PERFORMANCE EVALUATION OF WASTE STABILIZATION PONDS IN THE REMOVAL OF PHARMACEUTICALLY ACTIVE COMPOUNDS: A CASE OF LUBIGI WASTEWATER TREATMENT PLANT

BY

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DECLARATION

I, Asiimwe Brendah Patience, hereby declare that this dissertation is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree of the university or other institute of higher learning, except where due acknowledgement has been made in the text and reference list.

Signature: …………………………………… Date: ………………………………

APPROVAL

The undersigned approve that they have read and hereby recommend for submission to the Directorate of Research and Graduate Training, Kyambogo University, a dissertation entitled "*Performance Evaluation of Wastewater Stabilization Ponds in Removal of Pharmaceutically Active Compounds (PhACs); A case of Lubigi Wastewater Treatment Plant*", in fulfilment of the requirements for the award of Master of Science in Water and Sanitation Engineering Degree of Kyambogo University.

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LIST OF ACRONYMS

ASP	Activated Sludge Processes
BOD	Biochemical Oxygen Demand
CECs	Contaminants of Emerging Concern
DO	Dissolved Oxygen
DW	Dry weight
EC	Electrical Conductivity
EDC	Endocrine Disruptors
EDs	Endocrine Disruptors
FS	Faecal Sludge
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
IBP	Ibuprofen
KCCA	Kampala Capital City Authority
KCCA	Kampala Capital city Authority
LC	Liquid Chromatography
MBR	Membrane Bio Reactors
MFA	Material Flow Analysis
MoH	Ministry of Health
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NDP	National Development Plan
NEMA	National Environment Management Authority

NSAIDS	Non-Steroid Anti-inflammatory Drugs
PhACs	Pharmaceutically Active Compounds
SDGs	Sustainable Development Goals
SFA	Substance Flow Analysis
SPE	Solid Phase Extraction
TSS	Total Suspended Solids
UDDTs	Urine Diverting Dry Toilets
UDDTs	Urine Diverting Dry Toilets
WHO	World Health Organization
WSPs	Waste Stabilization Ponds
WW	Wastewater
WWTP	Wastewater Treatment Plants

ABSTRACT

Waste stabilization ponds (WSPs) have been extensively used for the treatment of wastewater due to their simplicity and cost-effectiveness. This study evaluated the performance of WSPs in removing Pharmaceutically Active Compounds (PhACs) at the Lubigi sewage and wastewater treatment plant. Three wastewater streams were sampled; the inlet for the domestic wastewater stream, the point of discharge for cesspool empties containing faecal sludge from septic tanks, and the gulper station receiving faecal sludge from pit latrines. Additionally, samples were collected at different treatment stages i.e. the inlet to the anaerobic pond, the outlet from the anaerobic pond, and the discharge point to the facultative pond. Fresh and dried sludge samples were also collected. Sample analysis was performed using Liquid Chromatography Mass spectrometry (LC-MS-MS). PhACs were present in median concentrations of 19.223 μgL^{-1} in wastewater from the sewer network, 13.429 μgL^{-1} in septage and 18.641 μgL^{-1} in faecal sludge from pit latrines respectively, with average concentrations of in the three source streams of up to 5300 μgL^{-1}. WSPs exhibited the ability to remove a variety of PhACs from wastewater at an overall removal efficiency of 76.15% with the highest removal efficiency of 70-99.99% for chlortetracycline, sulfapyridine, ampicillin, gentamicin, albendazole, ibuprofen, sulfachloropyridazine, sulfaquinoxaline, and penicillin, the moderate removal efficiency was 50-70% for like paracetamol, chloramphenicol and enrofloxacin, and the lowest removal efficiency of 1-40% for sulfadiazine, oxytetracycline, diclofenac, and ciprofloxacin. PhACs like sulfamethoxazole sulfamerazine and amoxicillin were more recalcitrant exhibiting negative removal efficiencies. The study found that faecal sludge (FS) from pit latrines (3.35 ton/year) and conventional sewer network systems (1.63 ton/year), contribute to substantial volumes of wastewater entering the Lubigi sewage treatment plant and therefore consequently reduce the treatment efficiency of WSPs in removing these PhACs as compared to septic tanks (0.74 ton/year). This study recommends the substitution of pit latrines with source-separated technologies, the addition of maturation ponds and other tertiary treatment mechanisms and the use of advanced treatment technologies to effectively remove PhACs, routine monitoring of PhACs, proper disposal and management of pharmaceutical waste, and further research on the behaviour and fate of PhACs particularly regarding their transformation and potential impacts when discharged into wetland environments.

Keywords: PhACs, Waste Stabilization Ponds, Lubigi, Wastewater, Faecal sludge

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Over the years, changes in lifestyles and rapid technological progress have led to numerous new products and materials categorized as Contaminants of Emerging Concern (CECs) being released into the ecosystem (Kumar and Kumar, 2020). One noted group is pharmaceutical residue-based CECs (Luo et al., 2014), whose existence could be attributed to a high incidence and burden of diseases, resulting from consumption of untreated water, poor human excreta disposal and faecal sludge management, and non-adherence to occupational health, and other safety and environmental measures (Carr, 2001). To reduce the burden of diseases and improve the health of individuals, developments in research and innovation have been realized which has led to more investment in the healthcare industry as well as large growth in the pharmaceutical field (Aus der Beek et al., 2016).

Currently, several pharmaceutically active compounds (PhACs) that include impotence drugs, painkillers, antibiotics, beta-blockers, antidepressants, x-ray contrast media, contraceptives and lipid regulators (Diamond et al., 2015; Rasheed et al., 2019) are available and easily accessible to people. In addition, unregulated agricultural outputs released to fields (Patel et al., 2019) and an increase in antibiotics administered to livestock animals in feed (OECD, 2019) are also sources of these PhACs.

However, it is crucial to note that pharmaceutical products are very stable and thus difficult to biodegrade and be completely assimilated into the body. After consumption unchanged forms of metabolites and conjugates of PhACs are excreted in urine and feces (Lindberg et al., 2014). These find their way into the toilets as wastewater or pit latrines forming faecal sludge that is later transported to the treatment plant and if not captured, the PhACs are released through the effluent into the environment. Pharmaceuticals have been linked to several negative effects on health and the aquatic environments, including their toxicity, endocrine disruption, the development of antibacterial resistance, and the inhibition of cell proliferation in aquatic organisms (Nantaba et al., 2020). Various studies worldwide have noted concentrations of PhACs in human excreta, wastewater, faecal sludge and various water bodies (K'oreje et al., 2016; Ilechukwu et al., 2021).

In Uganda, (Nantaba et al., 2020) examined the presence, distribution, and ecotoxicological threat of specific drug compounds in water sourced from Lake Victoria. Nantaba concluded that eighteen of the 24 pharmaceuticals occurred in quantifiable concentrations. Mafabi (2021) found paracetamol at concentrations of 0.038 ng/ml, 0.017 ng/ml, 0.046 ng/ml, and 0.031 ng/ml in communal stand taps, grey water, drainage channels, and protected springs, respectively. Ibuprofen was detected in drainage channels (0.0083 ng/ml), grey water (0.036 ng/ml), protected springs (0.083 ng/ml), unprotected springs (0.048 ng/ml), and solid waste dump sites (0.285 ng/ml) within the urban slum environment of Bwaise, Kampala, Uganda. Research has also found that the toxic risk of some of these substances is medium (ciprofloxacin, norfloxacin and ibuprofen) to high for sulfamethoxazole, oxytetracycline, erythromycin and diclofenac (Nantaba et al., 2020). Furthermore, the presence of these PhACs in the environment may also affect groundwater, particularly in countries like Uganda, where pit latrines are widely used. The existence of these PhACs in groundwater could be linked to their occurrence in human excreta and other onsite sanitation facilities. A study by Twinomucunguzi et al., (2021) examined the prevalence and seasonal fluctuations in the concentrations of developing organic pollutants in the shallow groundwater that underlies two peri-urban regions in Uganda, Bwaise and Wobulenzi. The study looked at 26 antibiotics and found that benzylpenicillin and ampicillin were highly detected though at minimum amounts that could not pose a threat to human life but whose presence could encourage the spread of genes that are resistant to antibiotics. The study concluded that long-term exposure to antibiotic and pesticide residues in shallow groundwater could have negative ecological effects.

This highlights the need to address the occurrence of these PhACs to prevent their potential side effects. One of these methods could be through the proper treatment of wastewater and faecal sludge.

1.2 Statement of the problem

Waste Stabilization Ponds (WSPs) are widely used in developing countries due to their simplicity, cost effectiveness in treating wastewater and faecal sludge (Kayombo, 2005; Gruchlik, Linge and Joll, 2018). While these systems efficiently remove microbial contaminants, organic matter, and nutrients (Tilley et al., 2008), with average removal efficiencies greater than 50% reported by (Rahmatiyar et al., 2014; Tornyi, 2017), they are not designed to eliminate pharmaceutical contaminants (Patel et al., 2019). This limitation results in the persistence of PhACs in the effluent, posing significant environmental and public health risks (Kanama et al., 2018).

Limited studies have evaluated the performance of WSPs in removing PhACs (Gruchlik, Linge and Joll, 2018; K'oreje et al., 2018; Kumar and Kumar, 2020; Edokpayi et al., 2021) with research mainly focused on WSPs that receive wastewater from conventional sewer systems. In Uganda, only (Dalahmeh et al., 2020) have investigated the removal of pharmaceutical contaminants in wastewater treatment plants, particularly in Bugolobi, Kampala. The study revealed poor removal of PhACs in the WWTP with PhACs in effluent (13,000-37,000 ngL-1) being greater than those found in the equivalent influent (4,000-28,000 ng-1).

The presence of these contaminants in effluents accounts for their potential entry into drinking water sources and agricultural systems, which poses a threat to human health and ecosystems (Kanama et al., 2018). Despite this, there is limited knowledge on the performance of WSPs in Uganda, especially for treatment plants like Lubigi, which handle both wastewater and faecal sludge from onsite sanitation systems.

This study aims to evaluate the performance of Lubigi WSPs in removing PhACs and to understand how different onsite sanitation systems influence the levels of these contaminants reaching the treatment plant as well as the removal efficiency. The findings help to guide the necessary improvements in treatment processes and the use of onsite sanitation technologies as well as inform relevant policy formulations to enhance wastewater management and protect environmental and human health.

1.3 Objectives of the study

1.3.1 Main objective

The main objective of the study is to evaluate the performance of Waste Stabilization Ponds in the removal of Pharmaceutically Active Compounds (PhACs). Lubigi WSP is considered in this study because it represents a typical scenario in middle- and low-income countries where treatment plants receive both wastewater and faecal sludge.

1.3.2 Specific objectives

1. To assess the variation of PhACs in wastewater and faecal sludge received at Lubigi WSPs.
2. To evaluate the efficiency of the Lubigi WSPs in removing PhACs.
3. To assess how onsite sanitation technologies could influence the removal of PhACs in the Lubigi WSPs.

1.4 Research Questions

1. What are the concentrations of PhACs in the wastewater and faecal sludge received at Lubigi WSPs?
2. What is the treatment efficiency of the WSPs in removing PhACs?
3. How do onsite sanitation technologies influence the removal of PhACs in the Lubigi WSPs?

1.5 Research Rationale

The discharge of raw and inadequately treated wastewater is one of the most significant point sources of surface water contamination (Edokpayi et al., 2021). The presence of

PhACs in receiving water bodies where effluent and sludge from WWTPs are discharged has continuously become a cause of the poor quality of drinking water and in turn affected public health in many developing countries (Maycock and Watts, 2011). Therefore, there is a need to quantify these PhACs and also understand their removal from the wastewater and faecal sludge in the influent from different human excreta disposal facilities. Understanding the variation in PhACs and their treatment efficiency in WSPs could guide the proper use and management of different human excreta disposal technologies. In addition, it will improve the effluent quality and therefore contribute to an overall improvement of water quality of receiving bodies where these effluents are discharged. This could contribute to improved public health and sanitation, protection of the ecosystems and improved agriculture through effluent re-use for irrigation and resource recovery and hence promotion of a circular economy.

1.6 Significance of the study

- This research will provide knowledge on the quantities and removal efficiencies of these PhACs by WSPs from different waste streams received at the WWTP. This will guide decision makers such as Kampala Capital City Authority (KCCA) on how to improve the treatment efficiencies of the WSPs at Lubigi WWTP. This will further assist the management of the human excreta disposal facilities to facilitate the removal of these PhACs based on the results of the study.
- This study will further help in improving the quality of receiving water bodies by improving effluent quality hence improving the drinking water quality and overall, the health of the people in Kampala. This will contribute towards attaining

Uganda's National Development Plan (NDP) III and Vision 2040 on water and sanitation as well as contributing to the Sustainable Development Goals (SDGs) No.3 and 6 which are; To ensure healthy lives and promote well-being for all at all ages and Ensure availability and sustainable management of water and sanitation for all respectively.

- This research will also serve as a partial requirement for the attainment of a Master's degree in Water and Sanitation engineering.
- Given the limited volume of research on Contaminants of Emerging Concern (CECs) in Uganda and even less on their removal from wastewater, this research will aid in setting the groundwork for any other possible future research on CECs.

1.7 Scope of the study

1.7.1 Content scope

This study focused on evaluating the performance of Waste Stabilization ponds in the removal of these PhACs. The study was restricted to only selected pharmaceutical contaminants. Waste streams considered were FS from septic tanks, FS from pit latrines, and wastewater from the sewer network, all delivered at the Lubigi WWTP, and flowing in, through and out of the WSP. In addition, human excreta disposal facilities used in typical low- and middle-income countries were considered during the systems analysis for the performance of WSPs.

1.7.2 Geographical scope

The scope of this study was limited to the Lubigi faecal sludge and wastewater treatment plant (GPS; N0.33998˚; E32.56032˚) which is located on Lubigi wetland in Kawempe division, Kampala district along the northern bypass Hoima Road approximately 5 km from Kampala city square in Uganda.

1.7.3 Time scope

The study was done for one year which is the period meant for Master's research.

1.8 Conceptual framework

The conceptual framework of this study is presented in Figure 1-1.

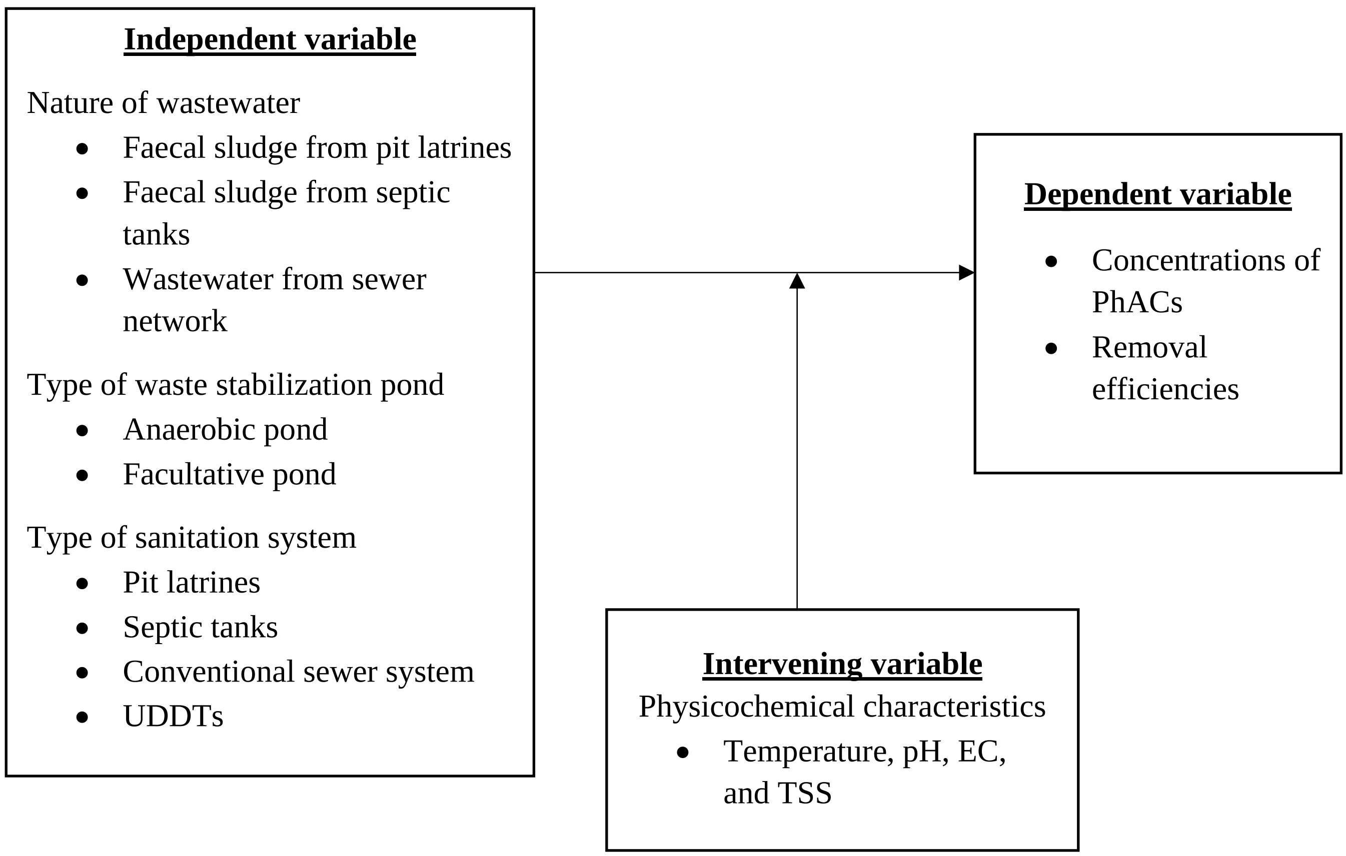


Figure 1-1: Conceptual framework

1.9 Chapter summary

This chapter consists of the study background, statement of the problem, research objectives, justification, significance, scope of the study and the conceptual framework.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter presents a review of existing literature related to this study.

2.2 Contaminants of Emerging Concern

Contaminants of Emerging Concern in the aquatic environment have become a worldwide issue of rising environmental concern over the last few years. Emerging contaminants, are a group of a wide range of human and natural compounds which are continuously becoming more prevalent and yet rarely regulated over the globe (K'oreje et al., 2020). CECs mainly include pharmaceuticals, personal care items, industrial chemicals, steroid hormones, insecticides, and other new substances. These microcontaminants are mostly found in minute concentrations in water, from parts per trillion (ng/l) to parts per billion (μgL-1). The limited quantities and variety of these microcontaminants make detection and analysis difficult and present difficulties in various processes of water and wastewater treatment (Luo et al., 2014). Many of these substances are present in discharges of wastewater treatment work since primary and secondary wastewater treatment were not expressly designed to eliminate them (Maruya, KeithVidal-Dorsch et al., 2012).

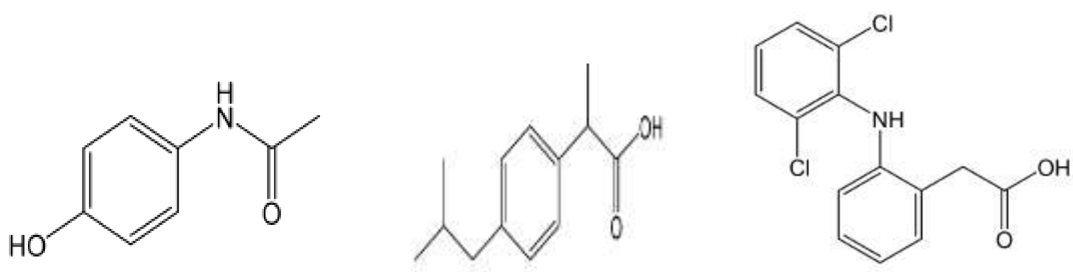
Despite being present in minimal concentrations, the adverse effects of these organic contaminants on humans, aquatic life and animals are emerging as a notable concern (Pal et al., 2010). The presence of these pollutants in the aquatic environment is linked to several deleterious consequences, encompassing disruption of endocrine functions, toxicity both in the short and long term and increased microbial antibiotic resistance (Luo et al., 2014; Saari, Scott and Brooks, 2017).

2.3 Pharmaceutically Active Contaminants (PhACs)

Ebele, Abou-Elwafa Abdallah and Harrad (2017) define pharmaceuticals as medicinal substances that can be obtained either through prescription or over-the-counter, as well as veterinary drugs used in the prevention and treatment of illnesses in both humans and animals. They are molecules that are meant to cure or provide therapy to the body as well as treat various illnesses or infections (Mahmood Aljamali, 2018). They include Antibiotics, painkillers, estrogens, birth control pills anti-inflammatories, steroids antiseptics, antidiabetics antihypertensive medications, tranquillizers, blood lipid regulators antineoplastic agents, impotence drugs, antiepileptics, beta-blockers, and other medicines (Nawaz and Sengupta, 2019).

Pharmaceutically active compounds (PhACs) can prevent, diagnose, and cure infections and disorders in people and animals, as well as correct or modify organic activities. Typically, these substances exhibit their activity even at extremely low concentrations, possess the ability to penetrate biological membranes, and have a prolonged duration of action, allowing them to remain effective without being deactivated prematurely (Bottoni, Caroli and Caracciolo, 2010). Figure 2-1 shows different molecular structures of commonly used PhACs.

Paracetamol Ibuprofen Diclofenac



Ampicillin Amoxicillin Ciprofloxacin

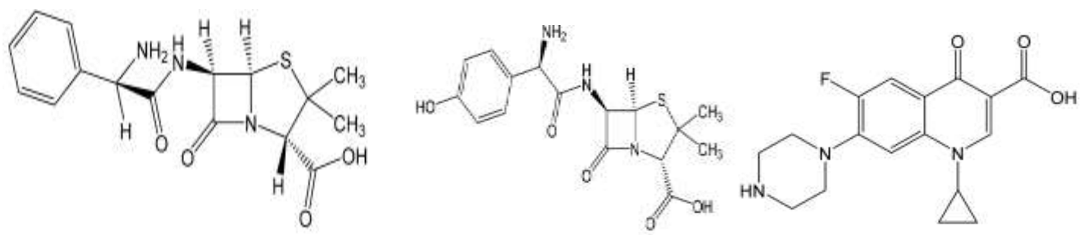


Figure 2-1: Molecular structures of common PhACs (Source: (Patel et al., 2019)

2.3.1 PhACs consumption rates

The consumption of pharmaceuticals is on a continual rise as it plays a very vital role in enhancing our quality of life. This is attributed to the increasing need for treating common, age-related, and chronic illnesses, along with advancements in clinical procedures. Generally, the consumption pattern of specific medications in the environment is one of the most crucial determinants of the occurrence trends of individual pharmaceutical elements (Boxall et al., 2012). The consumption trend in the area is influenced by the sickness pattern. For instance, low-income nations have more infections (World Health Organization, 2012) and more over-the-counter self-administration in general (Ayukekbong, Ntemgwa and Atabe, 2017). In developing nations, individuals consume an average of 15 grams of prescription drugs per person annually, while in developed nations, this figure ranges from 50 to 150 grams per person annually with an estimate of over 3000 distinct substances used as pharmaceuticals (Nawaz and Sengupta, 2019). This increase in medicine-induced demand has consequently increased the market of pharmaceuticals all over the world (González Peña, López Zavala and Cabral Ruelas, 2021) The worldwide pharmaceutical market is projected to increase from $1587.05 billion in 2022 to $2135.18 billion by 2026 (Globe Newswire, 2022), and is anticipated to rise in the upcoming years.

However, this optimism of the pharmaceutical business has been curtailed by the release of medicines and their derivatives into the environment as a result of poor management, treatment, and disposal, which is now a global threat to both the environment and human health (González Peña, López Zavala and Cabral Ruelas, 2021).

2.3.2 Origins of PhACs and their pathways into the environment

Pharmaceutical accumulation in the environment originates from human use of drugs that result in the release of both metabolized and unmetabolized drugs and the disposal of unused medication.

Pharmaceuticals have several sources from which these later become environmental contaminants. Pharmaceutical pollution in the environment mainly originates from unregulated domestic effluents, urban, hospital, and industrial wastewater effluents, discharges from wastewater treatment plants, the release of agricultural outputs to fields from intensive livestock farming sites where the use of veterinary medications is common and effluents from heavy aquaculture systems (Reddersen, Heberer and Dünnbier, 2002; Patel et al., 2019).

In addition, there are also indirect sources of contamination, such as the utilization of recycled wastewater from treatment plants for irrigation purposes, the improper disposal of expired medications, the release of manufacturing waste from pharmaceutical industries, the application of wastewater treatment plant sludge in agriculture to restore inorganic nutrients, the discharge of hospital wastewater, and the discharge of sewage from livestock farming sites onto farmland (Bottoni, Caroli and Caracciolo, 2010).

Pharmaceuticals and their derivatives are continuously discharged into the environment in various pathways. When pharmaceuticals are consumed after oral ingestion, some of them undergo metabolic transformations, which can occur through the action of gut microbes or enzymes in the host, while others may remain unchanged. Therefore, some of these pharmaceuticals are metabolized whereas others are excreted unaltered via human excreta into domestic water effluents (Patel et al., 2019). These medications, as well as their metabolites, enter the wastewater system and are processed by municipal wastewater treatment plants. However, typical wastewater treatment facilities do not easily eliminate these contaminants, which contributes to their continuous occurrence in the environment (Biel-Maeso et al., 2018).

PhACs from pharmaceutical industries, those in solid waste dumps and agricultural fields are washed off as surface runoff and land in wastewater treatment plants. Additionally, unused and expired drugs frequently end up in the environment after being disposed of with residential waste or flushed down sinks, toilets or drains. Pharmaceutical compounds dumped in with residential waste end up in landfills and turn into groundwater leachates especially if these landfill sites are not sealed off correctly.

Veterinarian antibiotics and hormones that are sprayed on fields or disposed of in lagoons for collecting animal waste eventually build up and seep into groundwater and surface water. Poor disposal of leftover livestock feed or veterinary medications and animal care are additional ways that these pollutants end up in the environment. Because animals excrete drugs and their metabolites, animal husbandry is the third-largest source of emissions (Ebele, Abou-Elwafa Abdallah and Harrad, 2017).

The major sources and pathways have been presented in Figure 2-2 below.

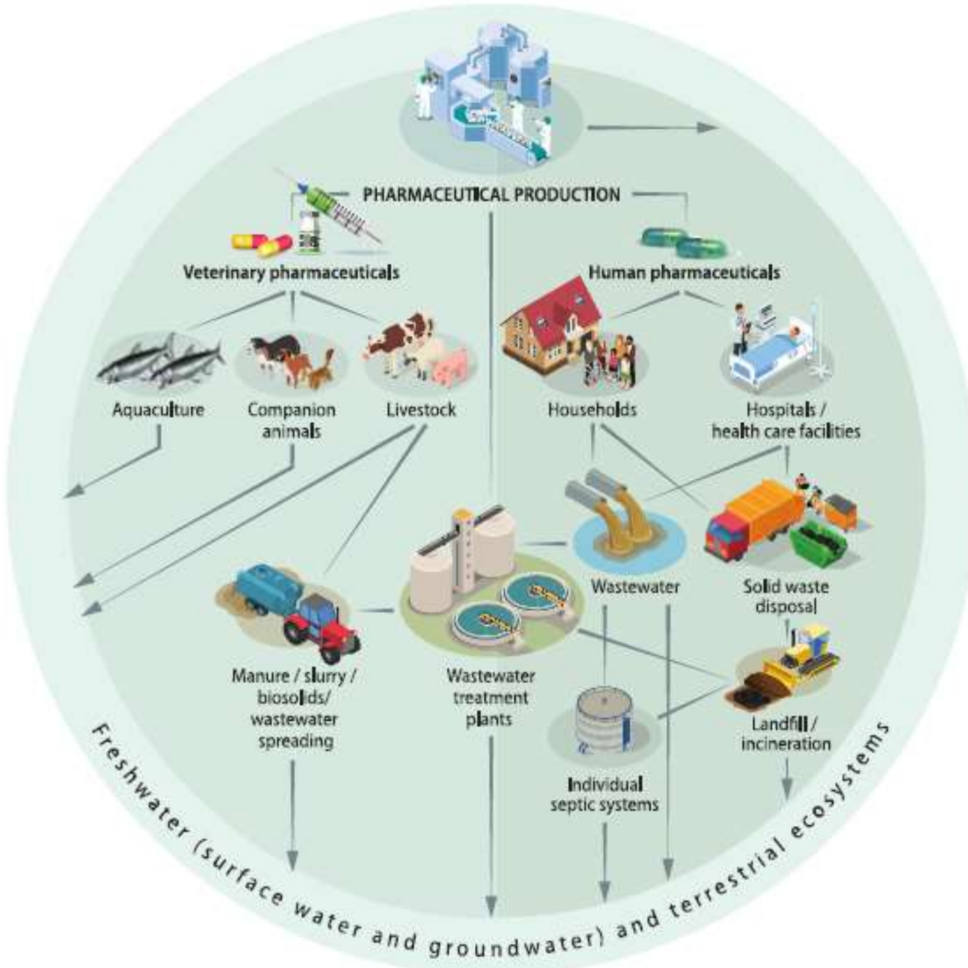


Figure 2-2: Major sources and pathways of PhACs to the environment
Source:(OECD, 2019)

2.3.3 Effects of PhACs on the environment

A significant impact of pharmaceutical elements on the environment is persistence. The existence of pharmaceutical components in drinking water underscores the challenge for conventional water treatment systems to effectively remove them, given their intricate physicochemical characteristics (Snyder, 2008). Therefore, most PhACs are considered 'pseudo-persistent' i.e. possess a high degree of environmental persistence. According to

(Houtman et al., 2004), this persistence is due to the fact that they are constantly replenished even when they have been biodegraded, photodegraded or sorbed and are continually released into the ecosystem. According to (Löffler et al., 2005), clofibric acid, and carbamazepine diazepam, have a high persistence (DT50 ¼ 119-328 days) paracetamol, 2- hyroxyibuprofen, ibuprofen and CBZ-diol show a low dissipation time (DT50 ¼ 3.1-7 days) hence are low persistent, while ivermectin, oxazepam and Iopromide have a moderate persistent level with (DT50 ¼ 15-54 days). The failure to completely remove these PhACs from wastewater treatment facilities is a threat to aquatic life and public health.

Another effect of PhACs is their ability to create strains of antibiotic resistance in naturally occurring microbial communities. The widespread antibiotic usage in treating both human diseases and animal infections has increased antibiotic-resistant bacteria which makes the treatment of diseases that are caused by pathogens difficult. (WHO, 2015). Antibiotics such as penicillin and sulfonamides have been found to cause resistance in bacterial pathogens, resulting in changes in microbial community structure while affecting their food chain (Bolong et al., 2009).

PhACs also result in long-term toxicity through endocrine disruption. The concern arising from the occurrence of PhACs in aquatic ecosystems stems from their potential to disrupt the endocrine system, leading to adverse effects or homeostasis disruption. According to the World Health Organization (WHO), Endocrine disruptors (EDs) are "exogenous substances that modify the function of the endocrine system and subsequently cause adverse health effects in an organism, or its subgroup." In a variety of consumer PhACs, there are several substances known as EDs, some of which are synthetic (like bisphenol) and others are natural in origin (like mycotoxins) (Wielogórska et al., 2015). Veterinary growth hormones, sex hormones, non-steroidal medicinal chemicals and glucocorticoids are examples of medications that disturb hormones. Furthermore, synergistic interactions may result from the harmful effects caused by complex PhACs combinations at low levels of concentration. Accordingly, PhACs combinations can have a high ecotoxicity even when individual PhACs may occur in minimal amounts that may not be majorly harmful on their own as stated by (Cleuvers, 2003).

In general, PhACs pose a threat to aquatic life that extends beyond the evident effects observed when therapeutic dosages are attained or surpassed. Recent research indicates that the impact of PhACs varies based on factors such as the developmental stage of exposure, the specific organism affected, the duration of exposure, the concentration of the pollutant, and the toxicity of the PhACs. Moreover, persistent, low-level exposure is more likely to contribute to observed anomalies in non-target organisms exposed, compared to acute, high-dosage exposure, especially during specific developmental phases (Ebele, Abou-Elwafa Abdallah and Harrad, 2017).

The occurrence of pharmaceuticals in freshwater and terrestrial ecosystems can also lead to intake by wildlife, which causes the potential for bioaccumulation. PHACs are found in freshwater environments at very low concentrations, but many of them, alongside their derivatives, are physiologically active and affect aquatic organisms that are not their intended targets (Ebele, Abou-Elwafa Abdallah and Harrad, 2017). According to (Bolong et al., 2009), PhACs found in contraceptive tablets, such as steroidal estrogens like Estrone and 17-estradiol and synthetic contraception like17-ethynylestradiol, promote feminization in fish in sewage treatment i.e. the discharge has the effect of simulating the estrogen/hormone impact on non-targeted cells. A study done by (Mimeault et al., 2005) examined the effect of PhACs on the exposure of goldfish to gemfibrozil at a concentration relevant to the environment with 113 as the plasma bioconcentration factor after 14 days. For instance, antidepressants have caused changes in fish behaviour, reducing their aversion to risk and making them more susceptible to predators. Fish and amphibians have become more feminine as a result of oral contraceptives. Furthermore, the issue of antimicrobial resistance has been exacerbated by the improper use and discharge of antibiotics into aquatic environments. (OECD, 2019).

