhia, T. G. T., Surata, I. W., Sucipta, M. and Hidajat, F. (2019). Activated Carbon Characteristics of Tabah Bamboo that Physically Activated

under Different Activation Time. In *IOP Conference Series: Materials Science and Engineering*, *539*(1), 012011.

Neil, S. (2016). Helicobacter Pylori Infection in the Era of Antibiotic Resistance. *Gastroenterology and Hepatology*, *12*(2), 122.

Ngumba,E., Kosunen, P., Gachanja, A. and Tuhkanen, T. (2016a). A Multiresidue Analytical Method for Trace Level Determination of Antibiotics and Antiretroviral Drugs in Wastewater and Surface Water Using SPE-LC-MS/MS and Matrix-Matched Standards. *Analytical Methods*, *8*(37), 6720–6729.

Nicholson, J. K., Connelly, J., Lindon, J. C. and Holmes, E. (2002). Metabolomics: A Platform for Studying Drug Toxicity and Gene Function. *Nature reviews Drug discovery*, *1*(2), 153-161.

Nxumalo, W. P. G. (2016). Improved Sample Preparation Ensures Accurate Quantification of Multiple Mycotoxins in Maize by Liquid Chromatography-Stable Isotope Dilution Assay-Tandem Mass Spectrometry *(LC-SIDA-MS/MS)* (Doctoral Dissertation, University of Pretoria).

Nyaga, M. N., Nyagah, D. M. and Njagi, A. (2020). Pharmaceutical Waste: Overview, Management and Impact of Improper Disposal.

Olives, A. I., Gonzalez-Ruiz, V. and Martin, M. A., 2012, 'Isolation of Non-Steroidal Anti-Inflammatory Drugs', *Anti-Inflammatory and Anti-Allergy Agents in Medicinal Chemistry, 11*, 65 - 95.

Otaru, A. J., Ameh, C. U., Abdulkareem, A. S., Odigure, J. O. and Okafor, J. O. (2013). Development and Characterization of Adsorbent from Rice Husk to Bleach Vegetable Oils. *Journal of Applied Chemistry*, *4* (2), 42 - 49.

Pakhre, V. and Srivastava, V. C. (2012, March). Adsorption of Benzoic acid and Salicylic acid onto Granular Activated Carbon. In *International Conference on Chemical, Civil and Environmental Engineering, Dubai*, *3*, 24-25

Pedrouzo, M., Borrull, F., Pocurull, E. and Marcé, R. M. (2011). Presence of Pharmaceuticals and Hormones in Waters from Sewage Treatment Plants. *Water, Air and Soil Pollution*, *217*(1), 267-281.

Pérez, S., Eichhorn, P. and Aga, D.S. (2005). Evaluating the Biodegradability of Sulfamethazine, Sulfamethoxazole, Sulfathiazole, and Trimethoprim at Different Stages of Sewage Treatment. *Environmental Toxicology and Chemistry 24*, 1361–1367

Petrović, M., Hernando, M. D., Díaz-Cruz, M. S. and Barceló, D. (2005). Liquid Chromatography–Tandem Mass Spectrometry for the Analysis of Pharmaceutical Residues in Environmental Samples: A Review. *Journal of Chromatography A, 1067*(1-2), 1-14.

Pia Arentsen, W., Jens J. Efsen, K. Ulrich and Bjarne Munk, H. (2005). Isolation and Taxonomic Affiliation of N-Heterocyclic Aromatic Hydrocarbon-Transforming Bacteria. *Applied Microbiology and Biotechnology*", *67*(3), 420-428.

Ponce, C., Chanona, J., Garibay, V., Palacios, E., Calderon, G. and Sabo, R. (2013). Functionalization of Agave Cellulose Nanoparticles and its Characterization by Microscopy and Spectroscopy Techniques. *Microscopy and Microanalysis*, *19*(2), 200–201.

Qulkowska, A., Leung, H. W., So, M. K., Taniyasu, S., Yamashita, N., Yeung, L.W.Y., Richardson, B.J., Lei., A.P., Giesy, J.P. and Lam, P.K.S. (2008). Removal of Antibiotics from Wastewater by Sewage Treatment Facilities in Hong Kong and Shenzhen. China. *Water Research, 42*(1-3), 395 – 403.

Rajvinder , K., Joginder S., Rajshree, K.Swaranjit, S. C . and Amjad A. (2013). Batch Sorption Dynamics, Kinetics and Equilibrium Studies of Cr(VI), Ni(II) and Cu(II) from Aqueous Phase using Agricultural Residues. *Applied Water Science, 3*(1), 207–218.

