ASSESSMENT OF SELECTED ANTIBIOTICS IN NGONG RIVER AND THEIR REMOVAL USING ACTIVATED CARBON

BY

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A thesis submitted to the Graduate School in partial fulfillment of the requirements for the award of the degree of Master of Science in Analytical Chemistry of the University of Nairobi.

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my own work has been used, this has been acknowledged and reference in accordance with the University of Nairobi's requirements.

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DEDICATION

To my father Gilbert Speke Opanga, my late mother Miriam Anyona Opanga and my wife Catherine Nasimiyu for their advice, inspiration, encouragement and motivation.

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ABSTRACT

Occurrence of antibiotics in aquatic environment has become of concern in the recent past due to development of antibiotic resistance among microorganisms. In addition, most Pharmaceuticals and Personal Care Products (PPCPs) are known to disrupt reproduction and hormone function of some aquatic organisms. The objective of this study was to determine the existence and distribution of three active ingredients of Amoxicillin (AMX), Sulfamethoxazole (SMX) and Trimethoprim (TMP) antibiotics in water from Ngong River Kenya, and their removal from water using activated carbon. Water samples were collected from seven sites along Ngong River profile constituting the up-, mid- and down-stream. Liquid/Liquid Extraction (LLE) method was used to extract antibiotics from the water samples. The extracts were analyzed for the presence of AMX, SMX and TMP using High Performance Liquid Chromatography (HPLC) coupled with UV/VIS detector. The levels of AMX, SMX and TMP were between <0.20 to 9.07±7.78 µg/l, 10.69±1.33 µg/L and 1.91±0.02 µg/L, respectively. Lower concentrations of antibiotics were detected during the wet season due to dilution by rain water. The detection limits for AMX, SMX and TMP were 0.20, 2.20 and 1.30 µg/l, respectively. The trend in the sum concentration (µg/L) was SMX>TMP>AMX in the wet season and SMX>AMX>TMP in the dry season. The presence of SMX in wastewater effluent from Ruai outlet showed that the treatment plant was ineffective at completely removing antibiotic contaminants from the wastewater. Adsorption of AMX, SMX and TMP was notably greater on Powdered Activated Carbon (PAC) than on Granulated Activated Carbon (GAC), suggesting that PAC was a better adsorbent for the antibiotics in both distilled and surface water. Adsorption data fitted better on Freundlich isotherm than Langmuir isotherm, suggesting a physical process. The results revealed contamination of Ngong River water by antibiotic residues, which has a potential impact on antibiotic resistance. In addition, the removal of antibiotic residues

from water could be improved by introducing activated carbon filters in the water treatment train. However, further studies should be conducted using water from the wastewater treatment plant to understand the effect of organic load on adsorption processes using PAC and GAC.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
Al3β	Aluminol group
AMX	Amoxicillin
BOD	Biological Oxygen Demand
DNA	Deoxy-ribonucleic acid
DWTPs	Drinking Water Treatment Plants
E- Coli	Escherichia Coli
EDCs	Endocrine Disrupting Chemicals
EDTA	Ethyl Diamine Tetracetic Acid
FDA	Food and Drug Agency
GAC	Granular Activated Carbon.
HIV	Human Immuno Deficiency Virus
HPLC	High Power Liquid Chromatography
Kd	Adsorption Coefficient
Mg2β	Magnesium group
NH_3β	Amine functional group
PAC	Powdered Activated Carbon.
pHpzc	pH at point of zero charge
PPCPs	Pharmaceuticals and Personal Care Products.
RNA	Ribonucleic Acid
Si4β	Silanol groups
SMX	Sulfamethoxazole
SPE	Solid Phase Extraction
TMP	Trimethoprim
WHO	World Health Organization
WWTPs	Wastewater Treatment Plants

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Fresh and safe drinking water access is considered to be one of the greatest challenges in this century (Asano *et al.,* 2007). This is due to the ever growing population and increased demand for water for agriculture, industry, energy and household use (FAO, 2011). In the arid areas, access to reliable and sufficient water supply to meet the population needs, sustainability of the natural surrounding and Agriculture is a problem (Rasul, 2016). In addition, low and unpredictable rainfall patterns experienced in many countries has left groundwater basins overdrawn. Climate changes and variability also represent an emerging trouble to the water resources and sustainability, globally (UNESCO-WWAP, 2009). Surprisingly in areas which receive high average rainfall, expected growth in population presents a threat to adequacy of the capacity of fresh water resource around the world. In this world, especially in Africa, drought has left many farmers with low yields. These challenges not only get worse as a climate change continues, but also reduce the fresh water availability (Alhassan and Hadwen, 2017). As a result, interest for countries to reclaim and reuse municipal wastewater have increased (Reuse, 2012). However, wastewater use to irrigate in agriculture and other domestic uses purposes require advanced treatment to eliminate deleterious substances (Shakir *et al*., 2017).

The commonness of active pharmaceutical compounds and other emerging micro-pollutants in the impaired wastewater sources is an issue of recent concern (Snyder *et al.,* 2005). Whereas the presence of pharmaceuticals in waters are at very low concentrations, they have been found in

drinking water and surface water systems in various places globally including the USA (Benoti, 2009), Europe and Asia (Boxall *et al*., 2012). Ternes *et al.* (2004) recognized the primary route of entry for these pharmaceuticals into the systems of supplying water is through release from wastewater treatment plants (WWTPs). They may also enter as metabolic excretes into the wastewater system, by means of discarding of excess drugs down the drainage (Ternes *et al.,* 2004). Once the pharmaceuticals are in the system supplying wastewater, a group of them emerge into the environment by passing through WWTPs, largely because they are not affected by primary and secondary treatment methods (Oulton *et al.,* 2010).

Currently, there is no law in place in Kenya to limit WWTPs effluent concentration of PPCPs. Furthermore, the likely effect of these Pharmaceuticals in drinking water on people fitness is not adequately researched. It is generally due to these unspecified effects that concerns the people over the existence of pharmaceuticals in sources of drinking water (WHO, 2011)

The focus of Primary and Secondary wastewater treatment is on removal of conventional components such as solids (Abdel-Raouf *et al.,* 2012). Biological oxygen demand (BOD) and nutrient levels are minimized by Secondary biological treatment while disinfection removes pathogens before being released (Lin *et al.,* 2009). Technologies of Conventional treatment are not devised in addressing micro contaminants, hence various studies show that many such micro contaminants are not affected or removed by the methods (Benoti, 2009).

Municipal drinking water are purified by activated carbon in many countries around the world. Public concerns and awareness on safety of drinking water has driven consumers to use carbon related systems to purify drinking water that they use daily at their homes (Tauxe-Wuersch *et al*., 2005).

Activated carbon filtration is the most common resent technologies used in the adsorption of contaminants in surface water. Nature of activated carbon, composition of water and operating parameters can affect the adsorption efficiency of Activated Carbon (Upadhyayula *et al*., 2009).

The source of Ngong River is Kikuyu springs and flows through at Mtoine Dam, Mbagathi, Matter Hospital, Donholm Bridge, Njiru Bypass sites and ends up at Ruai waste water Treatment Plants. Ruai sewage treatment plant treats wastewater generated from the city of Nairobi to ensure the water released back to the natural environment is clean. Ruai effluent finally joins the Athi River and drains into the Indian Ocean (Mundia and Aniya, 2005).

1.2 Problem Statement

Recently concern over the fate, occurrence and adverse effects of pharmaceuticals and personal care products (PPCPs) residues in the surrounding have increased. Examples of the frequently and widely used pharmaceuticals classes include antibiotics, analgesics, anti-inflammatory, antiseptics and antiepileptic drugs (Daughton and Ternes, 1999). Most of them are sold over the counter and the prescription are not there.

Before, PPCPs routine monitoring in water and wastewater treatment plants were not done, even their occurrence in effluent was not regulated (Sui *et al.,* 2015). However, public concerns and perception over possible ecosystem and negative health results related to PPCPs exposure have led to more study of their fate in the course of wastewater treatment in most developed countries (Rebekah *et al.,* 2010). Treated effluent discharge from wastewater treatment plants is the source for the establishment of PPCPs to the surface water (Daughton and Ternes, 1999).

A number of wastewater treatment plants are not devised to eliminate PPCPs. As a result , most of these pharmaceutical composites are disposed in aquatic environment, hence a threats to animals and troublesome to drinking water treatment industry (Castiglioni *et al.,* 2006; Lishman *et al.,* 2006; Paxeus, 2004; Santos *et al.,* 2007). The effects include long term and short-term poison, antibiotic resilience of microorganism and endocrine distorting effects (Luo *et al.,* 2014). In addition, monitoring actions and precautions for PPCPs have not been well known in most Wastewater Treatment Plants (WWTPs) especially in developing countries (Larson and Fick, 2009). Presently, discharge regulations and standards do not exist for most PPCPs (Luo *et al.,* 2014).

In Kenya the issue of PPCP is still new (Ebele *et al*., 2017). However, concern has been raised regarding the increasing level of antibiotic resistance that may compromise the fight against disease causing micro-organisms (Ventola, 2015). Data on environmental residues of PPCPs in Ngong River is limited, although the river water is widely used to irrigate vegetables along the river profile. The aim of this work was to ascertain the residue levels of selected antibiotics in Ngong River and their removal efficiency using activated carbon.

1.3 Research Questions

a) What are the levels of Amoxicillin, Sulfamethoxazole and Trimethprim in Ngong River and downstream of Ruai wastewater treatment plant?

b) What is the effect of seasonal variation on the concentration of Amoxicillin, Sulfamethoxazole and Trimethoprim in Ngong River and downstream of Ruai wastewater plant?

c) What is the performance of activated carbons on the removal of Amoxicillin, Sulfamethoxazole and Trimethoprim from natural water?

1.4 Hypothesis

1.4.1 Alternate Hypothesis

1.4.1.1 There is contamination of selected antibiotics in Ngong River and the concentrations are above the background levels.

1.4.1.2 AMX, TMP and SMX can be removed from natural water using activated carbon.

1.5Objectives

1.5.1 Overall Objective

To determine the impact of human activities on PPCPs contamination in Nairobi River Basin and their removal by adsorption process.

1.5.2 Specific Objective

i) To determine the levels of Amoxicillin, Sulfamethoxazole and Trimethoprim in Ngong River and downstream of Ruai wastewater treatment plant.
ii) To determine the effect of seasonal variation on concentration of Amoxicillin, Sulfamethoxazole and Trimethoprim in Ngong River and downstream of Ruai wastewater treatment plant.
iii) To determine the physicochemical parameters of natural water from Ngong River.
iv) To determine the performance of activated carbon on the removal of Amoxicillin, Sulfamethoxazole and Trimethoprim from natural river.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 General Overview of Pharmaceuticals and Personal Care Products

PPCPs comprise of a group of chemicals such as veterinary and human drugs, bioactive food supplements, investigative agents such as X-ray contrast media, and other end user chemicals such as cosmetics, sun screen agents, fragrances, as well as inert ingredients or recipients used in PPCP manufacture and formulation (Daughton, 2001). Table 1 shows a lists of some vital PPCP classes (Ellis, 2008).

Table 1.1 Emerging PPCP Classes

Class/Compound Group	Compound
Pharmaceuticals Human &Veterinary Antibiotics.	Amoxycillin,Trimethoprim,Lincomycin, Erytromycine, Chloramphenicol, Sulfamethoxazole, Flucloxacillin.
Anti-inflammatory & Analgesics Drugs.	Naproxen,Acetaminophen, Dichlofenac,Acetylsalicylic acid, Fenoprofen, Fluoxetine, Indometacine Ibuprofen, Ketoprofen, Mesalazine, Sufasalazine.
Psychiatric Drugs.	Carbamazepine, Diazepam, Salbutamol, Primidone,
Lipid Regulators.	Clofibric acid,Bezafibrate,Fenofibric acid, Etofibrate, Gemfibrocil, Metformin.
β-Blockers.	Timolol,Atenolol, Metoprolol,Sotalol, Propranolol.
X-ray Contrasts.	Diatrizoate, Iopamidol, Iopromide.
Hormones& Steroids.	Estradiol, DES, Estriol,, Estradiol.
Personal Care Products Fragrances Sun Screen Agents Insect Repellents	Macrocyclic Musk,Polycyclic, Phthalates N,N-diethloluamide,NitroBenzophenone, methylbenzylidene camphor.
Antiseptics	Chlorophene, Triclosan

(Source: Ellis, 2008)

Daughton (2004) proposed that approximately six million substances of PPCP may be present commercially all over the world. The same report showed that pharmaceuticals usage has increased

to 3- 4% per year by weight (Daughton, 2004). Globally, approximately over 600 tonnes of antibiotics are used by Germany per year, while France, Italy and Spain uses 300 tonnes per annum. According to Alder *et al*. (2007), 255 – 832 kilograms of diclofenac is consumed annually with a million populations within West European countries. Within UK licensed active substances used are over 3000 with, metformin (106 tons per annum), acetylsalicylic acid (770 tons per annum) and acetaminophen (or paracetamol; 2000 tons per annum) being the most consumed pharmaceuticals.

An estimate of around 170 pharmaceutical chemicals are consumed slightly above 1 tons yearly (Webb, 2000). Although, according to Alder *et al.* (2007) above 95% of the total active PPCP content consumed make up less than 50 PPCP compounds. With increase in development and related business related activities, and a growing interest with health and personal care, the awareness of PPCPs as a societal behavior in the contribution of water pollution is expected to introduce escalated risks.

2.2 Source, Pathway and Persistence in the Environment

A critical group of pharmaceuticals in modern day's medicine are antibiotics. The antibiotics have been discovered in different places of the aquatic environment, such as drinking water, ground water, surface water and wastewaters as well (Stolker *et al.,* 2006 and Perret *et al.,* 2006). They are viewed as "pseudo-persistent" pollutants because of their constant contribution into the surrounding. Consequently, the existence of antibiotics in the ecosystem has gathered noticeable interest. They are absorbed poorly by our body and hence eliminated either unaltered or changed, through faeces and urine (McArdell *et al.,* 2003). Attention is developing on their existence, effect

and persistence in the environment because lesser concentrations of antibiotics can benefit the creation of antibiotic resilient bacteria.

Antibiotics consumption in animal farming has been associated to the elevated development of resilient strains of infectious bacteria that are likely to affect the human health (Chang *et al.,* 2014). Antibiotic resilient bacteria and or resilient genes can be transmitted from animals to human beings. Bacteria can also evolve into cross-resistance between those of identical structures used entirely in human medicine with antibiotics used in veterinary medicine.

Discharged of antibiotics into the aquatic environment is by diverse routes. After administering, they are eliminated as metabolites. An abundant quantity is discharged in unaltered form as original compounds by the way of urine and feces into the sewage (Gros *et al.*, 2009). Lots of investigations have shown the incomplete discharge of pharmaceuticals by WWTPs (Jelic *et al.,* 2012), hence WWTPs are treated to be dominant contributors for the existence of pharmaceuticals in the environment.

Pharmaceuticals as well as their metabolites have been predicted in the effluents from WWTP (Gobel *et al.,* 2004; Schlüsener and Bester, 2005; Yang *et al.,* 2006). The fact that they can reach the ground water and surface water hints a possible exposure for the aquatic and soil organisms which are linked with the existence of minimum concentrations of these biologically active compounds. Additionally, health facilities are one of the greatest critical providers of the existence of the antibiotics into the aquatic habitat (Lindberg *et al.,* 2004; Schwartz *et al.,* 2011). The application of antibiotics in veterinary medication in the management of bacterial toxicities of animals besides preventive substitutes is another origination of contamination. In addition, animal dropping may also be a dominant basis of contamination, as much of these materials end up in

manure. The compost and sludge (feces and urine) are either reserved or directly utilized to the farmlands as agricultural fertilizers (Liu, 2012). The un-metabolized compositions existing in the compost or their bioactive metabolites may advance from the compost on the farmland to groundwater and finally go in surface water, like lakes and rivers, hence they can disturb the aquatic creatures relying upon their movement in the soil system (Wu *et al.,* 2009). The slurry from WWTPs can be used to enrich soils as well (Harrison *et al.,* 2003).

Fish farming also utilizes extensive amounts of antibiotics. They are applied as feed supplements or additives into the water. The outcome of an overfeeding is that several compounds arrive finally in the deposits where they are gently depraved or gradually seep away into the neighboring waters (Heidari *et al.,* 2013).

PPCPs may remain in solid ecological substances for a lengthy time. The permanence banks on their stability against photochemical change, adsorption capacity and binding, degeneration rate and filtrating into the water (Sosa-ferrera *et al*., 2013). Strongly adsorbing antibiotics tend to collect in sediment or soils however, highly mobile antibiotics have a probability to avoid deterioration and lead to leaching towards the groundwater and to be ferried with the drain water, run off from surface water and groundwater to surface waters (Babic *et al.,* 2006).

The sorptive altercation of chemicals separating a solid state and a water phase is described through the sorption coefficient *K*d, that is expressed as the proportion between the water at the equilibrium and the concentration of the compound in the substance that enable sorption (sorbent) (Huang *et al.,* 2004). Another sorption mechanism (processes of absorption and adsorption) is accumulating complexes separating metal ions like Fe^{3+} Ca^{2+} Al^{3+}or Mg^{2+} and antibiotics (Pils, 2005). Figure 2.1 shows the urban water cycle of PPCPs sources and pathways (Ellis, 2008).

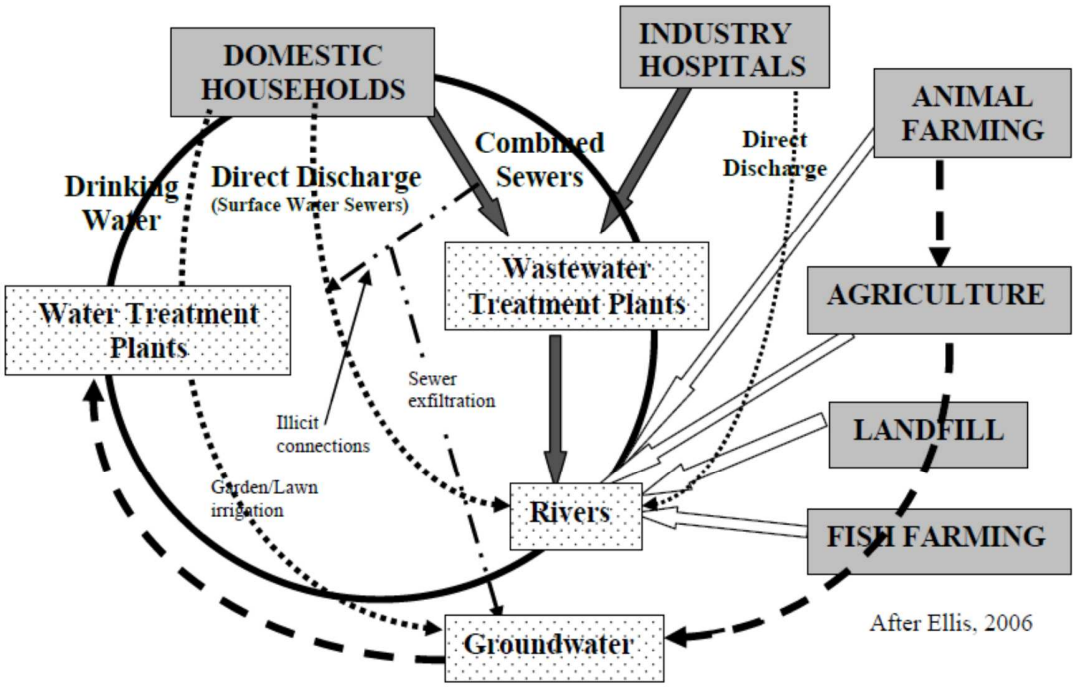

Figure 2.1 Pathways and Sources of PPCPs in the Urban Water Cycle

2.3. Pharmaceuticals and Personal Care Products Occurrence

PPCPs exists in drinking water, surface water, ground water, sewage effluent and sewage sludge (Blair *et al.,* 2013). It has also been found in soil irrigated with contaminated water, soil irrigated with recycled water and plants from soil applied with biosolids. Investigations on the existence and effects of PPCPs were conducted in the Europe and United States (Carter *et al.,* 2014).