Agricultural and veterinary practices, wastewater treatment plant technology, operation, and removal efficiency, the source and timing of pollution, the mobility, persistence, and poisoning potential of pharmaceuticals, and the sensitivity of the receiving environment, all influence pharmaceutical concentrations and their effects on the environment. Whereas certain medications have been shown to have minimal consequences on ecosystems, including mortality and physiology, behaviour, and reproductive alterations most of them have shown acute toxicological effects. While some drugs have minimal impacts on ecosystems, such as reproductive changes, behaviour, mortality and physiology, most of them have shown acute toxicological effects.

2.3.4 Concentrations of PhACs in wastewater

Pharmaceutical contaminants have been known to be present in wastewater in varying concentrations as confirmed by some studies as shown in Table 2-1.

A study by (Botero-Coy et al., 2018) in Colombia investigated the presence of 20 pharmaceuticals in wastewater. The findings indicated that all wastewater samples contained a majority of the targeted anti-depressants antibiotics, lipid regulators, analgesics, anti-inflammatories, statins that lower cholesterol, and anti-depressants. Acetaminophen was found in the greatest amounts, with up to 50 μg/L being recorded. Furthermore, the influent wastewater contained antihypertensive medications such as losartan and valsartan, as well as some antibiotics like norfloxacin, ciprofloxacin and azithromycin at levels exceeding μg/L.

A study conducted by (Sim et al., 2011) investigated twenty-four pharmaceuticals in wastewater sourced from several kinds of wastewater treatment plants (WWTPs), including wastewater treatment plants for pharmaceutical manufacture (4No.), livestock (4No.), municipal (12No.), and hospital applications (4No.). The highest concentrations of pharmaceutical pollutants were found in influent samples from livestock wastewater treatment plants, followed by pharmaceutical manufacturing wastewater treatment plants, hospital wastewater treatment plants and municipal wastewater treatment plants. Among these, municipal waste treatment plants showed the highest daily loads (0.404 to 1201 kg per day), followed by pharmaceutical manufacturing waste treatment plants (0.010 to 5.98 kg per day), livestock waste treatment plants (0.090 to 1.10 kg per day), and hospital waste treatment plants (0.002 to 0.042 kg per day). The study also discovered that antibiotics were predominately found in the influents from the livestock waste treatment plants while non-steroidal anti-inflammatory medicines (NSAIDs) were more prevalent in the incoming wastewater from the municipal and pharmaceutical manufacturing waste treatment plants. NSAIDs, coffee, and carbamazepine dominated the hospital plant influents. The effluents of municipal waste treatment plants contained pharmaceutical loads ranging from 0.101 to 23.0 kg per day, which were comparatively lower than the influent levels. Nevertheless, these effluent levels exceeded those of livestock waste treatment plants (0.00001 to 1.88 kg per day) and hospital waste treatment plants (0.0001 to 0.171 kg per day), except for pharmaceutical manufacturing waste treatment plants (0.00001 to 115 kg per day).

Gracia-Lor et al. (2012) investigated the presence and characteristics of about fifty medicines in urban wastewater treatment plants in the Castellon province of Spain, which is located in the Mediterranean region. Out of these, 17 substances were found in the samples; the most commonly found groupings were anti-inflammatories, lipid regulators, analgesics and statin medications that lower cholesterol. The most commonly detected substances were naproxen, venlafaxine, bezafibrate, diclofenac, gemfibrozil, ketoprofen and aminoantipyrine. Ibuprofen, acetaminophen, and salicylic acid had the largest quantities in influents with median concentrations of 12.4, 44.8 and 35.1 μgL^{-1} and maximum levels of 40, 201 and 277 μgL^{-1}, respectively. Cholesterol-lowering statin medications and lipid regulators were detected in effluents with median amounts less than 0.10 lgL^{-1}.

Sim, Lee and Oh (2010) studied 25 pharmaceutical contaminants in five rivers, one hospital and ten municipal waste treatment plants in Korea. The research findings indicated that in the influents of municipal wastewater treatment plant influents, caffeine, paracetamol and acetylsalicylic acid, (3.37 ± 1.94 μg/L), (6.80 ± 2.41 μg/L), and (6.29 ± 3.39 μg/L) respectively exhibited notably elevated levels. Conversely, paracetamol caffeine and ciprofloxacin were predominantly detected in hospital wastewater treatment plant effluents in concentrations of 41.9, 56.1 and 45.0 μg/L respectively.

(Roberts and Thomas, 2006) studied the presence of certain pharmaceutically active contaminants in surface water and wastewater effluent in the lower Tyne basin. His study found that all compounds from the wastewater treatment plants under study occurred in raw effluent at concentration ranges of 11 to 69,570 ng/L, except for sulfamethoxazole and acetyl-sulfamethoxazole. The surface water samples contained 4-2370 ng/L of trimethoprim, ibuprofen, Clotrimazole, propranolol, erythromycin, dextropropoxyphene and tamoxifen. Therefore, both wastewater effluents and influents contained pharmaceutical contaminants.

2.3.5 Concentrations of PhACs in faecal sludge

Gros et al. (2020) examined the presence and behaviour of 29 pharmaceuticals from various classes in two sanitation systems that employ source separation. The findings revealed that the untreated faecal sludge obtained from a latrine contained high concentrations of PhACs, with levels reaching several grams per litre (g/L) and grams per kilogram dry weight (g/kg dw) in the liquid and solid components, respectively. Of the

29 monitored PhACs, 16 were found in the solid fractions and 11 in the liquid fractions of faecal sludge. Concentrations of PhACs in liquid and solid fractions of faecal sludge were found to range from 1.6 to 180 μg/L and 76 to 7400 μg/L dw respectively.

A research study by (Conn et al., 2010) monitored the presence of 20 pharmaceuticals and chemicals found in consumer products in effluents of both raw wastewater and septic tanks. The investigation focused on six single-family homes utilizing onsite wastewater treatment systems. Among the compounds detected were ethylenediaminetetraacetic acid, caffeine, 4-nonylphenolmonoethoxylate and triclosan, including the stimulant which was present in all wastewater samples. The study found that over-the-counter anti-inflammatory medications were the most commonly identified pharmaceuticals. The pharmaceutical concentrations varied from less than 1 microgram per litre (<1 μg/L) to greater than 1,000 micrograms per litre (>1,000 μg/L).

2.3.6 Concentrations of PhACs in faeces and urine

In a study conducted by (Winker et al., 2008), a comparison was made between the concentrations of human pharmaceuticals in raw municipal wastewater and yellow water, which refers to urine. The study explores the significance of yellow water as a source of pharmaceuticals in the wastewater stream. The study reveals that while certain pharmaceutical substances exhibit significant excretion through faeces, most PhACs and their byproducts are largely eliminated by urine.

Faecal excretion data frequently provided no information on the proportions of parent medicines or their metabolites excreted. In the case of carbamazepine, only 28% of the parent medication is eliminated in the faeces, although only partially unaltered. Hence, it was estimated that 10% of carbamazepine is excreted through faeces in a partially unchanged state, accounting for 33% of the total excretion and 28% of the original amount (Winker et al., 2008).

In the examined literature, faecal excretion statistics for PhACs including paracetamol, azithromycin, albuterol, and clenbuterol were not mentioned. This could be interpreted as a sign that faeces excretion does not play a significant role for them. However, a greater proportion of the main substances and their metabolites are eliminated in the urine than in the faeces. For gemfibrozil, diclofenac and pentoxifylline, only minute fractions of the given dosage are removed; however, 90 per cent of consumed sotalol can be found in urine (Winker et al., 2008).

Bischel et al. (2015) studied the presence of pathogens and pharmaceuticals in source-separated urine from urine-diverting dry toilets in eThekwini, South Africa. The study evaluated 12 priority pharmaceuticals and revealed that the highest concentrations of antibiotics trimethoprim and sulfamethoxazole in urine were found to be 1280 µg/L and 6800 µg/L, respectively. Furthermore, the average concentration of the antiretroviral medication emtricitabine was found to be 100 ± 97 µg/L. Presented hereafter and summaries in Table 2-1 are the concentrations of PhACs in wastewater, faecal sludge, faeces and urine.

Table 2-1: Concentrations of PhACs in wastewater, faecal sludge, faeces and urine as documented from various studies

Citation	Country	Waste stream	Findings	Remarks
Botero-Coy et al., 2018	Columbia	Wastewater	All wastewater samples contained a majority of the targeted anti-depressants antibiotics, lipid regulators, analgesics, anti-inflammatories, statins that lower cholesterol, and anti-depressants. Acetaminophen was found in the greatest amounts, with up to 50 μg/L being recorded. Furthermore, the influent wastewater contained antihypertensive medications such as losartan and valsartan, as well as some antibiotics like norfloxacin, ciprofloxacin and azithromycin at levels exceeding μg/L.	PhACs were present in both wastewater influents and effluents.
Sim et al.,2011	Korea	Wastewater from treatment plants for pharmaceutical manufacturing (4No.), livestock (4No.), municipal (12No.), and hospital applications (4No.)	The highest concentrations of pharmaceutical pollutants were found in influent samples from livestock, followed by pharmaceutical manufacturing, hospital and municipal. Among these, municipal treatment plants showed the highest daily loads (0.404 to 1201 kg per day), followed by pharmaceutical manufacturing (0.010 to 5.98 kg per day), livestock (0.090 to 1.10 kg per day), and hospital (0.002 to 0.042 kg per day). Antibiotics were predominately found in the influents from the livestock waste while non-steroidal anti-inflammatory medicines (NSAIDs) were more prevalent in the incoming wastewater from the	The highest concentrations of pharmaceutical pollutants were found in influent samples from livestock wastewater treatment plants, followed by pharmaceutical manufacturing wastewater treatment plants, hospital wastewater treatment plants and municipal wastewater treatment plants.

Citation	Country	Waste stream	Findings	Remarks
			municipal and pharmaceutical manufacturing waste treatment plants. NSAIDs, coffee, and carbamazepine dominated the hospital plant influents. The effluents of municipal waste treatment plants contained pharmaceutical loads ranging from 0.101 to 23.0 kg per day, which were comparatively lower than the influent levels. These effluent levels exceeded those of livestock waste treatment plants (0.00001 to 1.88 kg per day) and hospital waste treatment plants (0.0001 to 0.171 kg per day), except for pharmaceutical manufacturing waste treatment plants (0.00001 to 115 kg per day).	
Gracia-Lor et al., 2012	Spain	Urban wastewater	Ibuprofen, acetaminophen, and salicylic acid had the largest quantities in influents with median concentrations of 12.4, 44.8 and 35.1 μgL^{-1} and maximum levels of 40, 201 and 277 μgL^{-1}, respectively. Cholesterol-lowering statin medications and lipid regulators were detected in effluents with median amounts less than 0.10 lgL^{-1}.	PhACs were present in both wastewater influents and effluents.

Citation	Country	Waste stream	Findings	Remarks
Sim, Lee and Oh, 2010	Korea	Ten municipal (WWTPs), one hospital WWTP	In the influents of municipal wastewater treatment plant influents, caffeine, paracetamol and acetylsalicylic acid, (3.37 ± 1.94 µg/L), (6.80 ± 2.41 µg/L), and (6.29 ± 3.39 µg/L) respectively, exhibited notably elevated levels. Conversely, paracetamol caffeine and ciprofloxacin were predominantly detected in hospital wastewater treatment plant effluents in concentrations of 41.9, 56.1 and 45.0 µg/L respectively.	PhACs were present in both wastewater influents and effluents.
Robert and Thomas, 2006	Lower River Tyne, UK	Wastewater	All compounds from the wastewater treatment plants under study occurred in raw effluent at concentration ranges of 11 to 69,570 ng/L, except for sulfamethoxazole and acetyl-sulfamethoxazole.	PhACs were present in both wastewater influents and effluents.
Gros et al.,2020	Sweden	Faecal sludge from Source-separated systems	Of the 29 monitored PhACs, 16 were found in the solid fractions and 11 in the liquid fractions of faecal sludge. Concentrations of PhACs in liquid and solid fractions of faecal sludge were found to range from 1.6 to 180 µg/L and 76 to 7400 µg/L dw respectively.	The untreated faecal sludge obtained from a latrine contained high concentrations of PhACs, with levels reaching several grams per litre (g/L) and grams per kilogram dry weight (g/kg dw) in the liquid and solid components, respectively.

Citation	Country	Waste stream	Findings	Remarks
Conn et al., 2010	Florida, Colorado, and Minnesota	Effluent from raw WW and septic tanks from six single-family homes	All wastewater samples contained 10 compounds inclusive of 4-nonylphenolmonoethoxylate, caffeine, triclosan and ethylenediaminetetraacetic acid were found in all WW samples. Concentrations of pharmaceuticals ranged from <1 µg/L to >1,000 µg/L.	Over-the-counter anti-inflammatory medications were the commonly identified pharmaceuticals. The pharmaceutical concentrations varied from less than 1 microgram per litre (<1 µg/L) to greater than 1,000 micrograms per litre (>1,000 µg/L).
Winker et al.' 2008	Germany	Raw municipal wastewater and yellow water.	In the case of carbamazepine, only 28% of the parent medication is eliminated in the faeces, although only partially unaltered. Hence, it was estimated that 10% of carbamazepine is excreted through faeces in a partially unchanged state, accounting for 33% of the total excretion and 28% of the original amount.	While certain pharmaceutical substances exhibit significant excretion through faeces, most PhACs and their byproducts are largely eliminated by urine.
Bischel et al., 2015	South Africa.	Source-separated urine from urine-diverting dry toilets	The highest concentrations of antibiotics trimethoprim and sulfamethoxazole in urine were found to be 1280 µg/L and 6800 µg/L, respectively. Furthermore, the average concentration of the antiretroviral medication emtricitabine was found to be 100 ± 97 µg/L.	PhACs can be present in urine.

2.3.7 An overview of the effectiveness of WSPs in removing PhACs

Conventional waste stabilization ponds (WSPs) comprise three categories of ponds, namely, anaerobic, facultative, and maturation ponds. These ponds differ in terms of biochemical processes, organic loading, pond geometry, and hydraulic (Gruchlik, Linge and Joll, 2018). Various factors influence the effectiveness of treatment in these WSPs, including the shape and layout of the ponds, the nature of the wastewater being treated, hydraulic residence time, and the quantity of organic loading (Sah, Rousseau and Hooijmans, 2012).

WSPs remove these micropollutants through biological, physical and chemical processes that take place within each pond such as biodegradation, sedimentation and adsorption (Sperling, 2007; Sah, Rousseau and Hooijmans, 2012). These processes are further affected by environmental aspects such as temperature, pH, algae presence, and light intensity (Gruchlik, Linge and Joll, 2018). Additionally, they are influenced by redox conditions, the presence of harmful compounds, wastewater composition, operational parameters during treatment, and characteristics of the pollutants including biodegradability, hydrophobicity and volatility (Pessoa et al., 2014).

2.3.8 Factors that affect the efficiency of WSPs in PhACs removal

The biological mechanisms within WSPs are primarily impacted by environmental elements like light levels, temperature, and pH, alongside factors such as algae presence, retention duration, organic load, and variations across seasons.

1. **Presence of algae,**

Algae presence is a significant contributor to the elimination of micro-contaminants within WSPs, given its high susceptibility to these harmful substances (Muñoz and Guieysse, 2006; Matamoros, Rodríguez and Albaigés, 2016). Utilizing suitable algae allows for the biodegradation of dangerous pollutants like phenolic chemicals, organic solvents and polycyclic aromatic hydrocarbons (Muñoz and Guieysse, 2006). According to (Matamoros et al., 2015), algae removes some pharmaceutically active microcontaminants.

2. **Light intensity,**

In WSPs, light is s source of sunlight which is used in the process of photosynthesis (Heaven, Banks and Zotova, 2005) since it enhances biodegradation by allowing ultraviolet light photodegradation (Rivera-Utrilla et al., 2013). Solar radiation greatly affects the removal of these pharmaceutical contaminants.

3. **pH**

In WSPs, the pH levels serve as indicators of the overall pond performance. A pH below 7 indicates a substantial organic load, resulting in lower oxygen in the dissolved state and eventual unpleasant smells (Sperling, 2007). Low pH can also signify that carbon dioxide was not fully consumed during photosynthesis.

4. **Temperature**

An increase in temperature enhances the removal of micro-pollutants. As indicated by (Matamoros et al., 2015), the elimination of degradable elements such as (5-methyl-benzotriazole , OH-benzothiazole, oxybenzone, methyl paraben, benzotriazole, triphenyl phosphate, naproxen, and benzothiazole), and compounds with modrate valatility (like tonalide and galaxolide) showed higher effectiveness during the summer season (with a 26°C mean temperature) in contrast to the winter season (with a 11°C mean temperature). Temperature also promotes breakdown by biological processes, thereby optimizing the efficiency of WSPs. In the research by (Li et al., 2011)), the ideal range of temperature for the breakdown rates of the ceftiofur antibiotic through biodegradation was determined to be approximately 35-40°C, closely matching the optimal temperature of 37°C for gut microbes.

5. **Retention Time.**

The effectiveness of contaminant removal also varies based on the retention times ie one for solids (SRT) and one for hydraulics (HRT). According to (Fernandez-Fontaina et al., 2012), micro-contaminants whose degradation kinetics quite take time, such as antibiotics or certain psychotropic drugs like fluoxetine exhibit lower biodegradability at reduced retention times or higher rates of loading. Additionally, a longer hydraulic residence time is linked to enhanced removal efficiency, as emphasized (Hijosa-Valsero et al., 2010). Garcia-Rodríguez et al., (2014) stress that as wastewater treatment systems become even more complex, and the number of treatment lagoons increase or even as the ponds are combined with other treatment systems that use biological processes like constructed wetlands, the removal efficiencies of these micro contaminants tend to be even higher. (Lesjean et al., 2004) noticed an uptick in PhACs removal with an extended 26-day SRT, but a reduction in removal with a shorter 8-day SRT.

6. Organic loading

(Graham, Giesen and Bunce, 2018) pointed out that overloading wastewater treatment facilities or the absence of a tertiary treatment compromises the treatment efficiency. Consequently, effluents from wastewater treatment plants serve as a major origin of PhACs, resulting in contamination of water resources, for example, groundwater, surface water, and lakes, when released since they are in significant amounts that may not be fully treated.

7. Seasonal changes

Seasonal changes especially in cold climates also affect the removal of these contaminants. The changes in the seasons consequently affect the temperature, sunlight intensity and biomass production (Matamoros, Rodríguez and Albaigés, 2016).

A study conducted by (Matamoros et al., 2015) discovered that during summer (with an average of 282 Wm^{-2} of sun radiation per day), the photodegradation of PhACs like diclofenac and ketoprofen was more effective in comparison to the winter season (with an average of 74 Wm^{-2} of sun radiation per day).

2.3.9 Mechanisms through which WSPs remove PhACs

According to (Garcia-Rodríguez et al., 2014), WSPs remove organic matter through photodegradation, biodegradation and sorption processes with the use of algae and water plants as shown in Figure 2-3. The mechanisms of removal in WSPs are explained below.

1. **Photodegradation**

Organic micropollutants can be removed through a process of photodegradation. This involves direct or indirect exposure of the contaminant to sunlight (Garcia-Rodríguez et al., 2014). In direct photodegradation, the contaminant directly absorbs the solar radiation, which causes an increase in it's the energy of the pollutant structure thereby breaking bonds and degrading it (Vione et al., 2014). In processes of indirect photodegradation, natural photosynthetic reactions involving elements like chromophoric dissolved organic matter or NO^{3-} and NO^{2-} anions produce active species like hydroxyl or singlet oxygen radicals. The photodegradation reactions in the water are then initiated by these reactive species. In indirect photodegradation, the reactions of photodegradation are triggered by these reactive species i.e singlet oxygen or hydroxyl radicals generated by natural photosynthetic processes involving chromophoric dissolved organic matter or NO^{3-} and NO^{2-} anions (Niu et al., 2016).

Photodegradation is essential in the elimination of microcontaminants in WSPs (Garcia-Rodríguez et al., 2014), although there is still a knowledge gap on how photodegradation works in WSPs to remove micropollutants as most studies have been carried out under controlled laboratory conditions stimulated by sunlight or in natural waters as in (Rivera-

Utrilla et al., 2013) and only (Jasper et al., 2014; Ryan et al., 2011 conducted the study in WSPs.

2. Biodegradation

Biological biodegradation involves the breaking down of organic micropollutants by microbes like fungi and bacteria into smaller and simpler compounds ultimately leading to complete breakdown under both anaerobic and aerobic circumstances (Garcia-Rodríguez et al., 2014). According to (Hijosa-Valsero et al., 2010), biodegradation in WSPs is faster in anaerobic than aerobic conditions. Aerobic and aerobic conditions coexist in WSPs and this promotes the decomposition of a variety of organic micropollutants.

There are also sophisticated biological associations that occur between bacteria, algae and other microbes in WSPs that also contribute to the elimination of such micropollutants (Coleman et al., 2010). Research by (Matamoros et al., 2015) shows that interaction between bacteria and algae leads to improved biodegradation of micropollutants than when these microorganisms exist individually where algae produce oxygen through photosynthesis that is then used by bacteria in the decomposition of organic matter.

Even though most studies like those done by (Verlicchi, Al Aukidy and Zambello, 2012; Wang and Wang, 2016) focus on the biodegradation of micropollutants using biological processes in conventional WWTPs, not many studies have focused on if this biodegradation of contaminants like PhACs in conventional WWTPs is due to some microorganisms. In addition, not many studies have focused on how interactions between microbes affect the effectiveness of WSPs in removal of these PhACs.

The type of the compound structure of the micropollutant and its functional group affect the biodegradability of the micropollutant (Luo et al., 2014). Substances with electron-donating functional groups, aliphatic compounds that are highly unsaturated, and short-side chain chemicals are not readily biodegradable.

Contrarily, substances with side chains that are lengthy, highly branched, saturated substances, and compounds containing sulfate, halogen, or substituents that withdraw electrons, are readily biodegradable (Luo et al., 2014). An example is diclofenac with two chlorine substituents making it difficult to degrade in WSPs, hence its fairly moderate removal efficiency (Hijosa-Valsero et al., 2010). The effectiveness of its removal could be attributed to photodegradation since it is highly photodegradable (Camacho-Muñoz et al., 2012) observed that keprofen and ibuprofen have a similar structure but different removal efficiencies with Ibrufen having slightly higher removal efficiency than Ketoprofen. Hence in some cases, the chemical structure does not correlate with removal efficiency.

3. Sorption

According to (Garcia-Rodríguez et al., 2014), the process of sorption mainly occurs through adsorption and absorption. In adsorption, micropollutant molecules or ions physically attach to the sorbent surface where as in absorption, the micropollutant and the sorbent mix. The pollutant's hydrophobicity greatly affects its adsorption rate and the octanol-water partition coefficient (K_{OW}) is used to quantify this. The major method for removing micropollutants in primary treatment is adsorption, which is often consistent with their phobicity (represented by the K_{OW}) value for the octanol-water partition coefficient). Substances having low octanol-water partition coefficient values i.e. log Kow<3, such as trimethoprim, atenolol, carbamazepine, sulpiride, and metoprolol are not anticipated to bind strongly to particulate matter but rather undergo breakdown in the solution phase (Wang et al., 2014).

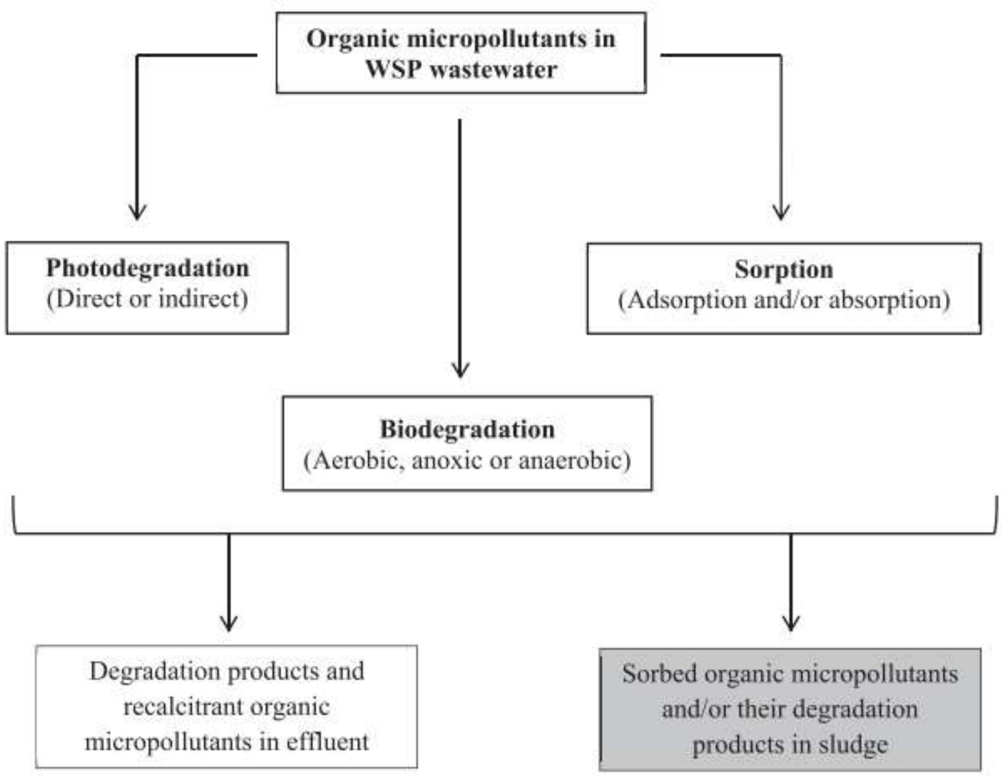


Figure 2-3: Mechanisms for removal of organic micropollutants in WSPs. Adapted from (Gracia-Lor et al., 2012)

2.3.10 Human Excreta Disposal technologies and their Impact on PhACs removal in Wastewater Treatment

The management of pharmaceutical contaminants in wastewater is significantly influenced by the type of human excreta disposal system employed. Systems such as septic tanks, pit latrines, and sewer networks each exhibit varying efficiencies in the removal of pharmaceutical compounds, ultimately affecting the quality of effluent that reaches wastewater treatment plants (WWTPs).

1. Septic Tanks

Septic tanks are commonly used in decentralized wastewater treatment systems, particularly in rural areas. While they play a significant role in reducing the concentration of some pharmaceutical contaminants, research highlights that their effectiveness is limited. Septic systems can reduce the levels of certain pharmaceuticals, but they often fail to eliminate these compounds entirely, posing environmental risks.

A study by (Arrubla et al., 2016) found that septic tanks demonstrated removal efficiencies of less than 50% for pharmaceuticals such as diclofenac and galaxolide. Other compounds, including caffeine and ibuprofen, showed no reduction during treatment. Specific removal efficiencies were reported as less than 50% for dihydrojasmonate, diclofenac, and galaxolide, while aspirin, naproxen, and tonalide exhibited only a 15% reduction. The inefficiency in removing these contaminants indicates that septic systems may not sufficiently prevent pharmaceuticals from entering the environment, which calls for further treatment processes.

Redox technologies have shown promise in enhancing the removal of specific antibiotics, such as cephalexin, with removal rates of up to 80% under optimal conditions(Zhang, Zheng and Tratnyek, 2023). However, these results suggest that septic tanks, even when complemented by additional treatments, require significant improvements to efficiently handle pharmaceutical contaminants before discharging treated water into the environment. Furthermore, the fate of emerging micropollutants (EMPs) in septic tanks is influenced by system maintenance and sanitary conditions, as poorly maintained systems are more likely to leak and exacerbate environmental contamination (Wardhani et al., 2024).

2. Pit Latrines

Pit latrines are another widespread sanitation solution, particularly in low-income regions. However, they are even less effective than septic tanks at removing pharmaceutical contaminants. Their design, which primarily focuses on solid waste decomposition, does not facilitate chemical degradation, allowing pharmaceuticals to persist in the effluent.

Research by (Koch, 2015) identified high concentrations of pharmaceuticals in blackwater from pit latrines, often exceeding those found in conventional wastewater systems. The study reported concentrations of pharmaceuticals reaching hundreds of µg L-1 in the liquid phase and µg g-1 in the solid phase. These elevated levels, coupled with poor removal rates, underscore the risk that pit latrines pose to both environmental and public health. Removal efficiency was found to be limited, with mixed microbial cultures providing some level of treatment but with inconsistent results (Tóth et al., 2023). Under mesophilic (37°C) conditions, pharmaceuticals such as PhACs achieved only 45% removal, which dropped to 31% under thermophilic (52°C) conditions (Koch, 2015). Overall, the pit latrines were found to contribute largely to lower removal efficiencies due to their design, which primarily focuses on solid waste decomposition rather than chemical degradation (Klanovicz et al., 2023).

3. Sewer Networks

Conventional sewer networks, which channel wastewater to centralized WWTPs, generally perform better at removing pharmaceuticals compared to decentralized systems like septic tanks and pit latrines. However, the overall removal efficiency of pharmaceuticals remains a challenge especially since these contribute to large volumes of wastewater reaching these wastewater treatment plants (Tilley, 2014; Gao et al., 2023).

The study by (Koch, 2015) noted that in blackwater treatment, the removal rate of pharmaceuticals was higher than in decentralized systems, with a 49% average removal rate reported. Certain compounds, particularly antibiotics, were removed almost completely, achieving up to 100% removal. Centralized WWTPs using biological treatments were found to reduce pharmaceutical concentrations by 23-54%, depending on the specific compounds being treated (Ulvi, Aydın and Emin, 2022). Despite these improvements, conventional WWTPs still struggle with the complete removal of several persistent pharmaceuticals, leading to their eventual discharge into the environment (Ying et al., 2009).

The effectiveness of human excreta disposal technologies in managing pharmaceutical contaminants varies significantly across different systems. Septic tanks and pit latrines show limited removal efficiencies, highlighting the need for enhancements and additional treatment processes to safeguard water quality. While sewer networks connected to centralized WWTPs demonstrate higher removal rates, they still face challenges with certain persistent pharmaceuticals. The development of advanced treatment technologies and better maintenance of decentralized systems is crucial to improving the removal of these contaminants and protecting environmental and public health.

Generally, there is still limited research on the effect of onsite sanitation technologies of PhACs removal especially in WSPs.

2.3.11 Techniques employed by various researchers in the removal of PhACs from wastewater

Jiang et al., (2014) highlight the effectiveness of Taramites Versicolor fungus in carbamazepine removal due to its ability to release enzymes such as peroxidase and laccase. Several strains of pseudomonas obtain their energy and carbon for growth from sulfamethoxazole, according to (Jiang et al., 2014). To identify the main mechanism for removing PhACs, a comparison of single substrate and co-metabolic degradation pathways was done. It was inferred that the main route of elimination for naproxen, bezafibrate, and ibuprofen was the process of co-metabolic biodegradation. On the other hand, the complete degradation of ketoprofen occurred only to a partial extent as a single substrate(Quintana, Weiss and Reemtsma, 2005).

Yang et al., (2016) observed that trimethoprim was effectively eliminated in both oxygen-deprived and oxygen-rich conditions. Furthermore, atenolol demonstrated removal potential in aerobic and anaerobic conditions. The EAWAG-BBD PPS (EAWAG Biodegradation Biocatalysis Database Prediction Pathway System) predicts that the major elimination of atenolol in aerobic circumstances is facilitated by microbial hydrolysis of the main amide into carboxylic acid.

Ozonation and activated carbon (AC) treatment have been identified as economically viable options for removing PhACs and are currently employed in some WWTPs (Rizzo et al., 2019).

Advanced oxidation processes (AOPs) utilized in the breakdown of these PhACs primarily employ hydroxyl radicals (OH•) as reactive agents, and the reaction mechanisms may include multiple parallel pathways (Hussain, Mahtab and Farooqi, 2020). The choice of suitable AOPs relies on several factors, such as the persistence of target compounds, wastewater characteristics, available resources, and economic considerations (Demirören et al., 2021).

Existing literature acknowledges the superior efficiency and environmental friendliness of integrated processes (Ibid). A study by (Sui et al., 2010) found that diclofenac, carbamazepine, and sulpiride exhibited remarkably high removal efficiencies (>95%) at an ozone dosage of 5 mg/L. However, each advanced oxidation process (AOP) has its limitations and disadvantages. Therefore, it is essential to create and implement suitable and optimized treatment procedures to achieve these desired removal efficiencies.