Rechab, S. O., Ngugi, C. M., Maina, E. G., Madivoli, E. S., Kareru, P. G., Mutembei, J. K. and Ruto, M. C. (2018). Antioxidant Activity and Antimicrobial Properties of Entada Leptostachya and Prosopis Juliflora Extracts. *Journal of Medicinal Plants for Economic Development, 2*(1), 1-8.

Roth, M. (2003). Pharmaceuticals in the Environment', Retrieved from Campus Ecology (WSU).

Ryan, C.C, Tan, D.T and Arnold, W.A (2011). Direct and Indirect Photolysis of Sulfa methoxazole and Trimethoprim in Waste Treatment Plant Effluent. *Water Resources 45*(3), 1280 – 1286.

Saadati, N., Abdullah, M. P., Zakaria, Z., Sany, S. B. T., Rezayi, M. and Hassonizadeh, H. (2013). Limit of Detection and Limit of Quantification Development Procedures for Organochlorine Pesticides Analysis in Water and Sediment Matrices. *Chemistry Central Journal*, *7*(1), 1-10.

Saito, Y. and Arima, T. (2007). *Carbon*, 45, 248-55.

Samsodien H, Bapoo M, Doms Tl, Harneker Z. and Louw A. S. (2017). FTIR, Dissolution and Anti-viral Activity of Nevirapine Cocrystals. *Pharm Analytical Acta 8*, 561.

Santos, L. S., Pavam, C. H., Almeida, W. P., Coelho, F. and Eberlin, M. N. (2004). Probing the Mechanism of the Baylis–Hillman Reaction by Electrospray Ionization Mass and Tandem Mass Spectrometry. *Angewandte Chemie International Edition*, *43*(33), 4330-4333.

Sarkar M, Perumal OP, Panchagnula R. (2008). Solid-State Characterization of Nevirapine. *Indian Journal Pharmaceutical Sciences,70*(5), 619–30.

Schaefer, N., Kröll, A. K., Körbel, C., Laschke, M. W., Menger, M. D., Maurer, H. H. and Schmidt, P. H. (2019). Distribution of the (Synthetic) Cannabinoids JWH-210, RCS-4, as Well as Δ 9-Tetrahydrocannabinol following Pulmonary Administration to Pigs. *Archives of Toxicology*, *93*(8), 2211-2218.

Schellinger, A. P. and Carr, P. W. (2006). Isocratic and Gradient Elution Chromatography: A Comparison in Terms of Speed, Retention Reproducibility and Quantitation. *Journal of Chromatography A*, *1109*(2), 253-266.

Semreen, M. H., Soliman, S. S., Saeed, B. Q., Alqarihi, A., Uppuluri, P. and Ibrahim, A. S. (2019). Metabolic Profiling of Candida Auris, A Newly Emerging Multi-Drug Resistant Candida Species, by GC-MS. *Molecules*, *24*(3), 399.

Shala, L. and Foster, A.D, (2010). Surface Water Concentrations and Loady Budgets of Pharmaceutical and other Cosmetics used as Chemicals in an Urban Watershed. *Environmental Contamination and Toxicology, 58*(3), 551 – 561.

Sharma, H. R., Chauhan, G. S. and Agrawal, K. (2004). Physico-Chemical Characteristics of Rice Bran Processed by Dry Heating and Extrusion Cooking. *International Journal of Food Properties, 7*(3), 603-614.

Sharma, K., Kaushik, G., Thotakura, N., Raza, K., Sharma, N. and Nimesh, S. (2020). Enhancement Effects of Process Optimization Technique while Elucidating the Degradation Pathways of Drugs Present in Pharmaceutical Industry Wastewater using Micrococcus yunnanensis. *Chemosphere, 238*, 124689.

Shirkhedkar, A. A., Bhirud, C. H. and Surana, S. J. (2009). Application of UV-Spectrophotometric Methods for Estimation of Tenofovir Disoproxil Fumarate in Tablets. *Pakistan Journal of Pharmaceutical Sciences, 22*(1), 27-30.

Silva, R.D.C.F.S., Almeida, D. G., Rufino, R. D., Luna, J. M., Santos, V. A. and Sarubbo, L. A. (2014). Applications of Biosurfactants in the Petroleum Industry and the Remediation of Oil Spills. *International Journal of Molecular Science, 15*, 12523-12542.