2.4. Effect of the Antibiotics in the Environment.

To date, a good number of the research has been attentive on marine environments. Male fish population can be feminized when they are exposed to wastewater consisting of low concentrations of estrogen from oral contraceptives. Some of the effects for this are Male fish trying to lay eggs, growing ovaries, egg proteins production and low sperm count (Kümmerer, 2010).

The incremented use of antibiotics has given life to antibiotic-resilient pathogens, while evidence of compounds from antibiotics may worsen the problem (Daughton, 2001). Fighting drug-resilience bacterial burdens will call for more expensive and more toxic changes, which, without PPCP removal, will only cause a periodical, escalating effect (Klaver and Mathew, 1994).

Besides the above mentioned side effects on public health, PPCPs are harmful to the food chain because they alter the nitrification process in the aquatic environment (Ziembiska, 2011). This effect is due to the sensitiveness of Nitrosomonas and Nitrobacterbacteria, for example to oxytetracycline (Gui *et al.,* 2010). Common wastewater treatment amenities, tertiary and secondary treatment plants are not invented to discharge PPCPs from wastewater. Still the PPCPs disposal is not strictly regulated as a consequence they are continually streaming into the surrounding with unspecified summative and concurring effects (Klaver and Mathew, 1994).

2.5 Removal Alternatives for the Pharmaceuticals and Personal Care Products

To date, different methods have been accepted to remove PPCPs from the wastewater or drinking water. Some of the method investigated so far include adsorption, biodegradation, bio filtration, Coagulation–flocculation, chlorination, ozonation, and photo degradation (Rahardjo *et al.,* 2011). On the other hand, it is disclosed that conventional treatments such as filtration, sedimentation and coagulation, may not discharge greater than 25% of PPCPs (Ghernaout, 2014). The discharge of PPCPs by adsorption is archiving much attention as this process is easy and cost effective. Up to the present moment, activated carbon is the generally used adsorbent for the discharge of PPCPs (Grassi *et al*., 2012).

As quoted above, most Drinking Water Treatment Plants (DWTPs) or conventional WWTPs are not invented for the discharge of highly polar pollutants in wastewater treatment plants (Michael

et al., 2013). Consequently, economical and practical solutions need to be realized in order to decrease the regular amounts of antibiotics removed into the surrounding. A broad range of physical and chemical methods for PPCPs discharge can be engaged, for instance, membrane techniques (nondestructive processes), chemical oxidation, liquid extraction, biodegradation (destruction methods), ozonation and activated carbon adsorption (Segneanu *et al.,* 2013).

Relying on the contaminant levels in the effluent and the expenditure of the process, different methodologies can be selected. These complex processes have mostly been assessed by use of laboratory batch tests. Limited investigations have tested PPCPs discharge in full-scale treatment plants consisting of the advanced wastewater treatment processes (Padhye and Tezel, 2012). Endocrine disturbing chemicals (EDCs) and PPCPs have been found inadequately discharged in different conventional WWTPs. Extensive availability of EDCs and PPCPs in WWTP effluents and in acquiring aquatic environments can alter quality of water and affect likely exposure to aquatic organisms and health of human beings (Luo *et al.,* 2014). Wastewater Treatment Plant effluents have again been thought out as a crucial foundation of micropollutants for aquatic environments. As a result, advanced treatment technologies like ozonation and activated carbon filtration are recommended to cut down the emission of micropollutants through WWTPs effluents (Yang *et al.,* 2012). The selected Antibiotics for this study are Amoxicillin, Sulfamethoxazole and Trimethoprim.

2.5.1 Sulfonamides

One of the first antimicrobial drugs commonly used is sulfonamide antibacterial group which was made in the middle of 1940s (Lindsey *et al*., 2001). At the moment, because of development of bacterial resilience, limited derivatives of sulfonamide still exists (Tilles, 2001). Sulfamethoxazole

(4-amino-N-(5-methyl-3-isoxazolyl) benzenesulfonamide, (SMX, $C_{10}H_{11}N_3O_3S$), a representation of antibacterial component of the sulfonamides class, is looked at as an evolving micro contaminant burden because of its possible negative outcomes on human health and ecology (Lindsey *et al.,* 2001).

The sulfamethoxazole was originally produced in early 1957 (Kasprzyk-Hordern *et al*., 2009) and accepted by the FDA in 1961. It is a group of sulfonamide antibiotic and its behavior of action is inhibition of folic acid production in a bacteria (Richard *et al*., 2008). It is water soluble, it has a 19 days half-life under daylight and is extremely resilient to additional decomposition by microorganism in the subsurface (Lam *et al.,* 2004). Its Kow is between 0.10 to 361.70 and is substantially polar and hydrophilic. These properties allow SMX to be carried over lengthy distances beyond sediments adsorbing it (Perez *et al.,* 2005). Moreover, under standardized ecological pH circumstances (pH ~ 7–8) sufamethoxazole has a negative charge around 95–100% a characteristic that can boost its shipment tempo in porous media as a result of anion exclusion. Below is the molecular structure of a SMX molecule (Figure 2.2).

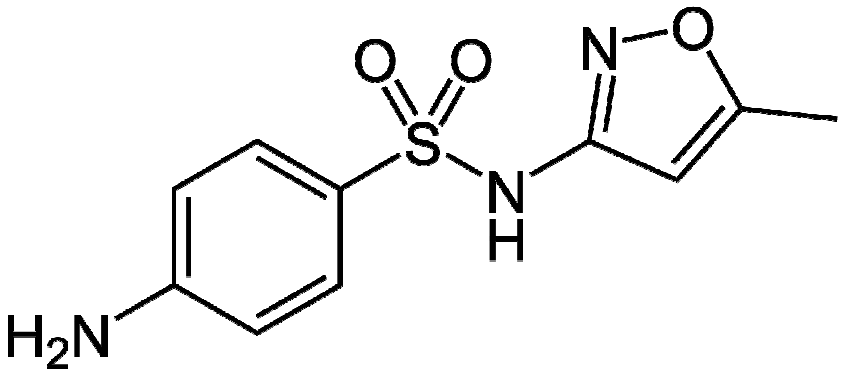

Figure 2.2: Sulfamethoxazole (4-amino-N-(5-methyl-3- isoxazolyl) benzene-sulfonamide)

The therapeutic benefit of SMX is in management of urinary tract infections, malaria and tuberculosis (Richard *et al.,* 2008). Evolvement of resilient bacteria has opposed SMX when it was consumed as a single constituent. Thus, SMX is currently mainly consumed when combined with TMP to cure, toxoplasmosis, *Pneumocystis carinii* pneumonia and Pneumocystis *jirovecii pneumonia* as well as the HIV-AIDS victims (Benson *et al.,* 2009). Similar to alternative sulfonamides, sulfamethoxazole disorganizes the biosynthetic folate route in bacteria that was lately recognized as alike to the one of plants, bringing up worries over non-target poisonousness (Richard *et al.,* 2008). Huang *et al*. (2001) approximated SMX concentrations to be between 4.39 ng/l to 0.027 mg/l in raw hospital wastewater. The free formations of TMP and SMX are well thought out to be the medicinally active forms (Kolpin *et al.,* 2002). The percentage average of the amount recouped in urine after a single oral dosage of TMP and SMX from 0 to 72 hours is 66.8% and 84.5% for free TMP and total Sulfonamide respectively (Kathryn, 2004). 30% of the total Sulfonamide is removed from the body as free SMX, whereas 70% is N4-acetylated metabolite (Huang, 2001). Maybe, this is because of the disparities of discharge from wastewater where TMP is decreased by around 69 % and SMX by about 20 % (Kathryn, 2004). In a research to establish out the concentration of SMX in sewage sludge, it was noted that SMX levels detected was between 0.028 and 0.068 mg/kg (Gobel, 2005).

2.5.2 Trimethoprim

Trimethoprim is a manufactured broad spectrum antibiotic that hinders the creation of dihydrofolic acid in bacteria. Deficiency of Dihydrofolate, may cause the bacteria die (Ryan *et al*., 2011). Figure 2.3 below shows the molecular structure of TMP molecule.

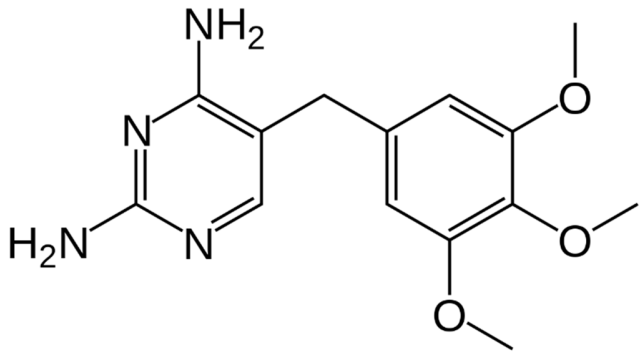

Figure 2.3 Trimethoprim [5-(3, 4, 5-trimethoxybenzyl) pyrimidine-2, 4-diamine]

Trimethoprim treats various distinctive bacterial diseases like middle ear infections, respiratory infections, urinary tract infections and respiratory diseases (Dauber-Osguthorpe *et al.,* 1988). The mixture of SMX and TMP hinders two successive stages in the metabolism of folic acid, hence disrupting the micro-organism production of DNA and RNA (Pérez *et al.,* 2005). In an investigation conducted by Ryan *et al.* (2011), the degree of SMX loss was established to be increased in wastewater effluent because of photodecomposition reactions which was not direct, especially effluent organic matters triplet excited state and hydroxyl radical reactions. Existence of natural organic matter photolysis, nonetheless, did not produce magnified degeneration of SMX.

Indirect photolysis was found to affect trimethoprim in wastewater effluents, with triplet excited effluent organic matter and hydroxyl radical remaining the important groups (Ryan *et al.,* 2011). Investigations done by Kathryn (2004) demonstrated a concentration of between 2.9-5 µg/L of TMP in not treated health facilities wastewater. Likewise investigations by Kolpin (2002) disclosed a low levels of between 0.18-0.59 µg/L of TMP in wastewater which is not treated. This is in agreement with the recent rise in the usage of TMP and SMX combined in the administration of opportunistic diseases amongst the immune implicated HIV/AIDS victims.

2.5.3 Amoxicillin

Amoxicillin is a broad spectrum antibacterial antibiotic which treats diseases of different infections. Example of these diseases are, bronchitis, urinary tract infections infection of the middle ear (otitis media), pneumonia and tonsils (Ralapanawa and Kularatne, 2015). The Figure 2.4 below shows the structure of amoxicillin. It treats of both animals and human being.

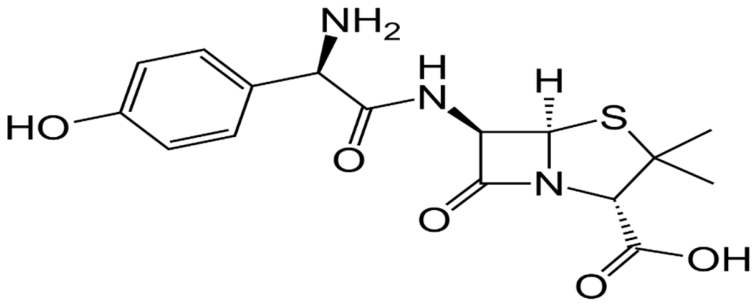

Figure 2.4 Chemical Structure of Amoxicillin

2.6 Antimicrobial Resistance in Kenya and its Comparison to Japan

In an investigation organized to analyze drug susceptibility and existence of virulence associated genes in diarrhoeagenic *Escherichia coli (E. coli*) sets apart from children of 5 years and below from Kenya and Japan established that *E. coli* sets apart from Kenya were more resilient to antimicrobials than those from Japan as shown in Table 2 (Bii *et al.,* 2005).

Table 2:1 E. coli isolates resistant to antimicrobials from Japan and Kenya

Antimicrobial agent	Conc. (µg/ml)	Susceptible (%)	Intermediately Susceptible (%)	Resistance (%)
Kenya				
Sulfamethoxazole-Trimethoprim	381; 0-19	25 (30.5)	1 (1.2)	56 (68.3)
Tetracycline	30	7 (8.6)	17 (20.7)	58 (70.7)
Ampicillin	30	27 (32.9)	1 (1.2)	27 (32.9)

Gentamicin	30	76 (8.6)	1 (1.2)	5 (6.1)
Norfloxacin	10	82 (100)	0 (0.0)	0 (0.0)
Cefotiam	30	77 (93.9)	2 (2.4)	3 (3.7)
Chloramphenicol	100	64 (78.0)	0 (0.0)	18 (22.0)
Fosfomycin	50	79 (96.4)	3 (3.6)	0 (0.0)
Japan				
Sulfamethoxazole-Trimethoprim	381; 0-19	46 (97.6)	0 (0.0)	1 (2.1)
Ampicillin	30	28 (59.6)	1 (2.1)	18 (38.3)
Gentamycin	30	47 (100)	0 (0.0)	0 (0.0)
Cefotian	30	41 (87.3)	1 (2.1)	5 (10.6)
Fosfomycin	50	45 (97.5)	0 (0.0)	2 (4.3)
Levofloxacin	100	45 (95.7)	0 (0.0)	2 (4.3)
Minocycline	30	42 (89.4)	0 (0.0)	5 (10.6)

From Kenya aboe 65% of *E. coli* isolates were resilient to SMX-TMP, Ampicillin and tetracycline (Bii *et al.,* 2005). 70.7% of isolates were resilient to tetracycline and 8.6% were fully susceptible; whereas 65.9 and 68.3% of the isolates were resilient to Ampicillin and TMP-SMX, respectively. In comparison, only 38.3 and 2.1% of isolates from japan showed resilience to Ampicillin and SMP-TMP, correspondingly, whereas resilience to Fosfomycin (4.3%) resilience and Cefotiam (10.6%) was not common (Bii *et al.,* 2005). No resilience to Norfloxacin and Fosfomycin was discovered amongst the isolates from Kenya. Only 6.1% of the latter isolates were Gentamicin resilient, whereas all the Japanese *E. coli* isolates were susceptible to this agent (Bii *et al.,* 2005). Table 2 shows *E. coli* isolates resistant to antimicrobials from Kenya and Japan. The existence of various resentment genes was linked with resilience to multidrug and this calls for more studies.

2.7 Antibiotic Analysis

The antibiotics analysis in the surrounding presents a challenging task both because of the high matrices complexities and low unusual (ng/L) at which aimed active compounds are in existence

in the surrounding waters (Seifrtovaa *et al.,* 2009). This understanding enacts the buildup of very responsive methods of analysis acceptable for the scrutinizing of the low level of the analytes. The average low levels of antibiotics established in the surrounding form a pre-concentration measure earlier before the detection is crucial and essential. For sample pre concentration off-line solid phase extraction (SPE) is a good method and liquid chromatography (LC) analysis come after. (Seifrtovaa *et al.,* 2009). Presently, there is a new method of water samples direct injection onto HPLC or Ultra-high-performance liquid chromatography (UHPLC) system with Mass Spectrometer (MS) detection. This is possible because of the MS detector is very sensitive (Seifrtovaa *et al.,* 2009)

Abundant antibiotics from diverse categories have been established in the environment which is aquatic. Consequently, multi residual methods of analysis are approved for the surveying and to determine the diverse antibiotics classes (Tylova *et al.,* 2013). These methods must be non-time-consuming, selective efficiently suitable to analyze environmental samples and sensitive. Reviews which dealt with the determining the distinct pharmaceuticals groups in diverse research journals have been reported (Hernandez *et al.,* 2007; Farre *et al.,* 2007). Identification of antibiotics is in accordance to their chemical and structural properties. Similar structures represents the same group, behave by mechanisms which are identical, and are expected to act equivalently in the surrounding. Because of the goal of this study these antibiotics groups were preferred: Amoxicillin, Trimethoprim and Sulfamethoxazole. This is because of their well-known use in the medicine and their existence in the marine environment. Their application is in human medicine as well as veterinary medicine (Prasanna *et al.,* 2015). Sulfamethoxazole are antibacterial promoters, regularly applied in animal treatment of infectious diseases (Serrano, 2005).

Trimethoprim is a dihydrofolate reductase hinderer which is structurally dissimilar from Sulfamethoxazole. It is frequently recommended in combined form with Sulfamethoxazole (as co-trimoxazole, which contains TMP: SMX in a 1:5 ratio) or it is recommended on its own (Huang and Renew, 2004). The lactam antimicrobials class which includes cephalosporins and penicillins, are used for the prophylaxis of both animals and humans. Nonetheless, because of the chemically instable lactam ring, representatives of the lactam antimicrobials group which undergo hydrolysis easily.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study Design

The work was implemented in two phases: Phase one included field sample collection and residue analysis, whereas phase two dealt with laboratory experiments to test the performance of activated carbons on AMX, TMP and SMX antibiotics.

3.2 Study Area

Samples were collected from Ngong River at seven different sites namely: Lang'ata Junction (upstream), Mbagathi Road Bridge, Enterprise Road Bridge, Industrial area (Donholm Bridge), Kangundo Road Bridge, Eastern Bypass Bridge and Ruai (downstream). Table 3.1 shows the GPS locations of the sampling points.

Table 3.1GPS Locations of the Sampling Points

Site	GPS Locations	Elevation (M)
Mtoine dam	01^{O} 18' 54S; 036^{O} 48' 12E	1675
Mbagathi bridge	01^{0} 18' 54S; 036^{O}48' 12E	1671
Enterprise road	01^{0} 18' 22S; 036^{0} 49' 35E	1648
Donholm	01^{0}18' 22S; 036^{0} 53' 00E	1621
Njiru	01^{0} 15' 00S; 036^{0} 56' 27E	1657
Bypass	01^{0} 14' 43S; 036^{0} 58' 56E	808
Ruai outlet	01^{0} 15' 02S; 037^{0} 01' 13E	1499

Figure 3.1 below show the specific locations of the sampling sites along the Ngong River profile, from upstream, midstream to downstream.

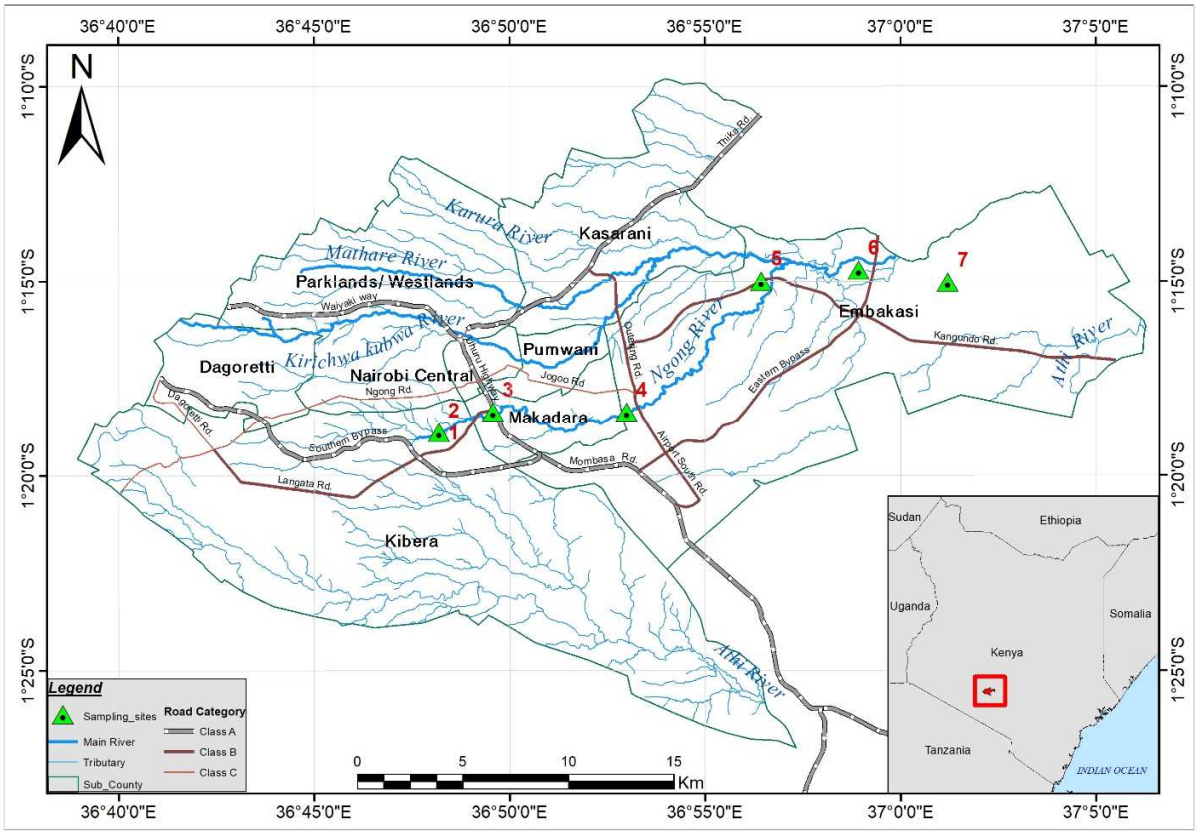

Figure 3.1Map Showing the Sampling Sites along the Ng'ong River

3.3 Chemicals and Reagents

Amoxicillin, Sulfamethoxazole, Trimethoprim references used were of high purity standards from Regal pharmaceutical limited, HPLC grade methanol from Sigma-Aldrich Company and acetonitrile from Merck Company. Analytical grade orthophosphoric acid from Horse and McGeorge limited, nitric acid, citric acid, disodium ethylenediaminetetraacetate (Na_2EDTA) from Rankem limited, Sodium hydrogen phosphate, sodium sulphate and dichloromethane were used.