(Hussain, Mahtab and Farooqi, 2020). The Fenton process has several drawbacks, including a requirement for low working pH and high sludge production, resulting in the buildup of pharmaceuticals within the iron sludge generated during treatment (Aziz et al., 2020). On the flip side, inadequate amounts of ozone can result in the creation of transformation byproducts. However, these byproducts' toxicity can be subsequently diminished through additional biological treatment (Mcardell et al., 2015). The combined approach of ozonation and biological processes has been identified to be a highly effective approach in removing pharmaceuticals from secondary urban effluent (Knopp et al., 2016).

Based on multiple studies, traditional treatment plants by themselves are insufficient to fully eliminate pharmaceuticals, necessitating additional steps for effective treatment. In addition, membrane bioreactor systems exhibited superior removal efficiencies for numerous pharmaceuticals than conventional activated sludge processes (Vickers, 2017). However, both ASP and MBR systems demonstrated poor removal efficiencies for carbamazepine and hydrochlorothiazide (Ibid).

O'Brien et al., (2017) discovered that certain compounds like atenolol, carbamazepine, and ibuprofen exhibit persistence in sewer systems. The enhanced performance of membrane bioreactor (MBR) systems in removing specific pharmaceuticals can be attributed to factors such as enhanced solid and microbial retention ability, extended solid retention time and increased biomass concentration (Vickers, 2017). Electrochemical membrane bioreactors (EMBR) represent an integration of membrane technology.

EMBRs have been found to exhibit greater efficiency and lower energy consumption in comparison to conventional activated sludge processes and MBRs (Asif, Maqbool and Zhang, 2020). However, advanced technologies are commonly limited to laboratory and pilot scales. Additionally, MBRs face challenges such as the high cost of membrane materials, high energy consumption and fouling of membranes, and costly membrane materials. It is therefore necessary to address these challenges for wider implementation of MBRs on a full-scale basis (Rout et al., 2021).

Zeng et al. (2015) used a cylindrical wetted-wall corona discharge reactor to study the degradation of pharmaceuticals, specifically ibuprofen (IBP), in an aqueous solution. They looked at things like pulse repetition rate and initial concentration. According to their research, after 80 minutes of plasma exposure, the most notable degradation (91.7% of 60 mg/L IBP) occurred, translating into a 6.9 g/kWh yield in energy. 30.3-10-3 min^{-1} was the first-order reaction rate at which ibuprofen was broken down. Ibuprofen was nearly completely broken down into H_2O, CO_2, and simpler salts, according to the molecular pathways of the degradation of the by-products of IBP that were detected by high-performance liquid chromatography (HPLC).

Another investigation by (Ajo et al., 2018) employed gas-phase pulsed corona discharge to oxidize a variety of medicines from real wastewater, including biologically treated wastewater from a healthcare facility and untreated wastewater from a public hospital. Their results showed that pharmaceutical residues could be removed from raw sewage by 87% with a moderate energy input of 1 kWh m^{-3}, whereas pharmaceutical toxins could be removed from biologically treated wastewater by an astonishing 99.99% with a lower energy input of 0.5 kWh m^{-3}.

In a related study, Banaschik et al. (2015) investigated the use of corona discharge for the treatment of wastewater containing a variety of medicines, including diclofenac, 17-ethinylestradiol, diazepam, diatrizoate, ibuprofen, carbamazepine and trimethoprim. This method improved the pharmaceutical elimination percentages to 99.99%, which was a substantial improvement.

The literature highlights how widely corona discharge is used to remove pharmaceuticals from wastewater (Crofton et al., 2016). However, in such corona discharge setups, there is a potential risk that the electrode of high voltage could come into contact with the treated effluent, making it susceptible to potential degradation caused by the oxidative species generated within the solution.

Previous studies have explored various treatment methods for pharmaceutical removal, including conventional, modern, and natural methods. The cost-effective natural processes include sorption, volatilization, dilution, biodegradation and photolysis (Rout et al., 2021). However, it has been demonstrated that natural processes are less efficient in removing pharmaceuticals (Barbosa et al., 2016). In contrast, traditional techniques including adsorption, ozonation, and membrane filtration have shown high effectiveness in removing pharmaceuticals (Pesqueira, Pereira and Silva, 2020).

Nevertheless, certain drawbacks are associated with these approaches. For instance, oxidation by-products of the ozonation process may be more dangerous than the original substances, and the membrane filtration process necessitates high operational costs and the proper disposal of the concentrate (Rizzo et al., 2019).

2.3.12 Policy and regulation concerning PhACs

While the pharmaceutical sector is becoming increasingly competitive worldwide, different nations are establishing regulatory bodies to oversee drug development procedures. These regulatory bodies are essential in complying with the legal obligations of a particular country regarding drug development processes. The main obligation of these regulatory bodies is to guarantee that medicines and medical devices are safe, effective, and of high quality, ensure synchronization of the legal processes surrounding the development of drugs and ensure adherence to the legal standards. Additionally, regulatory bodies have a crucial function in promoting and enforcing regulations in unregulated parts of the globe to ensure that individuals living in these regions safely use these drugs.

Globally, some of the international organizations that regulate pharmaceutical use include ICH (International Conference on Harmonization), WHO (World Health Organization), WTO (World Trade Organization), PAHO (Pan American Health Organization), and WIPO (World Intellectual Property Organization) (Geetanjali Sengar, n.d.). The WHO also provides guidelines for the safe disposal of unwanted drugs to ensure that they are not a threat to the environment (WHO, 2009).

In addition, every nation possesses a regulatory body tasked with the enforcement of regulations and the issuance of guidelines to oversee multiple facets of pharmaceutical products, including drug design, permitting, authorization, production, advertisement, and packaging. Examples of such regulatory agencies include USFDA in the United States, HEALTH CANADA in Canada, EMEA in the European Union, TGA in Australia, MHRA in the United Kingdom, MCAZ in Zimbabwe, SFDA in China, CDSCO in India, MCC in South Africa, MHLW in Japan, ANVISA in Brazil, NAFDAC in Nigeria, MEDSAFE in New Zealand, SWISSMEDIC in Switzerland, KFDA in Korea, and MoH in Sri Lanka (Geetanjali Sengar, 2019).

In Uganda, there have been various scientific and technological innovations in the areas of disease control and management such as the development of vaccines, therapeutics, diagnostics, biomedical ICT and Equipment. These have over time increased the level of drug use in the country. This calls for regulations and policies to ensure that these medicines are properly managed.

The major pharmaceutical regulatory bodies mandated to handle pharmaceuticals in Uganda are the National Drug Authority (NDA) and the National Medical Stores (NMS) (Byarugaba and Sewankambo, 2015). The use of drugs in Uganda is governed by the National Drug Authority and Policy Act Cap 206 of 1993 "to establish a national drug policy and a national drug authority to ensure the availability, at all times, of essential, efficacious and cost-effective drugs to the entire population of Uganda, as a means of providing satisfactory health care and safeguarding the appropriate use of drugs" (Government of Uganda, 1993). The national drug policy, as outlined in the NDA Statute

1993, aims, amongst other things to guarantee that all Ugandans have access to necessary, secure, efficient, and affordable medications to offer adequate medical care.

The issues related to pharmaceutical pollutants hold great significance as they align with various Sustainable Development Goals (SDGs). SDG Goal No.3 seeks to ensure good health and well-being for all, seeking to substantially decrease deaths and sicknesses caused by harmful chemicals, in addition to pollution and contamination of soil, water and air by 2030 (Target 9). Moreover, SDG Goal No.6 emphasizes providing universal access to clean water and adequate sanitation, with a target to enhance the quality of water by minimizing the release of dangerous chemicals and substances, stopping disposal and promoting global wastewater treatment and safe recycling by 2030 (Targets 2 and 3) (United Nations, 2015). Additionally, pharmaceutical contaminants contribute to SDG Goal No. 14, which is geared towards the conservation and sustainable use of seas, marine resources and oceans. The targets under this goal entail significant reductions in marine pollution, particularly from onshore activities like marine waste and nutrient pollution, by 2025 (Target 1). The goal also emphasizes the sustainable management, safeguarding, and revitalization of marine and coastal ecosystems to achieve thriving and productive oceans by 2020 (Target 2).

Proper use and disposal of pharmaceuticals also contribute towards attaining Uganda’s National Development Plan (NDP) III and Vision 2040 on water and sanitation as well as clean water and sanitation (United Nations, 2015).

The above-mentioned international and national development agenda will highly ensure that pharmaceuticals are properly developed, manufactured, used and disposed of with utmost safety and quality not to pose health risks to human health as well as endanger environmental ecosystems

2.4 Key findings and Knowledge gaps

Based on the literature study, several key issues related to PhACs have been identified, along with notable knowledge gaps, as shown in Figure 2-4.

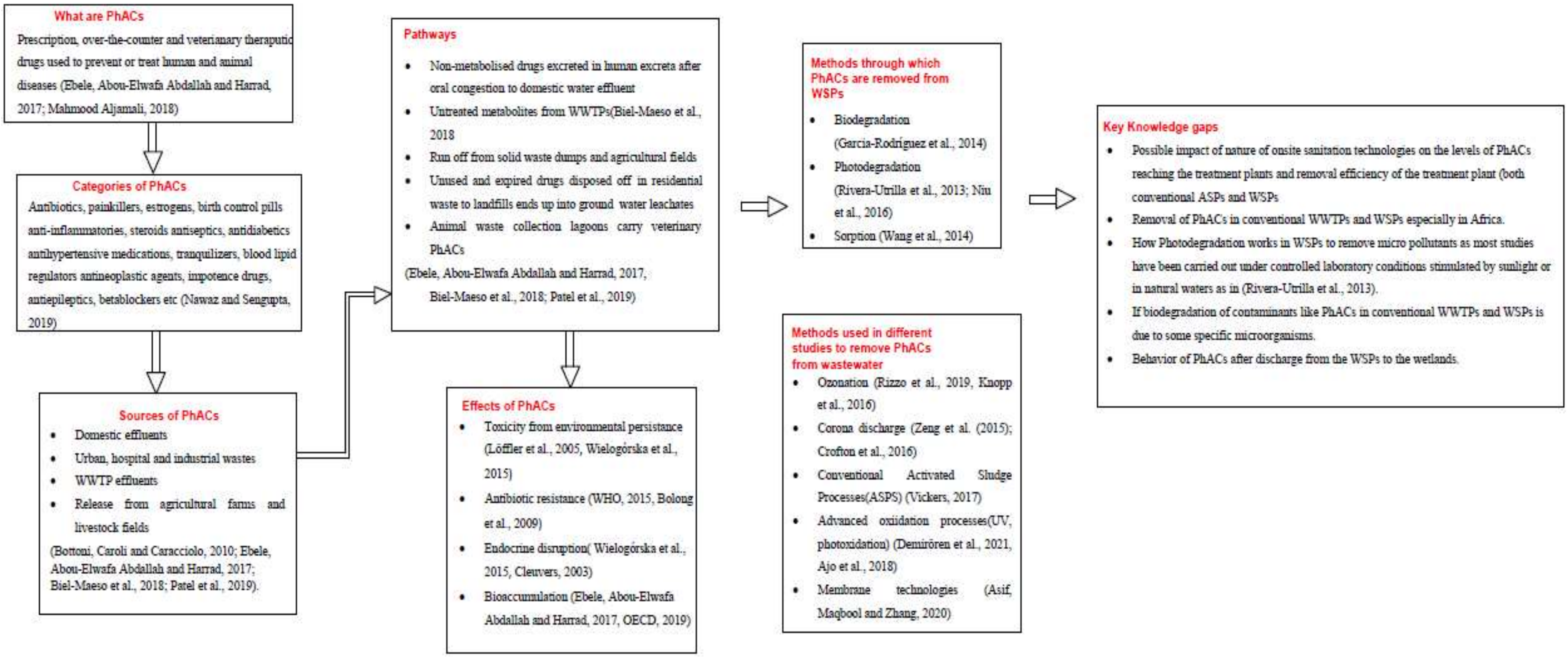


Figure 2-4: Key findings and knowledge gaps

CHAPTER THREE: RESEARCH METHODOLOGY

3.1 Introduction

The chapter will include the steps that were taken in selecting research subjects, developing the research tools, collecting and analyzing data, and interpreting the collected information. This study will incorporate the use of quantitative methods which enabled the acquisition of empirical data in the performance evaluation of WSPs in the removal of PhACs. The research took cognizance of the importance of credibility and validity of its findings. The various aspects of the research methodology used in this study are detailed below.

3.2 Research design and approach

3.2.1 Research design

This study was an analytical study. This study was carried away in such a way as to analyze the variations of PhACs at the different stages in the treatment chain to establish the treatment efficiency of the WSPs. The research was conducted in three phases, first a detailed literature review of past studies related to PhACs. This was followed by the collection of samples from the incoming waste streams to assess the levels of PhACs in these streams and along the treatment chain and sludge beds to analyze the treatment efficiency of these WSPs in removing these PhACs. Finally, the study sought to assess the effect of onsite sanitation technologies on the removal of PhACs in the Lubigi WSPs by using a material flow analysis and data from the existing literature.

3.2.2 Research Approach

The study was quantitative in which findings were expressed in numerical forms and data was analysed using statistical methods to explain the meanings of the outcomes.

3.3 Description of the Study Area

The research was conducted at the Lubigi faecal sludge and wastewater treatment plant in Kampala, Uganda, situated at GPS coordinates N0.33998˚ and E32.56032˚. Positioned on the Lubigi wetland in Kawempe division, Kampala district, the plant is about 5 km from Kampala city square along the northern bypass of Hoima Road (see Figure 3-1). Serving areas like Kawempe, Bwaise, Katanga, Makerere, and Nsooba, including key locations such as Mulago Hospital, Public Service, and Wandegeya, the Lubigi WWTP is the second facility of its kind in Kampala (Kyayesimira et al., 2019). Commissioned in May 2014, the plant can treat 400m^3 of faecal sludge and 5000m^3 of wastewater daily (KCCA-KFSM, 2017). It manages wastewater from the piped sewer network and faecal sludge brought in by private cesspool emptier trucks, with the latter originating from various onsite sanitation technologies like Dry toilets, septic tanks, pit latrines, aqua privies, and unsewered public ablution blocks (James Gideon and Bernard, 2018). This faecal sludge is raw or partially digested, presenting as a slurry or semisolid substance resulting from the collection, storage, or treatment of different combinations of excreta (Strande, Ronteltap and Brdjanovic, 2014). Furthermore, the Lubigi plant processes sewage sludge sourced from municipal wastewater treatment facilities, comprising a minimum of 80% water and nutrient-rich organic matter, alongside inorganic elements, which may include traces of non-biodegradable heavy metals (Kyayesimira et al., 2019).

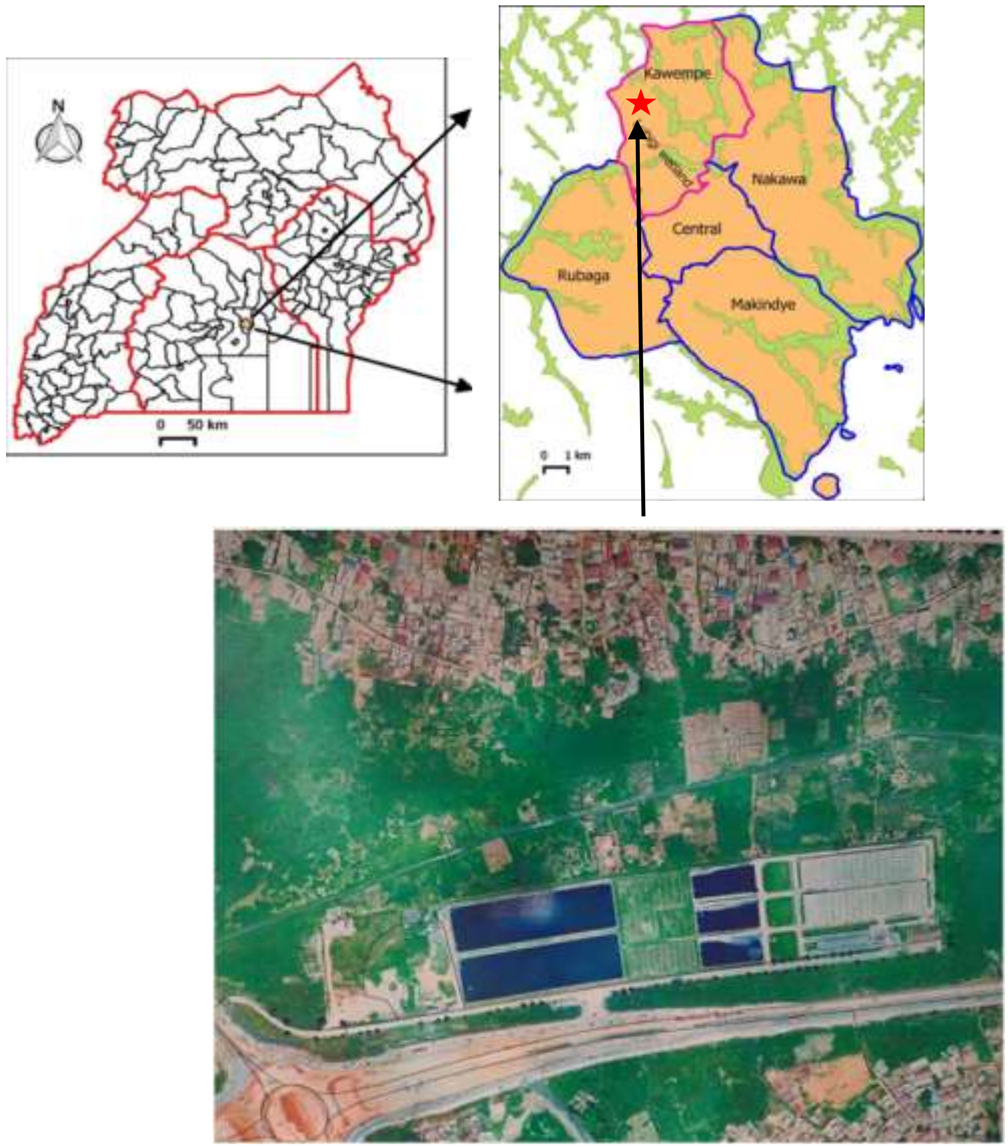


Figure 3-1: Map showing the location of Lubigi WWTP (Generated from QGIS 3)

3.4 Sample collection and sampling strategies

A total of 42 samples were selected from the different locations of the WSPs i.e. the incoming waste streams, along the treatment chain and sludge beds. The samples were collected from May 2022 to July 2022.

Grab samples were collected from seven locations within the treatment facility as shown in Figure 3.1. That is the inlet for the domestic wastewater stream; the point of discharge for cesspool empties discharging mainly faecal sludge from septic tanks; the gulper station that receives faecal sludge from pit latrines; inlet to the anaerobic pond; outlet to the anaerobic pond and discharge point (effluent from the facultative pond into the receiving environment). Samples were collected at intervals of two weeks for six weeks. A total of six samples were collected every two weeks, resulting in 18 samples from both the inlet streams and various treatment stages, yielding a combined total of 36 samples.

Additionally, two sludge samples were collected from each of the sludge drying beds of different sludge ages i.e., from fresh sludge, and sludge that has spent a considerable amount of time in the beds. In total, six sludge samples were collected. These samples were analyzed to determine the concentrations of pharmaceutical contaminants (PhACs) entering the sludge, which is essential for the Material Flow analysis.

From the biweekly samples collected from the inlet streams, three samples were taken in the morning and three in the evening, resulting in a total of nine samples for both morning and evening periods. These samples were intended to assess the diurnal fluctuations of pharmaceutical contaminants (PhACs) in the various inlet streams.

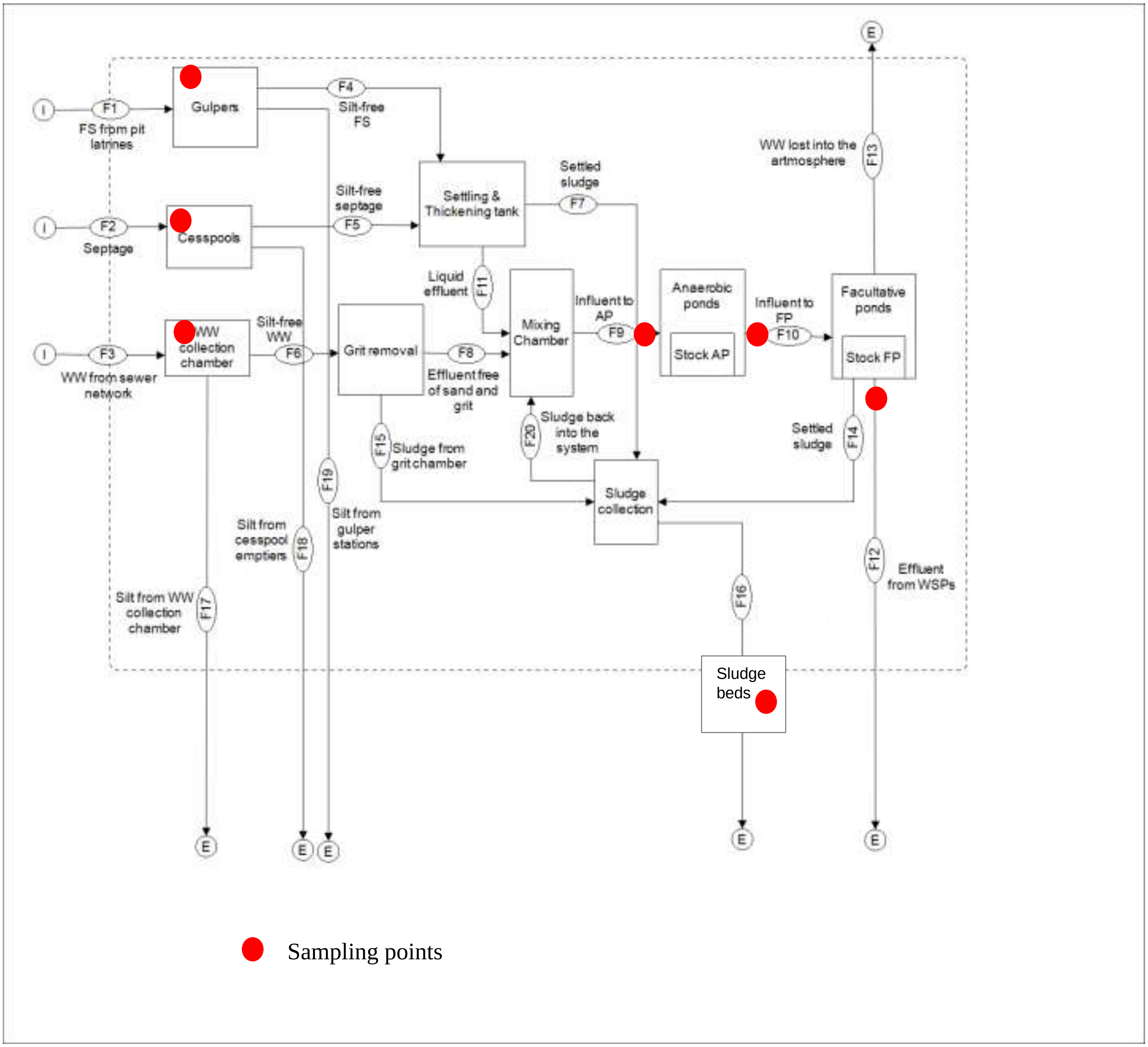


Figure 3-2: Schematic drawing of Lubigi WWTP showing sampling points (Adopted from STAN 2.7)

Sampling strategies

Prior to obtaining samples, the sampling containers were rinsed with distilled water to ensure that they were free of any contaminants. 1-litre of the composite sample was collected and placed in plastic bottles with gloved hands.

Additionally, sludge samples were also collected from the sludge beds and these were put in clean, 1-litre plastic containers. The samples were then labelled and transported to the laboratory in cool boxes and placed in a refrigerator at 4˚C for further analysis. In the laboratory, samples were tested for various physio-chemical properties and PhACs compositions.

Assessment of physicochemical properties

The samples collected were analysed for Total Suspended solids (TSS Dissolved Oxygen (DO),), Electrical Conductivity (EC) and pH according to (American Public Health Association, 2011). Briefly, TSS was analysed using the gravimetric method using a Sartorius MA35 Moisture Analyzer, and Dissolved Oxygen (DO), Electrical Conductivity (EC), and pH were analysed using a Hanna HI98194 Multiparameter portable digital meter.

3.5 Analysis for PhACs in wastewater and faecal sludge received.

Analysis for samples of PhACs was done using Liquid Chromatography in Mass Spectrometry (LC-MS-MS) technique.

Steps involved in the LC-MS-MS analysis

i. **Sample handling in the laboratory**.

Water samples were filtered through a 0.45µm acetate cellulose filter, acidified with 3.0M H2SO4, and treated with 0.2g Na2EDTA. Samples were stored at 4°C until extraction.

ii. Solid-Phase Extraction

SPE was performed using Oasis HLB cartridges (200mg/6mL) on a vacuum manifold. Samples were passed through the cartridge, cleaned, and dried. Extracts were reconstituted in a solvent mixture and stored at 15°C for analysis. Detailed steps for SPE can be found in (Grabic et al., 2012).

iii. Sample Analysis

Sample extracts were analyzed using a reverse-phase LC system coupled with an ESI source-equipped triple-quadrupole mass spectrometer. For detailed conditions and settings, refer to (Grabic et al., 2012).

3.6 Assessment of the treatment efficiency of WSPs in the removal of PhACs.

To evaluate the efficiency of WSP in the treatment of PhACs, an assessment of the inflows and outflows into various ponds was done. The overall performance was assessed by looking into the variation in concentration arriving at the treatment plant, at different points in the treatment chain and the effluent discharged from the plant.

3.7 Assessment of the impact of onsite sanitation technologies on the removal of PhACs in the Lubigi WSPs.

An investigation into the fate of PhACs through the treatment plant was conducted using a mathematical approach known as substance flow analysis (SFA) (Chèvre et al., 2013) taking into consideration the effect of removal of other contaminants. The goal of Material Flow Analysis (MFA) is to compute and visualize the movement of mass, including items like transferable goods such as PhACs, within a defined and typically open setup (Lederer, Karungi and Ogwang, 2015). In this research, the system of interest was the Lubigi sewage and wastewater treatment plant

This assessment relies on the mass balance principle considering how substances enter, are transported or transformed, and eventually leave a closed system. The SFA process involved defining temporal and spatial boundaries and identifying key substances. By understanding the system, relevant substance volumes and flows were determined. The system's relationships were converted into mathematical equations, representing substance flows and stock exchanges using parameters. Data from diverse sources, such as measurements and discussions, calibrated the model, which simulated current substance flows using a Monte Carlo simulation technique. Plausibility was confirmed by comparing simulations with primary data and other studies. Additionally, the study simulated and evaluated various mitigation measures (scenarios) to gauge their effectiveness (Chèvre et al., 2013).

Inside the system, the material movements (m) expressed as mass per unit of time (e.g. $kgyr^{-1}$) proceed via stages of conveyance, transformation, and stock exchange. ‘Input’ and ‘output’ flows relate to the entry and exit within a process, whereas ‘import’ and ‘export’ flows refer to the into and from the system. The principle of conserving mass applies to both processes and systems. Material Flow Analysis (MFA) typically employs two formulas, illustrated for a single process with various input and output flows, along with a change in stock (Lederer, Karungi and Ogwang, 2015). Below is the mass balance equation;

$$\sum_{i=1}^{k} \dot{\boldsymbol{m}}_{\boldsymbol{input,i}} = \sum_{j=1}^{k} \dot{\boldsymbol{m}}_{\boldsymbol{output,j}} \pm \dot{\boldsymbol{m}}_{\boldsymbol{storage}} \qquad \textbf{Equation 3-1}$$

Where;

$\sum_{i=1}^{k} \dot{m}_{input,i}$ is the total mass of k input material flows $\dot{m}_{input}$

$\sum_{j=1}^{k} \dot{m}_{output,j}$is the total mass of l output material flows$\dot{m}_{output}$ and

$\dot{m}_{storage}$ is the mass of the material flow per time unit from or to a stock located in a process per unit time.

The transfer formula is defined as;

$$\boldsymbol{TC_j} = \frac{\dot{\boldsymbol{m}}_{\boldsymbol{output,j}}}{\sum_{i=1}^{k} \dot{\boldsymbol{m}}_{\boldsymbol{input,i}}} \qquad \textbf{Equation 3-2}$$

Where;

TC_j is the transfer for output flow *j*

$\dot{m}_{output,j}$ is the mass of the output flow *j*

$\sum_{i=1}^{k} \dot{m}_{input,i}$ is the total mass of k input flows $\dot{m}_{input,i}$

The mass flows of the substance under study (i.e. PhACs) were obtained by multiplying the substance concentration in each of the material flows of goods by the material flows of goods containing the substance.

The quantities of wastewater and faecal sludge received at the treatment plant were obtained from records at the plant. All of this information is typically characterized by a degree of uncertainty (RSU) $u_{r,i}$ which is the percentage standard deviation in relation to the mean value as shown in Equation 3-3.

$$\boldsymbol{u_{r,i} = \frac{\sigma_i}{x_1} x100\%} \qquad \textbf{Equation 3-3}$$

Assuming independence of variables, RSU (relative standard uncertainty) u_r of a mass flow is obtained from Equation 3-4.

$$\boldsymbol{C = u_{r_{i=1}^{k}} = \sqrt{u_{r,1}{}^2 + u_{r,2}{}^2 + u_{r,k}{}^2}} \qquad \textbf{Equation 3-4}$$

The outcome is presented as in Equation 3-5.

$$\boldsymbol{\bar{x} \pm u_r} \qquad \textbf{Equation 3-5}$$

Where;

$\bar{x}$ is the mean value of the flow

$\pm u_r$ is the RSU in percent of the mean value

To determine the annal intake of PhACs, the average quantity of wastewater and FS received was multiplied by the PhACs concentration within each wastewater stream. The residual u_r was calculated by the $(u_{r,\mathrm{i},})$ of treatment plant $(u_{r,1})$ and concentration $(u_{r,2})$ as shown in Equation 3-4.

Using the MFA, different scenarios were modelled for the most likely interventions and mitigation measures.

3.8 Data analysis

The data obtained was analysed using various statistical methods using SPSS 26.0, R Studio 4.3.1, and Ms Excel 2016. Descriptive statistics is presented as mean ± standard deviation, median, maximum and minimum.

One-way analysis of variance (ANOVA) and a Two-sample t-test at a significant level of 0.05 were performed to determine whether the data exhibited significant differences. Comparative assessment of variables was done using correlation and simple linear regression analysis.

3.9 Data presentation

Data was presented graphically using bar charts, box and whisker plots, pie charts, and scatter plots to highlight the salient features of the data, compare data sets and observe trends of the distribution. The findings were depicted using tables and percentages.

3.10 Ethical consideration

The researcher sought approval or clearance from Kyambogo University through an introductory letter (Appendix I) obtained from the Department of Civil and Environmental Engineering, Faculty of Engineering, to facilitate clearance from the Government Analytical Laboratory (GAL) and ensure adherence to ethical guidelines throughout the process of collecting data.

At the start of the data collection process, the researcher obtained authorization from the Lubigi faecal sludge and wastewater treatment plant management to allow the researcher access to the study area to obtain any information for the study. The individuals and entities involved in this dissertation were assured of the confidentiality of the provided information. It was made clear that the study findings would be utilized solely for academic purposes and any required corrective actions within the plant.

CHAPTER FOUR: ANALYSIS AND DISCUSSION OF RESULTS

4.1 Introduction

This chapter shows the results obtained from the study. In addition, a discussion of the results is also presented here within. These results and discussions are presented as per the specific objectives of the study.

4.2 Quantities of PhACs in wastewater and faecal sludge received at Lubigi WSP.

4.2.1 Concentrations of PhACs in different incoming waste streams

Of the broad range of CECs identified at the Lubigi incoming waste streams, 44% (22 No.) of them were Pharmaceutically Active Compounds (PhACs). A summary of the physiochemical properties of the different streams and concentrations of PhACs received at the Lubigi WWTP is presented in Figure 4-1 and Table 4-1 hereafter. The wastewater streams received at Lubigi WWTP contained significant concentrations of high-consumption pharmaceutically active compounds up to maximum concentrations of 5300 $\mu gL^{-1,}$ 2600 μgL^{-1} and 700 μgL^{-}1, for antibiotics, analgesic/anti-inflammatory drugs and anthelmintic drugs respectively. Analysis of variance between the categories of PhACs found statistically significant differences between the means of the PhACs (*p=0.0293*) with anti-inflammatory drugs being significantly higher than antibiotics and anthelmintics.