Singer, A. C., Shaw, H., Rhodes, V. and Hart, A. (2016). Review of Antimicrobial Resistance in the Environment and its Relevance to Environmental Regulators. *Frontiers in Microbiology, 7*, 1728.

Sipma, J., Osuna, B., Collado, N., Monclús, H., Ferrero, G., Comas, J. and Rodriguez-Roda, I. (2010). Comparison of Removal of Pharmaceuticals in MBR and Activated Sludge Systems. *Desalination, 250*(2), 653-659.

Skoog, D. A., Holler, F. J. and Crouch, S. R. (2017). *Principles of Instrumental Analysis*. Cengage Learning.

Smýkalová, A., Sokolová, B., Foniok, K., Matějka, V. and Praus, P. (2019). Photocatalytic Degradation of Selected Pharmaceuticals using G-C3N4 and TiO_2 Nanomaterials. *Nanomaterials, 9*(9), 1194.

Soto, M. L., Moure, A., Dominguez, H. and Parajo, J. C.(2011). 'Recovery, Concentration and Purification of Phenolic Compounds by Adsorption: A Review', *Journal of Food Engineering,105*(1), 1 - 27.

Stackelberg, P. E., Gibs, J., Furlong, E. T., Meyer, M. T., Zaugg, S. D. and Lippincott, R. L. (2007). Efficiency of Conventional Drinking-Water-Treatment Processes in Removal of Pharmaceuticals and other Organic Compounds. *Science of the Total Environment*, *377*(2-3), 255-272.

Snyder, L. R., Kirkland, J. J. and Dolan, J. W. (2011). Introduction to Modern Liquid Chromatography. John Wiley and Sons.

Snyder, S. A., Adham, S., Redding, A. M., Cannon, F. S., DeCarolis, J., Oppenheimer, J. and Yoon, Y. (2007). Role of Membranes and Activated Carbon in the Removal of Endocrine Disruptors and Pharmaceuticals. *Desalination*, *202*(1-3), 156-181.

Sungpyo, K., and Aga, D. S. (2007). Potential Ecological and Human Health Impacts of Antibiotics and Antibiotic-Resistant Bacteria from Wastewater Treatment Plants. *Journal of Toxicology and Environmental Health, Part B, 10*(8), 559-573.

Stokvis, E., Rosing, H. and Beijnen, J. H. (2005). Stable Isotopically Labeled Internal Standards in Quantitative Bioanalysis using Liquid Chromatography/Mass Spectrometry: Necessity or Not? *Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry*, *19*(3), 401-407.

Sugumaran, P., Susan, V. P., Ravichandran, P. and Seshadri, S. (2012). Production and Characterization of Activated Carbon from Banana Empty Fruit Bunch and Delonix Regia Fruit Pod. *Journal of Sustainable Energy and Environment*, *3*(3), 125-132.

Swan, G. E., Cuthbert, R., Quevedo, M., Green, R. E., Pain, D. J., Bartels, P. and Wolter, K. (2006). Toxicity of Diclofenac to Gyps Vultures. *Biology Letters*, *2*(2), 279-282.

Takino, M., Daishima, S., Yamaguchi, K. and Nakahara, T. (2003). Quantitative Liquid Chromatography-Mass Spectrometry. Determination of Catechins in Human

Plasma by Automated On-Line Extraction using Turbulent Flow Chromatography. *Analyst*, *128*(1), 46-50.

Tan, X., Liu, Y., Zeng, G., Wang, X., Hu, X., Gu, Y. and Yang, Z. (2015). Application of Biochar for The Removal of Pollutants from Aqueous Solutions. *Chemosphere*, *125*, 70-85.

Tautenhahn, R., Böttcher, C. and Neumann, S. (2008). Highly Sensitive Feature Detection for High Resolution LC/MS. *BMC Bioinformatics*, *9*(1), 1-16.

Ternes, T. and Joss, A. (2006). Auman Pharmaceutical Hormes and Fragrances. The Micropolluntant Challenge for Urban Water Management. *Water 21*, 53.

Thomaidis, N. S, Asmakopoulos, A.G. and Bletsu, A. A. (2012). Emerging Contaminants, A Tutorial Mini- Review. *Global Nest Journal 14*(1), 72 – 79.

Tiyasha, K., Bhattacharjee, C. and Datta, S. (2015). Biodegradation of Acetamiprid from Wetland Wastewater using Indigenous *Micrococcus luteus* Strain SC 1204: Optimization, Evaluation of Kinetic Parameter and Toxicity. *Journal of Water Process Engineering*, *6*, 21-31.