3.4 Cleaning of Apparatus

4 M nitric acid was used to soak glass apparatus for 24 hours, apparatus were then washed with a detergent and double distilled water was used to rinsed them, they were then dried in an oven for four hours.

3.5 Sample Collection

Sediment and water samples were collected in Ngong River at five different sites along Ngong River profile covering the upstream, midstream to downstream and two other sites to cover Ruai WWTP and in Nairobi River Basin. This was from the same point/location of the sampling site.

Two liter amber glass bottles were used to collect water samples, while a stainless steel scooper collected sediment samples. A cooler box was used to transport the samples to the laboratory, where they were kept in a refrigerator with temperature range of between 0 °C and 8 °C prior to analysis.

3.6 Sample Preparation (Water Samples)

Liquid-liquid extraction (LLE) method was used to extract water. Each sample of water (2,000 ml) was quantitatively transferred into a 2 L separatory funnel. 30 ml of dichloromethane was used to rinse the sampling bottle and added to the separatory funnel. Dichloromethane (3x60 ml) was successively used to extract the combined contents. Plug wool was used to filter the organic layer. The wool contained anhydrous sodium sulphate (30 g) which was used for drying. Dichloromethane (2x3 ml) was later used to rinse Sodium sulphate. Rotary evaporator vacuo at 30 °C was used to concentrate the combined extract in readiness for analysis. The appearance of water

extract was clear and hence there was no need to subject them for further clean up. They were transferred to the 1.5 ml amber vial through 0.45 μm Millipore filters.

3.6.1 Analytical method for AMX analysis in water samples

Nexeira-I LC-2040C 3D liquid Chromatograph (Shimadzu, model LC-2040C 3D) with detector UV/VIS was used for the analysis of AMX antibiotics. Kromasil 100-5C18 column with dimensions of 0.25 m long and 4.6 mm diameter with silica gel package was used to perform all the separations. The mobile phase was made by dissolution of 2.5 g of NaH_2PO4 in 1,000 ml of double distilled water and the solution was mixed with phosphoric acid (1.94 ml) to make a solution of buffer solution. Isocratic mixture of 15:85 acetonitrile: buffer was used for elution. The flow rate was 1.0 ml/minute, injection volume of 20 μl at a wavelength of 229 nm. Determination of AMX concentration was made possible by comparing chromatograms of external reference standards and the samples.

3.6.2 Analytical Method for SMX and TMP Analysis in Water samples

Nexeira-I LC-2040C 3D liquid chromatograph (Shimadzu, model LC-2040C 3D) with detector UV/VIS was used for the the AMX antibiotics analysis. Kromasil 100-5C18 column with dimensions of 0.25 m long and 4.6 mm diameter with silica gel package was used to perform all separations. 0.05M KH_2PO_4 which made a phosphoric buffer was the mobile phase. Isocratic mixture of 10:15:75 for Methanol: Acetonitrile: buffer was used for elution. The flow rate was 1.0 ml/minute, injection volume of 20 ul at a wavelength of 275 nm. Determination of SMX and TMP concentrations were made possible by comparing the chromatograms of their respective external reference standards and the samples.

3.7 Sample Preparation (Sediment Sample)

10 grams of homogenized sample were weighed into acetone rinsed 100 ml glass beaker. They were transferred into a mortar. Approximately 30 g of baked out anhydrous sodium sulphate were added into the sample. These were grounded with pestle to a homogeneous powder. They were covered with Aluminium foil and left overnight to dry for further preparation.

The dry samples were transferred into the Soxhlet thimble. 180 ml of pentane: dichloromethane (1:1, v/v) were added to 500 ml round bottom flasks. A glass boiling rod/ glass chips were added to allow smooth boiling. Extraction was allowed to proceed for at least 16 hours.

After at least sixteen hours extraction was stopped. The inside of the condensers and extractors were rinsed three times with 1ml of pentane: dichloromethane mixture. The rinse was added to the sample extract. 2 ml of isooctane was added to the sample extracts. This acted as a keeper. The extracts were evaporated using rotary evaporator to 3 ml. The extracts were transferred to the test tubes. The flasks containing the extracts were rinsed three times with 1 ml of pentane: dichloromethane mixture. The extracts in the glass tubes were evaporated to 1 ml by a gentle stream of nitrogen gas. This was transferred into the 1.5 ml amber vials. They were stored in the fridge awaiting analysis by HPLC.

3.8 Adsorption Experiments

Adsorption experiments were carried out in duplicates. Batch equilibrium studies were carried out by adding a fixed amount (0.1 g) of activated carbon (PAC and GAC were used separately for each experiment) in to 15 ml centrifuge tubes containing 10 ml of different initial concentration (1,5, 10 and 20 mg/l) of AMX solution. The tubes were agitated in a water bath shaker at 150 rpm at

24^{O}C for 1, 2, 4,6,8,10,12 and 24 hrs. After equilibrium time had reached, the centrifuge of solution was done at 1500 rpm for 3 minutes and the clarified supernatant solutions were filtered through 0.45 µl acetate micro filters. They were transferred into 1.5 ml amber vials ready for analysis chromatographically. The initial and equilibrium AMX concentrations were determined by peak area measurement using HPLC (Shimadzu, Model LC-2040C 3D) at 229 nm. The peak areas were computed to AMX concentrations using standard calibration curve. The amount of adsorption at equilibrium, qe(mg/g) were calculated as shown in equation 1 below

$$qe = (Co-Ce)V/M \quad \text{.....Equation 1}$$

Where Ce and Co (mg/l) are the concentrations in liquid phase of AMX at equilibrium and initial respectively. V is the solution volume in litres (l) and M is the dry adsorbent mass used in grams (g). The experiment was repeated using sulphamethoxazole and Trimethoprim standard solutions.

HCl and NaOH were used for adjusting the initial pH of samples in order to study the effect of pH on removal efficiency. The pH measurements were made using a Metrohm 744 pH meter model for all experiment. Activated carbon 0.1g was added to 10 ml of 1,5,10 and 20 mg/l to solutions of AMX, SMX and TMP in centrifuge tubes. The pH values of the solution were set at pH 1, 2, 4, 6, 7, 9, 10, 11and 12.The centrifuge was agitated at room temperature on digital shaker at 150 rpm. Removals of antibiotics were measured after agitation at different time intervals and the concentrations of antibiotics analyzed on a Nexera-i LC-2040C 3DLiquid chromatograph model.

CHAPTER FOUR

4. RESULTS AND DISCUSSION

4.1 Overview of the Results

The results of the physicochemical parameters, antibiotic concentrations and adsorption studies of the three antibiotics on GAC and PAC in natural and distilled water are discussed below.

4.1.1 Physico-Chemical Parameters

Physical chemical parameter of river water is important for proper management of the quality of river water. Though it may not necessarily affect the pharmaceuticals occurrence in aquatic surrounding, their knowledge may help in explaining the observations made during analysis of antibiotics. The results of physico-chemical parameters measured at seven sites along the Ngong River are shown in Table 4.1 below.

Table 4.1 Physico-Chemical Parameters

Site	TDS (mg/L)	Conductivity (µS/cm)	NaCl (%)	pH	Temperature (^{0}C)
Mtoine dam	165	328	0.5	7.00	24
Mbagathi bridge	695	1,394	2.7	7.35	24
Enterprise road	386	767	1.3	7.72	24
Donholm	559	1,152	2.1	7.25	24
Njiru	650	1,288	2.3	7.99	23
Bypass	428	851	1.5	7.77	22
Ruai outlet	149	1,588	2.9	7.86	22

Temperature influences hydrolysis and other biological and chemical reactions in the aquatic system (Klinger and hardt, 2013). pH not only affects the activity of aquatic microorganisms but also metal speciation which in turn determines complexation and sorption processes. The total

dissolved solids levels is the sum of the anion and cations in the water. It ranged from 149 mg/L to 695 mg/L. The United States Environmental Protection Agency (EPA) standards for safe drinking water is up to 500 mg/L (USEPA, 1992), hence the concentration measured at Njiru, Donholm and Mbagathi Bridge were above the standard.

The conductivity ranged from 328 to 1,588 µS/cm, with the highest concentration registered at Ruai outlet site. This could be attributed to high concentrations of ions like, iron, calcium, magnesium, fluoride and sulphates at the site which were not removed during water treatment process. An increase in electric conductivity occurs due to higher anionic (carbonate, bicarbonate, chloride and nitrites ions) and cation (calcium and magnesium ions) contents in water (Abdel-Raouf *et al.,* 2012). The ions could be originating from the industrial waste discharges in the area and domestic waste to some extent.

Sodium chloride percentage ranged from 0.5 to 2.9%. The source could be from river stream beds with salt containing minerals, run off from industrial areas and agricultural lands, and chlorinated drinking water.

The temperature ranged from 22 °C to 24 °C. This variation can be explained by the differences in the sampling time of the day. Exposure of the sites to solar radiation and any thermal discharge into the rivers from industries could also be a factor.

The pH of the samples ranged between 7.00 and 7.86. The highest pH was measured in water from Ruai outlet site. This was within the normal pH for natural systems (6.50-8.50). The high pH in the effluent could be due to a reduced amount of carbon dioxide due to photosynthesis by algae and macrophytes, thus lowering the production of carbonic acid (Shoko *et al.,* 2014). The final

effluent from waste stabilization ponds often contains significant concentrations of algae (Abdennadher *et al.,* 2012). This shows that more algae are discharged into the river at of the WWTP.

Generally, all the parameters tested registered lower values at Mtoine site (upstream), suggesting that the site was less exposed to pollution sources, human activities and settlement. From these results it is evident that all the other parts of the river were more polluted than the WWTP effluent (Ruai outlet), with an exception of Mutoine site. Possible cause of the trend could be the effluent discharge from households, industries and urban runoff.

It was noted that there was a reduction in concentrations of parameters downstream especially at Ruai outlet site, that could be attributed to reduced human settlements and limited human activities in the area, and self-purification of the river.

4.2 Antibiotic Results

Antibiotics represent the most consumed pharmaceutical class in Kenya. This is because of their ease of access (non-prescription) and high prevalence of bacterial infections (Fent and Caminada, 2006; Zuccato *et al.,* 2010). Among the most consumed antibiotic drugs in Kenya are Co-trimazole and Amoxicillin. This is the reason behind the selection of the active content of these antibiotics for analysis. The calibration curves for SMX, TMP and AMX are shown in Appendix 1 Figures A1.1-A13 and Annex 3 Figure A3.1-A3.2).

The limit of detection for SMX, TMP and AMX were 2.20 µg/L, 1.30 µg/L and 0.20 µg/L, respectively. The retention times of SMX, TMP and AMX obtained during the analysis of this study were 16.87±0.02, 15.24±0.03 and 6.33±0.06 minutes, respectively. Table 4.2 shows the

concentrations of the antibiotics analyzed in samples collected along Ngong River at different sites.

Table 4.2 Concentrations of the Antibiotics analyzed in Ngong River Water

Site	Amoxicillin Conc. (µg/L)		Sulphamethoxazole Conc. (µg/L)		Trimethoprim Conc. (µg/L)	
	Dry	Wet	Dry	Wet	Dry	Wet
Mtoine	<0.20	<0.20	<2.20	<2.20	<1.30	<1.30
Mbagathi	1.85±0.89	<0.20	10.69±1.11	5.43±1.33	2.55±0.50	<1.30
Matter	<0.20	<0.20	3.97±0.60	3.20±0.03	2.10±0.14	<1.30
Donholm	9.06±7.78	<0.20	10.26±1.45	4.83±1.27	2.15±0.23	1.91±0.02
Njiru	<0.20	<0.20	5.67±0.44	3.43±0.35	2.03±0.20	1.90±0.01
Bypass	<0.20	<0.20	5.17±0.37	3.27±0.15	2.02±0.09	1.28±1.11
Ruai outlet	<0.20	<0.20	2.12±1.84	<2.20	<1.30	<1.30

In general the mean concentration for all the antibiotics analyzed ranged from below detection limit (BDL) to 10.69±1.11 ug/L. Mbagathi site recorded the highest mean concentrations for Sulfamethoxazole in both dry and wet seasons. This site also had the highest mean concentrations for Trimethoprim in dry season, while Donholm recorded the highest mean concentrations in wet season. Donholm also recorded the highest mean concentrations for Amoxicillin in dry season.

Figure 4.1 Shows the Bar Graph for Amoxicillin concentrations in the 7 sampling sites along Ngong River during both wet and dry seasons. Amoxicillin was detected in two sites out of the seven which were studied in dry season. Donholm site registered the highest concentration of Amoxicillin in dry season. The high concentration could be due to the discharge from the pharmaceutical industries found within the neighbouring areas (Lin and Tsai, 2009).

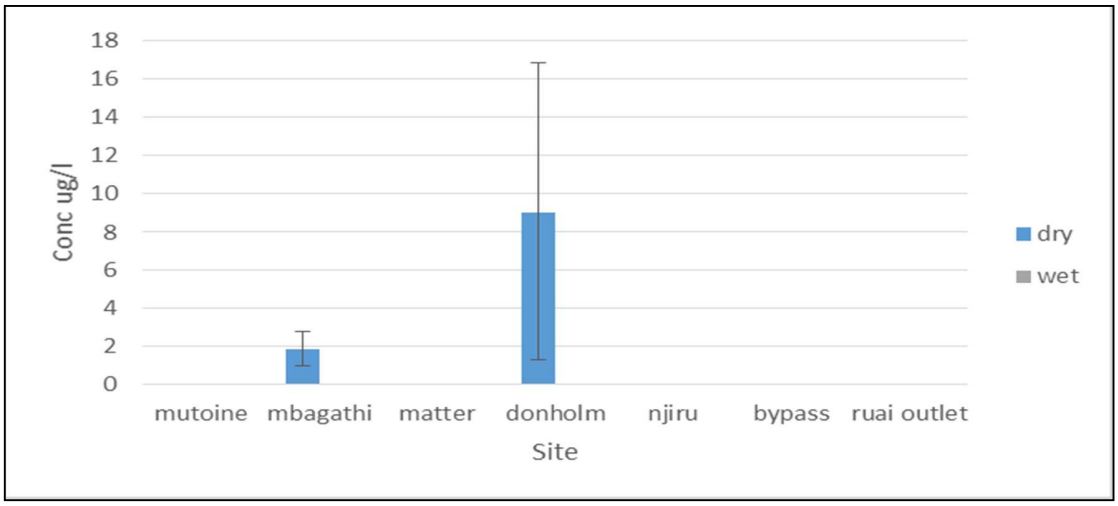

Figure 4.1 Concentration Amoxicillin in Ngong River water

High Amoxicillin concentration in Donholm site could also be due to high number of people living in the informal settlements around the site. These include Mukuru kwa Njenga, Mukuru Kayaba, Sinai, Kware, Mukuru kwa Reuben. The slum houses do not have sewer lines, hence these people either excreate directly in Ngong River, or if they use Pit latrine the waste products seeps in to the river. Overpopulation in informal settlement areas may lead to hygene problems, causing various infections. This leads to purchase of prescribed and unprescribed antibiotics to counter the infections. Amoxicillin being one of the commonly used antibiotics, it is excreated by the user either in conjugated or unchanged form, finds its way into the river hence increasing the

concentrations in river water. The other reason as to why the concentrations of Amoxicillin might be higher in Donholm is due to frequent bursts of the sewer lines along the outering road. This leads to the leakages of wastewater into Ngong River hence increasing the concentration of Amoxicillin. Source of antibiotics into river water is through the sewer line.

Mbagathi site also recorded a significant levels of Amoxicillin (1.85 ug/l). This could be due to the presence of Kibera informal settlement in the upstream of the sampling site. Most of the population in informal settlements do not have proper functioning sewerage systems. Hence most of the wastewater finds its way into the Ngong River, increasing the concentrations of Amoxicillin.

Presence of Amoxicillin concentration in Mbagthi may also be due to outbust of the sewer system at Mbagathi hospital. This leads to expired drugs and waste products to be drained into the river through the sewer line hence causing increase in antibiotics levels in the surface water.

The levels of Amoxicillin in water from Mtoine site were below the limits of detection. The finding could be attributed to the absence of domestic and industrial water discharge into the river. At Mater Bridge, Njiru, Bypass and Ruai outlet sites the concentrations of amoxicillin were below detection limits, suggesting the potential of Ngong River self cleaning process.

The other reason why Amoxicillin concentrations were not detected at Mater, Njiru, Bypass and Ruai outlet during the dry season is that Amoxicillin is susceptible to hydrolysis due to their unstable β- lactum ring, photolysis and biodegradation and hence, easily eliminated from surface waters.

In the wet season Amoxicillin concentrations were not detected in any of the seven sites. This could be due to the dilutions of the river water by the rains. The other reason could be due to the instability of the Amoxicillin as a result of the unlocking of the β- lactum ring.

Sulfamethoxazole concentrations were found in six of the seven sites sampled along Ngong River during the dry season (Figure 4.2). The highest concentrations were at Mbagathi site which recorded 10.69±1.11 ug/l, while the lowest concentration registered was at Ruai outlet (2.12±1.84 ug/l). It is also noted that the concentration of sulfamethoxazole decreased downstream. This could be because of the river self cleaning processes.

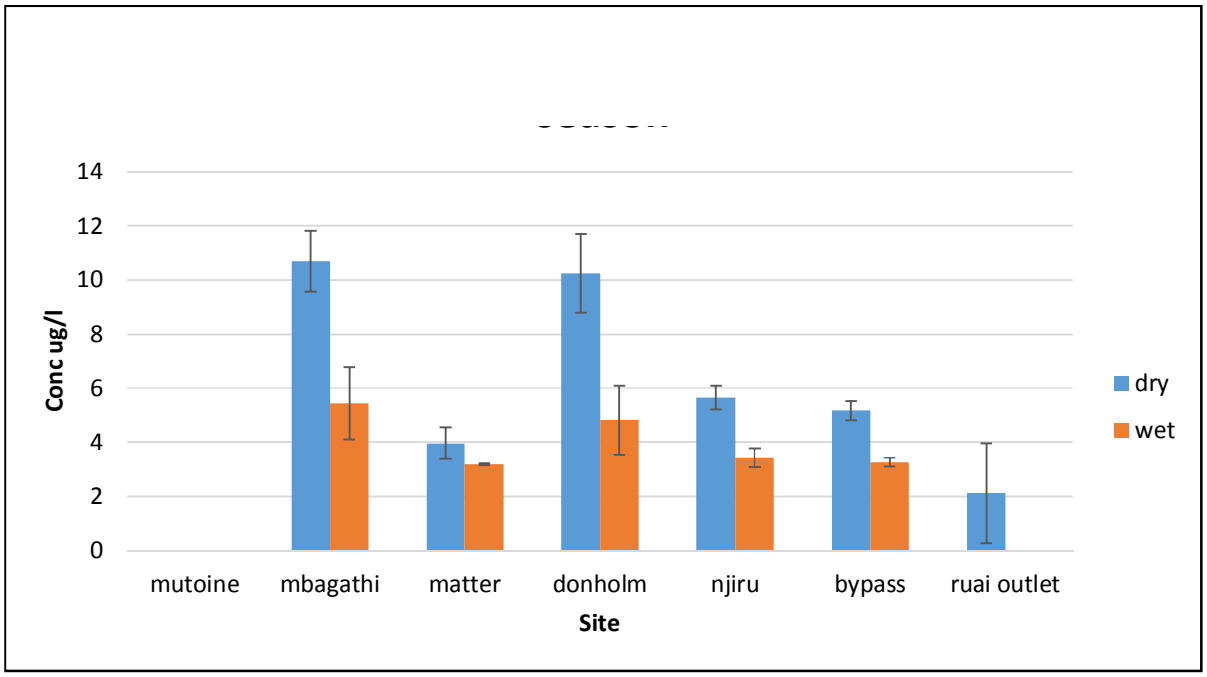

Figure 4.2 Sulfamethoxazole Concentrations along Ngong River Profile

Sulfamethoxazole concentrations were not detected at Mutoine site. This was expected since this was the source of Ngong River and the waters at this site were less contaminated due to limited human activities.