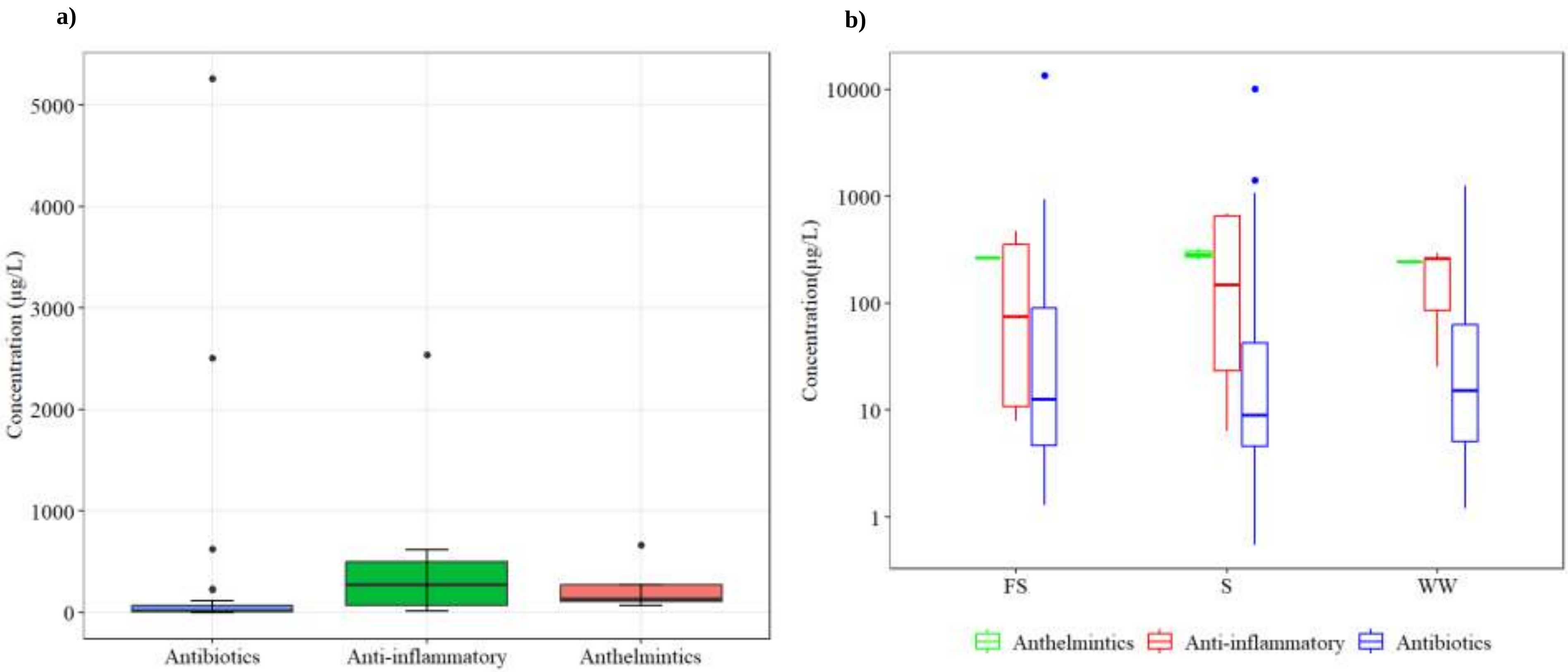


Figure 4-1: Concentration of different categories of PhACs; a) Variation in categories of PhACs entering the treatment plant, b) Variation in categories of PhACs in wastewater and FS sources. The box represents 50% of the data points, whiskers represent minimum and maximum, the line in the box represents the median. Concentrations presented on $\mathbf{Log_{10}}$ scale (n=18)

These findings are similar to those observed in a study by (K'oreje et al., 2018), where the concentrations of analgesic/anti-inflammatory and antiretroviral drugs were notably high in untreated influent across all wastewater treatment plants (WSPs). Paracetamol (ranging from 260 to 1090 μgL^{-1}) and ibuprofen (ranging from 8 to 81 μgL^{-1}) were particularly prevalent in the highest concentrations among the analgesic/anti-inflammatory drugs in the untreated influent.

This can be attributed to these compounds being among the topmost unregulated, commonly sold and consumed over-the-counter drugs in Kampala with anti-inflammatory drugs especially diclofenac among the most commonly prescribed drugs having a reporting rate of (24-100%) (Dalahmeh et al., 2020). A study by (Ziylan and Ince, 2011) also reports anti-inflammatory medications as the most widely used drugs, with an approximate annual consumption of several hundred tons in many developed nations. Additionally, the prominence of these drugs is attributed to their wide usage for addressing pain and inflammation which are common health issues in developing countries like Uganda (K'oreje et al., 2016). Notably, many analgesic/anti-inflammatory medications are not only readily available over-the-counter but are also consumed at considerable daily doses and without prescription (Ziylan and Ince, 2011). For instance, according to (K'oreje et al., 2018), the World Health Organization (WHO) has established the daily dose for paracetamol and ibuprofen as 3g and 1.2g, respectively.

The high occurrence of anthelmintics and antibiotics is associated with a high prevalence of worm and bacterial infections respectively (Verlicchi, Al Aukidy and Zambello, 2012).

An evaluation of various pharmaceutical categories in different sources reveals that anthelmintics are found in substantial quantities across all sources, including wastewater from sewer networks, faecal sludge from pit latrines, and septage. This phenomenon can be attributed to the high prevalence of parasitic worm infections, which affect both residents of slum areas and affluent neighbourhoods. According to (Hajare et al., 2021), over 3.5 billion people worldwide are affected by severe intestinal parasitic worm infections. In addition, a study by (Fuhrimann et al., 2016) reported the highest point-prevalence of intestinal parasite infections in Kampala at 75.9%. Furthermore, these drugs are cost-effective and readily accessible to individuals across different socio-economic strata.

In contrast, anti-inflammatory drugs and antibiotics are present in elevated concentrations in wastewater from the sewer network and septage. This can be attributed to the fact that these medications tend to be more expensive and are easily affordable to residents in urban areas of Kampala who predominantly use toilets for human waste disposal.

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Table 4-1: A summary of PhACs received at the plant, their concentrations

Source		Amoxicillin	Diclofenac	Erythromycin	Tylosin	Sulfadiazine	Paracetamol	Sulfapyridine	Sulfamerazine	Ciprofloxacin	Sulfamethoxazole	Tetracycline	Oxytetracycline	Enrofloxacin	Chloramphenicol	Sulfachloropyridazine	Ampicillin	Chlortetracycline	Ibuprofen	Penicillin	Gentamicin	Sulfaquinoxaline	Albendazole
Wastewater from the sewer network	**Median**	32.1	264.1	60.7	2.0	16.5	27.2	0.0	32.3	18.2	35.0	7.0	4.0	4.5	0.6	0.6	232.3	627.6	0.0	0.0	0.0	0.0	120.1
	Mean	46.9	270.1	61.7	65.8	16.9	76.1	1.0	33.3	19.2	105.6	11.6	4.4	4.4	1.6	1.6	304.1	628.6	0.0	0.0	21.4	3.6	121.1
	stdev	39.8	12.6	61.7	111.7	10.5	101.0	1.7	29.0	13.1	123.8	12.5	2.0	3.0	2.1	2.1	275.4	628.6	0.0	0.0	30.3	6.3	121.1
	Min	12.0	260.3	0.0	0.0	5.6	0.0	0.0	4.3	6.1	32.2	0.0	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Max	111.4	291.8	125.3	259.4	29.3	250.0	4.0	64.2	34.2	320.0	32.5	7.6	8.4	5.2	5.2	752.0	1259.2	0.0	0.0	64.2	14.5	244.2
Septage	**Median**	18.0	665.8	0.0	0.0	9.0	21.1	0.0	4.5	10.3	51.7	4.5	2.3	4.7	0.3	537.6	66.0	0.0	0.0	0.0	0.0	6.7	125.1
	Mean	30.0	618.3	42.3	0.0	9.0	24.3	1.4	5.1	19.2	115.1	9.8	2.6	3.6	0.9	621.4	69.3	2509.4	0.0	0.0	17.1	6.7	143.3
	stdev	23.2	98.7	73.2	0.0	1.6	16.0	2.5	1.3	18.1	118.9	9.7	1.0	2.1	1.3	632.6	67.6	4346.4	0.0	0.0	29.6	6.7	145.6
	Min	13.8	450.6	0.0	0.0	7.2	6.4	0.0	4.1	6.1	36.7	3.5	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Max	70.1	691.1	169.0	0.0	10.7	48.8	5.7	7.4	50.0	320.2	26.6	4.2	4.8	3.1	1410.5	145.4	10037.6	0.0	0.0	68.4	13.4	323.1
Faecal sludge from pit latrines	**Median**	16.6	367.4	0.0	0.0	8.3	10.4	2.7	20.5	8.3	44.1	4.6	2.6	4.7	0.7	67.1	80.5	391.0	0.0	2.2	0.0	0.0	0.0
	Mean	13.7	381.1	24.3	236.3	11.8	11.7	78.6	30.3	8.9	90.8	3.6	2.5	3.5	11.3	224.3	99.2	3557.2	0.0	14.8	22.5	0.0	66.5
	stdev	5.9	61.8	42.2	409.4	7.8	3.8	133.1	29.1	6.9	112.2	2.1	0.5	2.0	18.7	315.8	102.6	5718.7	0.0	23.1	39.0	0.0	115.1
	Min	3.6	314.8	0.0	0.0	5.5	8.0	0.0	4.4	0.0	0.0	0.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Max	18.1	474.7	97.3	945.4	25.0	17.9	309.1	75.8	19.1	274.8	5.2	3.1	4.7	43.7	763.1	235.7	13446.7	0.0	54.6	90.1	0.0	265.8

Of the PhACs identified, (Figure 4-2 a), chlortetracycline, diclofenac, sulfachloropyridazine, tylosin, ampicillin, albendazole, sulfamethoxazole, amoxicillin, erythromycin, and paracetamol were the dominant ones with average concentration ranging from 20-3000 μgL^{-1} as shown in Figure 4-2 b.

Looking at the individual streams (Figure 4-2 b), chlortetracycline, diclofenac, albendazole, ampicillin, erythromycin, and amoxicillin are dominant in all waste. Chlortetracycline, diclofenac, albendazole, and ampicillin were dominant in the wastewater stream from the sewer network while chlortetracycline, sulfachloropyridazine, diclofenac and albendazole were noted to dominate in septage. The concentrations of chlortetracycline and tylosin were found to be higher in FS from pit latrines than in the other two waste streams. Noted is the absence of penicillin in the wastewater stream and septage, the absence of tylosin in septage and the absence of sulfaquinoxaline in FS from pit latrines. The average concentrations of these dominant PhACs (Table 4-1) ranged from 30-700 μgL^{-1} (Wastewater from sewer network), 30-2500 μgL^{-1} (Septage) and 40-5300 μgL^{-1} (FS from pit latrines). Ibuprofen was also noted to exist in very minimal amounts in all the wastewater streams.

Overall, PhACs were found to exist to average concentrations of 190 μg/L, 70 μg/L, and 250 μg/L in wastewater from the sewer network, septage, and faecal sludge from pit latrines, respectively, received at Lubigi WSP. Median concentrations were measured at 19.223 μg/L, 13.429 μg/L, and 18.641 μg/L in wastewater from the sewer network, septage, and faecal sludge from pit latrines, respectively.

The maximum average concentrations were 700 μgL^{-1}, 2500 μgL^{-1} and 5300 μgL^{-1} in wastewater from the sewer network, septage and faecal sludge from pit latrines respectively, while the minimum average concentrations of 1.00 μgL^{-1}, 0.92 μgL^{-1} and 2.54 μgL^{-1} in wastewater from the sewer network, septage and in faecal sludge from pit latrines respectively.

Even at their minimum levels, these concentrations surpass the environmental limits recommended for discharges of faecal sludge and wastewater. The permissible limit for most pharmaceutical elements in the environment is less than 0.00001μgL^{-1} (Pivetta and Do Carmo Cauduro Gastaldini, 2019). For example, the permissible limits for Paracetamol, Diclofenac and Ibuprofen are 0.006μgL^{-1}, 0.00025μgL^{-1}, and 0.006μgL^{-1} respectively (National Association of Clean Water Agencies, 2011; Pivetta and Do Carmo Cauduro Gastaldini, 2019). This implies a potential risk if the water and faecal sludge are discharged untreated into the environment. An assessment for statistical differences of the data for the inlet streams returned a p-value of 0.5655 and hence statistical difference between the mean concentrations ($p>0.05$) implying that there was no significant difference.

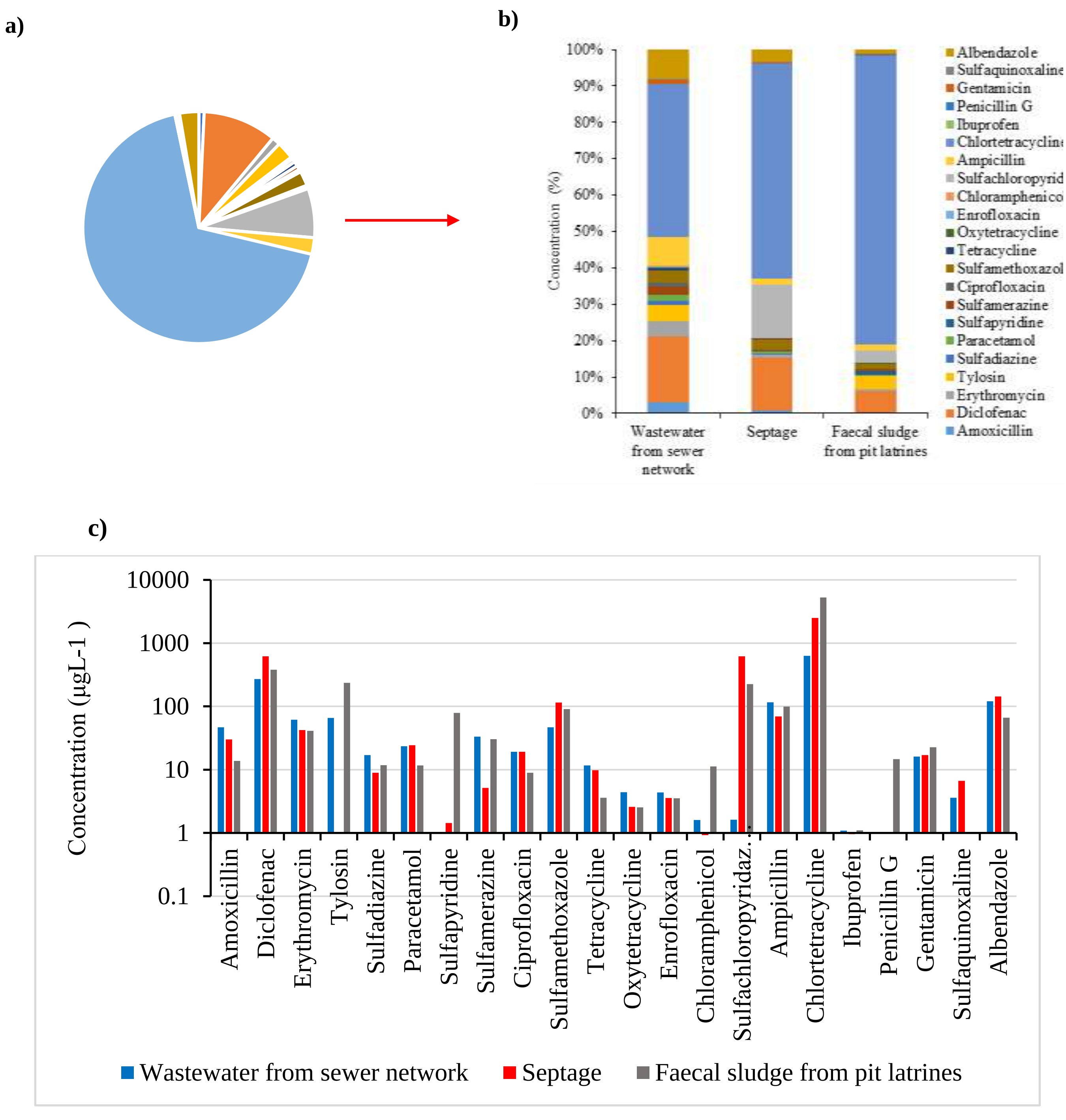


Figure 4-2: Composition (based on mean values) of PhACs in the waste streams received at the Lubigi WWTP; a) Percentage composition in all streams received, b) Percentage composition in individual streams and c) Variations of individual PhACs in the three waste streams into the WWTP (Concentrations presented on Log_{10} scale) (n=18)

The concentrations of individual pharmaceutical elements also vary significantly within each for each waste stream as shown in box and whisker plots in Figure 4-3.

The PhACs exist to median concentrations of 19.223 μgL^{-1}, 13.429 μgL^{-1} and 18.641 μgL^{-1} in wastewater from the sewer network, septage and faecal sludge from pit latrines respectively (Figure 4.3 a). Generally, the results in this study, indicate that the concentrations of these pharmaceutical contaminants were higher in wastewater from the sewer network and faecal sludge from pit latrines and lower in septage.

Further analysis for variation within individual streams notes significantly higher concentrations of chlortetracycline, ampicillin, diclofenac and albendazole in wastewater from the sewer network, while septage contains significant amounts of diclofenac, sulfachloropyridazine, diclofenac and albendazole. In FS from pit latrines, significant quantities of chlortetracycline and tylosin were noted.

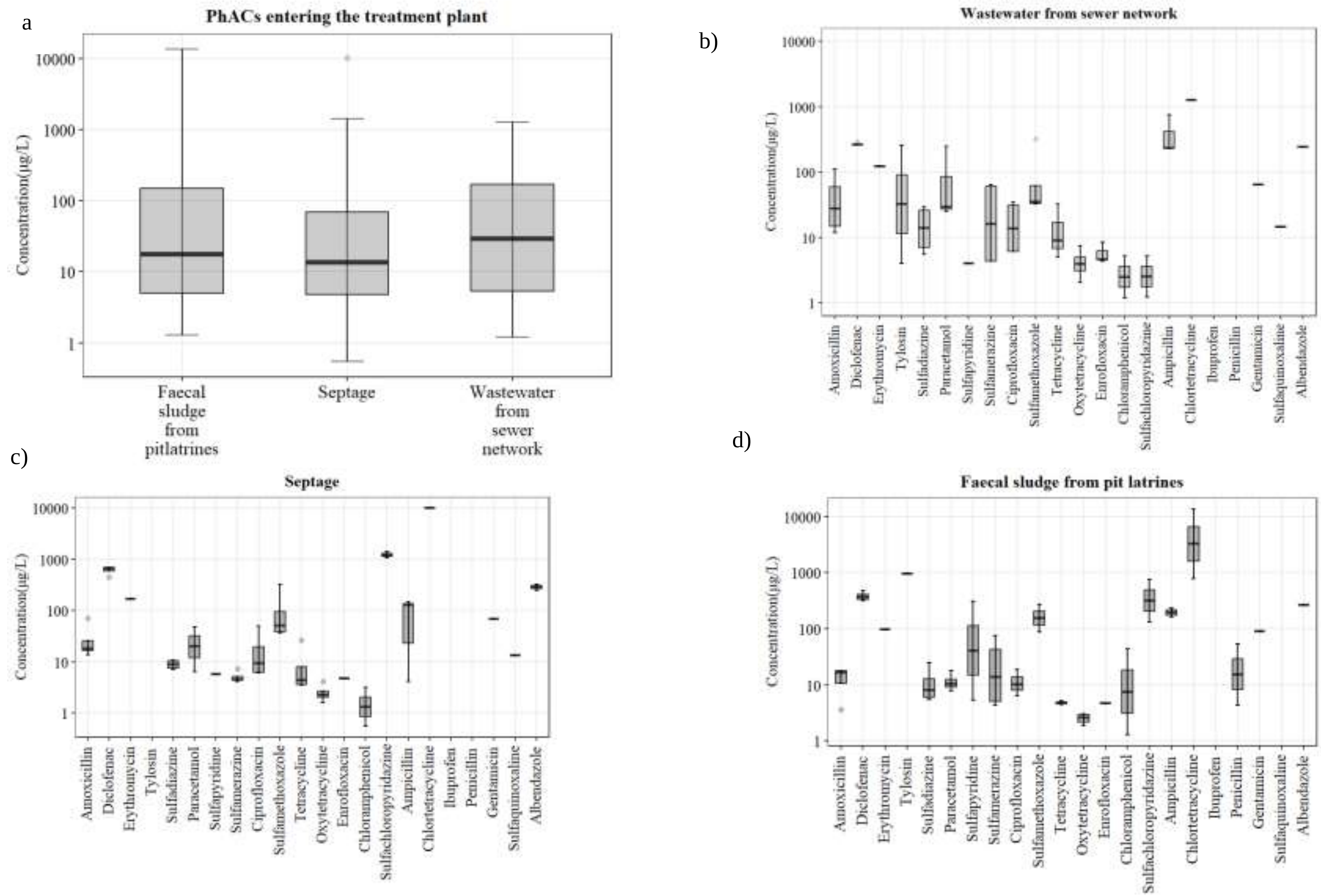


Figure 4-3: Concentrations of PhACs in the waste streams received at Lubigi WWT; a) overall concentration, b) concentration in wastewater received from the sewer network, c) concentrations of PhACs in septage and d) concentration at the gulper station that receives faecal sludge from pit latrines. The box represents 50% of the data points, whiskers represent the minimum and maximum, line in the box represents the median (Concentration is presented on Log_{10} scale) (n=18)

These results reflect those of (Sim, Lee and Oh, 2010; Sim et al., 2011; Gracia-Lor et al., 2012; Botero-Coy et al., 2018) which show ranges of <1 μgL^{-1} to >1000 μgL^{-1} for pharmaceutical concentrations in effluent for municipal wastewater. In addition, a study by (Conn et al., 2010) detected concentrations of pharmaceuticals in wastewater samples from septic tank effluents ranging from <1 μgL^{-1} to >300 μgL^{-1} while (Gros et al., 2020) detected concentrations of 76 to 7400 μgL^{-1} in faecal sludge solid fractions from pit latrines.

The high concentration of PhACs in faecal sludge from pit latrines could be attributed to the fact that pit latrines do not effectively treat wastewater. They simply collect and store human waste, which must then be periodically emptied and disposed of. Pit latrines only act as storage facilities for the sludge (Bakare et al, 2012), and processes required to degrade these PhACs may not be present in pit latrines hence their occurrence in high concentrations. Additionally, most pit latrines are located in slum areas of Kampala, similar to many other poor areas of Sub-Saharan Africa (SSA), where pit latrines are predominantly used for human excreta disposal (Nakagiri et al., 2015). Such areas are greatly affected by the high prevalence of illnesses especially bacterial infections as a result of poor hygiene and lack of access to sufficient water, sanitation, and hygiene (WASH) practices. It is reported that approximately 7.75% of all deaths caused by diarrheal diseases in SSA are linked to inadequate WASH practices with a high Risk Factor Attribution (RFA) percentage of 95.93% (Zerbo, Castro Delgado and Arcos González, 2021). Therefore, the consumption of drugs is highest in these areas explaining the high levels of PhACs in pit latrines.

The high concentrations of PhACs in wastewater from the sewer network are attributed to the fact that the sewer network receives large volumes of wastewater from many different wastewater sources which include; domestic wastewater from households that constitutes water from sinks, toilets, showers, baths, and laundry activities; wastewater generated by commercial establishments such as hospitals, clinics, restaurants, offices, and shops which comprises kitchen wastewater, restroom waste, and other sources specific to the business; industrial waste such as institutions such as schools, hospitals that discharge wastewater containing PhACs to the sewer network (Tilley, 2014; Gao et al., 2023). All of these sources contribute to an increase in the levels of these micropollutants in wastewater from the sewer network (Gao et al., 2023). However, these concentrations are lower than those in pit latrines. This could be attributed to dilution in sewer networks which occurs primarily due to the continuous flow of wastewater from various sources, including households, and commercial establishments. As wastewater from these different sources mixes, the overall concentration of contaminants, such as PhACs, is reduced. Additionally, stormwater inflow during rainfall or snowmelt can significantly increase the volume of water in the system, further diluting the pollutants. The mixing of different types of wastewater with varying levels of contamination, combined with the turbulence and flow dynamics in the sewer pipes, also contributes to the dilution of PhACs as wastewater moves through the network towards treatment facilities (Gao et al., 2023).

The low levels of PhACs in septage can be attributed to the role of septic tanks as pre-treatment facilities for wastewater. Septic tanks facilitate the anaerobic digestion of solid sludge (containing these PhACs), that settles at the bottom as the liquid portion settles at the top. This separation of solid and liquid waste in septic tanks allows for effective pre-treatment of these micropollutants before they reach the WWTP. A study by (Kang et al., 2019) looked at the presence and removal of microcontaminants in the sewage treatment tanks, commonly utilized in rural communities in Korea. The STTs demonstrated significant removal efficiencies for the micropollutants ranging from 86% for caffeine, 86% for acetaminophen, 72% for ibuprofen, and 63% for acetaminophen (naproxen). These findings highlight the important role of septic tanks in the elimination of micropollutants. Septic tanks employ automated operating systems and easy treatment methods, contributing to the removal of a portion of these micropollutants and enhancing the overall effectiveness of waste stabilization ponds in removing these contaminants. Therefore septic tanks provide a certain degree of treatment to wastewater, although they may not always be able to remove all of the contaminants present in wastewater (Tilley, 2014).

In addition, the lower levels of PhACs in septic tanks could be a result of dilution in septic tanks which occurs due to the continuous addition of wastewater from household activities such as bathing, laundry, and dishwashing. These large volumes of water mix with the sewage and faecal sludge, reducing the concentration of contaminants, including PhACs. As water enters the tank, it separates into three layers: the scum on top, the liquid in the middle, and the sludge at the bottom. The relatively higher volume of liquid compared to the solid sludge dilutes the concentration of PhACs reducing the overall concentration of PhACs in the FS from septic tanks (Tilley, 2014; Gao et al., 2023).

Furthermore, in septic tanks, some PhACs are lost through the liquid fraction that flows into the soak pit, further reducing the concentration of PhACs in the remaining sludge.

The higher concentrations of PhACs in pit latrines can be attributed to their function as containment units, where all waste is stored without significant dilution or loss of PhACs to a soak pit as in septic tanks. As a result, the waste, including PhACs, accumulates in the pit and is eventually transported directly to the treatment plant, leading to higher concentrations of these contaminants in the sludge compared to WW from sewers and FS from septic tanks.

It is important to note that other factors, such as types of drugs, patterns of drug use, population and demographics, environmental factors, condition and design of the sewer infrastructure, pharmaceutical manufacturing and disposal practices, as well as regulation and monitoring practices can also affect the concentrations of PhACs in wastewater (Gupta et al., 2022).

4.2.2 Diurnal fluctuations of PhACs in the different inlet streams received

Figure 4-4 below shows diurnal variations of the concentrations of PhACs received at Lubigi WWTP.

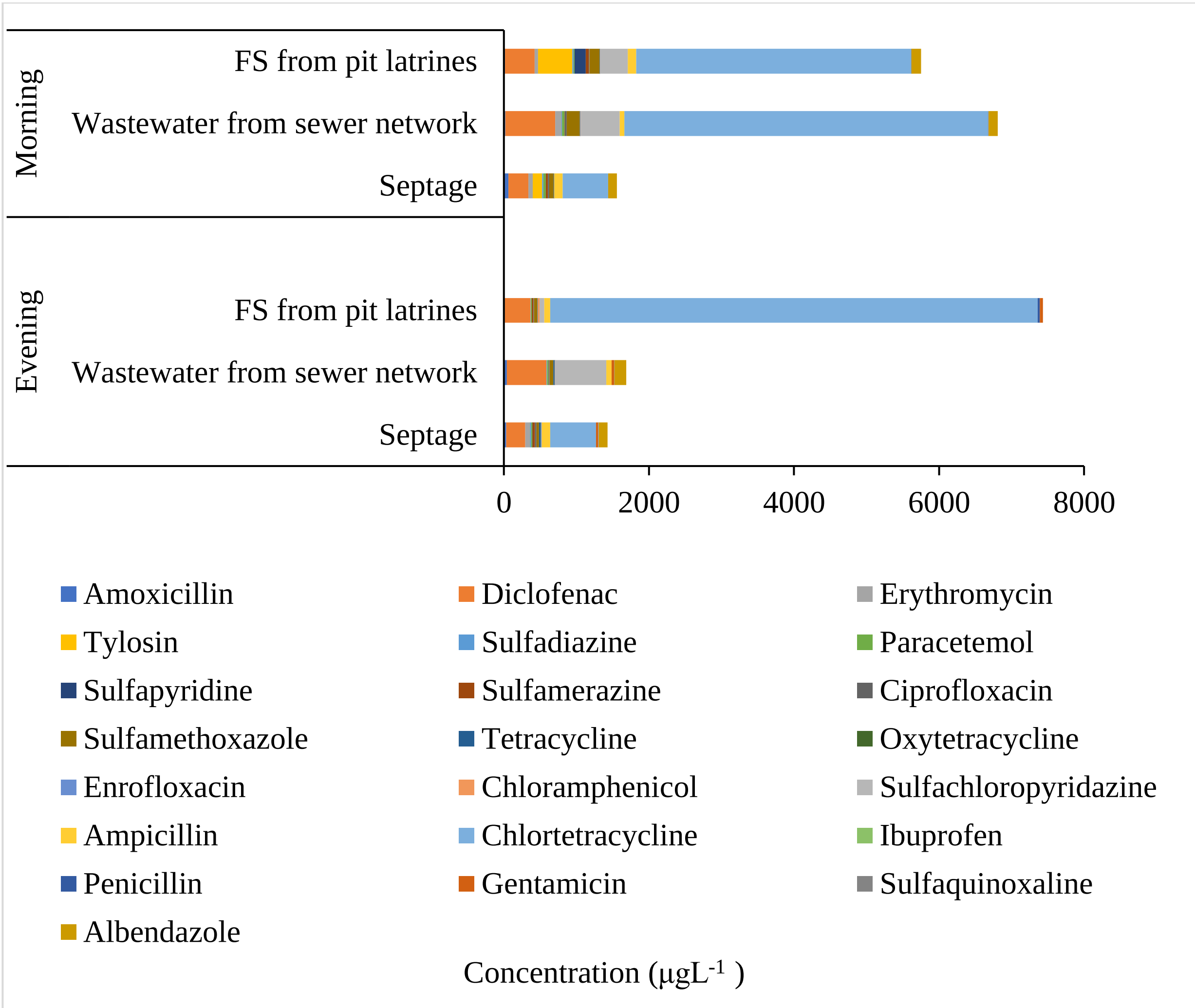


Figure 4-4: Variation of PhACs in wastewater received in the morning and evening (n=9)

The composition of PhACs in the wastewater from the sewer network, septic tanks and pit latrines was found to be generally lower in the evening than in the mornings with maximum PhACs concentrations of 630 μgL^{-1}, 5000 μgL^{-1}, 3800 μgL^{-1} for septage, wastewater from sewer network, and faecal sludge from pit latrines respectively in the morning to up to 600 μgL^{-1}, 700 μgL-1, 6700 μgL^{-1} for septage, wastewater from sewer network and faecal sludge from pit latrines respectively in the evening.

The total concentration of PhACs received at the Lubigi WWTP was 1600 μgL^{-1}, 6900 μgL^{-1} and 5800 μgL^{-1} for septage, wastewater from the sewer network and pit latrine faecal sludge respectively (received in the morning), to 1500 μgL^{-1}, 1700 μgL^{-1} and 7500 μgL^{-1} for septage, wastewater from the sewer network, and pit latrine faecal sludge, respectively (received in the evening).

The concentrations of PhACs in wastewater within the sewer network are generally lower in the evening compared to the morning due to several reasons. Firstly, the diurnal variation in activities where during the morning, there is typically a higher level of activity, such as high residential activity like personal hygiene routines, preparing meals, and household chores, high industrial and commercial activities since mornings are peak working hours (Gao et al., 2023). This increased activity contributes to higher concentrations of these micropollutants in wastewater during the morning. In the evening, however, these activities typically reduce resulting in lower volumes of wastewater entering the sewer network, leading to lower concentrations of PhACs.

Additionally, throughout the day, as more wastewater is generated from various sources enters the sewer network, dilution of the wastewater occurs hence a reduction in levels of these micropollutants present in the sewer as the day progresses. Furthermore, microorganisms present in wastewater can biodegrade certain pollutants over time (Dirckx et al., 2019). As wastewater remains in the sewer network throughout the day, there is more time for natural processes, including microbial degradation, to reduce the concentrations of PhACs, leading to lower levels in the evening.

These high levels of PhACs in faecal sludge collected from pit latrines and septic tanks in the morning could be due to the disturbances that occur between collection and delivery since the sludge is first stored throughout the night and then transported in the morning. However, the faecal sludge collected in the evening is delivered directly to the WWTP undisturbed. In addition, some micropollutants are susceptible to natural degradation processes. As the wastewater remains in the pit latrines or septic tanks throughout the day, microbial activity and other natural degradation mechanisms can break down and reduce the levels of certain micropollutants in faecal sludge collected in the evening (Tilley, 2014).

It is observed that the total concentration of PhACs in faecal sludge from pit latrines increases in the evening. This could be due to the high concentration of the sludge collected that has not had enough time for degradation of these micropollutants.