Tolls, J. (2001). Sorption of Veterinary Pharmaceuticals in Soils: A Review. *Environmental Science and Technology*, *35*(17), 3397-3406.

Tongpoothorn, W, Sriutha, M. Homchan, P. and Chanthai, S. (2011). Preparation of Activated Carbon Derived from Jatrophacurcas, Fruit Shells by Simple Thermo-Chemical Activation and Characterization of their Physicochemical Properties. *Chemical Engineering Journal 89*(3), 335 – 340.

Vaňková, M. (2010). Biodegradability Analysis of Pharmaceuticals used in Developing Countries; Screening with OxiTop ®-C 110

Vasiliadou, I. A., Molina, R., Martínez, F. and Melero, J. A. (2013). Biological Removal of Pharmaceutical and Personal Care Products by a Mixed Microbial Culture: Sorption, Desorption and Biodegradation. *Biochemical Engineering Journal*, *81*, 108-119.

Vera, M., Cruzat, C. and Vanegas, M. E. (2020). Low-Cost Crop Waste Biosorbent Technology for Removing Toxics and Pollutants from Wastewater.

In *Agricultural, Forestry and Bioindustry Biotechnology and Biodiscovery* 177-216.

Vergili, I. and Barlas, H., (2009). 'Removal of Selected Pharmaceutical Compounds from Water by an Organic Polymer Resin.' *Journal of Scientific and Industrial Research, 68,* 417-425.

Verlichi, P., Al-Aukidy, M. and Zambello, E. (2012). Occurrence of Pharmaceutical Compounds in Urban Waste Water.Removal , Mass Load and Environmental Risk after a Secondary Treatment. *Science of Total Environment, 10*(1), 4070 – 471.

Wadhah, H., Qais, Y., Ali, G. and Khalid, M. (2018). Biodegradation of Paracetamol by Native Bacterial Strains Isolated from Yemeni Pharmaceutical Wastewater Plant in Sana'a. *Chronicles of Pharmaceutical Science, 2,* 512-522.

Wanakai, S. I., Kareru, P. G., Makhanu, D. S., Madivoli, E. S., Maina, E. G. and Nyabola, A. O. (2019). Catalytic Degradation of Methylene Blue by Iron Nanoparticles Synthesized using Galinsoga Parviflora, Conyza Bonariensis and Bidens Pilosa Leaf Extracts. *SN Applied Sciences, 1*(10), 1-10.

Wang L, Guo Y, Zhu Y, Li Y, Qu Y, Rong C, Ma X. and Wang Z 2010. *Bioresource Technology,* 101, 9807-10.

Wang, Y., Zhang, C., Zhao, L. and Meng, G. (2017). Cellulose Based Porous Adsorbents with High Capacity for Methylene Blue Adsorption from Aqueous Solutions. *Fibres and Polymers*, *18*(5), 891-899.

Wang, Z., Ogata, H., Morimoto, S., Fujishige, M., Takeuchi, K., Hashimoto, Y. and Endo, M.
(2015). *Carbon,* 90, 154-9.

WHO. (2011). Pharmaceuticals in Drinking Water, WHO/HSE/WSH/11.05 WHO Press, Geneva.

Winker, M., Tettenborn, F., Gulyas, H. and Otterpohl, R. (2008). Comparison of Analytical and Theoretical Pharmaceutical Concentrations in Human Urine in Germany. *Water Research,* 42, 3633-3640.

Wong, K., Lee, C., Low, K. and Haron, M. (2003). Removal of Cu and Pb by Tartaric Acid Modified Rice Husk from Aqueous Solutions. *Chemosphere, 50*(1), 23–28.

Wu, S., Zhang, L. and Chen, J. (2012). Paracetamol in the Environment and its Degradation by Microorganisms. *Applied Microbiology and Biotechnology, 96*, 875-884.

Yahya, N., Aziz, F., Jamaludin, N. A., Mutalib, M. A., Ismail, A. F., Salleh, W. N. W. and Ludin, N. A. (2018). A Review of Integrated Photocatalyst Adsorbents for Wastewater Treatment. *Journal of Environmental Chemical Engineering, 6*(6), 7411-7425.

Yamada, M. K., Ohkawa, S., Akasaka, T. and Watari, F. (2004). Non-Contact Surface Morphology Analysis of CO_2 Laser-Irradiated Teeth by Scanning Electron Microscope and Confocal Laser Scanning Microscope. *Materials Transactions*, *45*(4), 1033-1040.

