CHARACTERIZATION OF EGERTON UNIVERSITY WASTEWATER STABILIZATION PONDS AND ASSESSMENT OF SUBSTRATE SIZE EFFICIENCY IN REDUCTION OF FAECAL POLLUTANTS IN A CONSTRUCTED WETLAND MESOCOSM.

Master of Science Thesis

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been submitted in part or whole for an award of a degree in any institution

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Recommendation

This thesis has been submitted with our approval as supervisors for examination according to Egerton University regulations

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DEDICATION

I would like to dedicate my work to my son Ted and my family members for their immense spiritual, emotional and material support during the entire study period**.**

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ABSTRACT

The aim of this study was to determine variations in concentration of Pathogen Indicator Organisms (PIOs) namely total coliforms *Escherichia coli* and heterotrophic bacteria; Biochemical Oxygen Demand (BOD) in Egerton University Wastewater Stabilisation Ponds (WSPs) and the effect of substrate sizes on wastewater treatment. Sampling at the WSPs was done on weekly basis for one month from mid-Nov to mid-Dec 2017 using standard procedures for examination of water and wastewater. A mesocosm study imitating a vertical sub surface flow constructed wetland was conducted to determine the most efficient substrate size in removal of these pollutant indicators. Total coliforms (TC) and *E. coli* were isolated using selective and differential media following membrane filtration method Colonies were enumerated on chromocult agar. Heterotrophic bacteria (HPCs) were enumerated using standard pour plate method on plate count agar. Biochemical Oxygen Demand was determined by incubating samples in a cabinet whose room temperature ranged between 20 $^{\circ}$C to 25 $^{\circ}$C for 5 days. For the mesocosm study, three sets of experiments with different gravel aggregate sizes were set up in triplicates. Wastewater from SMP was introduced and settled for six weeks to enable micro-organisms to establish and stabilize, before collection of water samples for analysis on weekly basis for eight weeks. The highest concentration of both PIOs and BOD was in the inlet, and this reduced along the pathway towards the outlet. Apart from BOD_5, there was a significant difference between the influent and effluent in all the parameters ($p<0.05$). The range for TC, *E. coli*, HPCs and BOD_5 was 5.5×10^6 - 2.9×10^{11}, 4.4×10^4 - 1.9×10^{10}CFUs / 100 ml, 4.5×10^6 - 5.0×10^9 CFUs / ml and 142.8 - 163.6 mg/l respectively. Removal efficiencies ranged between 99.8-99.9 % (3 log units) for both TC and *E. coli* in both First Maturation Pond and Second Maturation Pond. Heterotrophic Plate Counts reduced in concentration along the treatment pathway by 2 log units. In the mesocosm study, percentage reduction efficiency for TC for different substrate sizes was recorded as 95.3, 90.4 and 88.8 % for small, medium and large gravel aggregate respectively, while *E. coli* was recorded as 95.2, 94.3 and 88.4 % and HPCs was 99.8, 99.7 and 99.5 %. Furthermore, removal of organic matter was recorded as 15.9, 9.9 and 8.4 % for BOD_5 while TSS was 72.7, 56.6 and 52.4 % for small, medium and large sized gravel aggregates respectively. In conclusion, WSPs at Egerton University performed well in removal of PIOs. Heterotrophic bacteria levels indicated presence of pollution with easily degradable organic matter, while BOD_5 levels did not. In addition, none of the substrate sizes employed in mesocosm study performed better than the other in removal of PIOs and organic matter

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AOC	Assimilable Organic Carbon
APHA	American Public Health Association
BGLB	Brilliant Green Lactose Bile
BOD	Biochemical Oxygen Demand
BOD_5	Five-day Biochemical Oxygen Demand
CFUs	Colon Forming Units
CWs	Constructed Wetlands
DCEEN	Department of Civil and Environmental Engineering
DO	Dissolved Oxygen
DWSS	Department of Water, Sewerage and Sanitation
EPA	Environmental Protection Agency
FAP	First Anaerobic Pond
FC	Faecal coliforms
FFP	First Facultative Pond
FMP	First Maturation Pond
FWS	Free Water Surface
HPCs	Heterotrophic Plate Counts
HRT	Hydraulic Retention Time
HS	Hybrid System
HSD	Honest Significant Difference
HSSF	Horizontal Sub Surface Flow
MF	Membrane Filtration
MPN	Most Probable Number
NEMA	National Environmental Management Authority
PIOs	Pathogen Indicator Organisms
SFP	Second Facultative Pond
SMP	Second Maturation Pond
TC	Total Coliforms
USEPA	United States Environmental Protection Agency
VSSF	Vertical Sub Surface Flow
WHO	World Health Organization
WSPs	Wastewater Stabilization Ponds