Sulfamethoxazole concentrations were high at Mbagathi closely followed by Donholm site during the dry season. This could be as a result of high population of Kibera, Kwa Njenga and Sinai informal settlements, leading sanitation problems, which inturn leads to infections. The infections are usually treated by either Amoxicillin or Cotrimazole (Septrin). These two antibiotics are the most popularly purchased in the Pharmaceutical shops because they are cheap and also because of their availability. When ingested they are excreated unchanged or conjugated. Cotrimazole has two active ingredient Sulfamethoxazole and Trimethoprim. Sulfamethoxazole is very stable hence its presence in surface water.

Lack of sewer lines in these slums leads to people use the pit latrine where by the waste product find their way into the river through filtration and sipping. People who do not have pit latrines excreate directly to the river hence contaminating it. Sewer bursts along Outering road may also have contributed to the higher concentrations of sulfamethoxazole. The waste water from the sewer lines seeps into the river causing contamination.

The other reason why Sulfamethoxazole concentrations were high at Donholm site was due to the discharge from pharmaceutical factories in the vicinity (Lin and Tsai, 2009). Similar findings have been reported by other researchers such as Zuccato *et al*. (2010) who detected sulfamethoxazole in river water. In another study, K'oreje, (2012) detected Sulfamethoxazole in Nairobi River Basin with the highest concentration being 21.20 µg/l.

Ruai outlet had concentration of 2.12±1.84 ug/l for Sulfamethoxazole during the dry season suggesting that the Ruai WWTP was not efficient at removing the antibiotics. During the wet season the levels of sulfamethoxazole concentrations were lower compared to the dry season. This could be attributed to dilution by rain water. Mbagathi and Donholm sites recorded the highest concentrations due to precence of informal settlements and pharmaceutical factories which could be draining their discharges directly to the Ngong River. The concentration of the Sulfamethoxazole in the wet season also decreased downstream, due to the river self cleaning process.

Figure 4.3 shows the levels of Trimethoprim along the Ngong River Profile. Trimethoprim concentrations were found in five sites out of the seven sites sampled in dry season, unlike in the Sulfamethoxazole concentrations, Trimethoprim was not detected in the effluent (Ruai outlet).

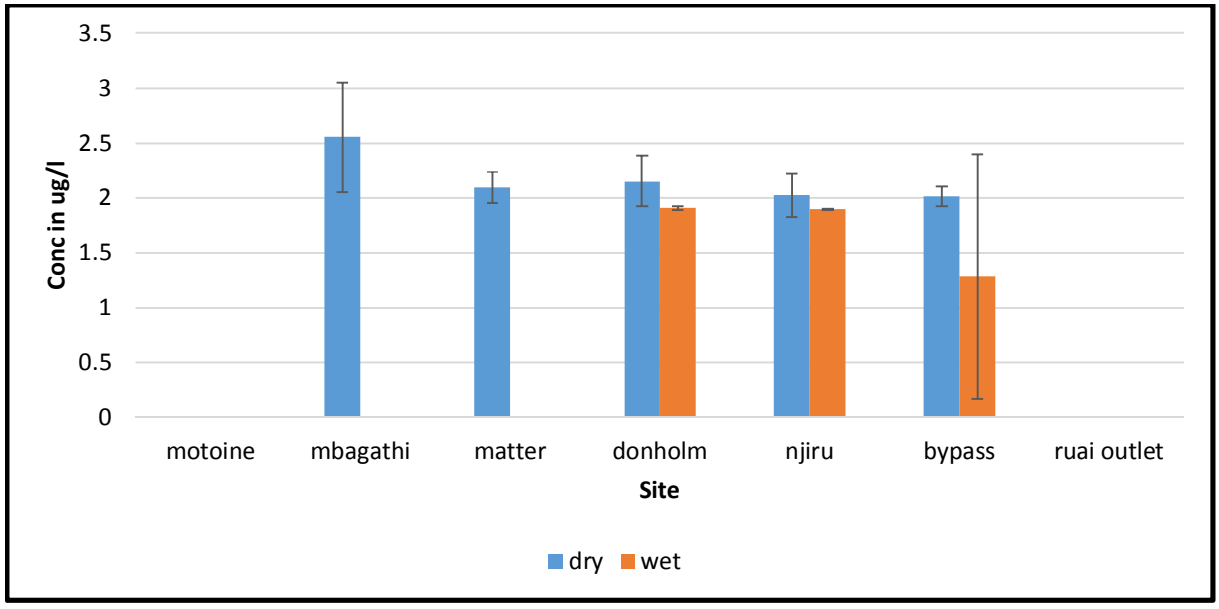

Figure 4.3 Trimethoprim Concentrations in Ngong River Water

The results were in agreement with the findings by Terzic *et al.* (2008) who noted that Trimethoprim and Sulfamethoxazole are administered as one drug (co-trimoxazole) in the ratio of 1:5, respectively. From the study their ratio of concentrations for the dry season varied from 1:2 to 1:5., suggesting that Trimethoprim and Sulfamethoxazole follow different removal processes in the environment. Kong *et al*. (2007) showed that trimethoprim is more susceptible to removal by photolysis (99%) than sulfamethoxazole (38%), which could explain the absence of trimethoprim in the effluent in contrast to sulfamethoxazole.

Carballa *et al*. (2010) detected that sulfamethoxazole at the frequency of 33% and trimethoprim at the frequency of 34% in the 87 samples analysed. However, Sulfamethoxazole had higher concentrations of 0.05 µg/l compared to trimethoprim which had 0.02 µg/l in surface water. They noted no correlation between the concentrations of these compounds in the environment and the ratio in which they existed in pharmaceutical formulations. Multiple factors influence the concentrations found in the aquatic environments. For instance, the percentage of sulfamethoxazole and trimethoprim excreted in unchanged form by humans is 15% and 60%, respectively.

Comparing the concentration of the three active contents of the antibiotics studied in Ngong River, Sulfamethoxazole had the highest concentration in the river, at 10.69 µg/l in dry season and 5.43 µg/l in wet season. This could be attributed to the fact that co-trimazole (contains Sulfamethoxazole and Trimethoprim in the ratio of 5:1) is the most commonly used antibiotics in Kenya (K'oreje *et al.,* 2012). This drug is always recommended for treatment and prevention of opportunistic infections in HIV- infected adults. The other reason as to why the concentration of

sulfamethoxazole might be high in Ngong River is that this active ingredient does not degrade easily in water (Keenan and Chaplin, 2015).

Trimethoprim had second highest concentration in the river water, which could be attributed to the fact that the active ingredient is commonly consumed together with sulfamethoxazole as Septrin drug. Septrin is commonly prescribed by doctors in Kenya due to its effectiveness against a broad range of infections. It's also commonly stored and bought in pharmaceuticals (chemists) without prescription from doctors at a low cost (Wuthiekanun *et al.,* 2016).

Amoxicillin concentration was higher in some sites but the levels reduced to below detection limit downstream. This could be due to its easy degradation in aquatic environments. Figure 4.4 below shows the comparison of the analyzed active ingredients in Ngong River in dry seasons.

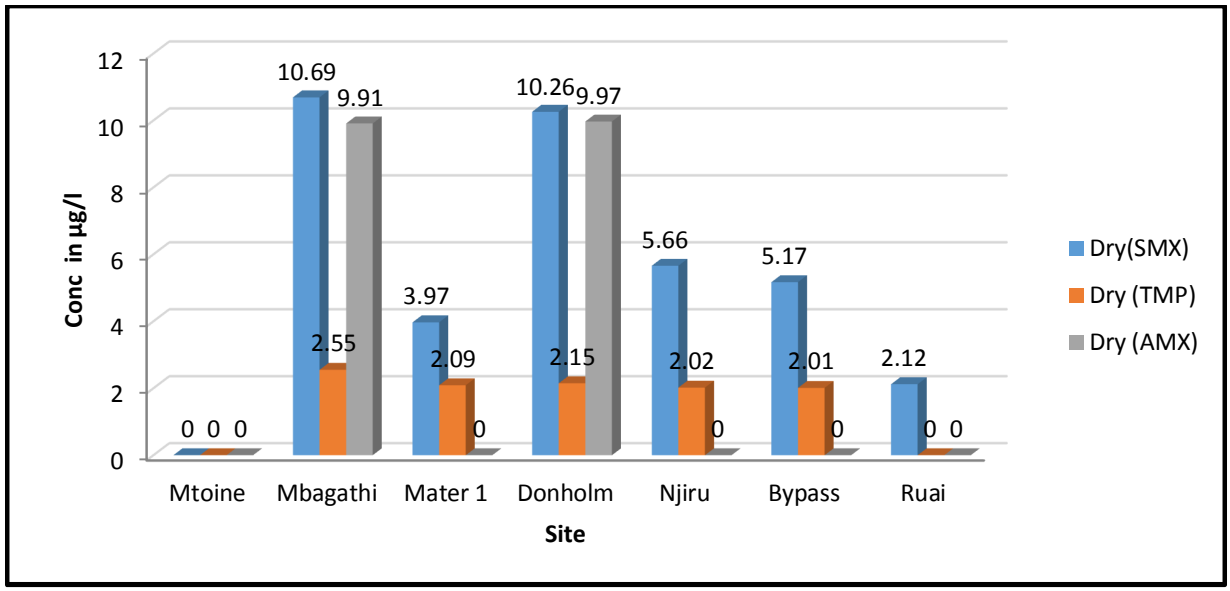

Figure 4.4 Comparison of Concentrations of Antibiotics in Ngong River in Dry Season

Figure 4.5 shows the bar graph for comparison of Amoxicillin, Sulphamethoxazole and Trimethoprim concentrations in the 7 sampling sites along Ngong River for wet seasons.

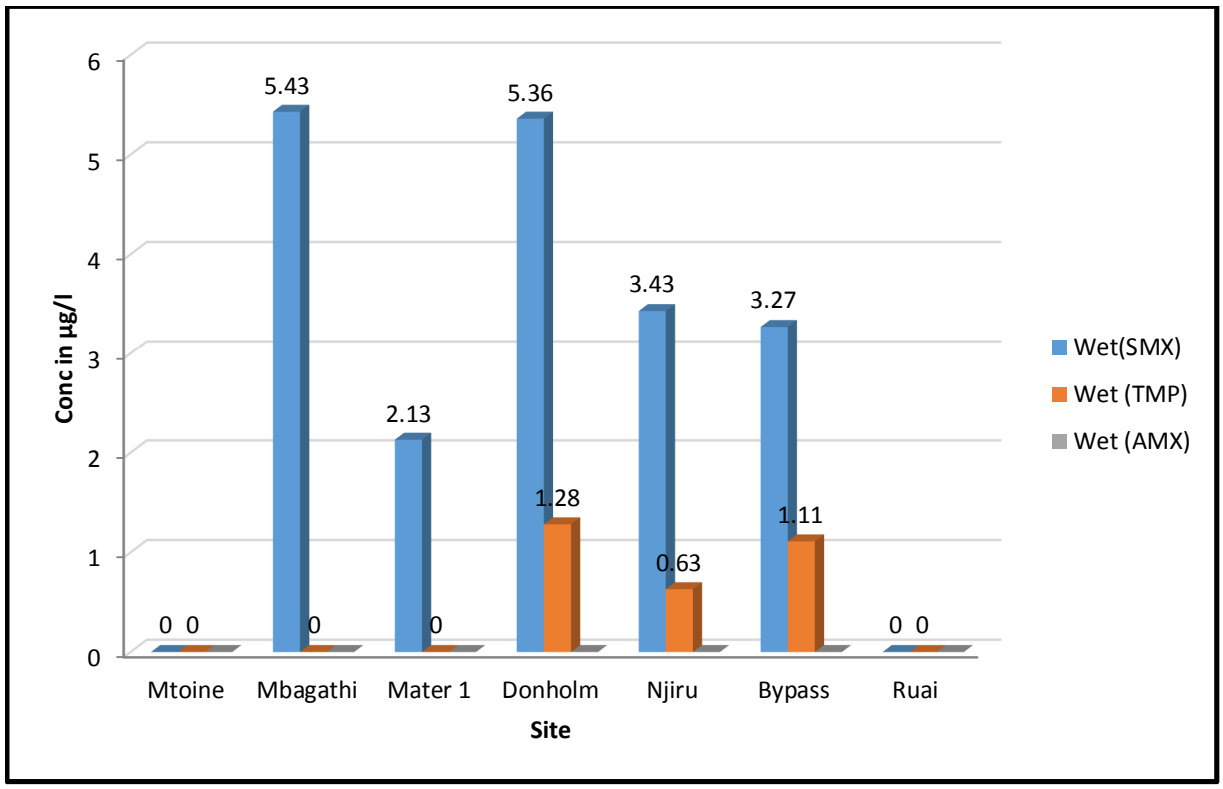

Figure 4.5 Comparison of Concentrations of Antibiotics in Ngong River in Wet Season

4.3 Antibiotics in Sediments

The concentrations of AMX, SMX and TMP were below detection limit in sediments collected along the Ngong River. Research done by Li *et al.* (2013) showed that Sulfonamides were the dominant antibiotics in water (0.86 -1,563.00 ng/l), while quinolones were prominent in sediments (65.50-1,166.00 ug/kg). Another study done in China reported the concentration of Oxytetracycline to be up to 712.00 mg/l and 262.00 mg/kg in surface water and sediments, respectively (Sengupta *et al.*, 2014).

4.4 Adsorption of antibiotics on activated carbon

4.4.1 Effect of Contact Time

The adsorption data for the uptake of AMX, SMX and TMP versus contact time at different initial concentrations used ranging from 1 mg/L to 20 mg/L (concentration of solutions used on activated carbon) is presented below. All experiments were conducted at pH 7 with constant agitation speed of 150 rpm. The amount of activated carbon which was the adsorbent was kept constant at 0.1 g. It can be observed that the adsorption capacity increased with increase in contact time. The sorption is increased rapidly at initial (due to rapid attachment of the active content to the surface of the activated carbon), and then gradually until the equilibrium (Figures 4.6 and 4.7).

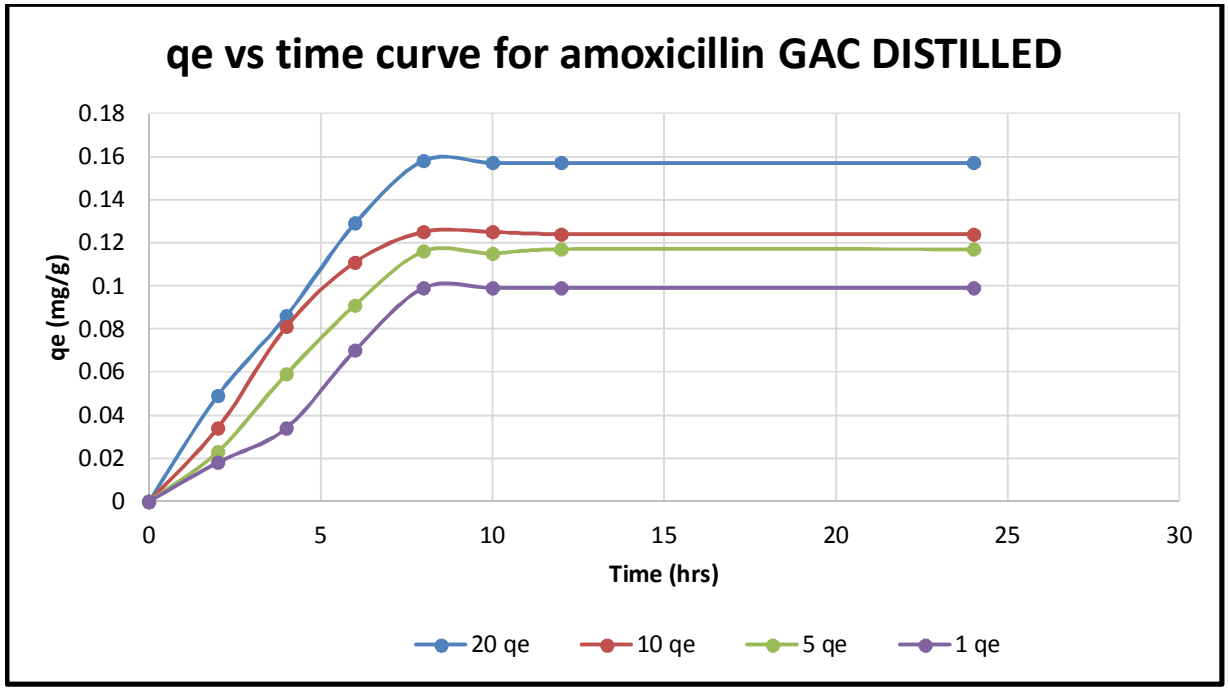

Figure 4.6 Contact time effect on adsorption of Amoxicillin antibiotics on GAC in distilled water.

Figure 4.6 shows contact time effect on amoxicillin adsorption on GAC, in surface water. The graph shows that adsorption of amoxicillin on GAC increased with concentration. Contact time of 8 hours was adequate to reach adsorption equilibrium. Figure 4.7 shows a line graph for the Contact time effect on Amoxicillin antibiotics adsorption on PAC in Surface water.

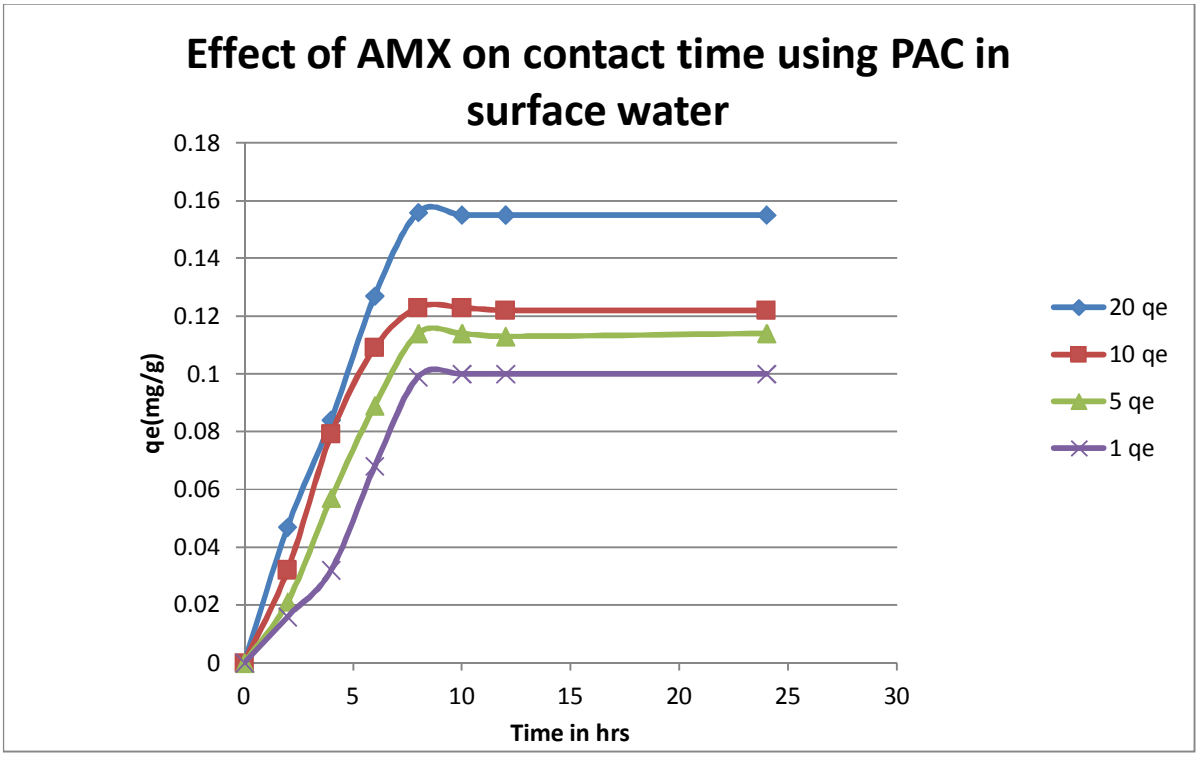

Figure 4.7 Contact time effect on antibiotic adsorption on PAC in surface water.