Yoon,Y. Westerhoff, P. Snyder, S.A. and Esparza, M. (2003). HPLC Fluorescence Detection and Adsorption of Bisphenol A, 17β-Estradiol, and 17α-Ethynyl Estradiol on Powdered Activated Carbon. *Water Research, 37*(14), 3530–3537.

Yu, C., Hao, D., Chu, Q., Wang, T., Liu, S., Lan, T. and Pan, C. (2020). A One Adsorbent QuEChERS Method Coupled with LC-MS/MS for Simultaneous Determination of 10 Organophosphorus Pesticide Residues in Tea. *Food Chemistry*, *321*, 126657.

Zhang, D., Wang, F., Yi, W., Li, Z., Shen, X. and Niu, W. (2017). Comparison Study on Pyrolysis Characteristics and Kinetics of Corn Stover and its Digestate by TG-FTIR. *BioResources*, *12*(4), 8240-8254.

Zhang, L., Song, X., Liu, X., Yang, L., Pan, F. and Lv, J. (2011). Studies on the Removal of Tetracycline by Multi-Walled Carbon Nanotubes. *Chemical Engineering Journal, 178*, 26-33.

Zhang, L., Hu, J., Zhu, R., Zhou, Q. and Chen, J. (2013). Degradation of Paracetamol by Pure Bacterial Cultures and their Microbial Consortium. *Applied Microbiology and Biotechnology*, *97*, 3687-3698.

Zhang, Y., Ghaly, A. E. and Li, B. (2012). 'Physical Properties of Rice Hull Residues as Affected by a Variety and Climatic and Cultivation Conditions in 3 Continents's. *American Journal of Engineering and Applied Sciences, 9*(11), 1757- 1768.

Zhu, X., Wang, P., Peng, C., Yang. Y. and Yan, X. (2014). Activated Carbon Produced from Paulownia Sawdust for High-Performance CO_2 Sorbents. *Chinese Chemical Letters*, 25, 929 –932.

Zuccato, E., Castiglioni, S., Fanelli, R., Reitano, G., Bagnati , R. Chiabrando, C., Pomati, F., Rossetti, C. and Calameri, D. (2006) Pharmaceutical in the Environment in Italy, Cause, Occurence, Effects and Control. *Environment Science and Pollution Research,13*(1), 15 -21.

APPENDICES

Appendix I: ANOVA results for the effect of pH on adsorption of trimethoprim

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.00226	4	0.000565	2.973684	0.131515	5.192168
Within Groups	0.00095	5	0.00019			
Total	0.00321	9				

Appendix II: ANOVA results for the effect of pH on adsorption of paracetamol

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.00226	4	0.000565	2.973684	0.131515	5.192168
Within Groups	0.00095	5	0.00019			
Total	0.00321	9				

Appendix III: ANOVA results for the effect of pH on adsorption of nevirapine

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.00846	4	0.002115	6.220588	0.035292	5.192168
Within Groups	0.0017	5	0.00034			
Total	0.01016	9				

Appendix IV: ANOVA results for the effect of contact time on adsorption of trimethoprim

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	0.084715	4	0.021179	19.70116	0.002913	5.192168
Within Groups	0.005375	5	0.001075			
Total	0.09009	9				

Appendix V: ANOVA results for the effect of contact time on adsorption of paracetamol

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.03814	5	0.007628	32.11789	0.002521	6.256057
Within Groups	0.00095	4	0.000238			
Total	0.03909	9				

Appendix VI: ANOVA results for the effect of contact time on adsorption of nevirapine

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.1529	4	0.038225	112.4264706	4.4E-05	5.192168
Within Groups	0.0017	5	0.00034			
Total	0.1546	9				

Appendix VII: ANOVA results for the effect of sorbent mass on adsorption of trimethoprim

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.08024	4	0.02006	17.75221	0.003702	5.192168
Within Groups	0.00565	5	0.00113			
Total	0.08589	9				

Appendix VIII: ANOVA results for the effect of sorbent mass on adsorption of paracetamol

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.025794	5	0.005159	32.15844	0.000293	4.387374
Within Groups	0.000963	6	0.00016			
Total	0.026756	11				

Appendix IX: ANOVA results for the effect of sorbent mass on adsorption of nevirapine

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.024667	5	0.004933	29.6	0.000371	4.387374
Within Groups	0.001	6	0.000167			
Total	0.025667	11				