4.2.3 An assessment of the physicochemical parameters and how they affect the levels of PhACs in the wastewater streams arriving at Lubigi WWTP

4.2.3.1 An assessment of physicochemical parameters in the wastewater streams

Four physicochemical parameters i.e. Electrical Conductivity (EC), Total Suspended Solids (TSS), Dissolved oxygen (DO) and pH were tested for the different wastewater streams to assess their influence on the level of concentration of pharmaceutical contaminants received at the Lubigi WWTP (Table 4-1). The results in Figure 4-5 show box and whisker plots of the variations of physiochemical parameters in the different waste streams received at Lubigi WWTP with average values of these parameters shown in Table 4-2.

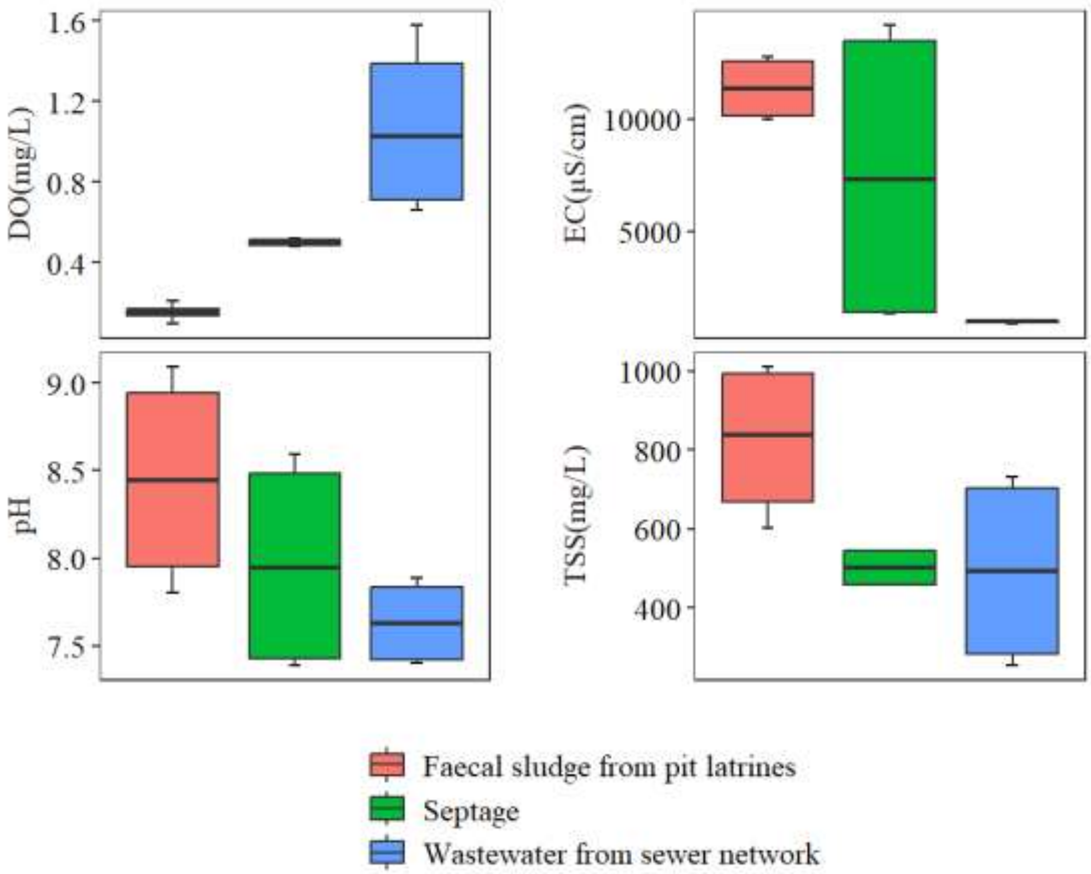


Figure 4-5: Box plots showing the variation of physiochemical parameters in different WW and FS streams (n=9)

Table 4-2: A summary of physicochemical parameters of the waste streams

Wastewater and FS stream	Statistic	EC (µs/cm)	pH	DO (mg/l)	TSS (mg/l)
Wastewater from the sewer network	Mean	979.50	7.64	1.07	492.75
	median	989.00	7.63	1.03	493.00
	stdev	30.51	0.22	0.39	220.09
	minimum	930.00	7.40	0.66	254.00
	maximum	1010.00	7.89	1.58	731.00
Septage	Mean	7552.25	7.97	0.50	501.00
	median	7336.50	7.95	0.50	501.00
	stdev	6176.76	0.55	0.02	43.00
	minimum	1346.00	7.39	0.48	458.00
	maximum	14190.00	8.59	0.52	544.00
Faecal sludge from pit latrines	Mean	11361.25	8.45	0.16	822.25
	median	11352.50	8.45	0.16	839.00
	stdev	1278.20	0.55	0.04	178.87
	minimum	9990.00	7.80	0.10	601.00
	maximum	12750.00	9.09	0.21	1010.00

The results indicate that TSS, EC and pH are generally higher in faecal sludge from pit latrines, followed by septage and lastly wastewater from the sewer network. The DO is higher in wastewater from the sewer network, followed by septage and then faecal sludge from pit latrines.

These findings align with those present in existing literature. A study by (Doglas, Kimwaga and Mayo, 2021) examined the physical-chemical parameters of FS to determine their potential as indicators for dewatering performance across various on-site sanitation facilities in unplanned settlements situated in Dar es Salaam, Tanzania. The outcomes revealed that the average values of the measured physical-chemical indicators were notably higher in FS originating from pit latrines compared to other sources ($\alpha <$

0.005). Particularly, the EC values in pit latrine-derived FS were 1.2–2 times greater than those found in other types of containment i.e. septic tanks and soak-away pits. Furthermore, the FS from pit latrines exhibited a higher total solids (TS) content in contrast to that observed in other containment systems.

This could be attributed to the presence of high contamination of organic matter in faecal sludge from pit latrines since pit latrines only act as storage facilities and do not treat the faecal matter effectively, followed by septage because it allows for sedimentation and settling of sludge solids and partial degradation before the septage is collected and lower in wastewater from the sewer network as a result of continuous dilution of wastewater as more is added as well as natural degradation that occurs in the sewer system.

4.2.3.2 Relationship between physicochemical parameters and concentration of PhACs in the wastewater stream

An assessment of the relationship between the physiochemical parameters (TSS, EC, DO and pH) and the PhACs selected from at least each pharmaceutical category was done. The PhACs selected were amoxicillin, paracetamol, diclofenac, and albendazole.

The correlation coefficients for all parameters were less than 0.5 indicating a weak relationship. Regression analysis yielded R-squared values of 0.0069, 9.9×10^{-6}, 0.0032 and 0.0036 for TSS, EC, DO and pH respectively indicating that less than 0.4% of these physicochemical parameters in these wastewater source streams arriving at the plant can be explained by the levels of concentration of PhACs.

The relationship between the physicochemical parameters and concentration of pharmaceutical elements in the incoming wastewater streams is shown in Figure 4-6 below.

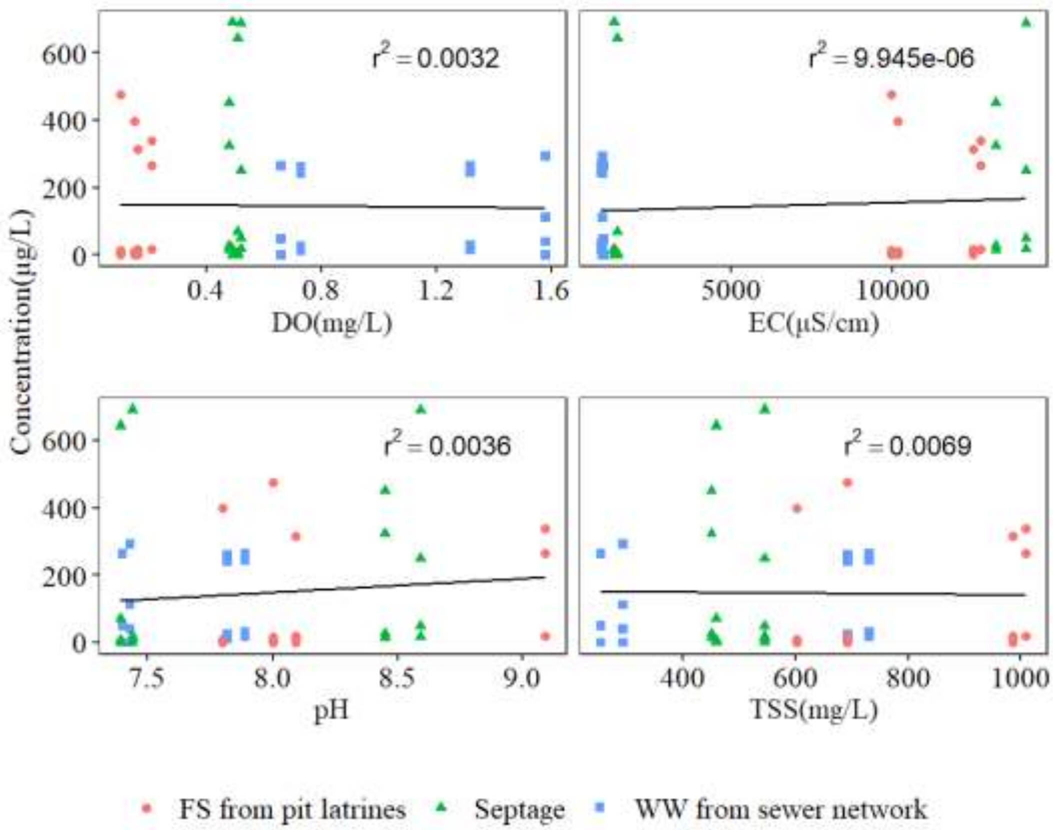


Figure 4-6: Relationship between physicochemical parameters and concentration of PhACs for different sources of wastewater and faecal sludge (n=9)

Therefore, this study found a weak correlation between these physicochemical parameters and the concentration of PhACs in the waste streams arriving at Lubigi WWTP. This low extent of correlation may be attributed to the independent occurrence of these pharmaceutical contaminants in wastewater, irrespective of other parameters. The occurrence of pharmaceutical micropollutants in wastewater is influenced by various factors, including but not limited to physicochemical parameters such as total suspended solids (TSS) and electrical conductivity (EC) (Fawzi, Khasawneh and Palaniandy, 2021). However, it is important to note that the presence of these PhACs in wastewater is not solely determined by these physicochemical parameters.

Pharmaceutical micropollutants enter wastewater via several pathways, including domestic sewage, agricultural runoff and industrial discharges (Patel et al., 2019). The occurrence of pharmaceutical micropollutants in wastewater is influenced by factors such as population density, usage patterns, prescribing habits, and improper disposal of medications. These factors contribute to the overall concentration of pharmaceuticals in wastewater, irrespective of the physicochemical parameters.

While physicochemical parameters can affect the transport, fate, and behaviour of pharmaceutical micropollutants in wastewater treatment processes (Olabode, Olorundare and Somerset, 2020), their presence in wastewater is primarily determined by the patterns of human consumption and disposal (Ebele, Abou-Elwafa Abdallah and Harrad, 2017; Biel-Maeso et al., 2018; Patel et al., 2019). The physicochemical parameters of wastewater may indirectly influence the degradation or removal of pharmaceuticals during treatment (Olabode, Olorundare and Somerset, 2020), but they do not play a direct role in determining their occurrence.

Therefore, the weak relationship between physicochemical parameters and the concentration of PhACs suggests that the presence of these contaminants in wastewater is largely independent of these specific parameters and is driven by other factors related to human activities and usage patterns.

However, it is key to note that this scenario may be different when looking at the relationship between individual pharmaceutical compounds and physiochemical parameters.

4.3 Assessment of the removal efficiencies of PhACs of the Lubigi WSPs.

4.3.1 Variations of these PHACS along the treatment chain of WSPs

The results of this study showed that most of the PhACs that arrive at the wastewater treatment plant through various sources from sewer networks, septage and faecal sludge from pit latrines reach the waste stabilization ponds. Out of the 22 PhACs identified in the waste streams received at the Lubigi WWTP, 82% (18 No.) arrive at the waste stabilization ponds i.e. the inlet of the anaerobic pond. Figure 4-7 shows PhACs from the incoming waste streams that reach the WSPs.

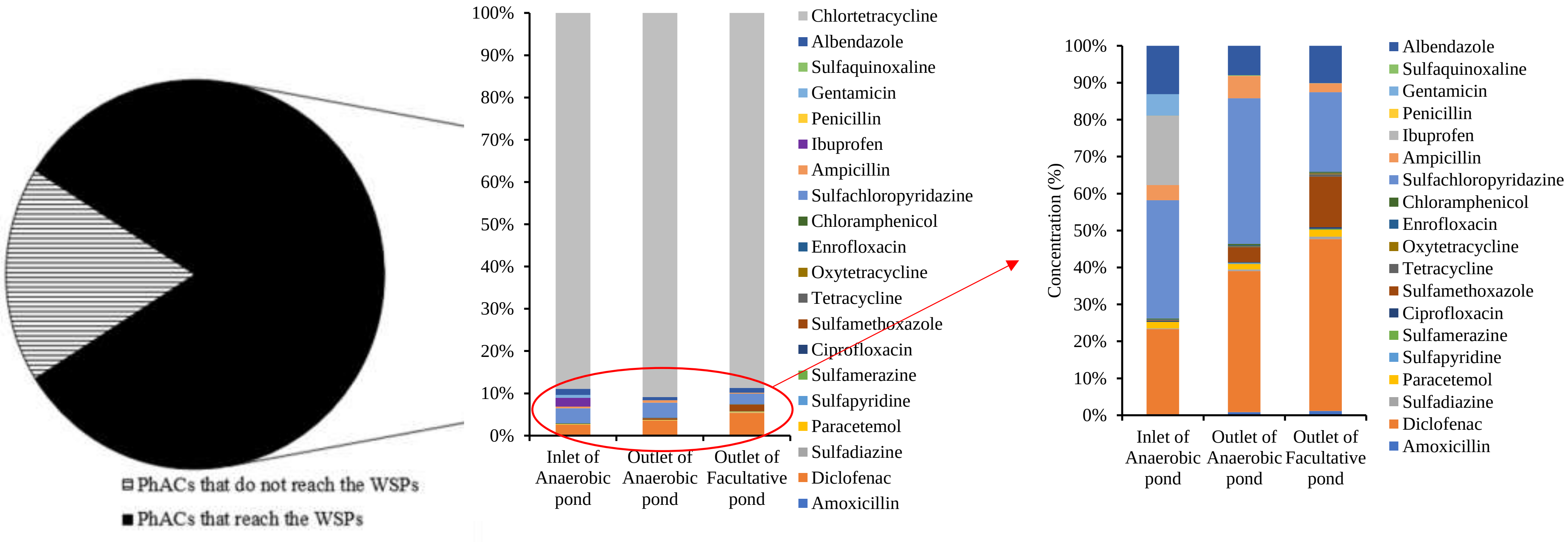


Figure 4-7: PhACs from the incoming waste streams that reach the WSPs (n=18)

This means that some of the PhACs are lost even before the treatment process by the WSPs begins as in the case of tylosin and erythromycin. These PhACs could be lost through; preliminary treatment steps such as screening or grit removal where some PhACs may be physically removed or lost (Gunarathne, Ashiq and Ginige, 2018). For example, small particulate matter or insoluble substances may settle out or get trapped in the screenings or grit, effectively reducing their concentration in the wastewater. Other PhACs are lost through sorption to surfaces where these micropollutants can adsorb or adhere to surfaces within the treatment plant infrastructure such as pipes, equipment, or tank walls (Gracia-Lor et al., 2012). Furthermore, volatile PhACs could be lost as they vaporize into the air through aeration or mixing that involves agitation or exposure to atmospheric conditions. A study by (Namkung and Rittmann, 1987) found that over 30 tons of volatile organic compounds (VOCs) are emitted annually at two publicly owned wastewater treatment plants while a study by (Mihelcic et al., 1993) revealed that among 7 out of 589 plants in California had VOC emissions exceeding 25 tons.

The large, hydrophobic ring structure of macrolides like tylosin and erythromycin might contribute to their sorption to surfaces or particulates, enhancing the possibility of volatilization and loss during the pre-treatment process. These compounds have high hydrophobic characteristics make them volatile, especially when they associate with organic matter or are present in aerosolized water droplets. This could lead to their loss before reaching the WSPs.

These PhACs can be lost through biodegradation by naturally occurring microorganisms in the wastewater where these microbes can utilize these substances as a food source, resulting in their transformation or degradation before reaching the treatment process (Hijosa-Valsero et al., 2010).

Overall, ampicillin, ibuprofen, diclofenac, sulfachloropyridazine, chlortetracycline and albendazole exist in the highest concentration (>100 μgL^{-1}), paracetamol, amoxicillin, gentamicin and sulfamethoxazole moderate amounts of 10-100 μgL^{-1} and penicillin, oxytetracycline, sulfapyridine, tetracycline, enrofloxacin, ciprofloxacin, chloramphenicol, sulfadiazine, sulfaquinoxaline, and oxytetracycline exist in the lowest concentrations of <10 μgL^{-1}.

A look at the treatment stages (Figure 4-8), showed that diclofenac, ampicillin chlortetracycline, paracetamol, sulfachloropyridazine, sulfamethoxazole, and albendazole were the most abundant (20-30,000 μgL^{-1}) in both the raw influent and final effluent (Figure 4-12). These drugs are commonly used in Uganda to treat bacterial and inflammatory infections (Dalahmeh et al., 2020).

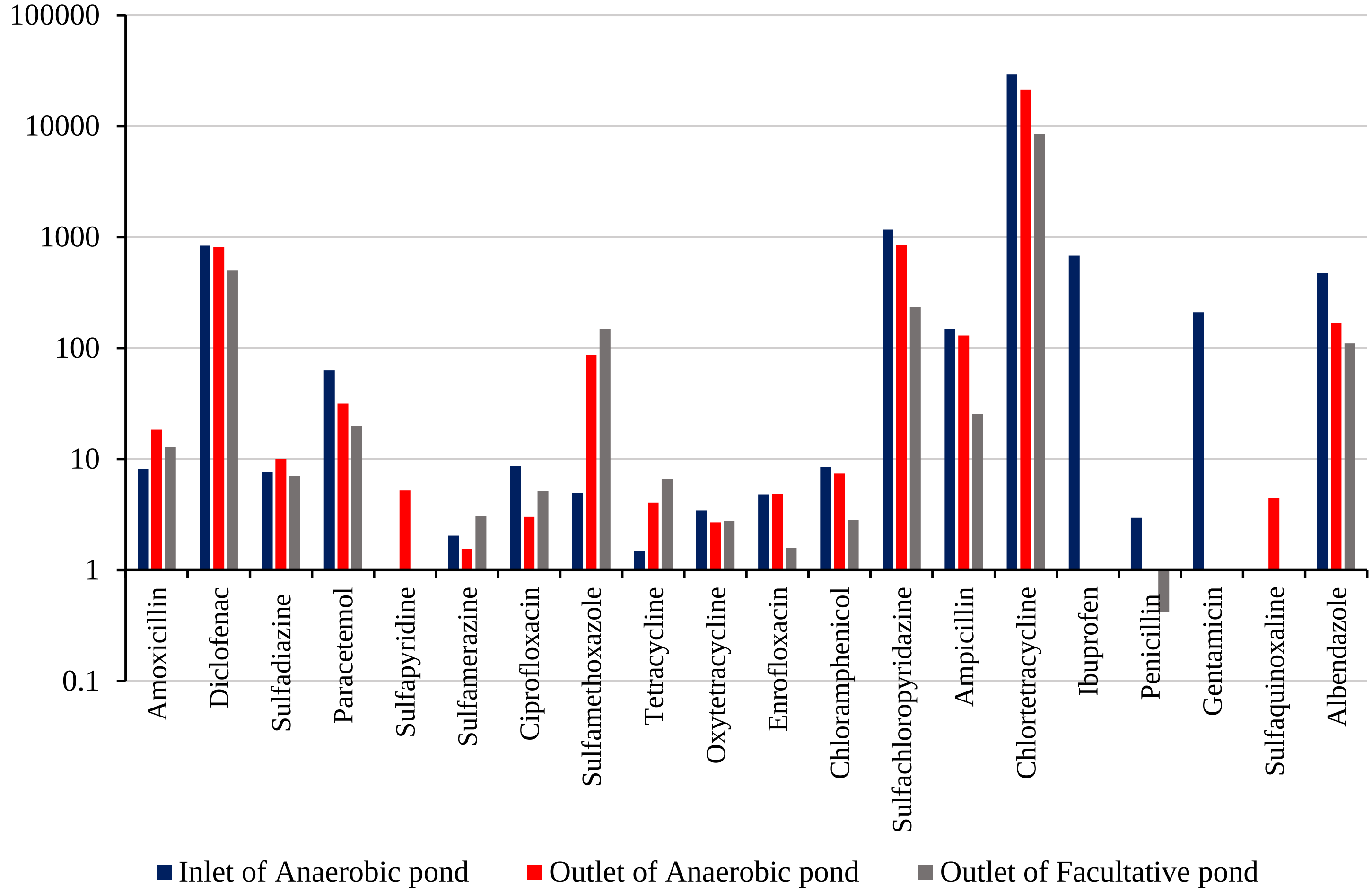


Figure 4-8: Concentrations of individual PhACs along the treatment stages (n=18)

Figure 4-9 below shows box and whisker plots showing variations of individual pharmaceutical elements along the treatment stages of the WSPs.

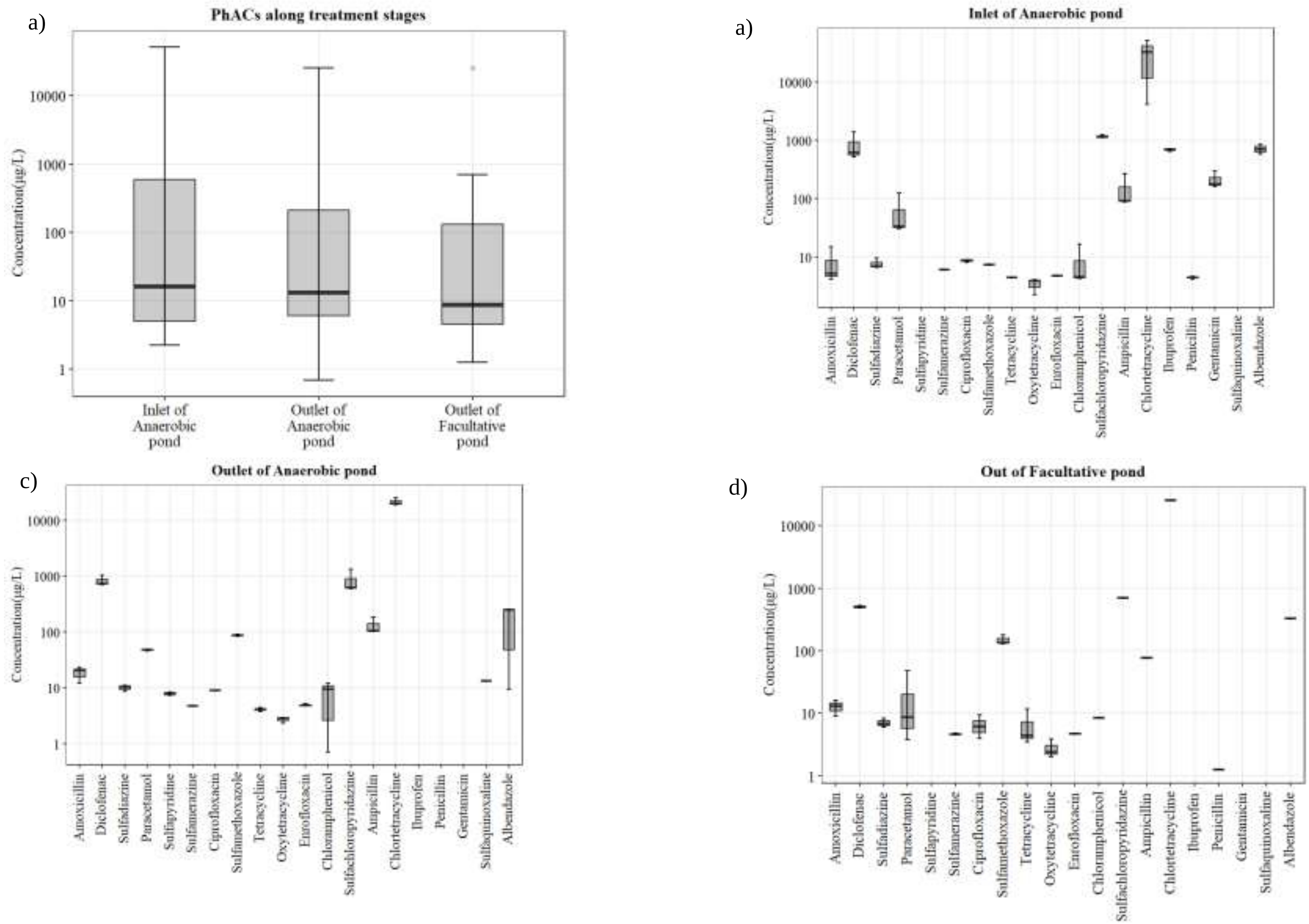


Figure 4-9: Concentrations of PhACs along the treatment stages of WSPs; a) overall concentration, b) concentration at the inlet of anaerobic pond, c) concentrations of PhACs at the outlet of anaerobic pond and d) concentration at the outlet of facultative pond. The box represents 50% of the data points, whiskers represent the minimum and maximum, line in the box represents the media. (Concentration presented on Log_{10} scale) (n=18)

PhACs along the treatment chain vary from up to 30,000 μgL^{-1} at the inlet of the anaerobic pond to up to 8,500 μgL^{-1} at the outlet of the facultative pond. This trend of reduction in levels of concentration between anaerobic and facultative ponds is also observed in studies conducted by (Gruchlik, Linge and Joll, 2018; K'oreje et al., 2018; Kumar and Kumar, 2020).

The raw influent at Lubigi WWTP is highly dominated by high-consumption PhACs like diclofenac, ibuprofen, paracetamol (analgesic/anti-inflammatory drugs); chlortetracycline, gentamicin, sulfachloropyridazine, ampicillin (antibiotics) and albendazole (anthelmintic). This can be attributed to the fact that these drugs are unregulated, readily available over-the-counter and are often taken in high doses. These anti-inflammatory and antibiotic drugs are commonly used to alleviate pain and inflammation and treat bacterial infections respectively whereas anthelmintics are mainly for treating parasitic worm infections, all of which are frequently experienced health issues in developing countries like Uganda (Verlicchi, Al Aukidy and Zambello, 2012).

The effluent is highly comprised of chlortetracycline, sulfachloropyridazine, diclofenac, albendazole and sulfamethoxazole. These drugs have the highest concentrations in the effluent due to their ineffective removal during WSPs as noted by for example as noted by (Vree et al., 1994) for sulfamethoxazole. Their concentrations remain way higher than the recommended discharge limits for faecal sludge and wastewater discharges even at the end of the treatment chain. The permissible limit for most pharmaceutical elements in the environment is less than 0.00001μgL^{-1} (Pivetta and Do Carmo Cauduro Gastaldini, 2019). For example, the permissible limits for Paracetamol, Diclofenac and Ibuprofen in drinking water where these discharges end up are 0.006μgL^{-1}, 0.00025μgL^{-1}, and 0.006μgL^{-1} respectively (National Association of Clean Water Agencies, 2011; Pivetta and Do Carmo Cauduro Gastaldini, 2019). Therefore, these pharmaceutical concentrations in the discharges can pose a huge threat to human life and environmental ecosystems if they are not minimized to the allowable environmental limits.

The total concentration of the influent of all the PhACs summed up to 32,000 μgL^{-1} and the effluent concentration at the discharge point summed up to 9,500 μgL^{-1} implying that the influent concentration is almost 4 times the effluent. This shows that WSPs are capable of eliminating some PhACs as they go through the different treatment stages.

Figure 4-10 shows how concentrations of PhACs vary from entry to exit of the WWTP.

4.3.2 Determination of removal efficiencies of PhACs of the Lubigi WSPs.

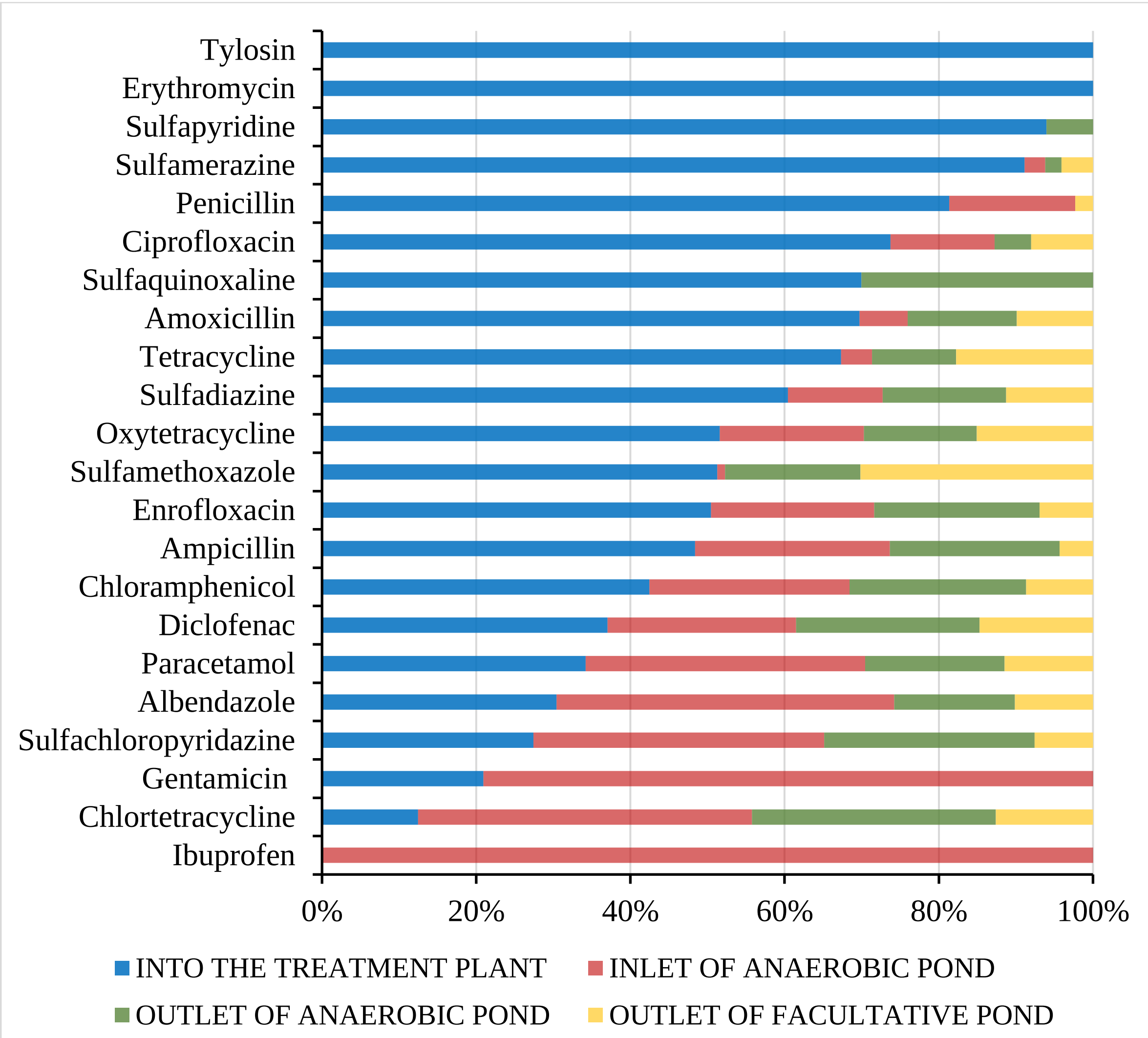


Figure 4-10: Bar chart showing how concentrations of PhACs vary from entry to exit of the WWTP (n=18)

4.3.3 Removal efficiencies before and after the WSPs

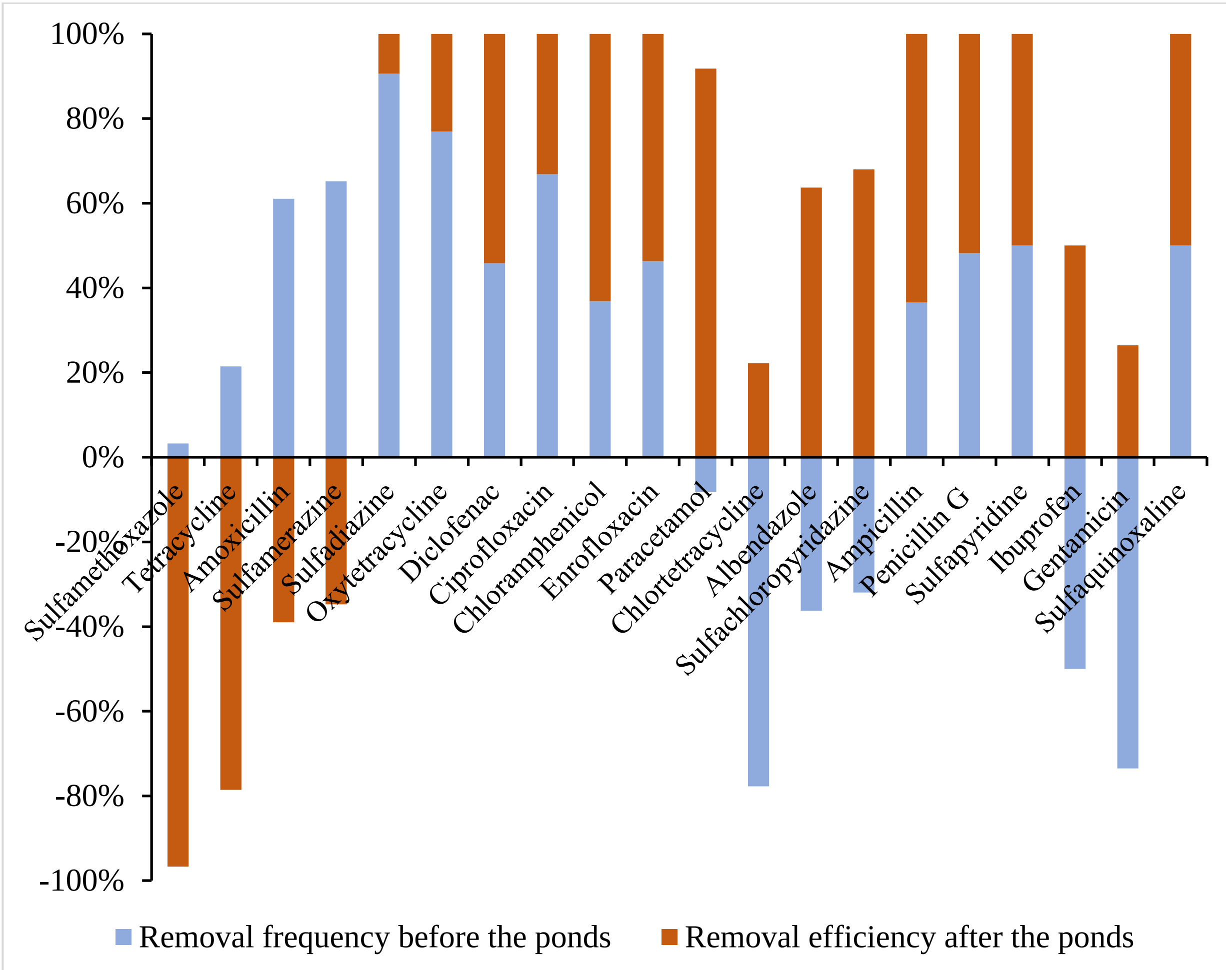


Figure 4-11: Removal efficiency before and after the WSPs (n=18)

The graphs above demonstrate that the pretreatment process in the treatment plant effectively removes a portion of contaminants. This initial treatment stage involves a simple procedure where solid particles present in the untreated influent settle and separate.