Figure 4.8 below shows the contact time effect on Amoxicillin antibiotics adsorption on GAC in surface water. The results showed that adsorption capacity increased with increase in contact time until equilibrium in 8 hours.

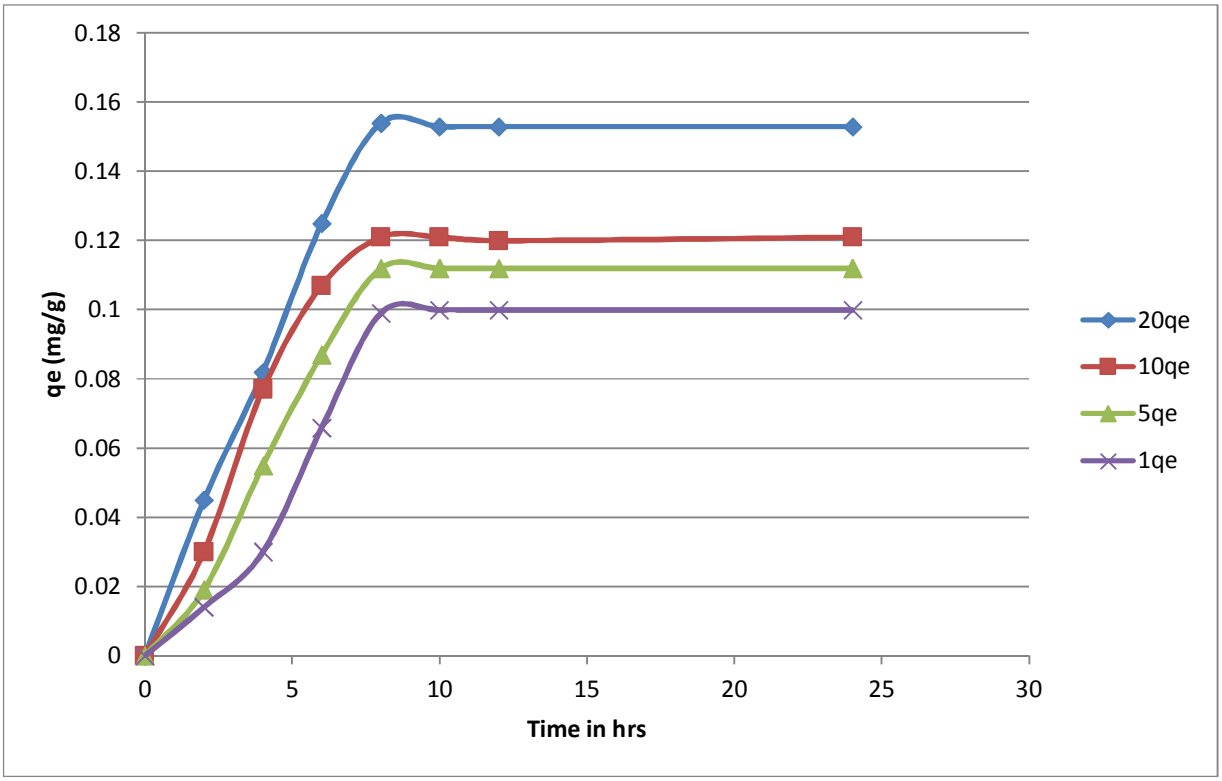

Figure 4.8 Contact time effect on AMX antibiotics adsorption on GAC in surface water

Figure 4.9 shows the contact time effect on amoxicillin antibiotics adsorption on PAC in distilled water. Adsorption on PAC increased with increase in concentrations of Amoxicillin in distilled water, from 1 mg/L to 20 mg/L.

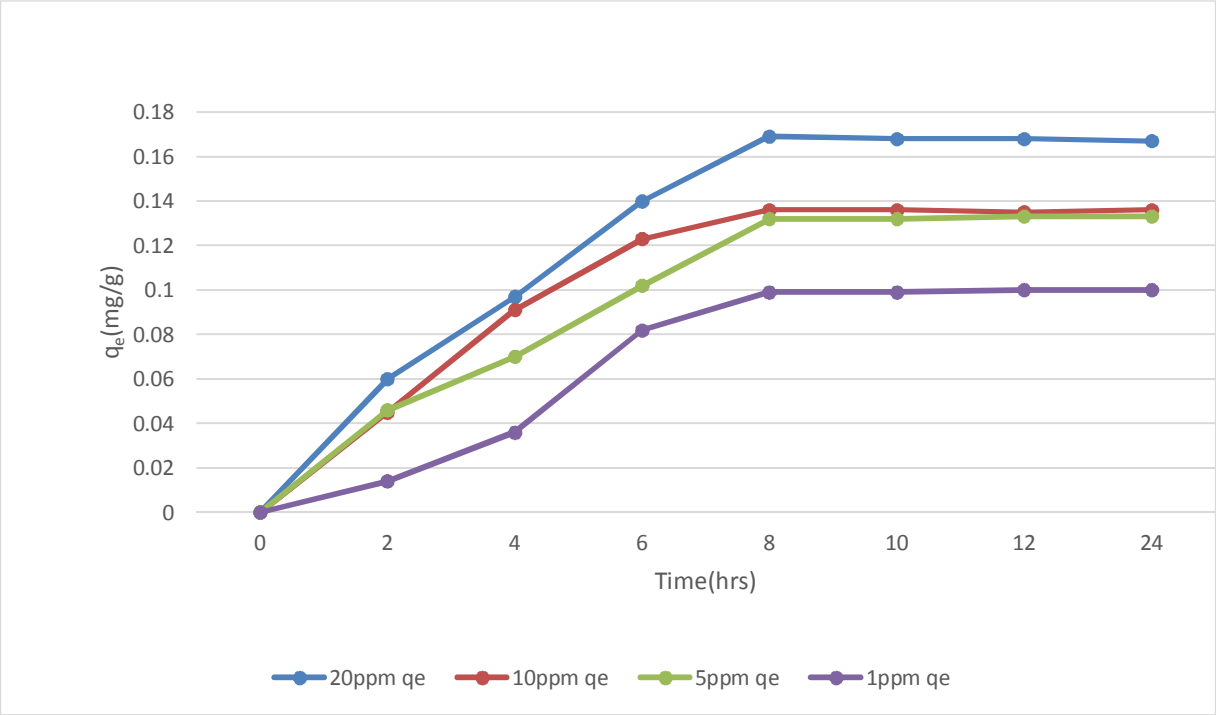

Figure 4.9 Contact time effect on AMX antibiotics adsorption on PAC in distilled water

The results of adsorption of Sulfamethoxazole antibiotics on PAC in distilled water are shown in Figure 4.10. Adsorption increased with contact time until equilibrium, within 6 hours. In addition, adsorption capacity on PAC also increased with increase in concentrations of Sulfamethoxazole in distilled water.

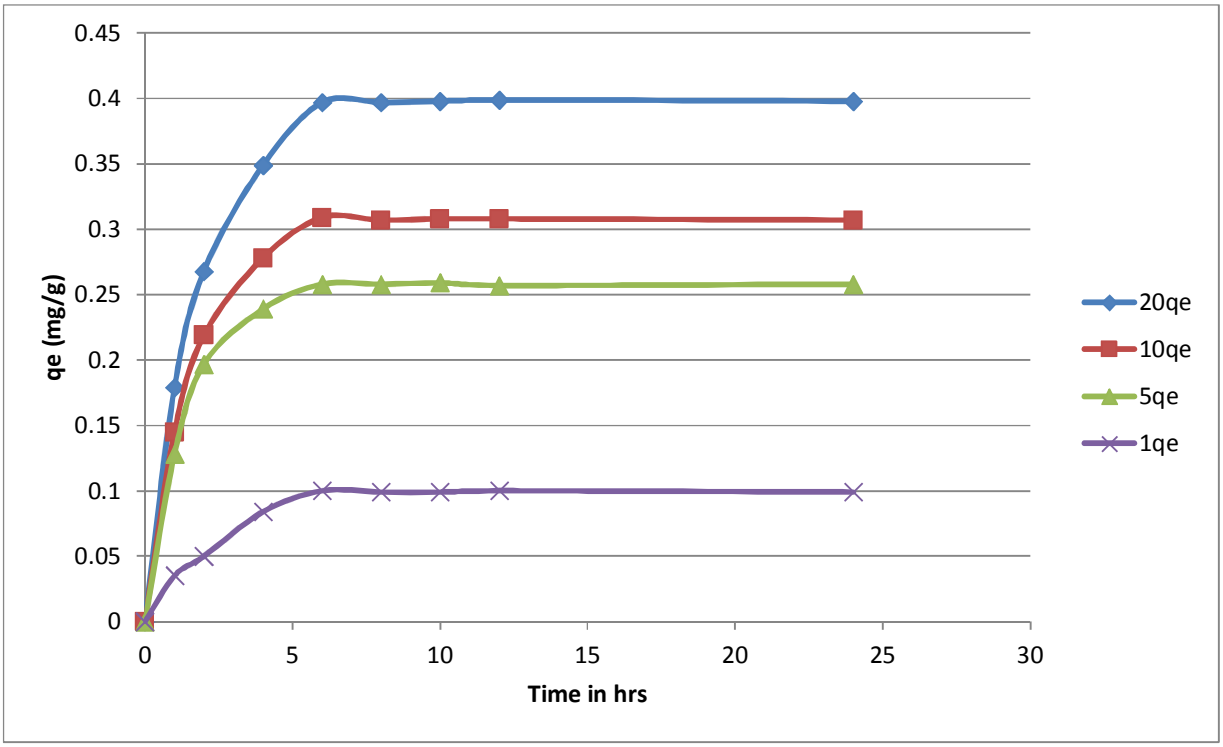

Figure 4.10 Effect of Contact time on adsorption of SMX antibiotics on PAC in distilled water

The results of adsorption of Sulfamethoxazole antibiotics on GAC in distilled water are shown in Figure 4.11. The data shows that adsorption increased with increase in contact time until equilibrium was attained within 6 hours. Adsorption of sulfamethoxazole on GAC increased with concentrations in distilled water.

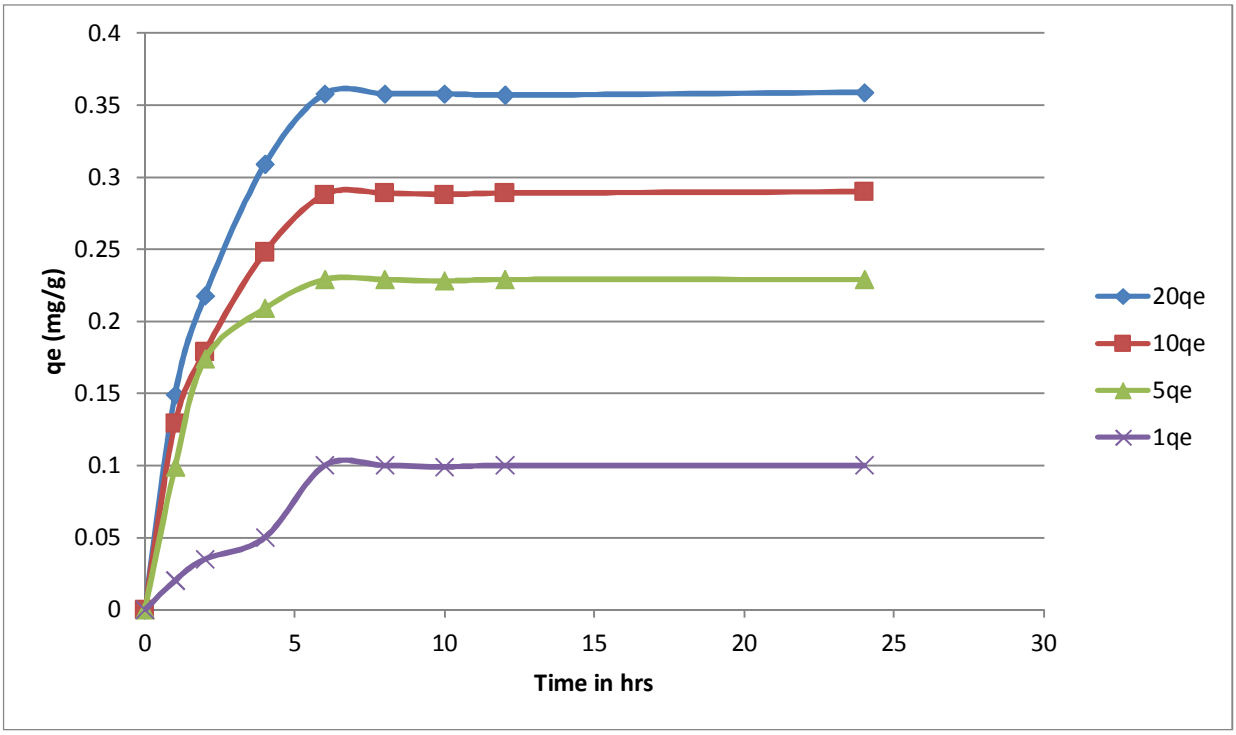

Figure 4.11 Contact time effect on SMX antibiotics adsorption on GAC in distilled water

Figure 4.12 shows adsorption of Sulfamethoxazole on PAC in surface water. Adsorption increased up to 6 hours and plateaued. The initial fast rate is due to the available space of the adsorbent. Once the active sites got occupied adsorption rate decreased. For higher concentrations there were more ions in the solutions competing for the active sites of the adsorbent, hence increased adsorption capacity compared to lower concentrations.

Comparison of the data in Figure 4.12 and Figure 4.13 showed that at a concentration of 20 ppm adsorption capacity of PAC for Sulfamethoxazole in surface water (0.30 mg/g) was higher than that of GAC in the same medium (0.28 mg/g). This suggests that PAC is a better adsorbent compared to GAC.

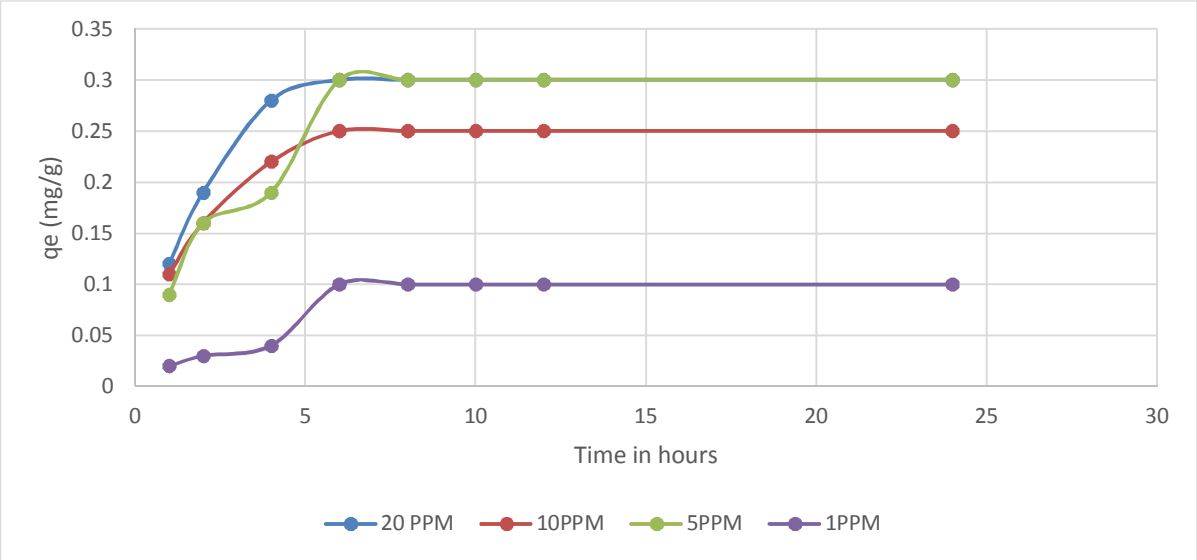

Figure 4.12 Contact time effect on SMX antibiotics adsorption on PAC in surface water

Figure 4.13 shows the behavior of SMX adsorption on GAC in surface water. Rapid adsorption was observed at the beginning up to 6 hours and plateaued. In addition, higher concentrations achieved higher adsorption rates as shown in Figure 4.13.

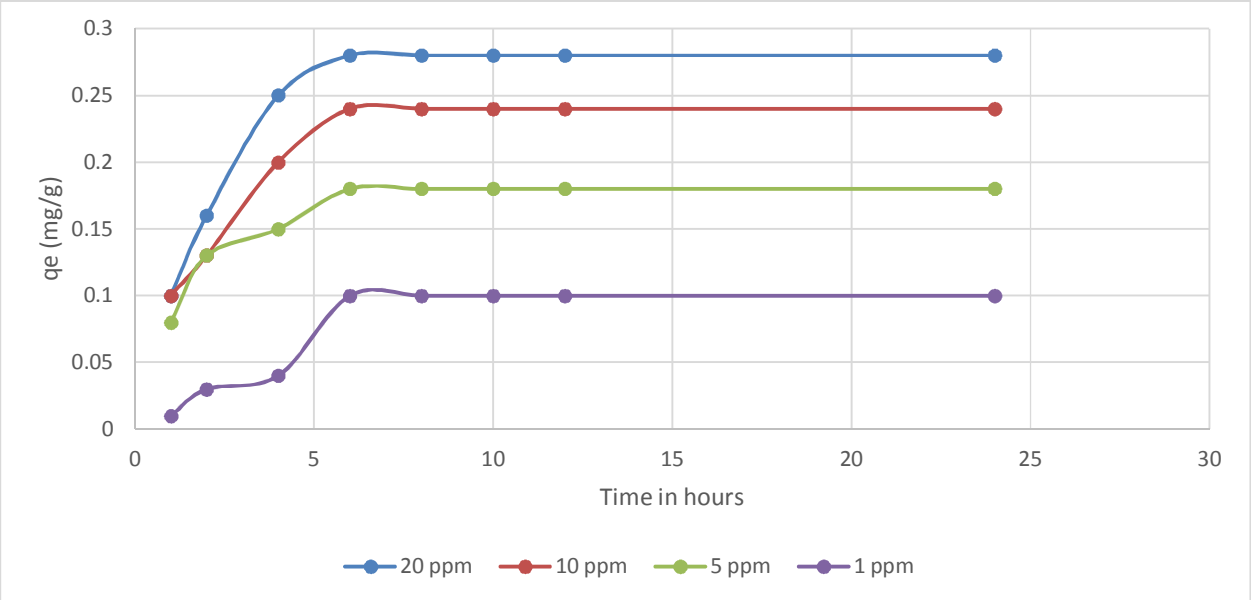

Figure 4.13 Contact time effect on SMX antibiotics adsorption on GAC in surface water

Figure 4.14 shows the Contact time effect on Trimethoprim antibiotics adsorption on PAC in Distilled water. Adsorption equilibrium was established within 6 hours of the experiment.