Appendix X: ANOVA results for the initial drug concentration on adsorption of trimethoprim

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.031394	5	0.006279	20.22685	0.001081	4.387374
Within Groups	0.001863	6	0.00031			
Total	0.033256	11				

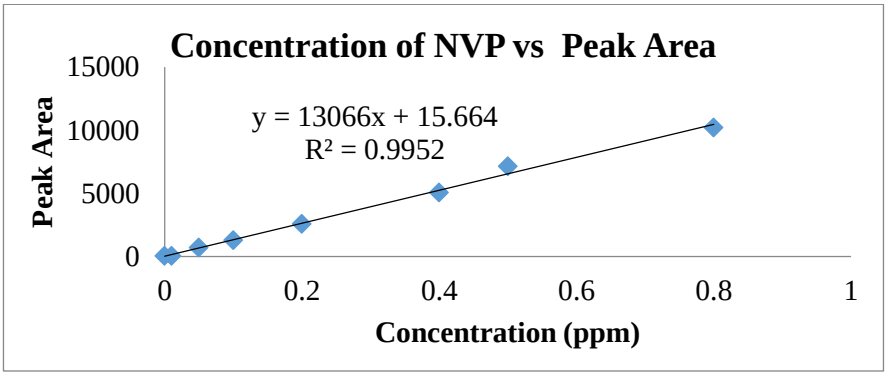
Appendix XI: ANOVA results for the initial drug concentration on adsorption of paracetamol

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.067467	5	0.013493	106.1538	1.66E-09	3.105875
Within Groups	0.001525	12	0.000127			
Total	0.068992	17				

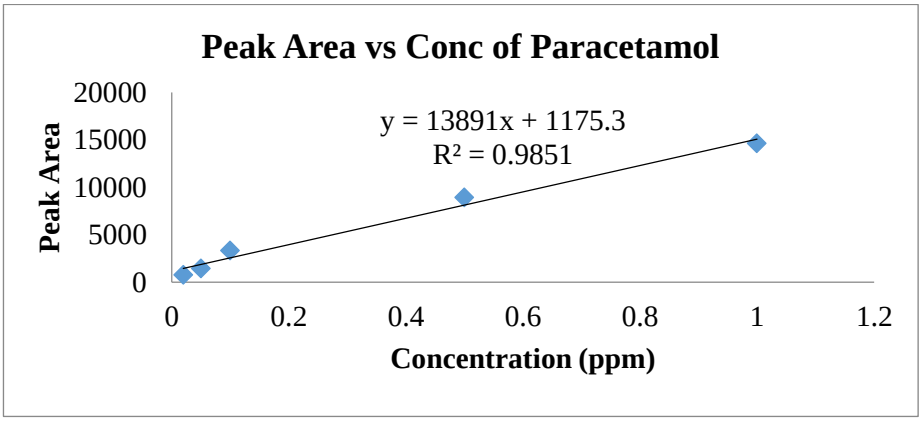
Appendix XII: ANOVA results for the initial drug concentration on adsorption of nevirapine

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.067467	5	0.013493	106.1538	1.66E-09	3.105875
Within Groups	0.001525	12	0.000127			
Total	0.068992	17				

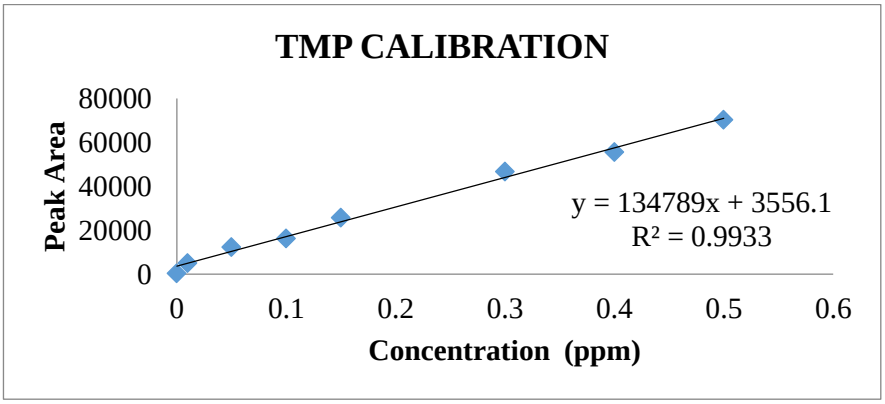
Appendix XIII: Regression graph of peak area against concentration of nevirapine