This process eliminates large particles through screens and also rids the system of grit. It additionally includes a settling and thickening tank that removes substantial, dense particles that are too small to be captured by the bar screens.

The removal of PhACs during pretreatment happens through both adsorption and sorption mechanisms (Garcia-Rodríguez et al., 2014). In adsorption, micropollutant molecules or ions physically adhere to the surface of a sorbent material, whereas in absorption, the micropollutant mixes with the sorbent material

The hydrophobicity of a pollutant influences its rate of adsorption, and this is quantified using the octanol-water partition coefficient (K_{OW}). The primary method for eliminating micropollutants during primary treatment is adsorption, which often correlates with their hydrophobicity (indicated by the K_{OW} value). Compounds with high $\log K_{OW}$ values (>3.0) strongly attach to the particles during settling, while those with low $\log K_{OW}$ values (<3.0) disperse in the aqueous phase (Wang et al., 2014) and do not bind to the particles (Thomas and Foster, 2005; Wang et al., 2014).

The predominant portion of compound removal takes place during the subsequent phase of treatment. This secondary treatment is a procedure where a combination of aerobic and anaerobic microbial activities within distinct zones degrades the majority of remaining organic matter, which evaded removal in the primary treatment. Moreover, secondary treatment eliminates a substantial quantity of fine particulate matter to which pharmaceuticals might have adhered (Thomas and Foster, 2005).

The primary treatment phase showed relatively lower removal effectiveness when compared to the removal observed in the WSPs. This suggests that the degradation of PhACs in the anaerobic and facultative ponds is more pronounced than the adsorption of pharmaceutical substances onto particles during the pre-treatment stage.

4.3.4 Removal efficiencies along the anaerobic and facultative ponds

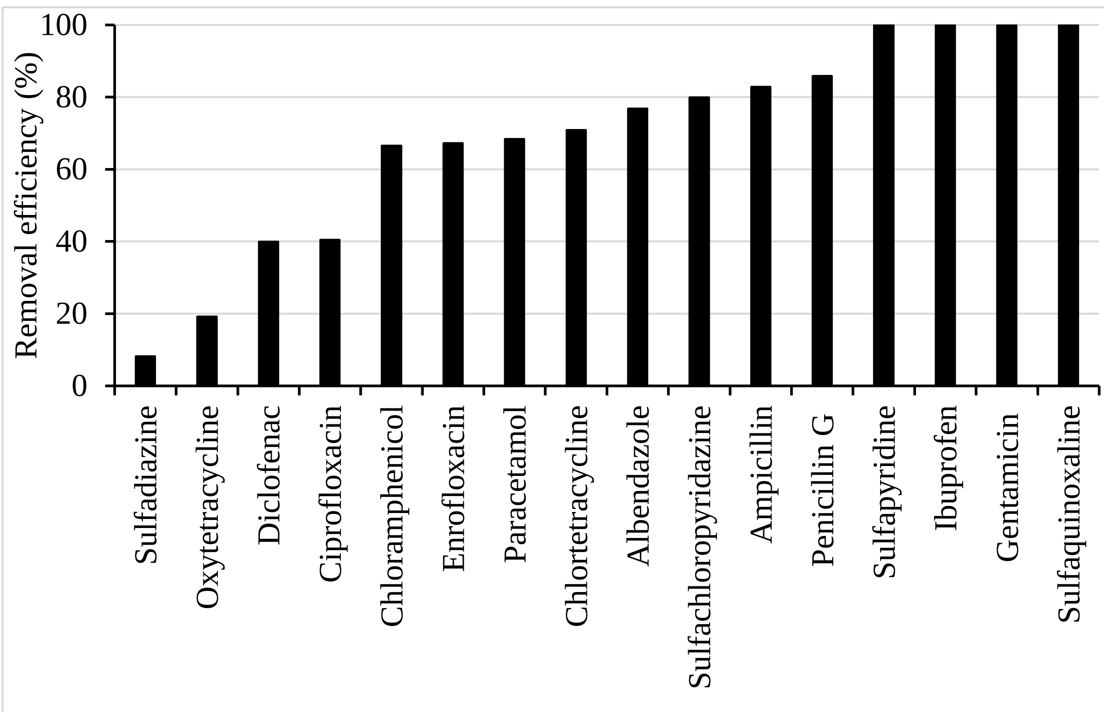


Figure 4-12: Removal efficiencies of PhACs along the WSPs (n=18)

The findings of this study indicate that the Lubigi WSPs have the ability to remove various PhACs from wastewater, with an overall removal efficiency of 76.15%. The highest removal efficiency was in the range of 70-99.99% for chlortetracycline, sulfapyridine, ampicillin, gentamicin, albendazole, ibuprofen, sulfachloropyridazine, sulfaquinoxaline, and penicillin, the moderate removal efficiency was 50-70% for paracetamol, chloramphenicol and enrofloxacin and the lowest was 1-40% for sulfadiazine, oxytetracycline, diclofenac, and ciprofloxacin. These removal efficiencies are only limited to the WSPs. Available literature has noted similar removal efficiencies as reported by (Gruchlik, Linge and Joll, 2018; Kumar and Kumar, 2020; Samal, Mahapatra and Hibzur Ali, 2022). Within the WSPs, the concentrations of the majority of the PhACs decreased by more than 80% as shown in Figure 4.12.

Removal efficiencies are attributed to various factors, such as the characteristics of the substances, including whether they are water-soluble or insoluble, hydrophilic or hydrophobic, as well as their half-life, log Kow. Overall, WSPs have the potential to remove some PhACs from wastewater. This is attributed to their long hydraulic retention time i.e. Hydraulic Retention Time (HRT) of up to 90 days and SRT of up to one year, different stages of waste treatment with both anaerobic and aerobic zones that offer diversity in microorganism populations (Phuntsho et al., 2016), and a large surface area to volume ratios that provide large solar irradiation exposure for photooxidation (Norvill, Shilton and Guieysse, 2016). However, these WSPs are not able to eliminate some of these pharmaceutical compounds since they are not designed to eliminate these contaminants of emerging concern implying that they only remove these PhACs to some extent (Kumar and Kumar, 2020).

Paracetamol was found to have a high removal efficiency of approximately 70%, which is due to its high biodegradability and a high biodegradation constant (Kbiol) that is greater than 102gss $^{-1}$ d^{-1} (Kumar and Kumar, 2020). This observation aligns with the findings of a prior investigation conducted by (Mohapatra et al., 2016), which found paracetamol removal efficiency ranging from 45-90% in a WTP with four facultative ponds in series with a hydraulic retention time (HRT) of two days each.

On the other hand, diclofenac exhibited a slightly lower removal efficiency (<40%) as reported in a similar study by (Kumar and Kumar, 2020). This is probably due to diclofenac's high log Kow i.e. greater than 4, signifying its hydrophobic nature and low solubility in water. Moreover, research by (Kimura, Hara and Watanabe, 2007) revealed that diclofenac poses challenges in terms of biodegradation, with sorption being the predominant removal mechanism. A study done by (Radjenović, Petrović and Barceló, 2009) also found that diclofenac had slow biodegradability, although it exhibited slightly better removal (31%) in WSPs than in activated sludge processes (ASP) (25%).

Although the concentrations of most pharmaceutical contaminants were found to decrease along the treatment process i.e. from the inlet to the outlet of the WSPs, some elements exhibited an increase in concentration. Specifically, compounds such as sulfamethoxazole, amoxicillin, sulfamerazine, and tetracycline showed an increase in concentration during the treatment process. As a result, higher levels of these pharmaceuticals were detected in the effluent at the outlet of the facultative ponds than in the influent at the inlet of the anaerobic ponds, indicating negative removal efficiency. This is because these pharmaceuticals may be excreted in the form of conjugates, which are then biodegraded by enzymatic processes in the facultative ponds, leading to additional amounts of these pharmaceuticals in the effluent (García Galán, Díaz-Cruz and Barceló, 2012).

Sulfamethoxazole was found to be the most recalcitrant compound of all the pharmaceuticals studied with low removal efficiencies. Comparable findings have been documented for carbamazepine and nevirapine in different treatment systems, such as aeration lagoons (Conkle, White and Metcalfe, 2008), activated sludge (Verlicchi, Al Aukidy and Zambello, 2012) as well as constructed wetlands (Verlicchi, Al Aukidy and Zambello, 2012). The rise in the concentration of the effluent of these compounds may be attributed to the biological deconjugation of the conjugates of biotransformation. For instance, Sulfamethoxazole is excreted in urine as HPLC:5-methyl hydroxy sulfamethoxazole (SOH), N4-acetyl-5-methyl hydroxy sulfamethoxazole (N4SOH), and sulfamethoxazole-N1-glucuronide (Sgluc), which may become attached to the solids while the wastewater is in the ponds (Vree et al., 1994).

An assessment for statistical differences of the data using the ANOVA analysis test for concentrations of PhACs in anaerobic and facultative ponds returned a p-value of 0.7415 which is greater than 0.05 hence there were no statistically significant differences between the mean concentrations of PhACs along the WSPs.

An assessment for statistical differences of the data using ANOVA analysis test for removal efficiencies returned a p-value of 0.2792 and hence there were no statistically significant differences between the mean removal efficiencies ($p>0.05$), implying that there was no sufficient evidence to say that these WSPs completely remove these PhACs. This study, therefore, infers that although WSPs do remove PhACs from wastewater, this removal is not significant since they do not completely eliminate them. In fact, out of the

20 Pharmaceutical compounds that enter the treatment chain at the inlet of the anaerobic pond, sixteen of them were found to arrive at the discharge point i.e. the outlet of the facultative pond (Figure 4-10).

4.3.5 Comparative Evaluation of Removal Efficiency of PhACs in Anaerobic and Facultative Ponds in Lubigi WWTP

A comparison of removal efficiencies was done for a few selected PhACs that showed significance in their removal efficiencies in the two ponds as shown in Figure 4-13. The study found that facultative ponds offer better removal of PhACs than anaerobic ponds with an average removal efficiency for anaerobic and facultative ponds estimated at 42.7% and 44.1% respectively. From the t-test analysis, the p-value of the anaerobic pond ($p = 0.7955$) is greater than 0.05 than the p-value of the facultative pond ($p=0.306$). This implies that the facultative pond offers better removal of PhACs than the anaerobic pond.

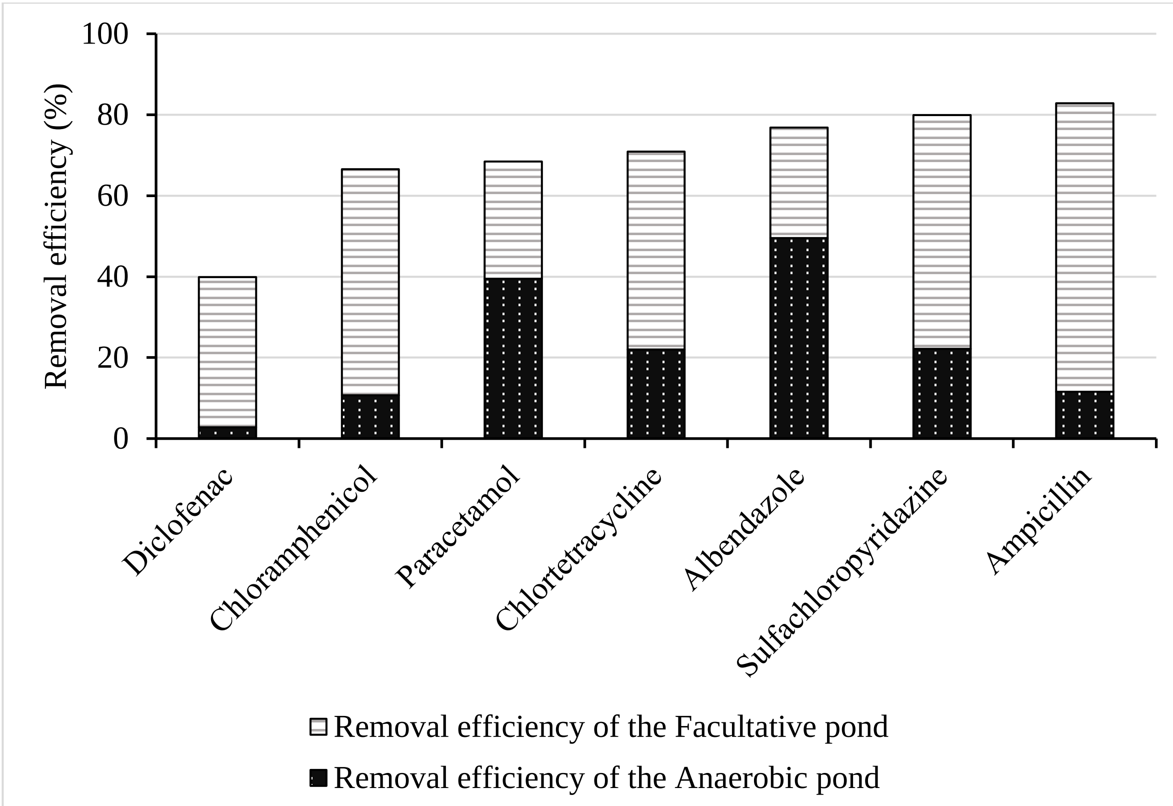


Figure 4-13: A comparison of removal efficiency of Anaerobic and Facultative ponds (n=18)

This is because facultative ponds support both aerobic and anaerobic conditions, providing a more diverse and versatile microbial community which enables a wider range of PhACs to be degraded, including those that require oxygen for biodegradation (Edokpayi et al., 2021). In contrast, anaerobic ponds only support anaerobic conditions, limiting the types of microbial processes that can occur. As a result, facultative ponds are more effective in removing a broad spectrum of PhACs (Trevino Quiroga, 2011). In addition, a study done by (Guerra et al. 2014) demonstrated the superior performance of facultative ponds (with a median removal rate of 90%) over the Activated Sludge Process (ASP) for 62 PhACs is attributed to longer HRTs (180 days) in facultative ponds.

However, (K'oreje et al., 2018) suggested that a combination of both anaerobic and facultative ponds can result in the highest removal (with an average log Removal Factor of 0.43–1.1 or RE of 63–92% after the first two stages) for all PhACs, which is consistent with the findings of (Hijosa-Valsero et al., 2010). In addition to the primary anaerobic and facultative ponds, it is important to incorporate secondary facultative and maturation ponds to achieve effective removal of PhACs residues that may not be efficiently eliminated during the initial treatment steps, or may even exhibit negative removal values (such as sulfamethoxazole). Maturation ponds are particularly important because they aid in photodegradation, which is made possible by algae growth, a high surface area, little sludge, and low turbidity (Wang et al., 2014; Bai and Acharya, 2017).

4.3.6 An assessment of the relationship between physical parameters and concentration of PhACs along the WSPs

4.3.6.1 An assessment of the physical parameters along the WSPs

Tests of various physicochemical parameters were carried out along the treatment chain and the results are shown in Figure 4-14 below.

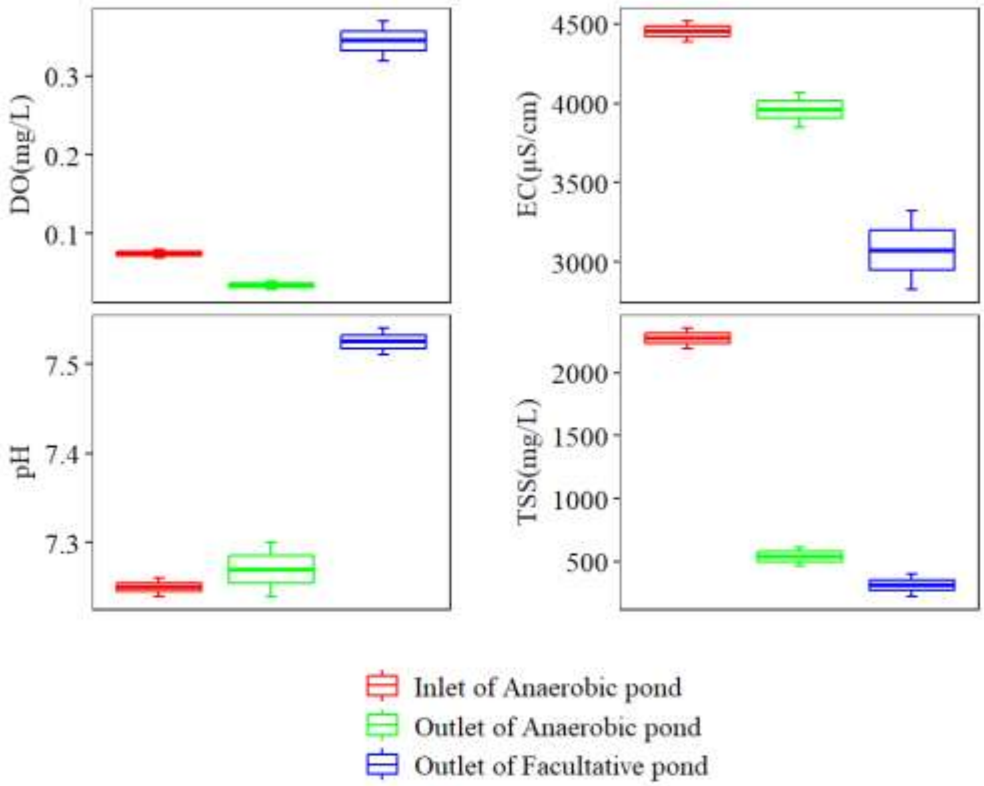


Figure 4-14: Variation of physicochemical parameters along the treatment stages of the WSPs (n=9)

These results indicate that the EC, and TSS, reduce along the treatment chain while pH and DO increase. This indicates a reduction in organic contamination along the treatment chain. Anaerobic ponds contain microorganisms that aid in the breakdown of wastewater by the use of oxygen. The aerobic conditions in facultative ponds, along with the growth of algae and other aquatic plants lead to the breakdown of organic matter which results in gradual decomposition and transformation of wastewater components. This process reduces TSS, increases oxygen production, and removes acidic compounds i.e. raises the pH and lowers the concentration of dissolved ions and salts, resulting in decreased electrical conductivity.

An assessment of the relationship between the physiochemical parameters (TSS, EC, DO and pH) and the PhACs selected from at least each pharmaceutical category was done for amoxicillin, paracetamol, diclofenac, and albendazole. The relationship is demonstrated in the figure 4-15 below.

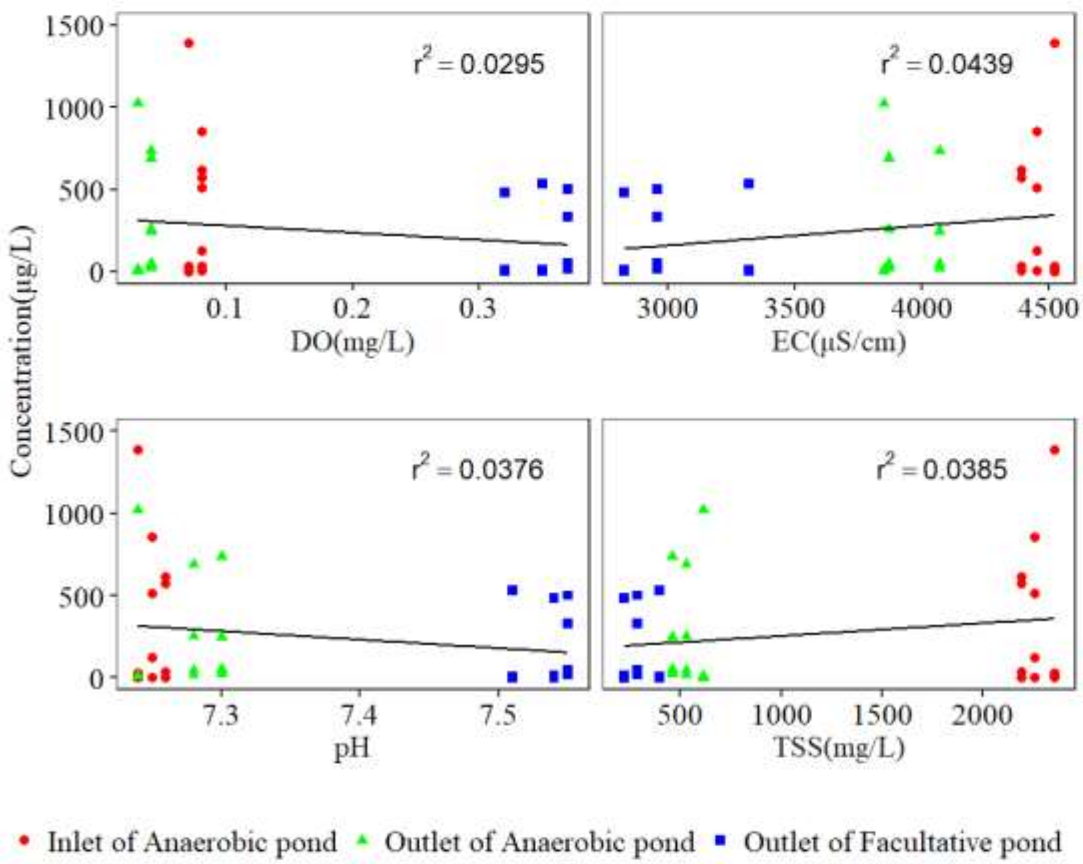


Figure 4-15: Relationship between Physicochemical parameters and Concentration of PhACs along the WSPs treatment stages (n=9)

The correlation coefficients for all parameters were less than 0.5 indicating a weak relationship. Regression analysis yielded an average determination coefficient of 0.04, indicating that 4% of these physicochemical parameters along the treatment ponds can be explained by the levels of concentration of PhACs.

This relationship may be attributed to the science of treatment where treatment processes along the different stages occur. As wastewater flows through ponds, the presence of aerobic processes and longer hydraulic retention times allow for the settling and sedimentation of suspended solids, including micropollutants. The settling process helps separate and remove these solids from the water, resulting in a decrease in TSS and subsequently lowering EC.

Additionally, WSPs provide an environment conducive to the growth of aerobic bacteria and algae. These organisms contribute to the biological degradation and transformation of micropollutants, including pharmaceuticals. As the micropollutant concentration decreases due to degradation, the TSS associated with these particles also reduces, leading to a decrease in EC. As the concentration of micropollutants decreases due to biodegradation, the demand for oxygen decreases as well. This reduction in oxygen demand allows for a higher concentration of dissolved oxygen in the water.

Physical and Chemical Processes such as sedimentation, filtration, and adsorption occur in the WSPs. These processes can effectively remove suspended particles, including micropollutants like pharmaceuticals, from the wastewater, resulting in a reduction in both TSS and EC.

Furthermore, as wastewater flows from anaerobic ponds to facultative ponds, aerobic conditions become more prevalent. The introduction of oxygen through aeration or natural air exposure promotes the growth of aerobic bacteria and algae, which break down the PhACs leading to increased oxygen levels and higher DO concentrations. In addition, Algae in facultative ponds carry out photosynthesis in the presence of sunlight, which leads to the production of oxygen. This process contributes to the increase in dissolved oxygen levels in the water.

Lastly, micropollutants, including pharmaceuticals, can contribute to the presence of acidic compounds in wastewater. As the concentration of PhACs reduces through biodegradation and treatment, the removal of these acidic compounds occurs. This removal increases pH, as the acidic influence decreases.

Overall, as the wastewater moves through the different stages of treatment aerobic conditions promote the biological degradation of micropollutants which in turn increase the oxygen levels through photosynthesis, leading to the removal of acidic compounds, and lowering the electrical conductivity.

4.3.7 Concentrations of PhACs in sludge

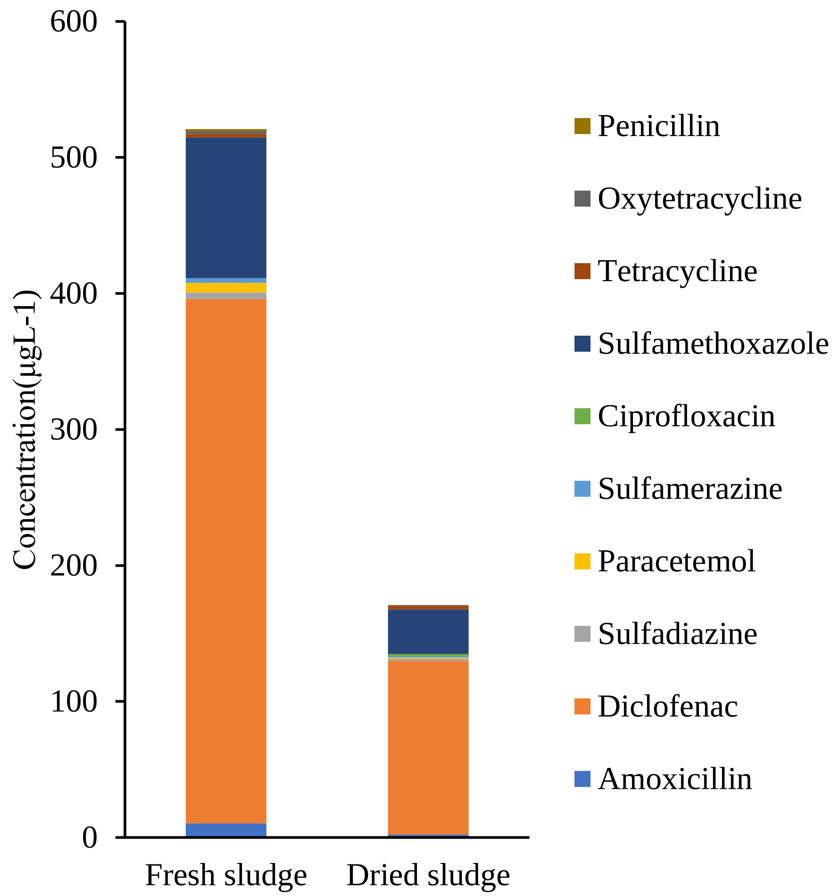


Figure 4-16: Variations of PhACs concentrations in sludge (n=6)

The study indicates that there are some pharmaceutical compounds present in sludge though the concentrations are lower than those along the treatment chain as shown in Figures 4-17 and 4-18.

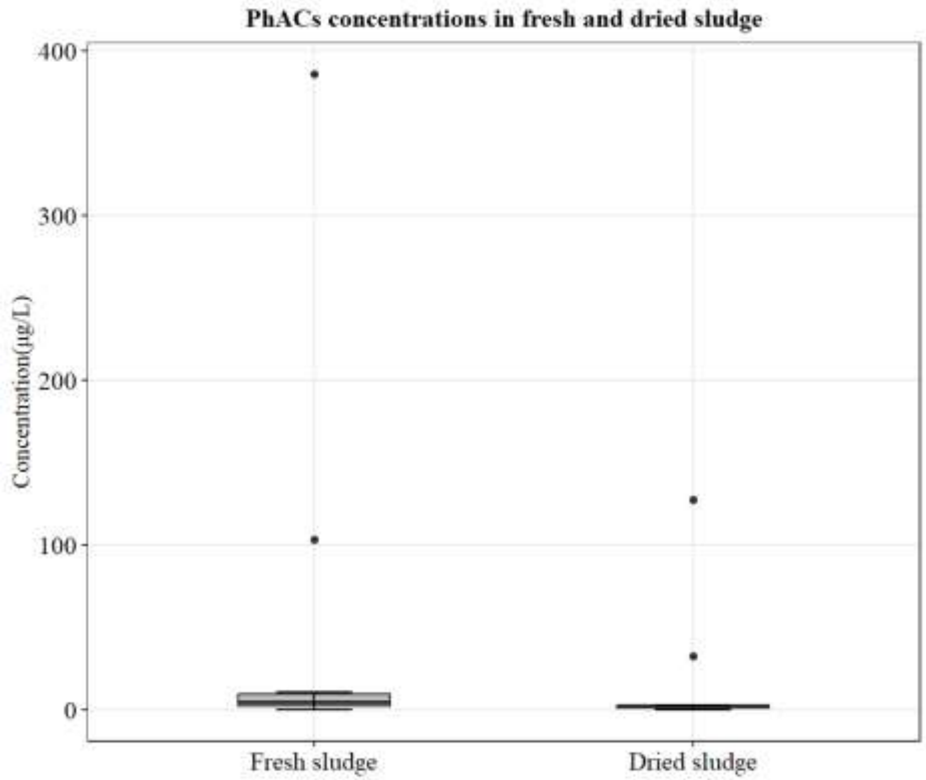


Figure 4-17: Variations of concentration of PhACs in fresh and dried sludge (n=6)

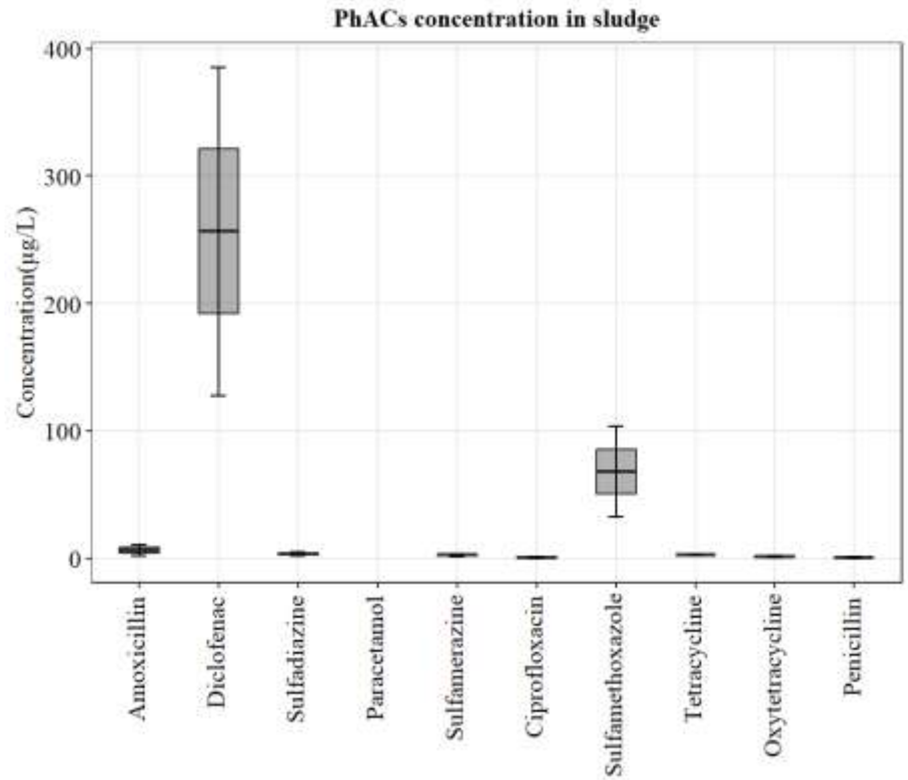


Figure 4-18: Concentrations of individual PhACs in sludge (n=6)

PhACs are present in maximum concentrations of 400 μgL^{-1} for raw sludge and 130 μgL^{-1} for dried sludge. The PhACs dominant in sludge were diclofenac and sulfamethoxazole. For diclofenac, this is attributed to its low removal efficiency (Kumar and Kumar, 2020), its high insolubility in water (high log Kow > 4) and its slow biodegradability (Kimura, Hara and Watanabe, 2007; Radjenović, Petrović and Barceló, 2009). The recalcitrant nature of sulfamethoxazole is explained by the biological deconjugation of the conjugates of biotransformation which may become attached to the solids while the wastewater is in the ponds (Vree et al., 1994).