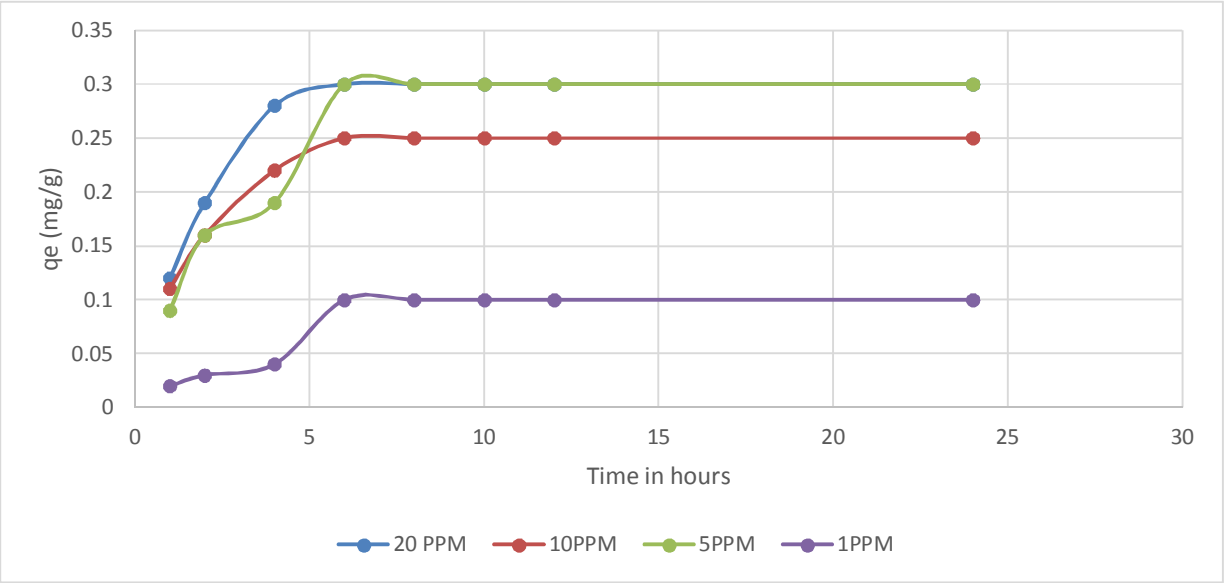

Figure 4.14 Contact time effect on TMP antibiotics adsorption on PAC in distilled water

Figure 4.15 shows adsorption of Trimethoprim antibiotics on GAC in distilled water. The data shows that adsorption rate increased very fast at the beginning, but plateaued after 6 hours suggesting attainment of the equilibrium conditions. Similarly, increase in adsorption was observed with increase in solution concentration from 1 mg/L to 20 mg/L.

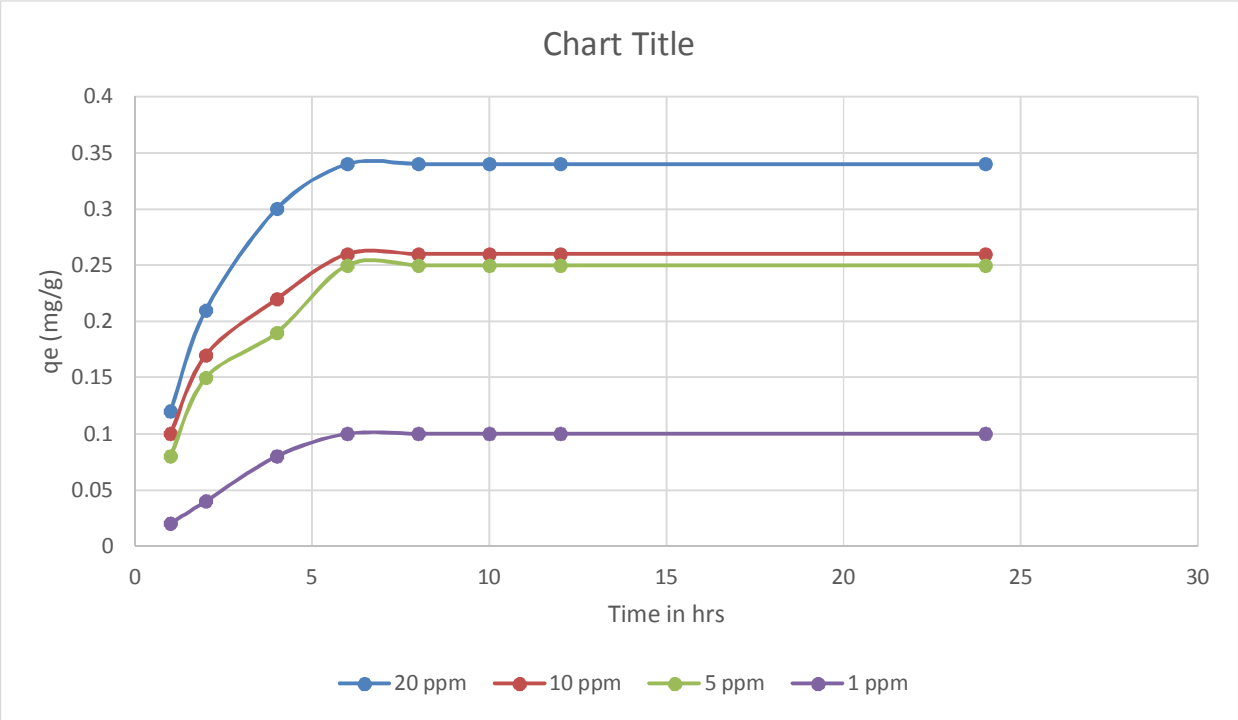

Figure 4.15 Contact time effect on TMP antibiotics adsorption on GAC in distilled water

Figure 4.16 shows the contact time effect on Trimethoprim antibiotics adsorption on PAC in Surface water. Adsorption capacity increased with increase in contact time up to the 6th hour and plateaued. The data also revealed that increase in concentration of line graph also shows that Trimethoprim increased adsorption on PAC.

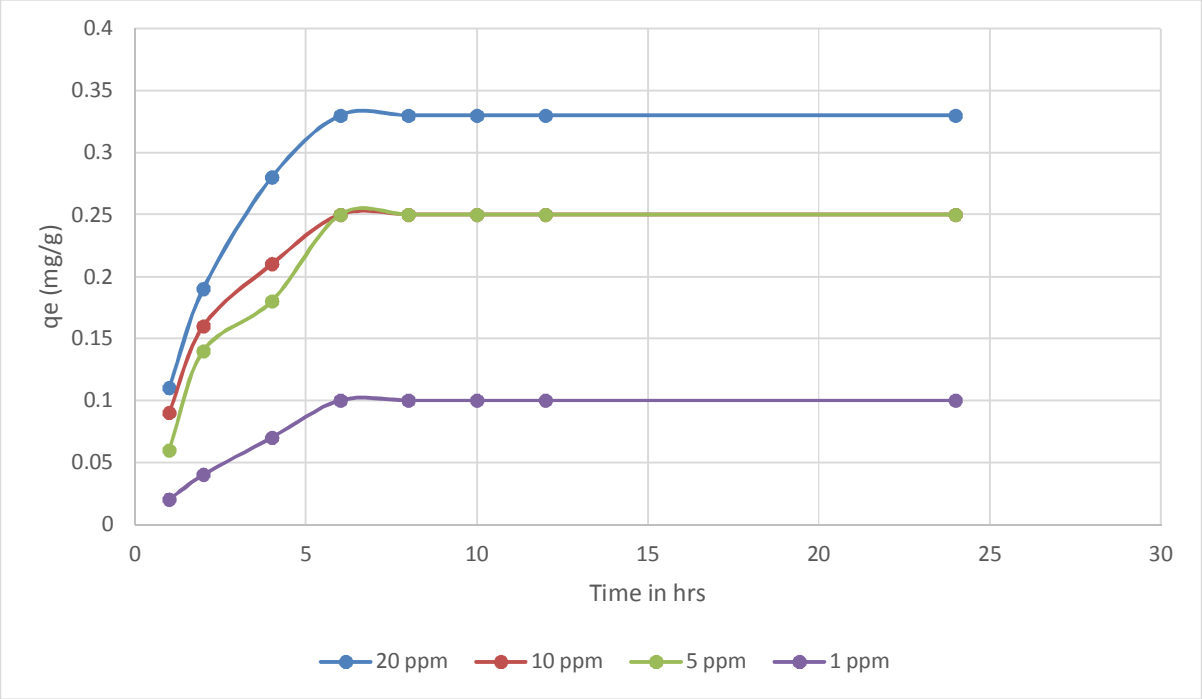

Figure 4.16 Effect of Contact time on adsorption of TMP antibiotics on PAC in surface water

The contact time effect on Trimethoprim antibiotics adsorption on GAC in Surface water is shown in Figure 4.17 below. The data shows that adsorption capacity increased with increase in contact time until equilibrium. In addition, adsorption of Trimethoprim on GAC in surface water increased with increase in concentrations up to the 20 mg/L.

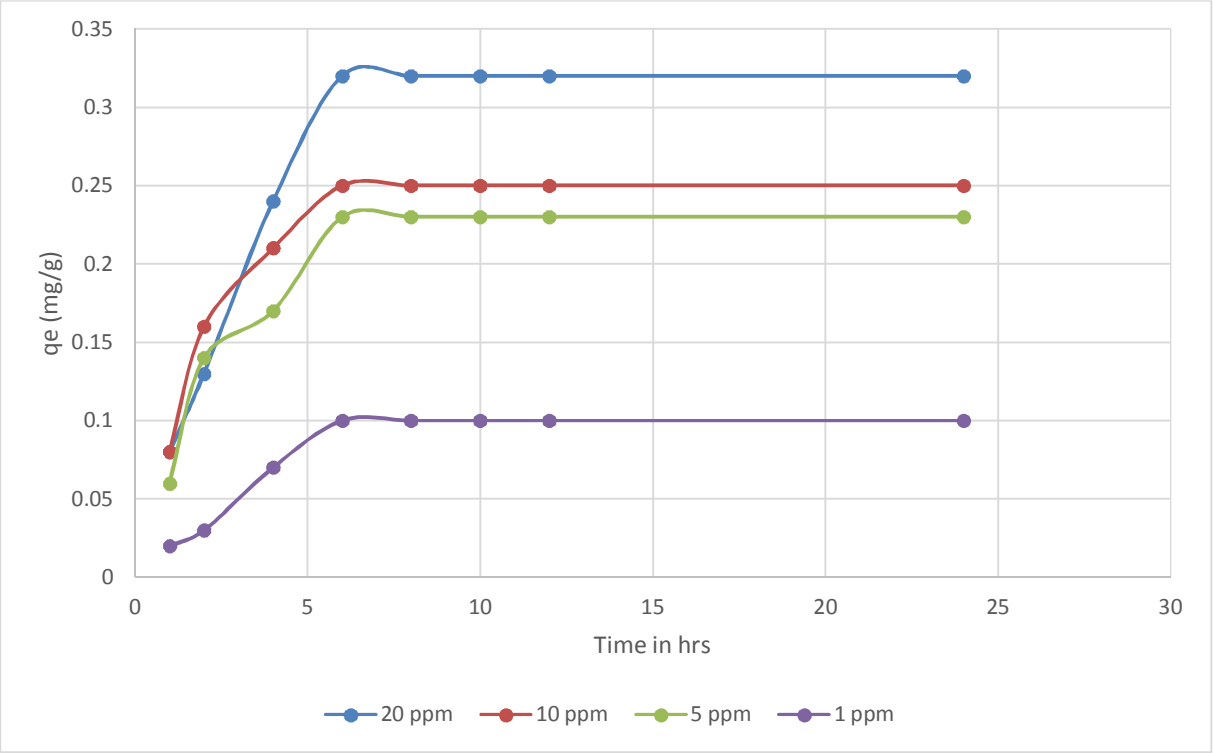

Figure 4.17 Contact time effect onTMP antibiotics adsorption on GAC in surface water.

Adsorption of AMX, SMX and TMP were notably greater on PAC than on GAC. This showed that PAC was a better adsorbent than GAC in removal of antibiotic from both distilled and surface water. Maximum removal of AMX, SMX and TMP at initial concentration of 1mg/l using PAC and GAC in both surface and distilled water was 100%. Maximum removal efficiencies decreased as the concentrations of the various active contents increased. Table 4.3 below summarizes maximum removal rate of GAC and PAC after 24 hours adsorption for AMX, SMX and TMP. The decrease in maximum removal efficiency as concentration increases could be attributed to increase in the number of particles in the solution as concentration increased. There was competition for attachment of the particles to the fixed number of active sites on the adsorbent (GAC or PAC). This means that the fewer the particles the more the adsorption of the particles by the adsorbent hence higher removal efficiency.

Table 4.3 Removal rate of GAC and PAC after 24 hours Adsorption for AMX, SMX and TMP

Active Content	Type of Water	Type of Activated Carbon	Concentration (ppm)	% Removal
AMX	Distilled	PAC	1	100
			5	27
			10	13.7
			20	8.5
		GAC	1	100
			5	23.6
			10	12.6
			20	7.9
	Surface	PAC	1	100
			5	23
			10	12.4
			20	7.8
		GAC	1	100
			5	22.6
			10	12.2
			20	7.7
SMX	Distilled	PAC	1	100
			5	52
			10	31
			20	20
		GAC	1	100
			5	46
			10	29
			20	18
	Surface	PAC	1	100
			5	59.9
			10	25.4
			20	15.2
		GAC	1	100
			5	35.9
			10	23.8
			20	14.1
TMP	Distilled	PAC	1	100
			5	53.8
			10	27.2
			20	18.6
		GAC	1	100
			5	50.6
			10	26.3
			20	17.2
	Surface	PAC	1	100
			5	50.2
			10	25.8
			20	16.5
		GAC	1	100
			5	47.3
			10	25.3
			20	16.2

High removal rate for PAC were was observed compared to GAC. This could be explained by the way each activated carbon was packaged. The PAC contained small particles, hence larger surface area for the adsorption compared to GAC which had larger particles, hence smaller surface area.

From Table 4.3, GAC and PAC adsorbed less AMX, SMX or TMP in surface water compared to distilled water. This could be explained by the fact that surface water from the river contained organic matter, competing for the active sites on the adsorbent in the solution. On the contrary, distilled water was perceived to be free of other particulates.

4.5. Effect of pH on Adsorption

In amoxicillin adsorption, the solution pH is key to the adsorption process, since amoxicillin molecule charge can be altered. Generally, when the pH of amoxicillin molecule lies below its isoelectric point it has a positive charge. It shifts to negative charge when the pH of the solution rises above its isoelectric point (Bhattacharjee, 2016). The occurrence of this phenomenon is due to its functional groups ionization. The ionization groups in the amoxicillin structure are phenolic hydroxyl (pKa 9.63)), amine (pKa 7.49) and carboxyl (pKa 2.68) (Jang *et al.,* 2002).

The protonation for both activated carbon adsorbent and amoxicillin molecule at low pH enhances the adsorption capacity of activated carbon. pH of the solution strongly influences the adsorption of the amoxicillin molecule onto the activated carbon surface in aqueous solution, hence there could be several adsorption mechanisms for amoxicillin molecule onto activated carbon which can be proposed. One of them is cation-exchange mechanism in which principally, cations of low valence in activated carbon structure are exchanged by amoxicillin protonated functional groups which are positively charged. The positive charge of amoxicillin is due to the protonated carboxyl

and amine groups by the presence of free hydrogen (H^+) ions in the solution (Andreozzi *et al.,* 2005).

On the other part, protonation or deprotonation does not take part on the phenol groups of the amoxicillin. The interchange between cations of low valence in activated carbon structure and protonated amine groups and carboxyl in the amoxicillin during process of adsorption is indicated by the cations presence in the solution after adsorption.

Another possible mechanism is the interactions between silanol and aluminol protonated groups in activated carbon and carboxyl group which contain negatively charged oxygen atoms. Functional groups of alumina and silica on activated carbon surface are protonated as the pH of thesolution is below its pHpzc, because of the presence of free hydrogen ($H^{+)}$ ion in the solution (Liu *et al*., 2010).

Moreover, on the surface of activated carbon the aluminol groups can be protonated (Bajpai and Sachdeva, 2002). Since activated carbon is negatively charged on its surface because of its isomorphous substitution of Al3þ for Si4þ in tetrahedral layer and Mg2þ for Al3þ in octahedral sheet, it can attract molecules of amoxicillin which are positively charged from amine groups protonation.

At pH of 7.01, deprotonation on activated carbon has provision of repulsive force to the molecule of amoxicillin which is negatively charged. The deprotonation of carboxyl group causes the amoxicillin molecule to be negatively charged. Neutral form of amoxicillin is in the pH ranges of between is 3–6) (Lavniewski *et al.,* 1998). Researchers (Dutta *et al.,* 1997a, b, 1999) also noted similar observations. Adsorbent at pH pzc (pKa1 <pH soln <pKa2, pKa3), functional groups of

amine in amoxicillin present in the form of $–NH_3$ þ and functional groups of carboxyl are found in the form of groups of carbonyl. In these conditions, the forces of electrostatic enhances the adsorption capacity because negatively and positively charged activated carbon functional groups will attracts the negatively and positive charged amoxicillin functional groups. Another mechanism, is the reaction between carbonyl functional groups on activated carbon with amoxicillin amine functional groups. Carbonyl functional groups are suppressed by the NH_2 groups to become nitrile functional group while H_2O is being released to the solution.

4.5.1 Effect of pH on Sulfamethoxazole

Figure 4.18 shows that above pH 7, there is decrease of adsorption of SMX on the surface of GAC and PAC. This is because increase in pH means increase of hydroxyl ions in the solution. This causes the surface of the PAC and/or GAC to be negatively charged. The increase of the negativity of the charge of the surface of GAC and /or PAC increase as the pH increase. There is also negatively charged SMX in the solution. The anionic SMX- is the only species in the solution, hence repulsive electrostatic interactions which did not favour adsorption when the pH was increased from 7 to 11.

Between pH 6 and 7 the adsorption of antibiotic on PAC and GAC was at its maximum. This is because the surface charges of PAC and/or GAC was positive and the anionic SMX- was the predominant species at pH 7 and this enhances electrostatic attraction which favours the adsorption capacity.

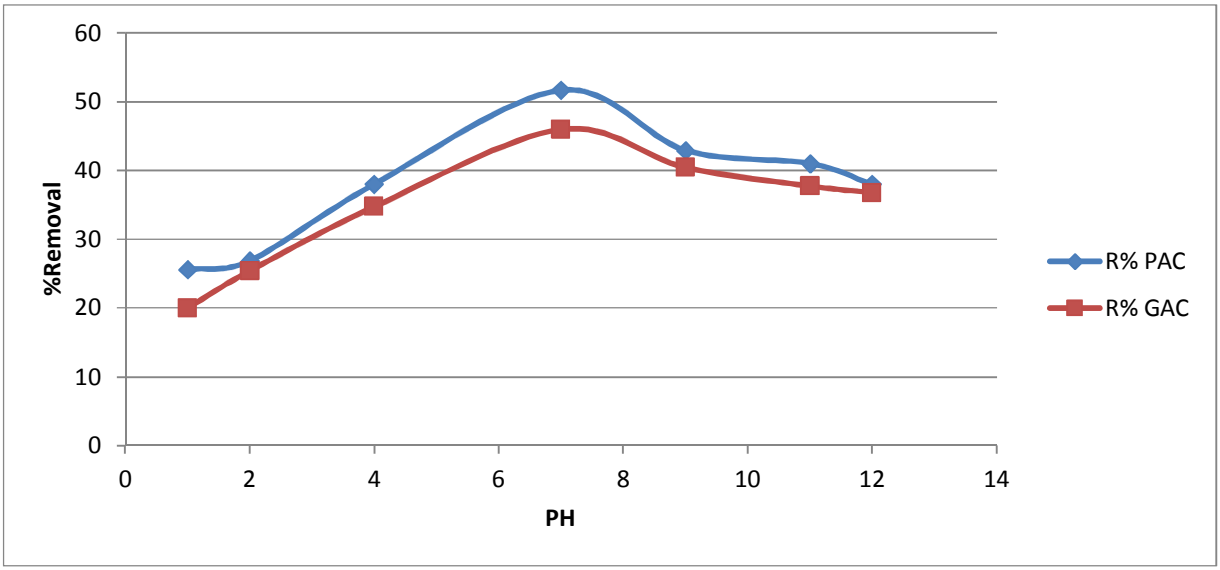

Figure 4.18 Effect of pH on SMX using PAC and GAC as an adsorbent

From pH 2 to 5, adsorption process was moderate, but it reduced as pH decreased. At low pH range, the electrostatic interaction was not important. Surface charge of GAC and/or PAC was positive and the main species of SMX was neutral at these pH ranges. The results suggest that adsorption was not maximum since there was no electrostatic interaction. As a result of all these changes of pH on adsorption, it could be concluded that the effect of the electrostatic interactions was significantly influenced by solution pH.