Appendix XIV: Regression graph of peak area against concentration of paracetamol



Appendix XV: Regression graph of peak area against concentration of trimethoprim



Appendix XVI: ANOVA result for effect of autoclaving on trimethoprim biodegradation

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00135	1	0.00135	13.5	0.021312	7.708647
Within Groups	0.0004	4	1E-04			
Total	0.00175	5				

Appendix XVII: ANOVA result for effect of autoclaving on nevirapine biodegradation

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.67E-05	1	1.67E-05	0.1	0.767644	7.708647
Within Groups	0.000667	4	0.000167			
Total	0.000683	5				

Appendix XVIII: ANOVA result for effect of autoclaving on paracetamol biodegradation

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00015	1	0.00015	1.5	0.287864	7.708647
Within Groups	0.0004	4	0.0001			
Total	0.00055	5				

Appendix XIX: ANOVA results for biodegradation of trimethoprim by different bacteria

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13.5	1	13.5	2.7	0.175693	7.708647
Within Groups	20	4	5			
Total	33.5	5				

Appendix XX: ANOVA results for biodegradation of paracetamol by different bacteria

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	150	1	150	7.317073	0.053814	7.708647
Within Groups	82	4	20.5			
Total	232	5				

Appendix XXI: ANOVA results for biodegradation of nevirapine by different bacteria

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	16	1	16	0.615385	0.514929	18.51282
Within Groups	52	2	26			
Total	68	3				

Appendix XXII: ANOVA results on biodegradation by *E. coli* on variation of different pharmaceutical molecules

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	96	1	96	7.68	0.050265	7.708647
Within Groups	50	4	12.5			
Total	146	5				

Appendix XXIII: ANOVA results on biodegradation by *B. subtilis* on variation of different pharmaceutical molecules

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	204.1667	1	204.1667	8.277027	0.04515	7.708647
Within Groups	98.66667	4	24.66667			
Total	302.8333	5				

Appendix XXIV: ANOVA results on biodegradation by *P. aeroginosa* on variation of different pharmaceutical molecules

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	64	1	64	3.764706	0.191878	18.51282
Within Groups	34	2	17			
Total	98	3				

Appendix XXV: ANOVA results on biodegradation by *S. aureus* on variation of different pharmaceutical molecules

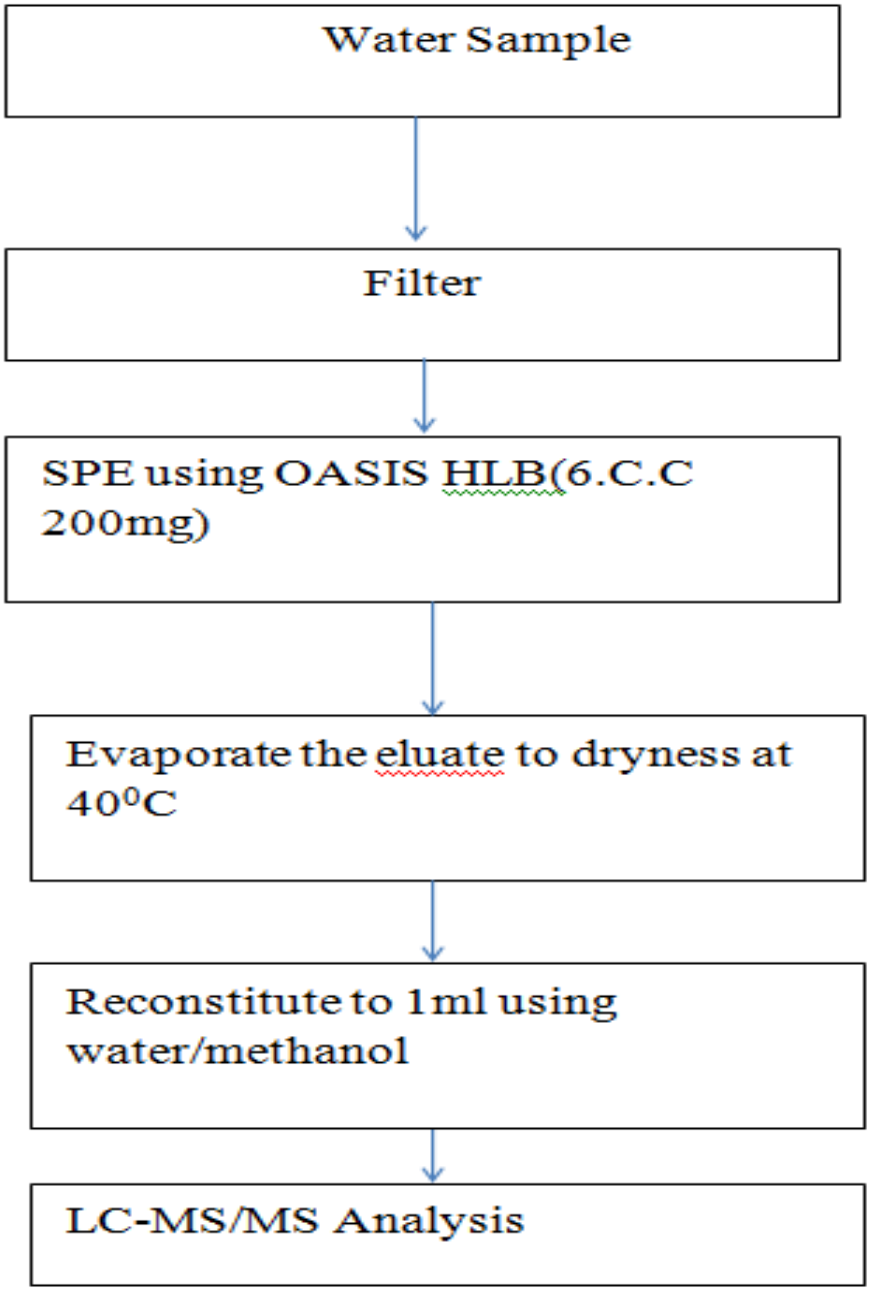
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	196	1	196	24.5	0.038476	18.51282
Within Groups	16	2	8			
Total	212	3				