This implies that PhACs continue to exist in the sludge in various concentrations although these concentrations are lower than the ones for wastewater. The quantity of pharmaceuticals present in sludge can differ according to several factors, such as the types and quantities of these micropollutants present in the wastewater, the treatment methods employed, and the characteristics of the sludge itself (Ekpeghere et al., 2017).

Studies have shown that a wide range of pharmaceuticals can be detected in sludge from municipal wastewater treatment plants, including antibiotics, antifungals, analgesics, hormones, and antidepressants. A study by (Ekpeghere et al., 2017) showed a total concentration of PhACs in sludge from municipal sewage treatment plants of 2.622-422.8 mg kg^{-1} with oxytetracycline (34.54-86.39 mg kg^{-1}) as the most abundant element. In their analysis, (Lindberg, Fick and Tysklind, 2010) examined the presence of various antifungal agents in sludge and discovered concentrations of econazole and ketoconazole in raw sludge at 240 and 1300 ng/g DM, respectively. A study by (Jia et al., 2012) found that concentrations of certain antibiotics and the antiseptic lipemic acid ranged from 10 to 70 ng/g DM, with no significant difference between raw and primary sludge. Dry sludges have garnered considerable attention for the groups of non-ionic surfactants and psychiatric medications, with six compounds each, followed by antifungals with four compounds. Research by (Mailler et al., 2014) indicates that NP1EO and NP have the highest concentrations at 50,000 and 31,000 ng/g DM respectively. Following these, diphenhydramine is reported at 6000 ng/g DM by (Peysson and Vulliet, 2013), tonalite at 5000 ng/g DM reported by (Kinney et al., 2006), triclosan at 3700 ng/g DM reported by (Kinney et al., 2006), and caffeine at 2100 ng/g DM reported by (Malmborg and Magnér, 2015).

These studies and more show that pharmaceuticals can be found in sludge from wastewater treatment plants. There is, therefore, a need for proper sludge treatment to ensure that these PhACs are eliminated before it is used as manure or disposed of.

Overall, pharmaceutical contaminants are present in wastewater from various sources and sanitation technologies i.e. FS in septic tanks and pit latrines and the wastewater in the conventional sewer system. It is, therefore, essential to establish proper disposal and management practices for human, commercial, industrial, and institutional wastewater to minimize the levels of these contaminants in wastewater. Regulatory efforts are necessary to enhance the management of pharmaceutical waste, to reduce the treatment load of these microcontaminants on WWTPs and improve their overall treatment efficiency. Failure to control the levels of these contaminants imposes a greater treatment burden on WWTPs, requiring additional tertiary treatment stages and more complex removal mechanisms. Consequently, this leads to increased costs for wastewater treatment to effectively eliminate these contaminants.

The findings indicate that WSPs are capable of removing PhACs to a certain extent. However, these contaminants are not completely eliminated, as evidenced by their presence in the effluent and sludge. Consequently, there is a need for additional treatment stages, such as maturation ponds, and the implementation of advanced treatment technologies like ozonation and membrane processes to more effectively remove persistent PhACs and reduce their concentrations in the effluent and sludge to permissible limits. This could help minimize their threat to human and animal life, as well as other ecosystems in the environment particularly if the effluent or sludge is utilized for land application or other disposal methods. In addition, further research is required to better understand the fate and impacts of a broader range of pharmaceuticals and to develop comprehensive strategies for their proper management and control.

4.4 Assessment of the effect of onsite sanitation technologies on the removal of PhACs in the Lubigi WSPs.

The SFA technique was implemented within the Lubigi WWTP. The treatment process at the Lubigi WWTP involves preliminary stages such as grit removal and screening, followed by secondary treatment carried out in waste stabilization ponds which include anaerobic and facultative sections, as well as sludge drying beds.

This system accepts diverse waste sources, including faecal sludge from pit latrines (20m^3/day), faecal sludge from septic tanks (350m^3/day), and wastewater from the conventional sewer network (4200m^3/day). It then releases effluent wastewater (4113 m^3/day) into the surrounding wetland and disposes of sludge (50m^3/day) into the sludge beds as shown in Table 4-3 below.

Table 4-3: Data for MFA calculation

Source	**Quantity of WW and FS received (m3/day)**	**Concentration of PhACs in each stream (μgL^{-1})**
Inflows		
Waste received from pit latrines via gulpers	20	6700
Waste received from septic tanks by cesspools	350	1500
Wastewater received via the conventional sewer network	4200	4300
Outflows		
Wastewater effluent produced/ discharged from the waste stabilization ponds	4113	9600
Sludge produced from the waste stabilization ponds per day	50	400

The primary components of this system encompass three streams of wastewater and faecal sludge, initial treatments like screens and grit separation, secondary treatments involving anaerobic and facultative processes, as well as the utilization of sludge beds. The representation of this system is provided in Figure 4-19, and a one-year timeframe has been taken into account for the analysis.

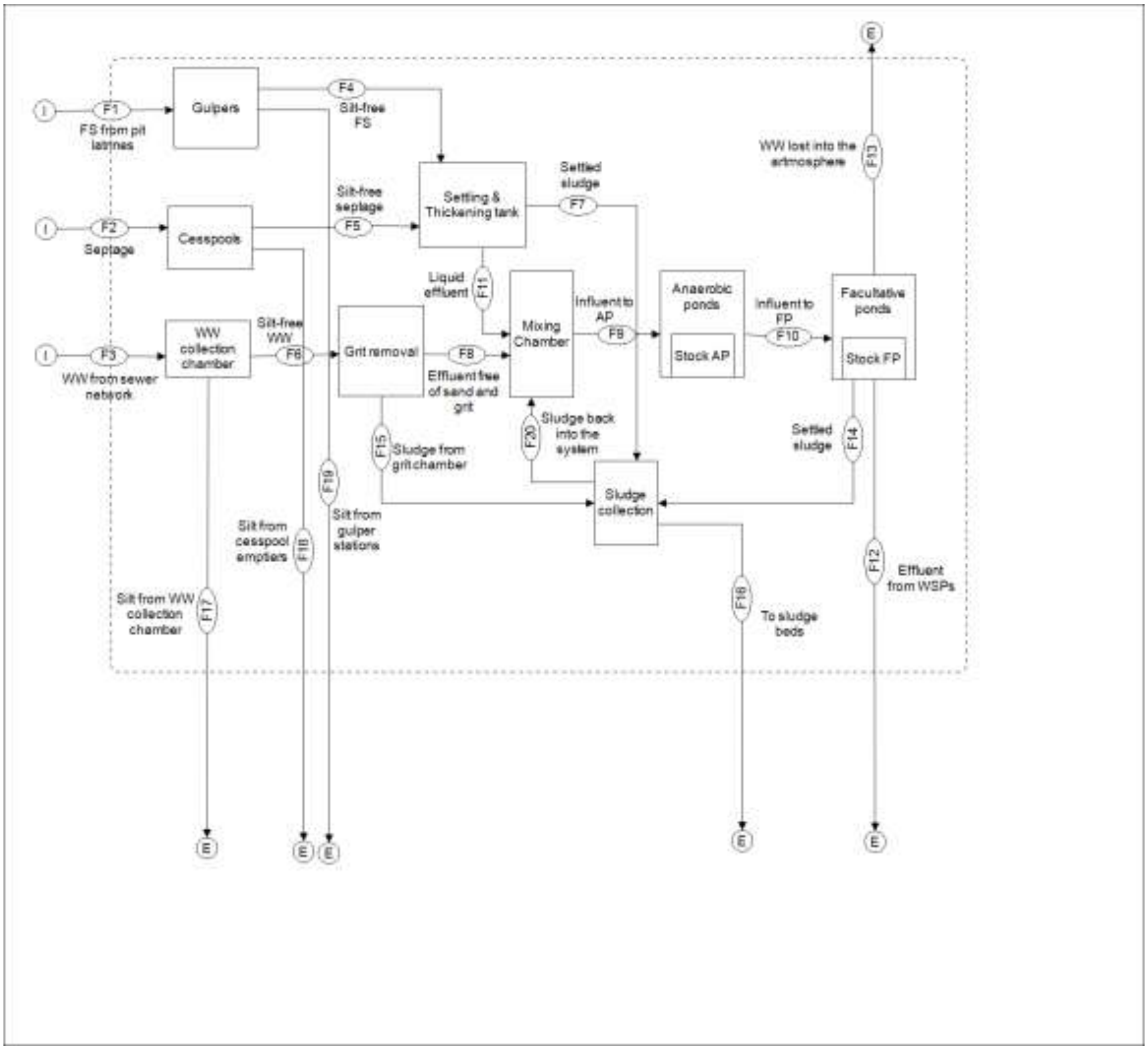


Figure 4-19: MFA model for WW and FS flows in Lubigi Sewage Treatment Plant

4.4.1 Simulation of the current scenario at Lubigi WWTPs

First, the present scenario was simulated, and the outcomes are depicted in Figure 4-20 and Figure 4-21 below.

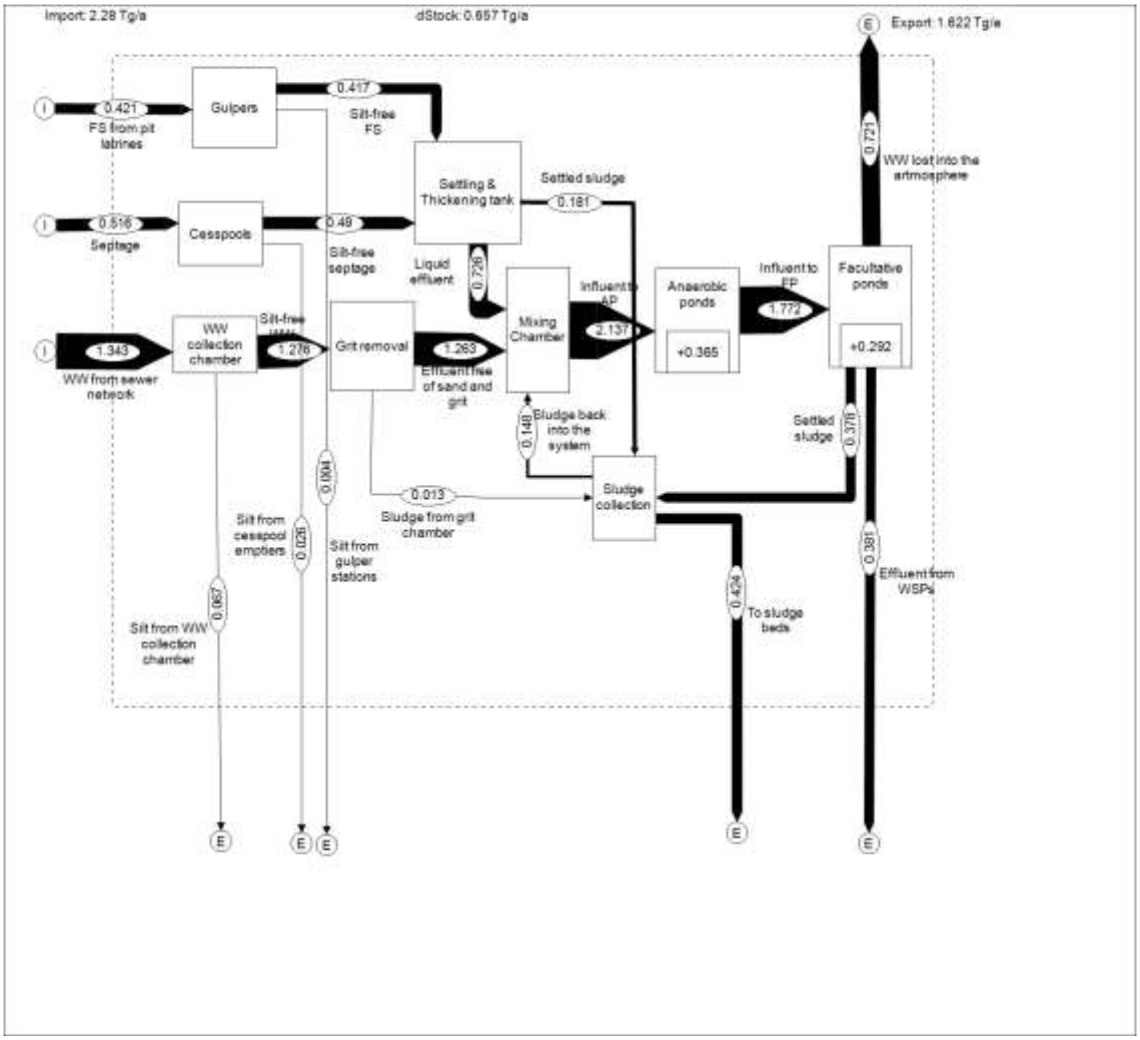


Figure 4-20: MFA for material flows of goods (i.e. WW and FS) in Lubigi WWTP in Tgyr^{-1} (Current scenario)

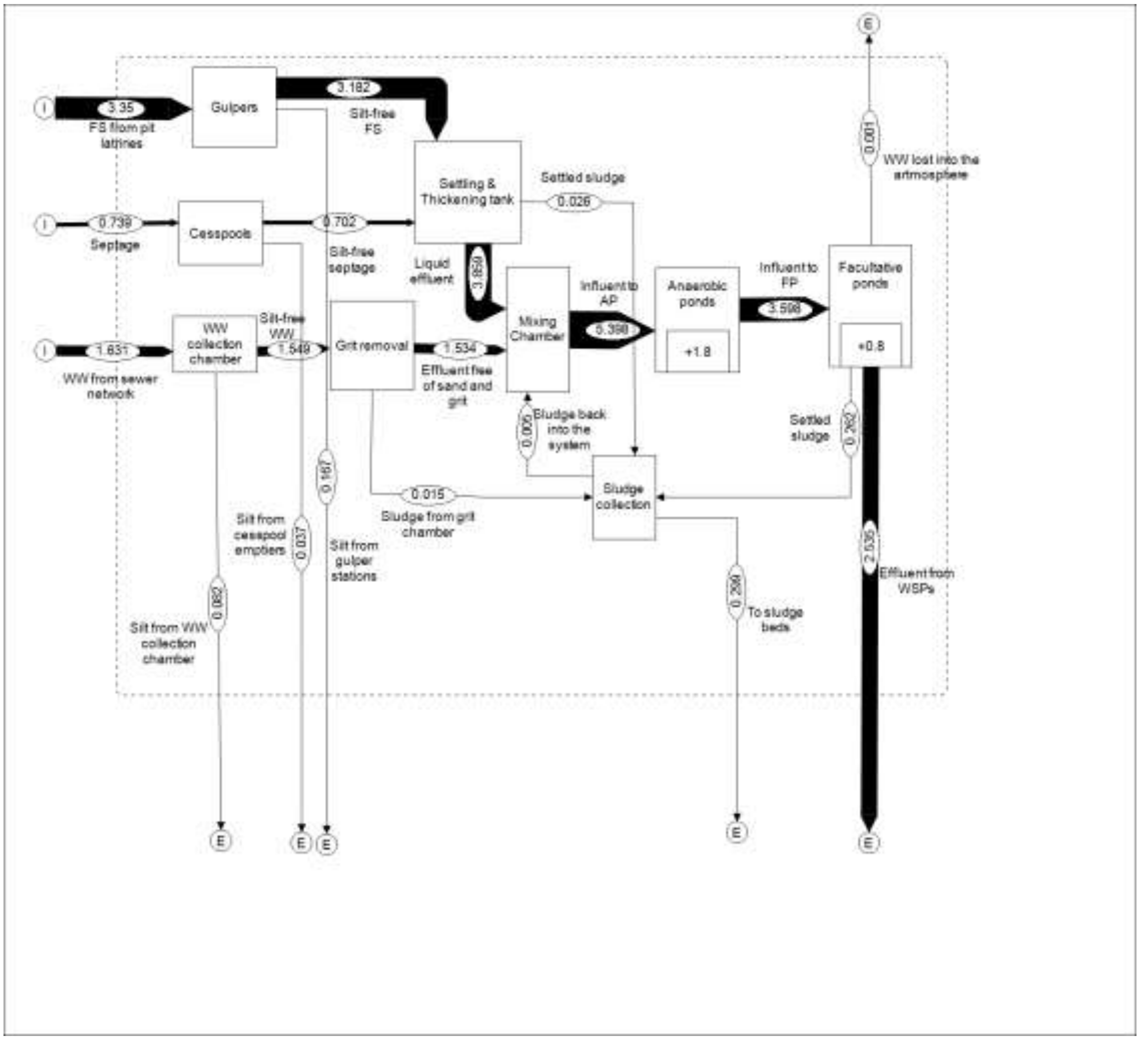


Figure 4-21: MFA for PhACs in Lubigi WWTP in ton/year (Current scenario)

The current situation at Lubigi WWTP shows that the conventional sewer system and septage contribute substantial volumes of wastewater and FS entering the Lubigi sewage treatment plant.

Interestingly, despite the majority of material flows (i.e. WW and FS) coming from the conventional sewer system and septage, MFA presented in Figure 4-21 reveals that FS from pit latrines contributes the highest pharmaceutical loads, surpassing contributions from the WW from the conventional sewer system and FS from septic tanks.

The simulation reveals that the majority of PhACs in the influent originate from FS from pit latrines, followed by WW from the sewer system, and then FS from septic tanks. Consequently, it implies that both pit latrines and sewer network systems lower the treatment efficiency of WSPs in eliminating these pharmaceuticals as they contribute to higher pharmaceutical loads in the WWTP influent.

The significant abundance of pharmaceutical loads in faecal sludge of pit latrines arriving at the WWTP can be attributed to the inadequate treatment of wastewater in these systems. Pit latrines serve as storage units for human waste and lack the processes needed to break down these PhACs, necessitating periodic emptying and disposal of the collected waste (Bakare et al, 2012). Furthermore, the prevalence of high PhACs levels in FS from pit latrines can be linked to the location of these facilities, primarily in impoverished areas like slums in Kampala where pit latrines are commonly used for human waste disposal (Nakagiri et al., 2015). These regions grapple with a heightened burden of diseases, due to inadequate hygiene and limited access to WASH practices. Research indicates that about 7.75% of all diarrheal disease-related deaths in SSA can be attributed to insufficient WASH practices (Zerbo, Castro Delgado and Arcos González, 2021). Consequently, the consumption of medications is highest in these underserved areas, elucidating the elevated levels of PhACs observed in FS from pit latrines.

The considerable influx of wastewater from many sources (i.e. include domestic wastewater from homes such as from sinks, toilets, showers, baths, and laundry, commercial establishments such as hospitals, restaurants, and offices and industrial facilities like schools and hospitals into the sewage network (Tilley, 2014; Gao et al., 2023) explains the high pharmaceutical loads in arriving at the WWTP from via sewer wastewater.

The minimal presence of PhACs loads in septage arriving at the WWTP can be ascribed to the function of septic tanks as initial wastewater treatment facilities. Septic tanks promote the anaerobic digestion of solid sludge, which contains these PhACs, causing it to settle at the tank's bottom while the liquid portion settles at the top. This segregation of solid and liquid waste within septic tanks allows for efficient pre-treatment of these micropollutants before they enter wastewater treatment plants (WWTPs). According to (Kang et al., 2019) sewage treatment tanks (STTs), exhibited substantial removal rates for micropollutants, ranging from 86% for caffeine, 86% for acetaminophen, 72% for ibuprofen, to 63% for acetaminophen (naproxen). These results underscore the vital role of septic tanks in mitigating micropollutants. Septic tanks contribute to the partial removal of these micropollutants hence enhancing the overall efficiency of WSPs in eliminating these contaminants (Tilley, 2014).

There is, therefore, a pressing need to embrace alternative onsite sanitation technologies to reduce the level of PhACs reaching the treatment plant to increase the treatment effectiveness of WSPs in the removal of these micropollutants. Completely eliminating the conventional sewer system may not be feasible. Therefore, the most practical approach is to substitute ordinary pit latrines. This could be done by more people adopting the utilization of septic tanks through the use of flush toilets or replacing ordinary pit latrines with UDDTs.

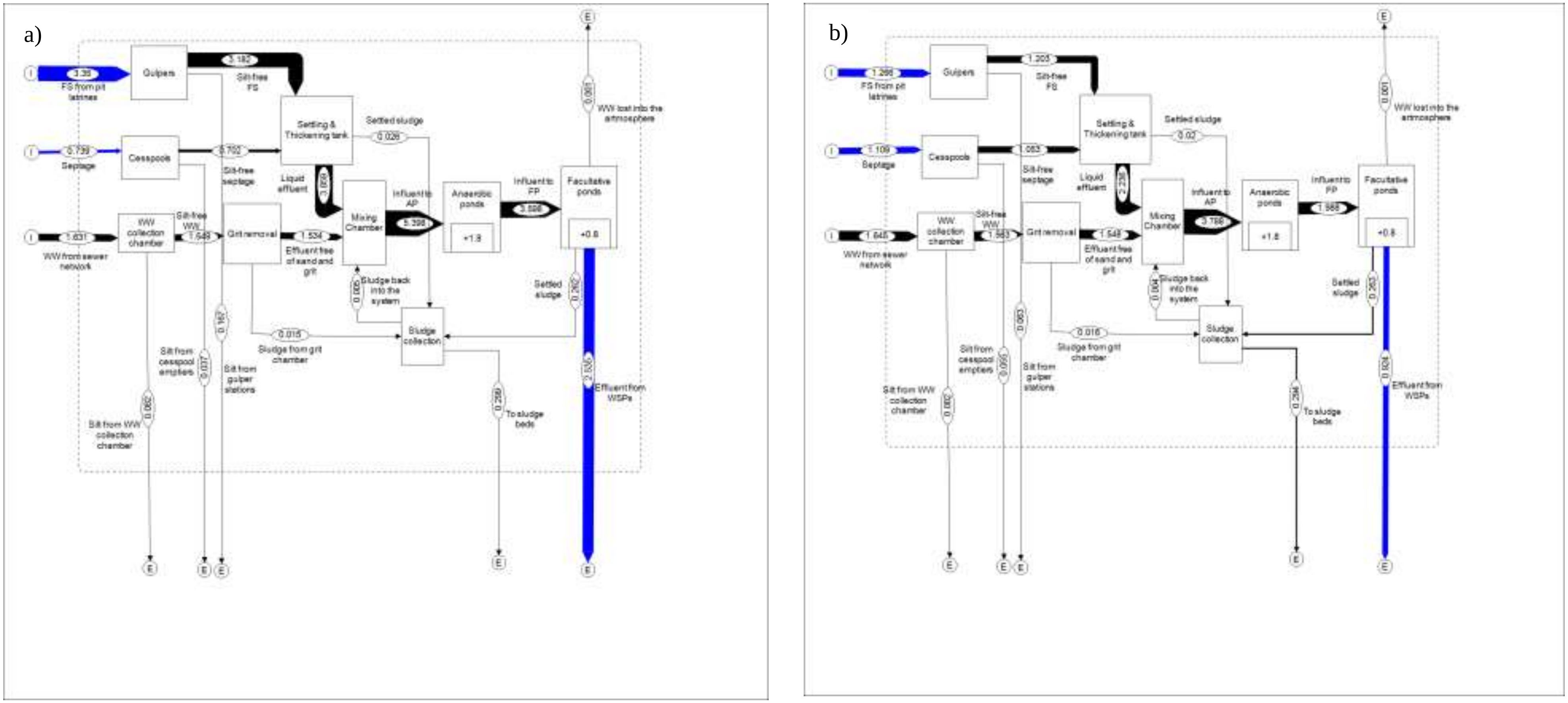
4.4.2 Scenario of reduction in use of pit latrines and a higher utilization of septic tanks

A simulation was carried out to assess the scenario where more people adopt the use of flush toilets and septic tanks. This substitution in the MFA model implies that more waste will enter the WSPs via septage than FS from pit latrines.

Shifting from conventional pit latrines to higher utilization of septic tanks (as depicted in Figure 4-22 and Table 4-4), the MFA simulation indicates that a 75% reduction in pit latrine usage (equivalent to a 75% increase in septic tank usage) leads to a proportional decrease or increase in the pharmaceutical load reaching the wastewater treatment plant (WWTP) through pit latrines or septic tanks respectively. This transition results in an 88% reduction in the concentration of pharmaceuticals discharged into the environment in WWTP effluent.

Table 4-4: Effect of a reduction in use of pit latrines to a higher utilization of septic tanks

Percentage reduction in use of pit latrines/ Increase in use of septic tanks	Quantity of waste received via gulpers from pit latrines per capita per day) (m^3/day)	Waste received via cesspools from septic tanks (m^3/day)	Concentration of PhACs in the influent of FS pit latrines (μgL^{-1})	Concentration of PhACs in effluent (ton/year)	Percentage reduction in concentration of PhACs in the effluent
0%	20	350	6700	2.535	0%
50%	10	525	3350	0.924	64%
75%	5	613	1675	0.311	88%


a)
Gulpers
FS from pit latrines
Silt-free FS
Septage
Cesspools
Silt-free septage
Settling & Thickening tank
Settled sludge
Liquid effluent
WW from sewer network
WW collection chamber
Silt-free WW
Grit removal
Effluent free of sand and grit
Mixing Chamber
Influent to AP
Anaerobic ponds
+1.8
Influent to FP
Facultative ponds
+0.8
WW lost into the atmosphere
Settled sludge
Sludge back into the system
Sludge collection
Sludge from grit chamber
Silt from cesspool emptiers
Silt from gulper stations
Silt from WW collection chamber
To sludge beds
Effluent from WSPs
b)
Gulpers
FS from pit latrines
Silt-free FS
Septage
Cesspools
Silt-free septage
Settling & Thickening tank
Settled sludge
Liquid effluent
WW from sewer network
WW collection chamber
Silt-free WW
Grit removal
Effluent free of sand and grit
Mixing Chamber
Influent to AP
Anaerobic ponds
+1.8
Influent to FP
Facultative ponds
+0.8
WW lost into the atmosphere
Settled sludge
Sludge back into the system
Sludge collection
Sludge from grit chamber
Silt from cesspool emptiers
Silt from gulper stations
Silt from WW collection chamber
To sludge beds
Effluent from WSPs

c)

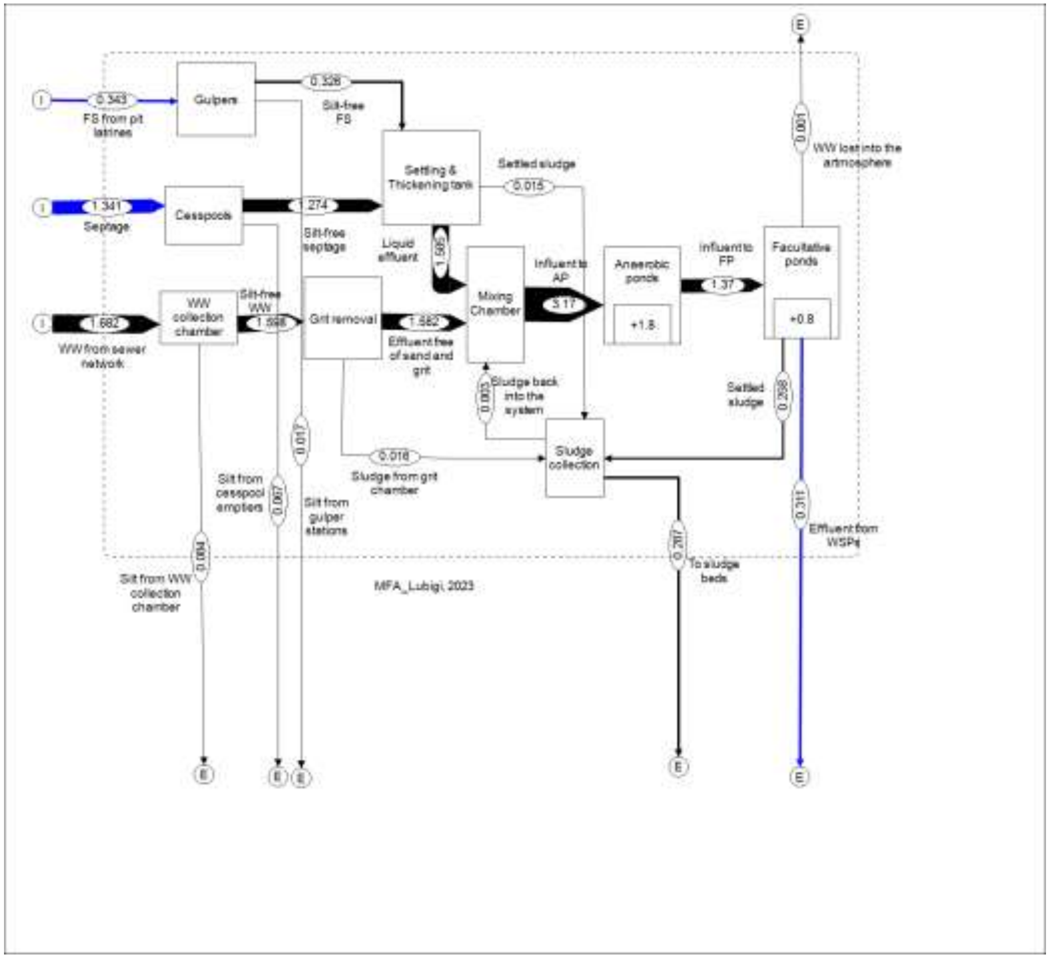


Figure 4-22: Effect of reduction in the use of pit latrines to higher utilization of septic tanks; a) Current situation; b) 50% reduction in the use of pit latrines (equivalent to a 50% increase in septic tank usage; c) 75% reduction in the use of pit latrines (equivalent to a 75% increase in septic tank usage). Pharmaceutical concentrations presented in ton/year

However, it's essential to acknowledge that not everyone, particularly those residing in slum areas of Kampala, can readily adopt this approach due to the cost and water requirements associated with flush toilets. As a more viable alternative, this study suggests the implementation of Urine Diverting Dry Toilets (UDDTs).

4.4.3 Scenario of use of UDDTs as a more feasible substitution for ordinary pit latrines

A very good option for replacement of ordinary pit latrines is the Urine Diverting Dry Toilets (UDDTs). UDDTs exploit human anatomy's natural separation of urine and faeces. A simulation was carried out to assess the scenario where ordinary pit latrines are replaced with UDDTs. This substitution in the MFA model implies that only dried faeces are transported to the WWTP.

The MFA simulation results revealed that transitioning from ordinary pit latrines to UDDTs (Figure 4-23), results in a notable decrease of around 90% in pharmaceutical load entering the wastewater treatment plant. Consequently, this leads to a 94% reduction in the discharge of PhACs into the environment from the WWTP effluent.