4.5.2 Effect of PH on Trimethoprim

Figure 4.19 shows the effect of pH on adsorption of Trimethoprim, with optimal adsorption at pH 7. pH affects the surface charge on activated carbon, since above pH 7 there is an increase in hydroxyl ions. This causes the activated carbon and the TMP species in the solution to be negatively charged and hence reduces the sorption process by electrostatic repulsion. At higher pH values there is also production of aqua-complexes which reduces the adsorption capacities of activated carbon.

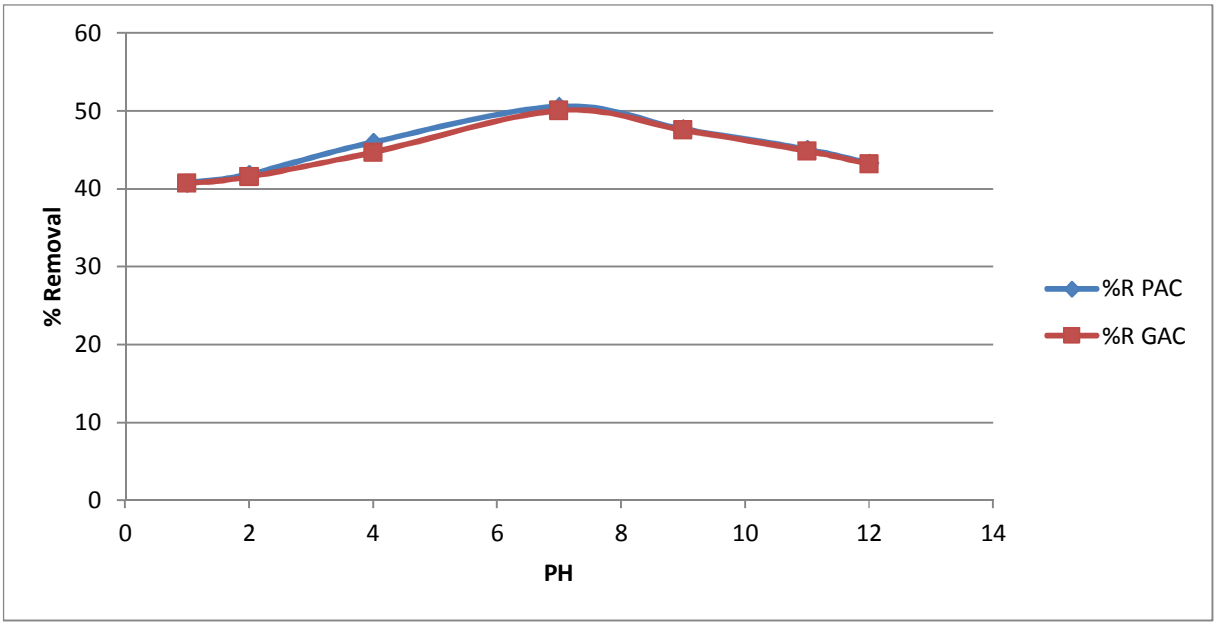

Figure 4.19 Effect of pH on TMP using PAC and GAC as an adsorbent

4.6 Adsorption Experiments

Adsorption isotherms show the relationship between the amount of substance adsorbed at equilibrium and the remaining concentration in aqueous media. In this study, two adsorption models were applied; the Langmuir and Freundlich isotherms.

4.6.1 Langmuir Isotherm

Langmuir isotherm described by the equation 2 (Ternes *et al*., 2002) below was used to determine the values of adsorption maximum.

$Ce/Qe=1/BQe+ Ce/Qm$ …… Equation 2.

Where B is a Langmuir constant (L/g); Qm is the maximum sorbate uptake per unit mass of the adsorbent (mg/g); Qe is the adsorbed amount on unit mass of the adsorbent mg/g and Ce is the equilibrium concentration of sorbate in solution.

The values of Q_m and B computed from the slopes and y intercepts are shown in Table 4.4 and Appendix 2 Figures 2.4-2.6, where Q is the maximum adsorbate that can be adsorbed on the surface (adsorption capacity) and B is the isotherm constant.

From Table 4.4 the results yielded values of QB less than one. According to Musa *et al.* (2011) larger values of b and QB suggest favorable adsorption process. Therefore, since the value of QB was less than 1 for AMX, SMX and TMP on activated carbon was not favorable.

Table 4.4 Maximum uptake for antibiotics on PAC and GAC in distilled and surface water

Active Content	Water type	Activated Carbon Type	Equation	Q (mg/g of activated carbon)	QB	B(l/g)	R^2
AMX	Surface	GAC	Y=10.64x+6.52	0.15	0.09	0.60	0.81
		PAC	Y=7.10x+6.77	0.15	0.14	0.93	0.78
	Distilled	GAC	Y=7.26x+6.58	0.16	0.14	0.88	0.80
		PAC	Y=14.31x+5.73	0.17	0.07	0.40	0.89
SMX	Surface	GAC	Y=18.93x+2.55	0.39	0.05	0.13	0.98
		PAC	Y=6.59x+2.38	0.42	0.15	0.36	0.77
	Distilled	GAC	Y=10.77x+2.45	0.41	0.09	0.22	0.93
		PAC	Y=3.42x+2.38	0.42	0.29	0.69	0.89
TMP	Surface	GAC	Y=12.51x+1.96	0.51	0.08	0.16	0.73
		PAC	Y=10.84x+1.97	0.51	0.09	0.18	0.76
	Distilled	GAC	Y=12.51x+1.96	0.51	0.08	0.16	0.73
		PAC	Y=9.08x+1.95	0.50	0.11	0.22	0.88

From the table it can be concluded that the results yielded fairly goof correlation coefficient. It could also be noted that there was no significant difference in maximum adsorption uptake between distilled water and surface water. This means that the surface water had minimal organic matter dissolved into it. Adsorption capacities of TMP and SMX were higher than that of AMX, which could be attributed to the fact that TMP and SMX are smaller molecules as compared to AMX.

4.6.2 Freundlich Isotherm

The Freundlich isotherm is based on the log transformation of the equation 3 and 4 below.

$$q_e = k_f c_e^{1/n} \text{.....equation 3}$$

$$\text{Log } q_e = \text{Log } k_f + 1/n \text{ Log } c_e \text{.....equation 4.}$$

Where,

k_f = Freundlich constant related to the bonding energy 1/n is the heterogeneity factor, n (g/l) is a measure of the deviation from linearity of adsorption or the degree of nonlinearity between solution concentration and adsorption. The Freundlich isotherm supposition is based on uniformity of active sites energy, or the fact that different functional groups are adsorbed on the surface by different energies. In this case sorption can happen in multilayer manner. Graphs of log Qe versus log Ce were plotted for AMX, SMX and TMP. The values of K_f and n computed from the slopes and y intercepts of the graphs are shown in Table 4.5 and Appendix 2 Figures 2.1-2.3.

Table 4.5 shows the summary of the calculations done to obtain Freundlich isotherms values

Active Content	Water type	Activated Carbon Type	Equation	K_F	1/n	N	R^2
AMX	Surface	GAC	Y=0.14x-1.02	0.36	0.14	7.14	0.87
		PAC	Y=0.14x-1.01	0.36	0.14	7.14	0.91
	Distilled	GAC	Y=0.15x-1.01	0.36	0.15	6.67	0.92
		PAC	Y=0.21x-1.09	0.34	0.21	4.76	0.93
SMX	Surface	GAC	Y=0.37x-0.20	0.82	0.37	2.70	0.99
		PAC	0.29x-0.84	0.43	0.29	3.45	0.69
	Distilled	GAC	Y=0.24x-0.75	0.47	0.24	4.17	0.99
		PAC	Y=0.23x-0.68	0.51	0.23	4.35	0.97
TMP	Surface	GAC	Y=0.38x-0.92	0.40	0.38	2.63	0.83
		PAC	Y=0.37x-0.91	0.40	0.37	2.70	0.78
	Distilled	GAC	Y=0.39x-0.90	0.41	0.39	2.56	0.79
		PAC	Y=0.38x-0.90	0.41	0.38	2.63	0.79

For Freundlich isotherms if the value of n is below unity, then the adsorption is a chemical process; otherwise the process is a physical process. In Table 4.5 which summarized the results of this study the n value obtained for AMX, SMX and TMP were above one, suggesting that the adsorption was a physical process.

The values of regression coefficients R^2 are regarded as a measure of goodness of fit of the experimental data to the isotherm model. Hence the R^2 of Langmuir and Freundlich isotherm in this study, the data fitted better in Freundlich isotherm model, suggesting that adsorption of AMX, SMX and TMP followed multilayer adsorption.

CHAPTER FIVE

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Motoine Dam (upstream) was the least contaminated site while subsequent sites along the river profile recorded higher TDS, Electrical conductivity, suggesting input of pollutants from households, industries and commercial activities.

The concentrations of antibiotics in river water samples increased downstream, suggesting anthropogenic sources of antibiotic contamination into the Ngong River. The three tested antibiotics AMX, SMX and TMP were all detected in the river water with higher levels in the mid and downstream compared to upstream.

The dry season recorded higher antibiotic concentrations compared to the wet season. The sum concentrations in wet season was 20.17 ug/L; 6.99 ug/L and bdl for SMX, TMP and AMX, respectively, while sum concentrations in the dry season was 37.88 ug/l, 10.84 ug/l and 10.91 ug/l.

The trend in sum concentration was SMX>TMP>AMX during the wet season and SMX>AMX>TMP, during the dry season, suggesting that seasonal variations had an effect on the occurrence and concentrations of AMX, SMX and TMP in the River water. The presence of SMX in waste water effluent from Ruai outlet showed that the wastewater treatment plant was not effective at completely removing antibiotic contaminants from the waste water.

Adsorption of AMX, SMX and TMP was greater on PAC than on GAC, showing that PAC was a more effective at removing these antibiotic contaminants from water than GAC. Adsorption was

strongly influenced by the electrostatic interactions as evidenced by changing adsorption capacities at different solution pH values. In addition, adsorption was also influenced by the physic-chemical properties of the antibiotic in question such as the pka values.

5.2 Recommendations

5.2.1 Policy recommendations

1) The study has established the presence of antibiotics in Ngong River Water. Bacterial exposure to low doses of antibiotics could contribute to antibiotic resistance and make it difficult to treat bacterial infections using these antibiotics. Hence immediate action should be taken to stop antibiotic discharges into the river system.

2) There is need to enforce NEMA regulations to minimize discharge of untreated effluents into the River, to reduce high loads of physico-chemical contamination in Ngong River water.

5.2.2 Research recommendations

Further studies should:

1) Analyze antibiotics from other tributaries of Nairobi River Basin since they join and discharge into Athi River which is used a source of drinking water to the downstream cities like Mombasa.
2) Analyze and characterize dissolved organic carbon contents in water from Ngong River using FTIR and other technique to show the interactions and effects on adsorption of antibiotics.
3) Investigate influence of organic matter from wastewater treatment plants on adsorption of AMX, SMX and TMP in order to gain more information on the performance of PAC and GAC under real time application in removal of antibiotics in wastewater treatment plants.

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APPENDIX 1: CALIBRATION CURVES

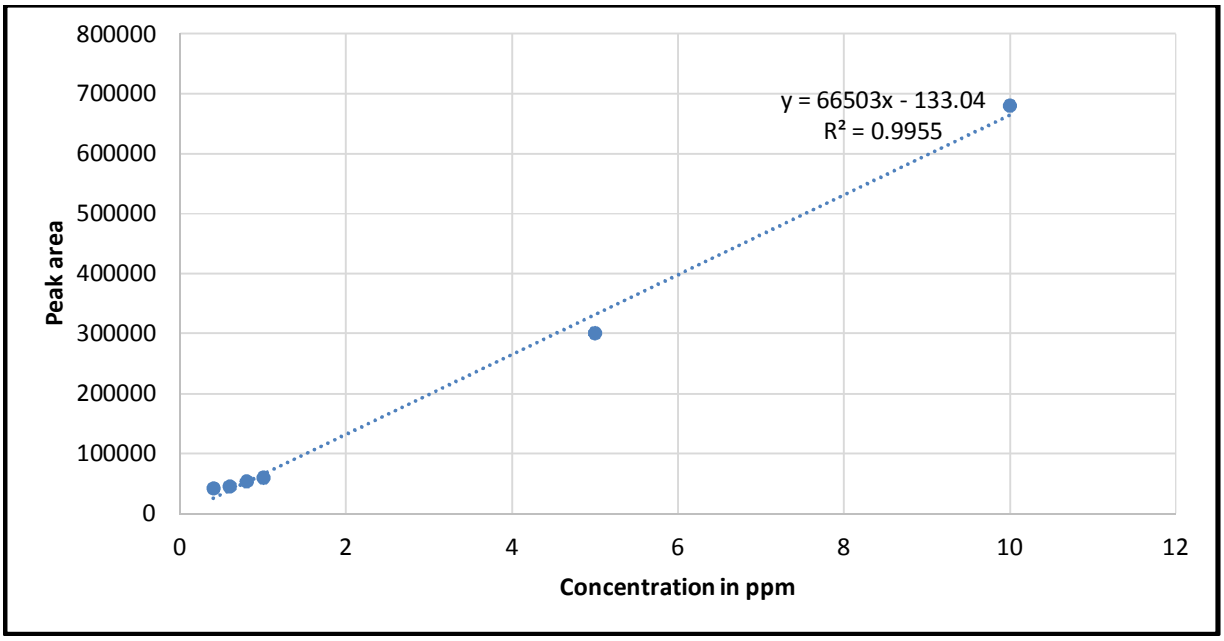


Figure A1.1 Calibration curve for Sulfamethoxazole

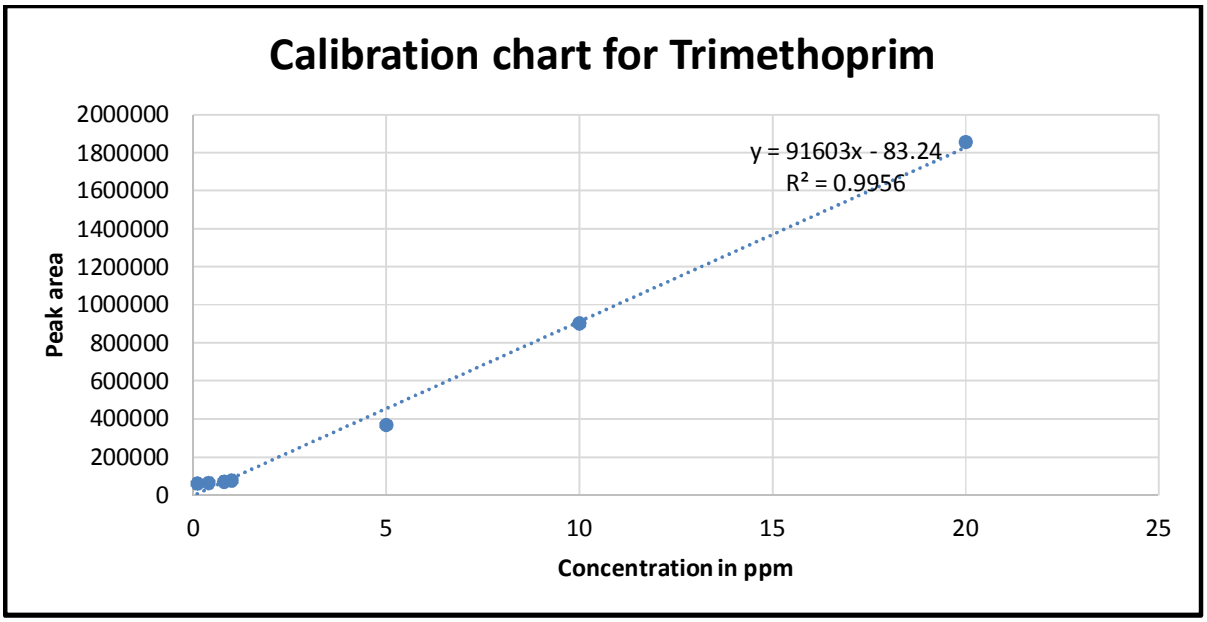


Figure A1.2 Calibration curve for **Trimethoprim**

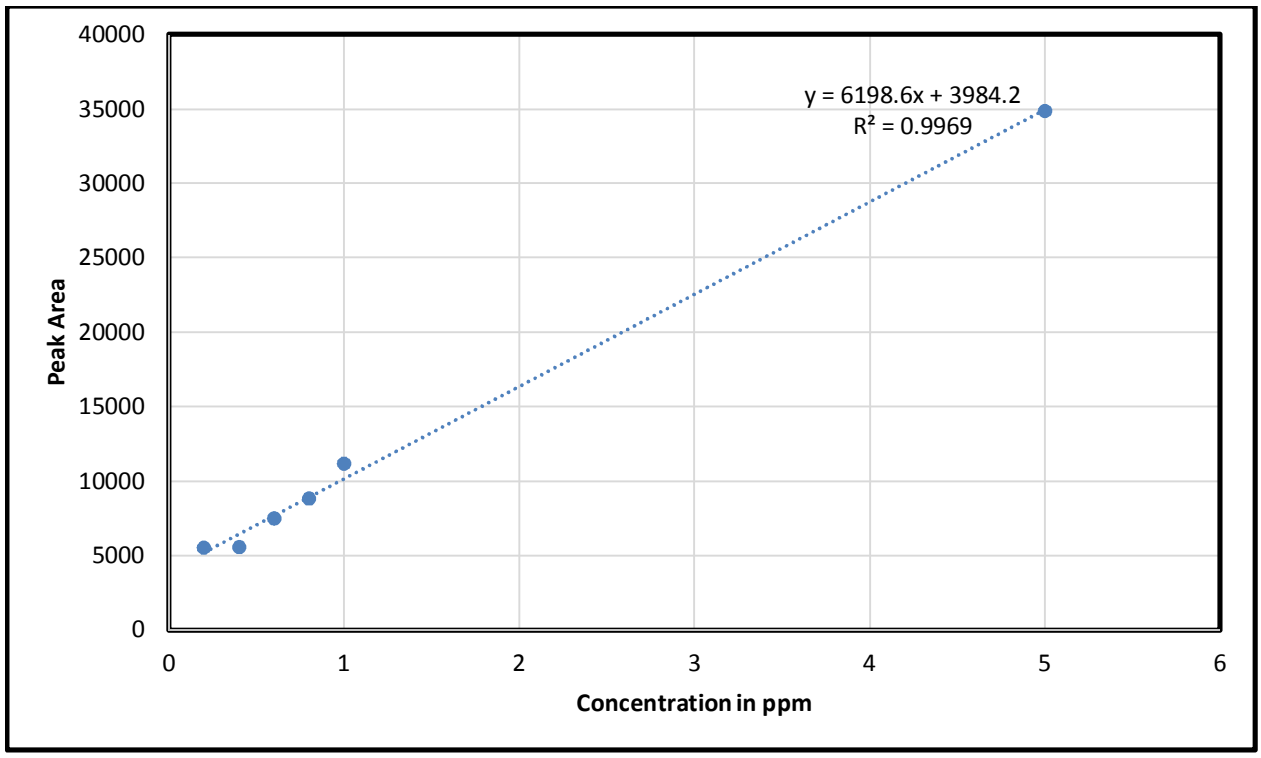


Figure A1.3 Calibration curve for Amoxicillin

APPENDIX 2: FREUNDLICH AND LANGMUIR ADSORPTION ISOTHERMS

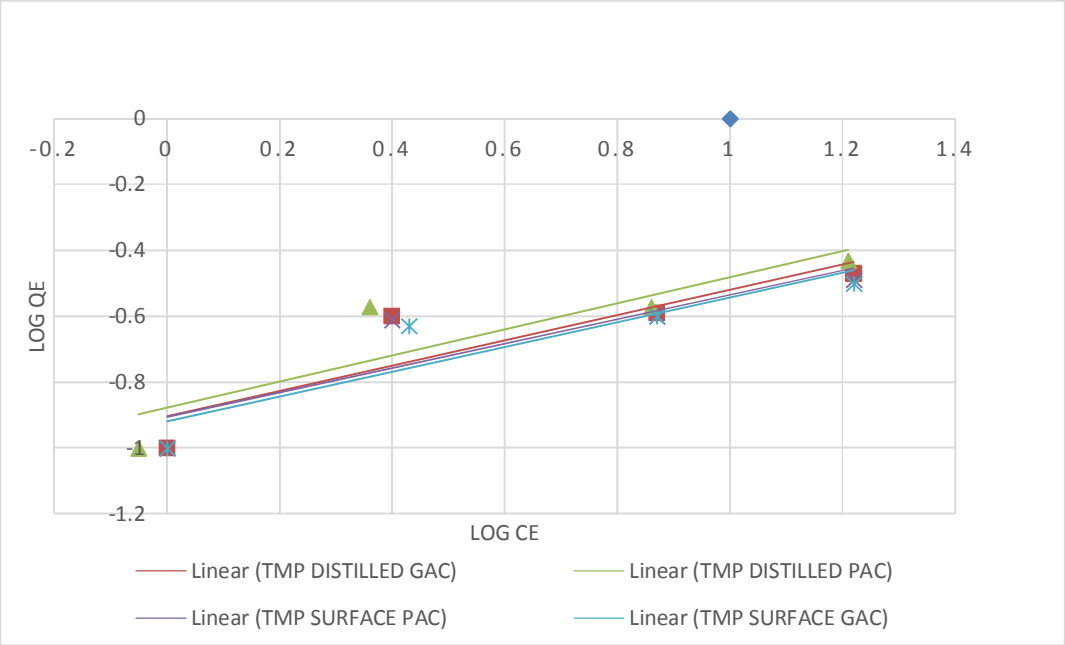


Figure A2.1 Freundlich Isotherm for TMP on GAC and PAC in surface and distilled water