Appendix XXVI: Formula for Calculation of LOD and LOQ

L.O.D = 3.3 σ/s

L.O.Q = 10 σ/s

Appendix XXVII: Work flow diagram for solid phase extraction of pharmaceuticals from wastewater



Appendix XXVIII: Published papers

Chemical Science International Journal

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Removal of Pharmaceutical Residues from Wastewater using Activated Carbon from Rice Husks

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Original Research Article

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ABSTRACT

The presence of pharmaceutical residues in discharges that end up in rivers is a growing concern for the disruption of aquatic ecosystems and human health. The risk of exposure to these medical wastes becomes greater because they are not biodegradable even after sewage treatment. This study aimed to remove trimethoprim (antibiotic), paracetamol (painkiller), and nevirapine (anti-retroviral) from wastewater using activated carbon made from rice husks, an agricultural waste that was investigated as a potential adsorbent. The instrument used for analysis was a liquid chromatography-tandem mass spectrometer (LC-MS/MS). The powdered carbon of rice husks was carbonated at a temperature of 500°C and then activated by phosphoric acid to increase its porosity. After activation, it was successfully characterized by the use of Scanning electron microscopy which showed irregular cavities with open fine pores. Fourier transform infrared showed different functional groups which determined adsorbent- adsorbate interactions while X-ray diffraction revealed amorphous particle arrangement. The effects of the adsorbent dose, contact time, pH, and initial drug concentration were studied. Freundlich and Langmuir's isotherms were used in the evaluation of adsorption phenomena. Thus, obtained results showed that rice husks activated carbon is an effective adsorbent.

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Keywords: Paracetamol; trimethoprim; nevirapine; biodegradation; biotransformation; pharmaceuticals.

Degradation of Nevirapine and Trimethoprim from Aqueous Solutions using Selected Microorganisms

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Abstract— *Together with pharmaceutical residues, personal care products encompassing prescription drugs, fragrances, and cosmetics have been detected in groundwater and other aquatic environments, hence compromising the quality of water. Their classification as micropollutants is due to their antibacterial resistance potential, persistence, and ecotoxicity. Biodegradation has been identified as a potential mechanism in their removal. The focus of this study was to use Bacillus subtilis, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeroginosa) to enhance the degradation of Nevirapine and Trimethoprim in model aqueous solutions. A liquid chromatography-tandem mass spectrometer (LC-MS/MS) was used to determine the pharmaceuticalsconcentrations. The efficacy of the bacterial strains to degrade selected drugs was evaluated by making the two drugs the sole source of energy and carbon. From the experimental data, the highest percentage biodegradation was recorded; Pseudomonas aeroginosa (86 %) and Staphylococcus aureus (79 %) for TMP and NVP respectively.*

Keywords—Biodegradation, efficacy, LC-MS/MS, model solutions, pharmaceutical.