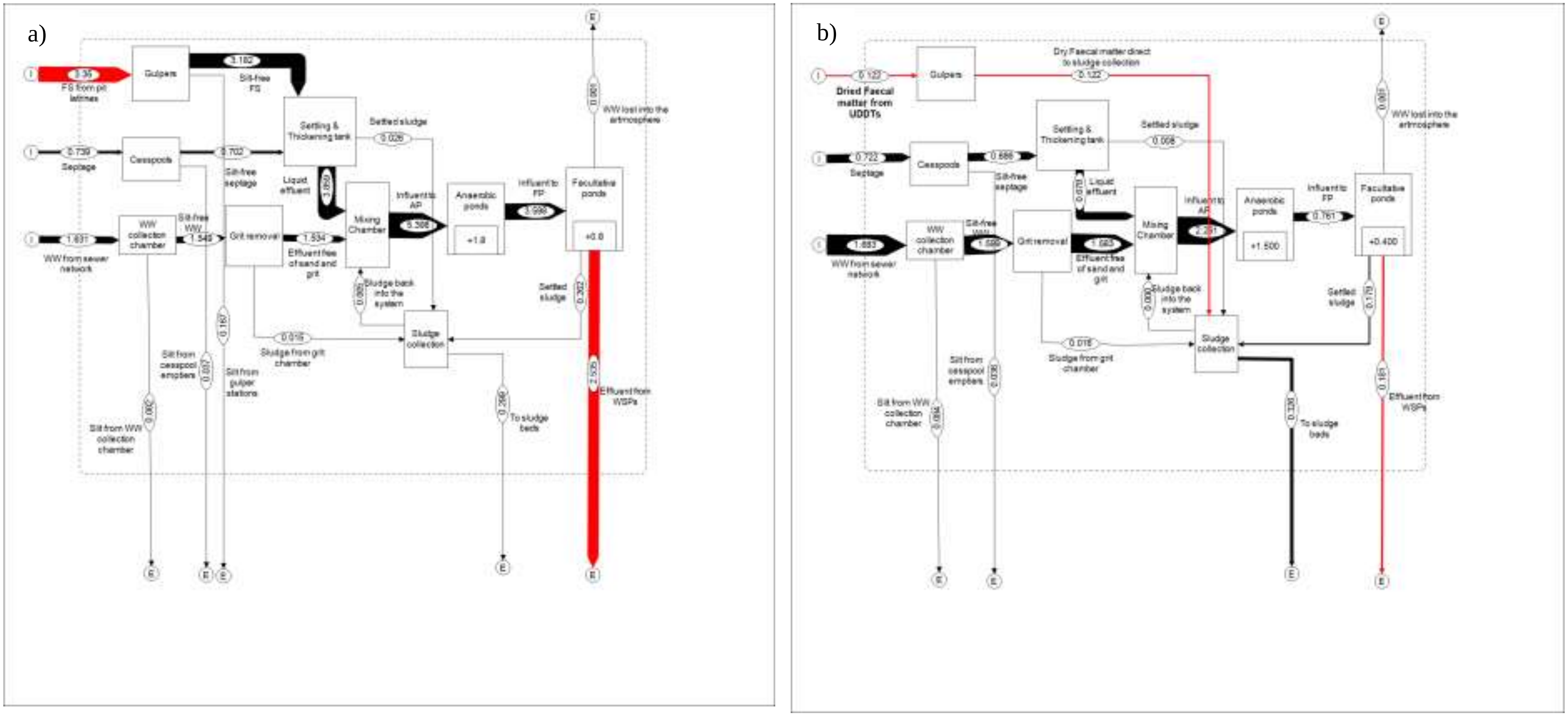


Figure 4-23: MFA for PhACs after partial substitution of ordinary pit latrine with UDDTs; a) Current situation; b) Scenario after 90% replacement of ordinary pit latrine with UDDTs. Pharmaceutical loads presented in ton/year.

In UDDTs, urine is diverted separately and drained, while faeces are collected and dried within the processing vault using natural evaporation and ventilation, without requiring water for flushing. UDDTs are adaptable to various climates, particularly advantageous in water-scarce and arid regions. The urine collected from UDDTs can serve as a valuable fertilizer in agriculture after appropriate storage, effectively sequestering essential nutrients. (von Münch, 2009). Separation of urine from faeces in UDDTs also enhances the dehydration of faeces stored in dry toilets. The dried faecal material from UDDTs, after a storage period, is more sanitized and contains fewer PhACs than the original faecal matter, although secondary treatment like WSPs may still be necessary (Bischel et al., 2015).

Moreover, research indicates that urine contains higher concentrations of PhACs than faeces. In a research conducted by (Bischel et al., 2015), trimethoprim and sulfamethoxazole, with peak concentrations of 1280 g/L and 6800 g/L, respectively, were found in urine. A study by (Winker et al., 2008) found only 28% of the original carbamazepine drug is eliminated in feces demonstrating that pharmaceuticals and their metabolites are largely expelled through urine rather than feces. All this explains the reduction in pharmaceutical loads entering the WWTP via dried faecal sludge from UDDTs.

The findings of this study suggest that septic tanks present superior choices for the management of wastewater containing pharmaceutical pollutants since they contribute less load of Pharmaceuticals on the WWTP compared to pit latrines and conventional sewer systems. The study emphasizes that septic tanks outperform both the conventional sewer system and pit latrines and are more suitable options for enhancing the treatment efficiency of PhACs in WSPs.

Furthermore, the study suggests that the substitution of ordinary pit latrines with source-separated technologies could offer the best solution in both reducing the load of PhACs that reach the WSPs and increasing the treatment efficiency of WSPs, thereby reducing PhACs concentration in the effluent that is discharged into the environment hence promoting enhanced human health, and safeguarding ecosystems.

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

This study set out to evaluate the performance of wastewater stabilization ponds in the removal of Pharmaceutically Active Compounds in the Lubigi Wastewater Treatment Plant. The study offers fresh insights into the concentrations of PhACs in the waste streams received at the plant, the removal efficiencies of WSPs and how onsite sanitation technologies could influence the removal of PhACs in the Lubigi WSPs.

PhACs were present in median concentrations of 19.223 μgL^{-1} in wastewater from the sewer network, 13.429 μgL^{-1} septage and 18.641 μgL^{-1} in faecal sludge from pit latrines received at Lubigi WWTP. PhACs were also present in average concentrations of up to 5300 μgL^{-1} in the three WW and FS sources.

This study found that WSPs have the ability to remove a range of PhACs from wastewater to a certain extent with an overall removal efficiency of 76.15%. WSPs are good in getting rid of PhACs like chlortetracycline, sulfapyridine, ampicillin, gentamicin, albendazole, ibuprofen, sulfachloropyridazine, sulfaquinoxaline, and penicillin with a high removal efficiency of 70-99.99%, moderately removing PhACs like paracetamol, chloramphenicol and enrofloxacin with a removal efficiency of 50-70% while PhACs like sulfadiazine, oxytetracycline, diclofenac, and ciprofloxacin proven to be more tenacious with lower removal efficiencies of was 1-40% with others like sulfamethoxazole, amoxicillin and sulfamerazine being even more recalcitrant with negative removal efficiencies.

Generally, WSPs have a high potential to remove PhACs from wastewater with at least 70% removal for about 60% of the compounds although they do not effectively eliminate these PhACs.

The study found that faecal sludge (FS) from pit latrines (3.35 ton/year) and conventional sewer network systems (1.63 ton/year) contribute to substantial volumes of wastewater entering the Lubigi sewage treatment plant and therefore consequently reduce the treatment efficiency of WSPs in removing these PhACs as compared to septic tanks (0.74 ton/year).

This study enhances our understanding of PhACs in the different wastewater sources and their removal from the wastewater and faecal sludge by WSPs. The findings provide valuable guidance on the choice of appropriate onsite sanitation technologies to improve the influent quality and suggest measures to improve the treatment efficiency and effluent quality of WSPs. This consequently increases the overall water quality of receiving bodies where these effluents are discharged, contributing to improved public health and sanitation and the protection of the ecosystems.

5.1 Recommendations

5.1.1 Technical recommendations

To improve the elimination of pharmaceutical contaminants from wastewater at the Lubigi treatment plant, the following recommendations are proposed;

i. Septic tanks should be unequivocally utilized as better sanitation technology choices for the management of wastewater containing pharmaceutical pollutants due to their demonstrated superior performance in effectively handling such wastewater and increasing the treatment efficiency of WSPs than conventional sewer systems and pit latrines.

ii. Substitution of ordinary pit latrines with source-separated technologies could help in reducing the load of PhACs that reach the WSPs and increasing the treatment efficiency of WSPs in the removal of these PhACs.

iii. Introduce additional treatment stages such as maturation ponds and constructed wetlands to effectively remove more persistent PhACs. For example, a study done by (Hijosa-Valsero et al., 2010) indicates that a combination of facultative ponds and constructed wetlands removed around 15-20% more diclofenac than waste stabilization ponds that are composed of two anaerobic ponds, a facultative pond, and a maturation pond.

iv. Implement regular monitoring programs specifically targeting pharmaceuticals to track their presence and concentrations at the Lubigi treatment plant.

v. Explore the utilization of advanced treatment technologies like ozonation and membrane processes, which have shown effectiveness in the removal of PhACs from wastewater.

5.1.2 Sociological recommendations

To address the issue of pharmaceutical contaminants in wastewater, the following recommendations are proposed:

i. Sensitization of the public to educate people on proper disposal and management of pharmaceutical waste particularly in urban slums connected to the sewer network and those using pit latrines since these contribute to high levels of concentrations of PhACs at the treatment plant.
ii. There is a need to promote the use of septic tanks as an alternative to pit latrines, as septic tanks contribute to lower concentrations of PhACs in wastewater.
iii. Continued efforts are necessary to increase public awareness of the pathways of the PhACs to the human body, potential health risks associated with exposure to these elements and ways of preventing these health risks. This will help individuals take preventive measures to reduce their exposure to PhACs and protect their health.

5.1.3 Policy Recommendations

To ensure proper disposal and management of pharmaceutical waste, the following actions are recommended:

i. KCCA (Kampala Capital City Authority), NEMA (National Environment Management Authority) and National Drug Authority (NDA) should collaborate to develop comprehensive guidelines specifically addressing the disposal of pharmaceuticals and the appropriate management of pharmaceutical waste. It is crucial to enforce strict adherence to the guidelines established by implementing penalties for non-compliance with the prescribed rules. This will emphasize the importance of proper pharmaceutical waste management.

ii. Pharmaceuticals should be recognized as significant environmental pollutants and included within the scope of existing KCCA policies for regulation.

iii. There is a definite need to implement robust sampling programs as part of future monitoring strategies to effectively monitor the presence and concentrations of pharmaceuticals in the environment.

5.1.4 Recommendation for further research

Additional research is needed for a deepened understanding of the behaviour and fate of PhACs throughout the treatment process, particularly regarding their transformation and potential impacts when discharged into wetland environments. The research should also encompass a broader range of CECs since this study was limited to a few selected PhACs. This could help to improve these environments where WSP effluent is discharged to minimize the effects of these pharmaceutical elements on the natural ecosystems.

A further study should also look into the generation and toxicity of transformed byproducts derived from these micropollutants within WSPs. This is because the transformed byproducts of certain CECs may exhibit greater toxicity compared to the original compounds.

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APPENDICES

Appendix I:

Introductory letter

KYAMBOGO UNIVERSITY

Department of Civil and Environmental Engineering

P.O.BOX 1, KYAMBOGO-P.O.BOX 7181 KAMPALA UGANDA

Website: www.kyu.ac.ug, Email: civil@kyu.ac.ug

TEL:+256-41-4287340, FAX: +256-41-4289056/4222643

January 31, 2022

The Director,
Uganda Government Analytical Laboratory,
Plot 2 Lourdel Road,
Kampala – Uganda.

RE: REQUEST FOR SUBSIDIZED RATES FOR LABORATORY TESTS TO BE CONDUCED BY ASIIMWE BRENDAH PATIENCE

Ms. Asiimwe Brendah Patience is a student of Kyambogo University undertaking Master of science in Water and Sanitation from Department of Civil and Environmental Engineering (DCEE). She is conducting a research on "Performance evaluation of Waste Stabilization Ponds (WSPs) in removal of Pharmaceutically Active Compounds (PhACs)". The researcher is being supervised by Eng. Dr. Anne Nakagiri and Dr. Charles Onyutha, all from DCEE.

This research is very vital regarding the decision on whether waste water treatments should take into consideration these emerging contaminants of concern. However, because the pertinent laboratory tests are quite expensive in nature, I hearby write to your office in line with the above reference. The necessary support to enable the researcher timely conduct the laboratory tests and complete her research will be duly acknowledged in the dissemination of the results. The researcher will also require from your office (if available) the current standards against which the results from the laboratory tests will be compared.

I shall be grateful for your cooperation and support toward this research.

Yours sincerely,

HEAD OF DEPARTMENT
31 JAN 2022

Lawrence Muhwezi (PhD)
Head, Department of Civil and Environmental Engineering

CC. Dean, School of Graduate Studies, Kyambogo University
Dr. Charles Onyutha - Department of Civil and Environmental Engineering, Kyambogo University
Eng. Dr. Anne Nakagiri - Department of Civil and Environmental Engineering, Kyambogo University

The specific objectives of this study are;

1. To assess the variation of PhACs in waste water, septage and faecal sludge received at Lubigi WSPs.
2. To evaluate the efficiency of the Lubigi WSPs in removing PhACs.
3. To analyze the extent to which different human excreta disposal technologies affect the treatment efficiency of WSPs in removal of PhACs.

Appendix II:

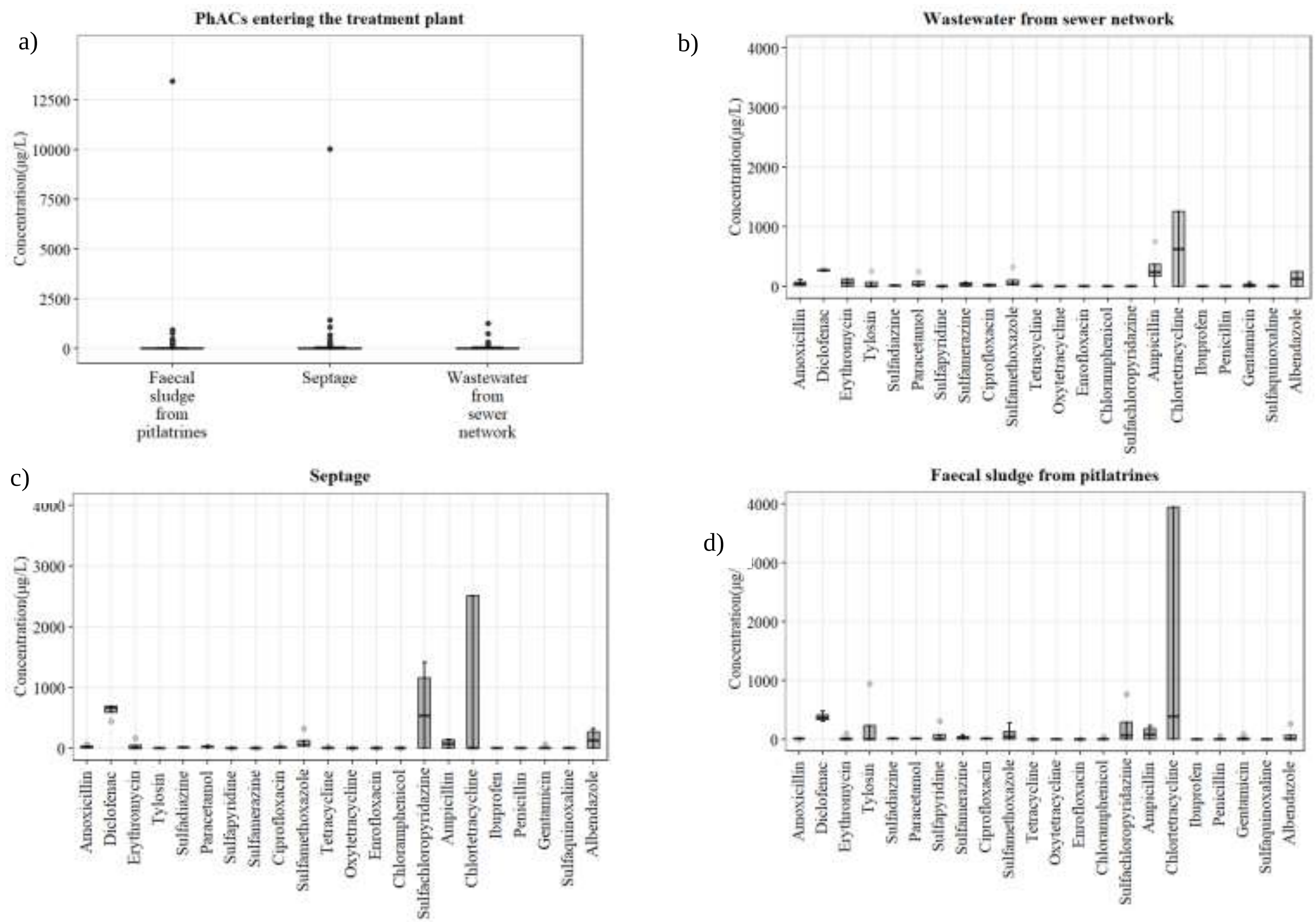

Figure A.1: Concentrations of PhACs in the waste streams received at Lubigi WWT; a) overall concentration, b) concentration in wastewater received from the sewer network, c) concentrations of PhACs in septage and d) concentration at the gulper station that receives faecal sludge from pit latrines. Box represents 50% of the data points, whiskers represent minimum and maximum, line in box represents the median (n=18)

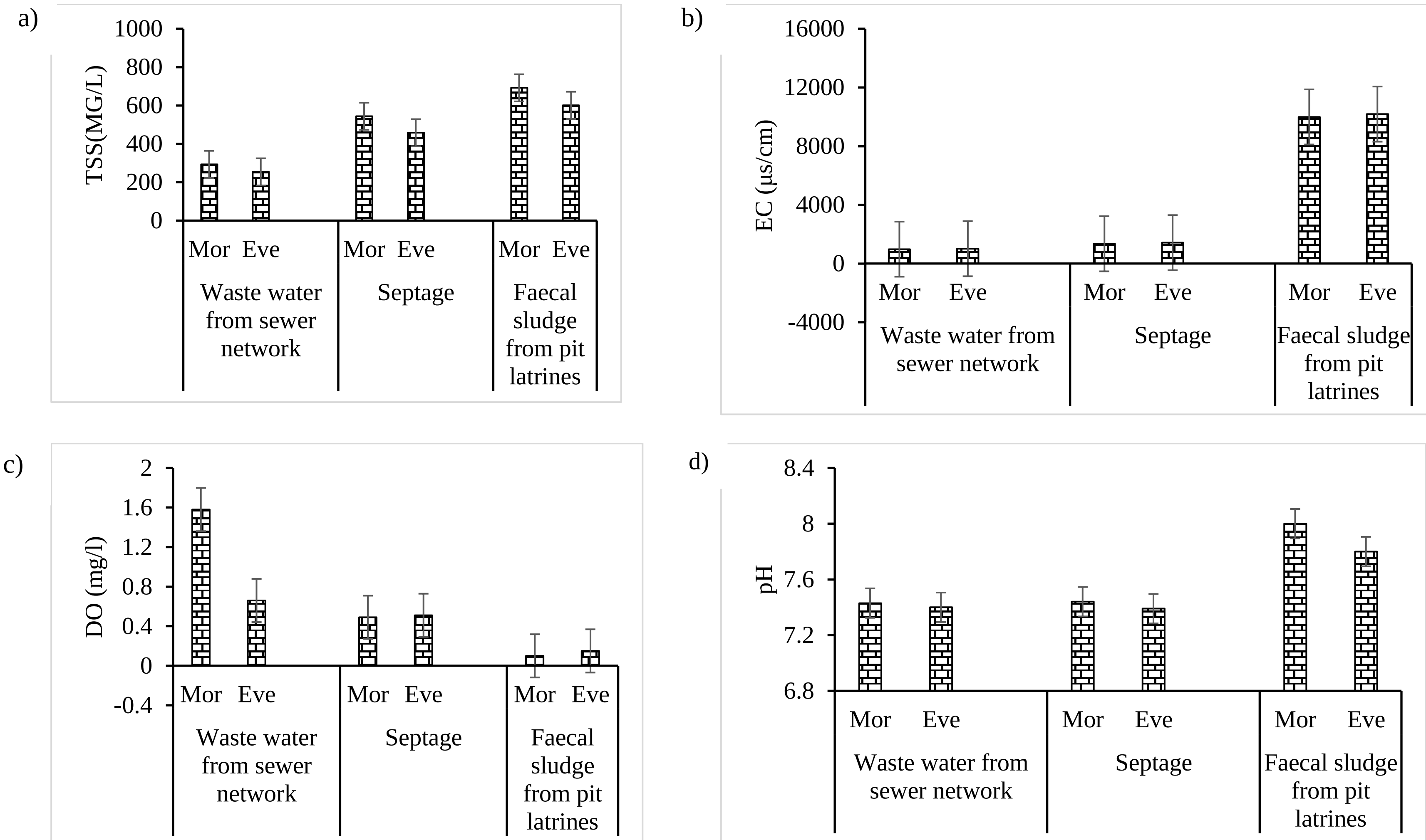

Figure A.2: Physicochemical parameters in WW and FS streams; a) Total Suspended Solids b) Electrical Conductivity c) Dissolved Oxygen and d) Hydrogen Potential

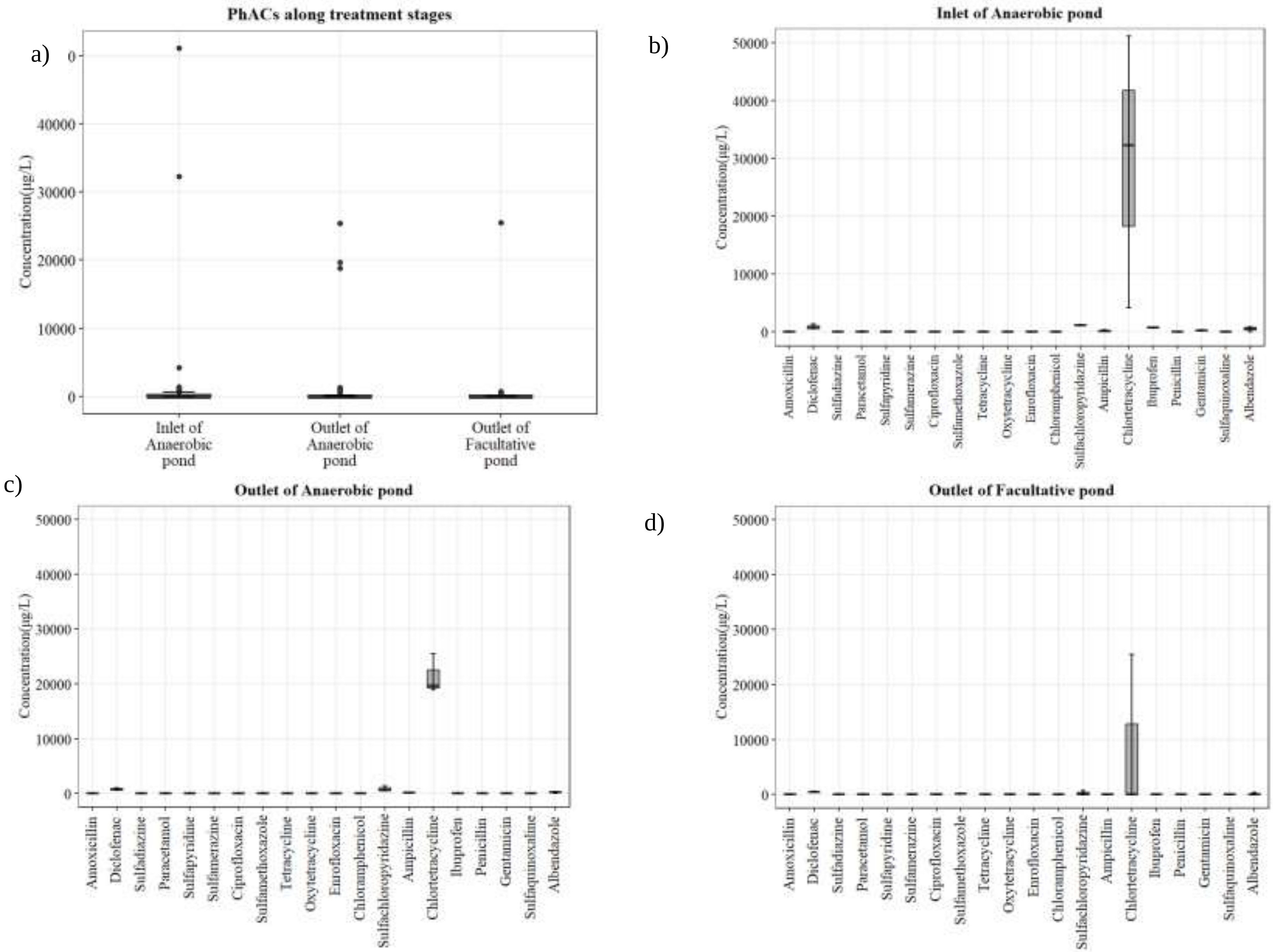

Figure A.3: Concentrations of PhACs along the treatment stages of WSPs; a) overall concentration, b) concentration at the inlet of anaerobic pond, c) concentrations of PhACs at the outlet of anaerobic pond and d) concentration at the outlet of facultative pond. Box represents 50% of the data points, whiskers represent minimum and maximum, line in box represents the median

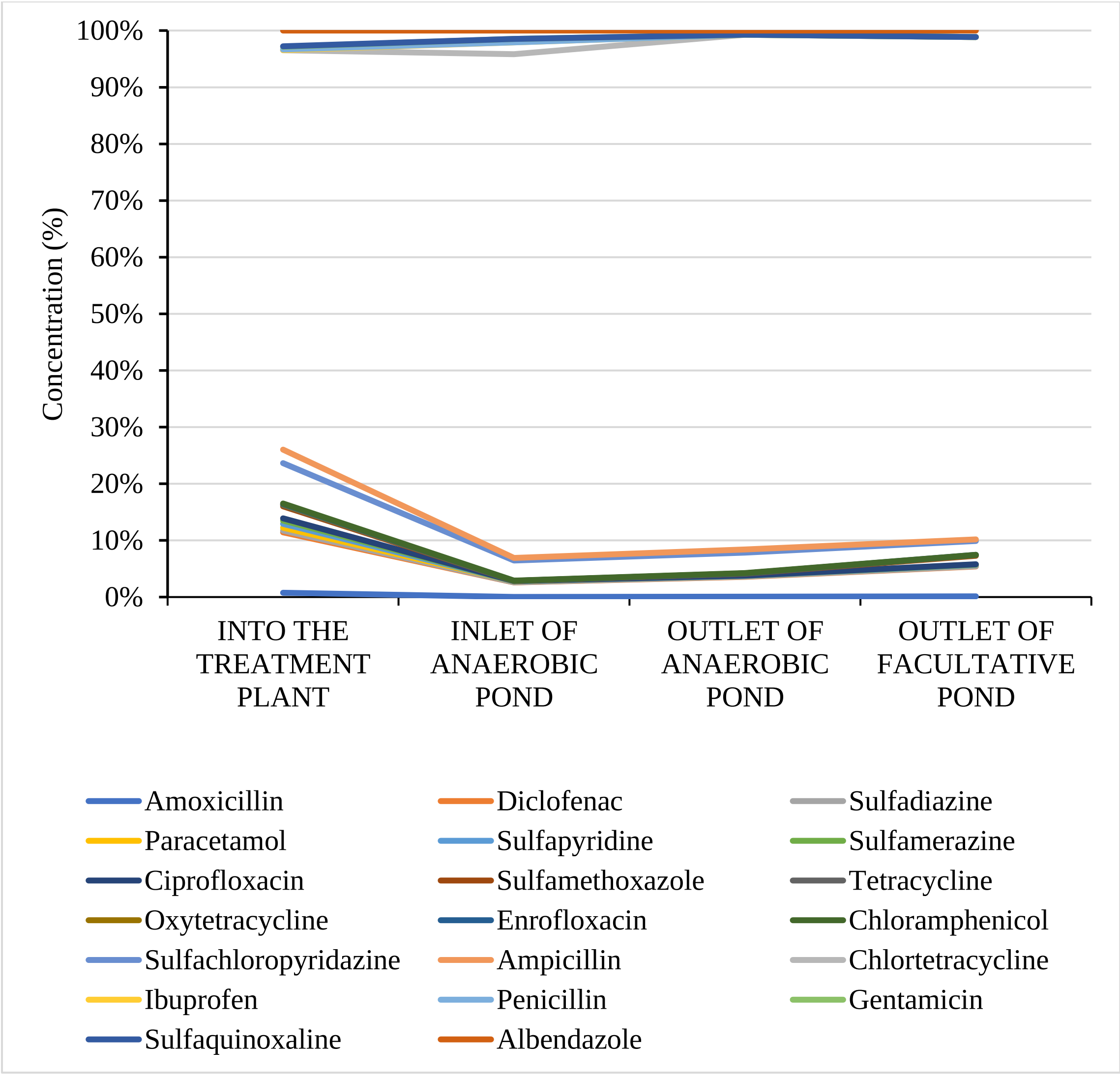

Figure A.4: Variation in percentage concentrations of PhACs from entry to exit of the WWTP (n=18)

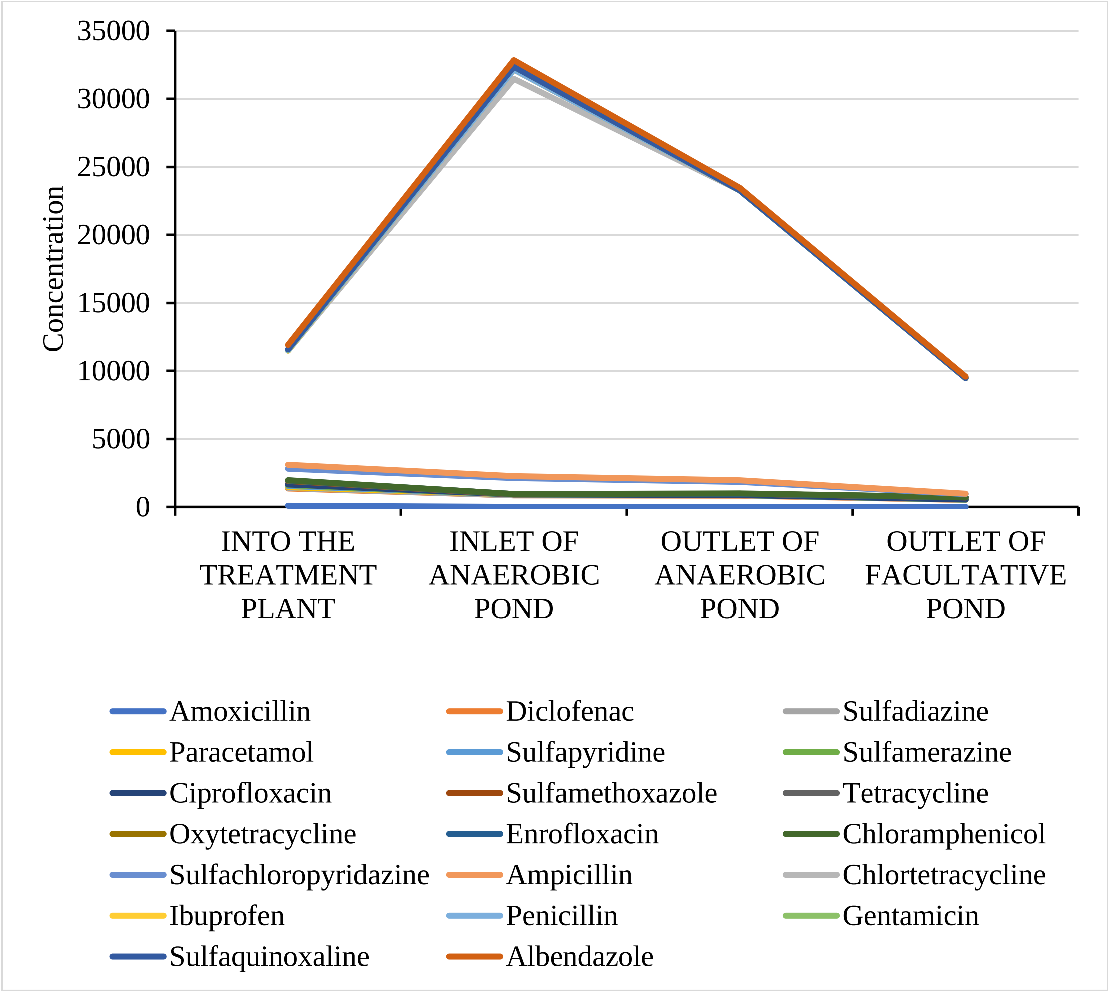

Figure A.5: Variation in concentrations of PhACs from entry to exit of the WWTP (n=18)

Appendix III

Table A.1 ANOVA for Inlet wastewater and FS streams

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Wastewater from sewer network	22	1494.088	67.913108	19526.18
Septage	22	4248.768	193.12583	299418.2
Faecal sludge from pit latrines	22	6609.472	300.43053	1235057

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	595883.7612	2	297941.88	0.575177	0.565532	3.142809
Within Groups	32634032.5	63	518000.52			
Total	33229916.26	65				

Table A.2 ANOVA along the treatment stages

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Inlet of Anaerobic pond	20	32842.6896	1642.134	42202267
Outlet of Anaerobic pond	20	23450.6471	1172.532	22535199
Outlet of Facultative pond	20	9581.6787	479.0839	3576182

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	13693890	2	6846945	0.300684	0.741479	3.158843
Within Groups	1.3E+09	57	22771216			
Total	1.31E+09	59				

Table A.3 ANOVA for the removal efficiencies

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
		-	-	
Removal efficiency of the Anaerobic pond	20	1571.976	78.598819	142194.3
Removal efficiency of the Facultative pond	20	306.1237	15.306186	4133.662

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	88181.5	1	88181.5	1.205258	0.279181	4.098172
Within Groups	2780231.8	38	73163.995			
Total	2868413.3	39				