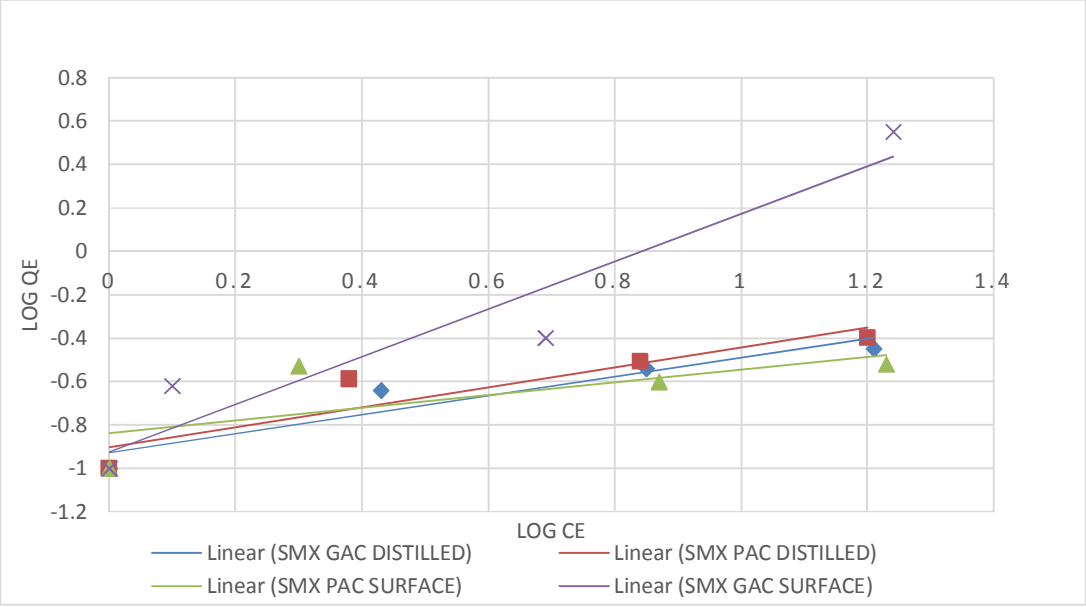


Figure A2.2 Freundlich Isotherm for SMX on GAC and PAC in surface and distilled water

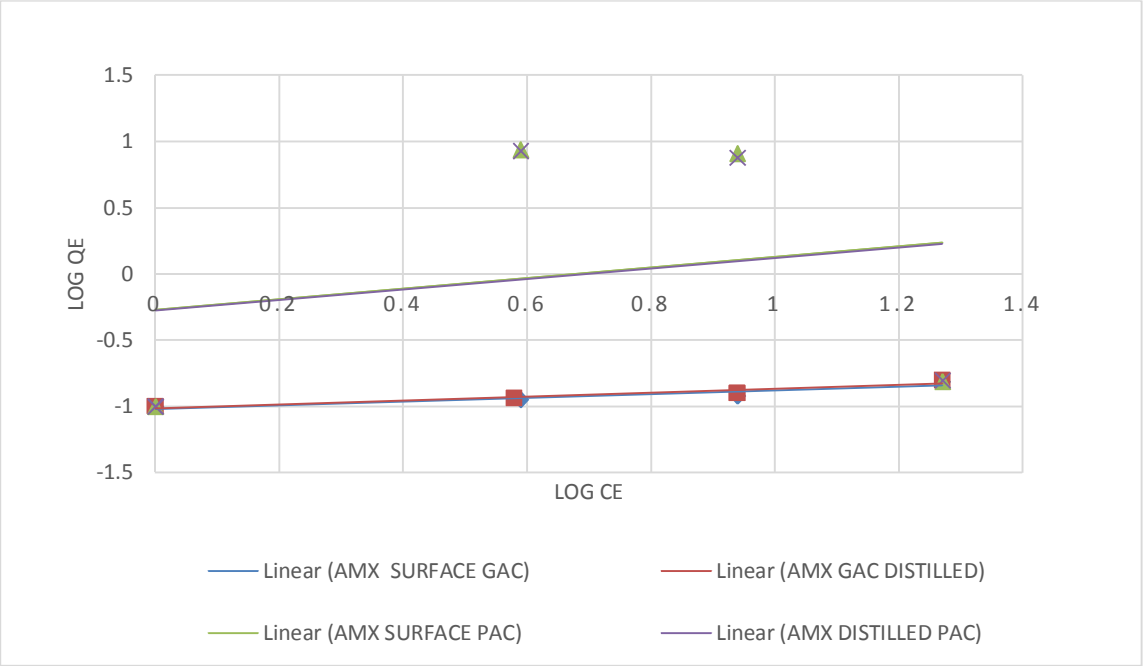


Figure A2.3 Freundlich Isotherm for AMX on GAC and PAC in surface and distilled water

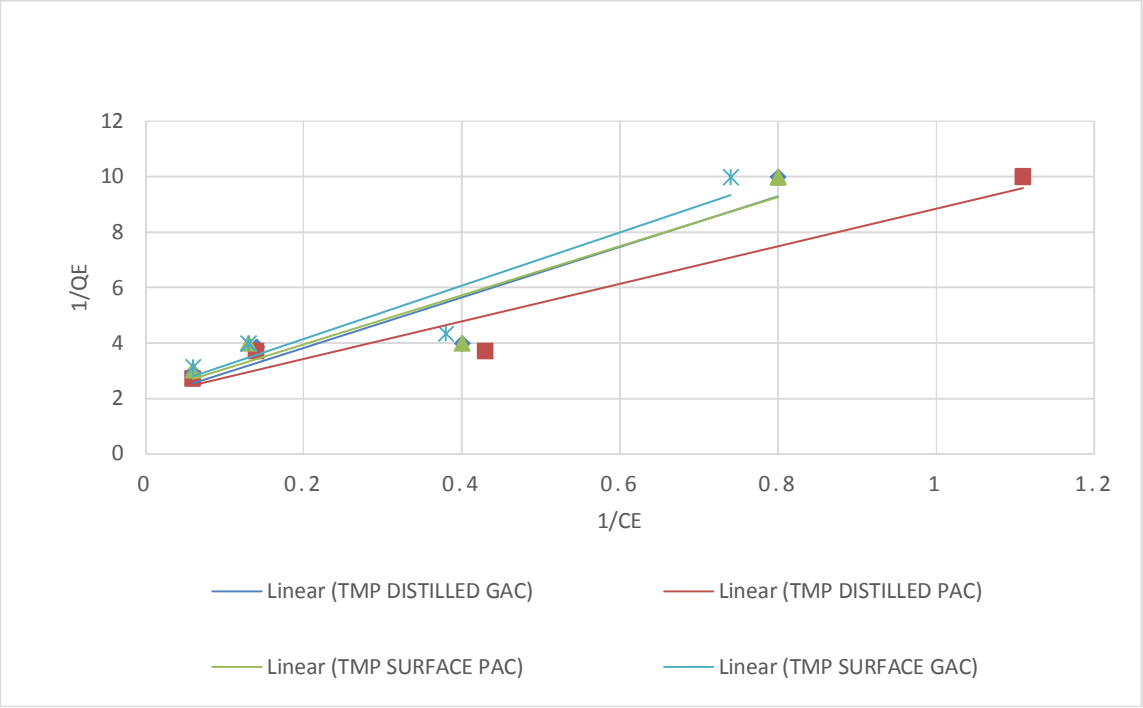


Figure A2.4 Langmuir Isotherm for TMP on GAC and PAC in surface and distilled water

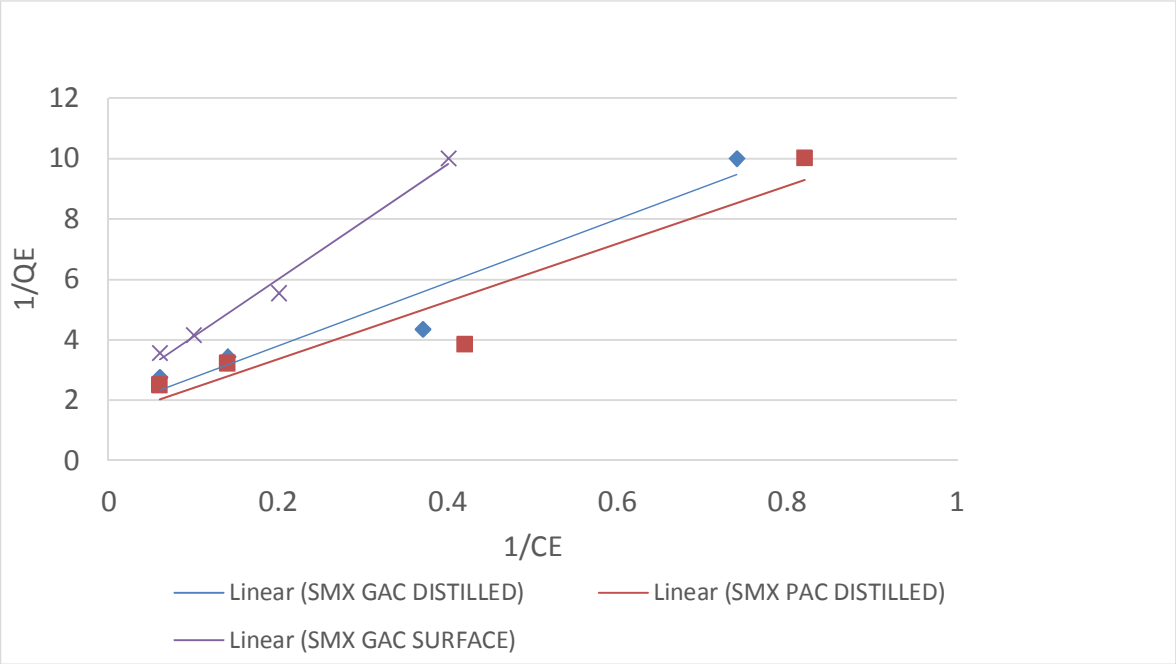


Figure A2.5 Langmuir Isotherm for SMX on GAC and PAC in surface and distilled water

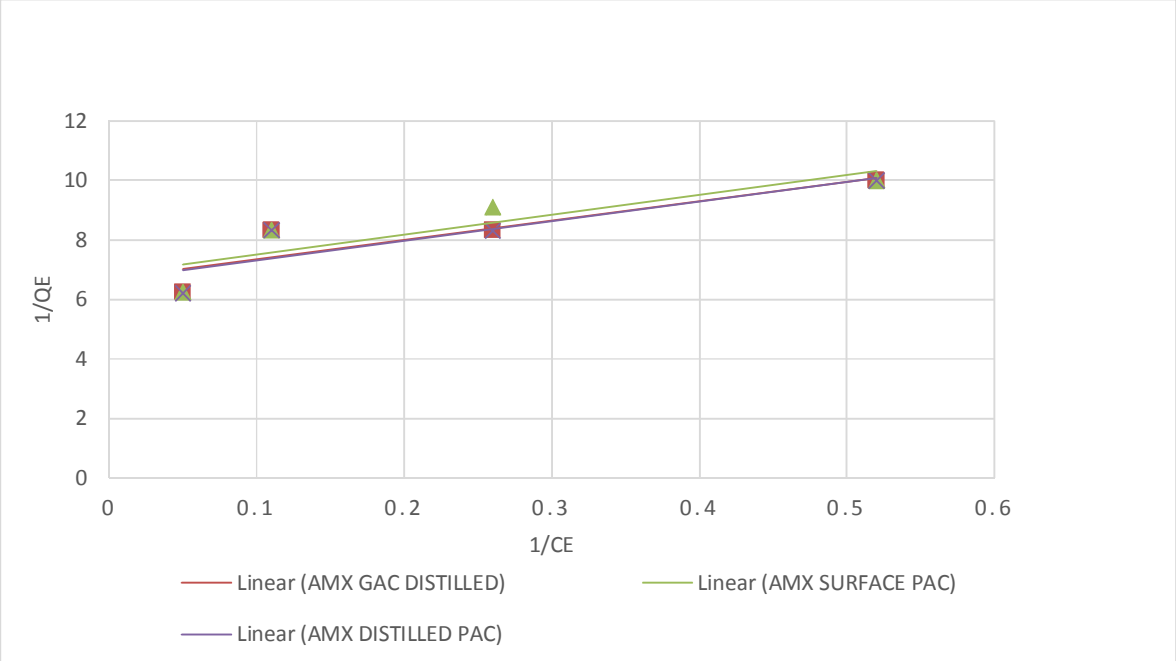


Figure A2.6 Langmuir Isotherm for AMX on GAC and PAC in surface and distilled water

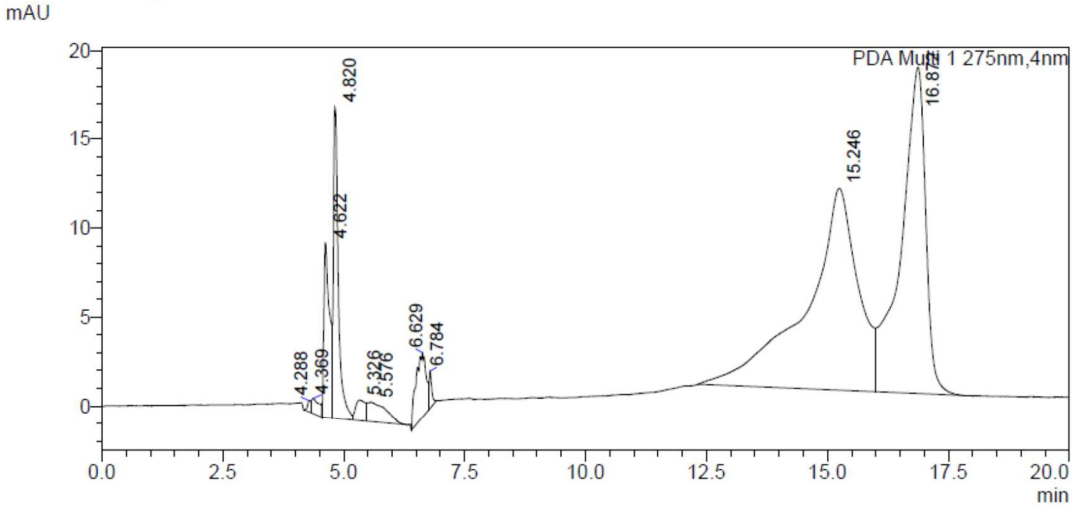
ANNEX 3: CHROMATOGRAMS

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: 14		
Sample ID	: water analysis013		
Data Filename	: 014 20052029.lcd		
Method Filename	: smx and tmp analysis.lcm		
Batch Filename	: smx and tmx analysis 20052016.lcb		
Vial #	: 1-14	Sample Type	: Unknown
Injection Volume	: 20 uL	Level	: 1
Date Acquired	: 20-May-16 4:36:34 PM	Acquired by	: System Administrator
Date Processed	: 20-May-16 4:56:35 PM	Processed by	: System Administrator

<Chromatogram>



<Peak Table>

PDA Ch1 275nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	4.288	2951	651	0.000			
2	4.369	9991	868	0.000		V	
3	4.622	79368	9817	0.000			
4	4.820	124166	17497	0.000		V	
5	5.326	17084	1179	0.000		V	
6	5.576	30426	1114	0.000		V	
7	6.629	54318	3622	0.000			
8	6.784	7488	2046	0.000		V	
9	15.246	842845	11338	0.000			
10	16.872	649579	18352	0.000		V	
Total		1818216	66483				

Sulphamethoxazole(16.87) and Trimethoprim(15.24)

Figure A3.1 Chromatogram of standard SMX and TMP

SHIMADZU LabSolutions **Analysis Report**

<Sample Information>

Sample Name	: 14		
Sample ID	: water analysis013		
Data Filename	: 014 20052029.lcd		
Method Filename	: smx and tmp analysis.lcm		
Batch Filename	: smx and tmx analysis 20052016.lcb		
Vial #	: 1-14	Sample Type	: Unknown
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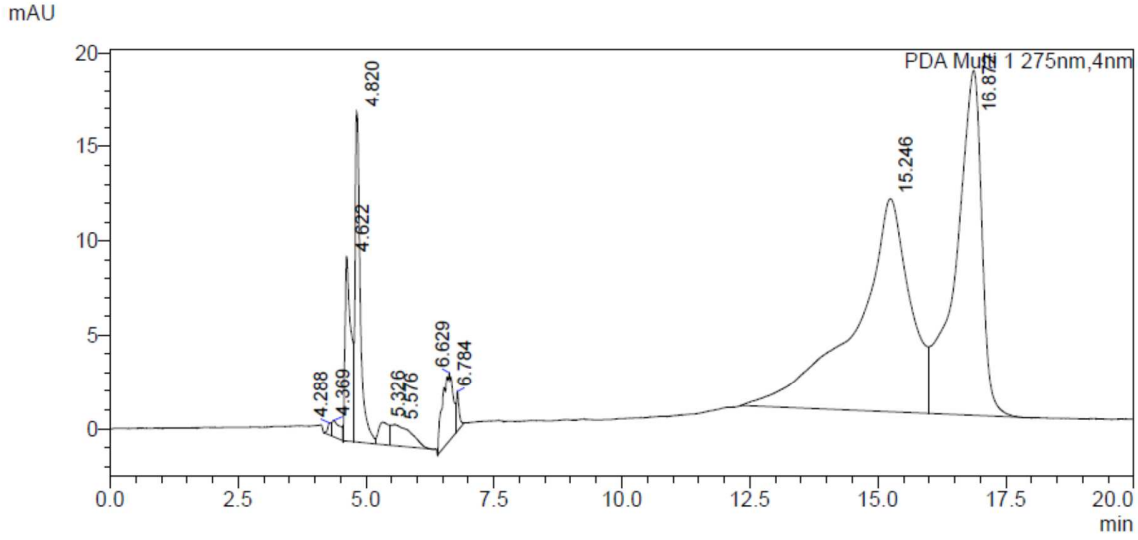
<Chromatogram>



<Peak Table>

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Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
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5	5.326	17084	1179	0.000		V	
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7	6.629	54318	3622	0.000			
8	6.784	7488	2046	0.000		V	
9	15.246	842845	11338	0.000			
10	16.872	649579	18352	0.000		V	
Total		1818216	66483				

Figure A3.2 Chromatogram of a water sample