CHAPTER ONE

INTRODUCTION

1.1 Background information

Wastewater management is still a challenge in many developing countries, and untreated or partially treated wastewater finds its way into aquatic systems (Drechsel et al., 2015). Surface waters are contaminated with organic matter, total suspended solids (TSS), heavy metals, nutrients and micro-organisms (Mbwele, 2006). Such scenarios are the basis for contamination with faecal matter in receiving water bodies, which contain total and faecal coliforms on the order of 10^8-10^{10} and 10^7-10^9 colony forming units (CFU) L^{-1}, respectively (George et al., 2002). On average, high-income countries treat about 70 % of the wastewater they generate, while that ratio drops to 38% in upper middle-income countries and to 28% in lower middle-income countries. In low-income countries, only 8% of industrial and municipal wastewater undergoes treatment of any kind (Sato et al., 2013). Wastewater treatment involves conventional methods including activated sludge systems, trickling filters, slow sand filtration, rotating biological contractors, among others. Activated sludge systems are the most commonly used systems worldwide, although they do not completely eliminate bacterial pathogens and organic matter (Okoh et al., 2007). According to Akratos & Tsihrintzis, (2007), in spite of the wastewater of human origin containing various pollutants, more attention is given to organic matter and nutrients, with less consideration of pathogenic micro-organisms and their potential risks to public health.

The most commonly used methods for wastewater treatment in developing countries are wastewater stabilization ponds (WSPs) and constructed wetlands (CW) (Kivaisi, 2001). Wastewater stabilisation ponds are shallow basins that naturally treat water using decomposition and autotrophic activities of aerobic and anaerobic microorganisms. They are preferred in tropical environment due to the temperature and sunlight that provides excellent conditions for wastewater processing by microbes (Kayombo, 2005). A study carried out in Kenya, Egerton University WSPs showed a reduction in faecal pathogens from facultative to maturation ponds (Kimani et al., 2009).

The ability of a CW ecosystem to improve water quality has been recognized since the 1970’s (Knight, 1999; Vymazal, 2005; Fernando & Quiroga., 2011). The idea behind the use of a CW is to ensure that it mimics the properties of a natural wetland ecosystem, treating contaminants as they pass through the system before they are released into the receiving aquatic systems (Vymazal, 2005). Constructed Wetlands have been adopted in most developing countries, remote areas, in cities and at household levels due to their low capital and low operational cost requirements (Kivaisi, 2001). They prove to be the most cost-effective alternative in wastewater treatment in comparison to the technical and expensive tertiary processes (Morsy et al., 2007).

Pathogen indicator organisms include total and faecal coliforms (with *E. coli* as the most predominant species), intestinal *Enterococci, Clostridium perfringens* and heterotrophic bacteria. They are used to indicate faecal contamination due to their presence in the intestines of humans and warm - blooded animals and their excretion through faeces (Kavka et al., 2002). They are not necessarily pathogenic to humans, but their existence is an indication of potential presence of pathogenic bacteria, viruses and parasites as well (USEPA, 2006; Morgan et al., 2008). On the other hand, heterotrophic bacteria and Biochemical Oxygen Demand (BOD) are useful indicators for organic pollution with easily degradable organic matter found in domestic wastewater.

Bacteriological identification of PIO employs a host of techniques including Most Probable Number (MPN) method, Membrane Filtration Technique (MF) and Heterotrophic Plate Counts (HPCs) determinations among others (USEPA, 2006). Organic matter concentrations are indirectly measured by BOD determinations. Biochemical oxygen Demand is the amount of dissolved oxygen (DO) consumed by aerobic microorganisms for the oxidation of organic matter; thus, it provides a measure of the organic content of wastewater and indicates how much oxygen is required to break it down (Lee et al., 2014). The mechanisms of BOD removal include settling of organic matter and bacterial decomposition of BOD causing substances (Cronk, 1996).

This study involved a combined system, characterising the inlet and five WSP at Egerton University with respect to concentration of TC, *E. coli*, and easily degradable organic matter. The study further assessed the effect of substrate size in removal of PIO, organic matter and using coliform, BOD, TSS and HPCs tests respectively in a mesocosm experiment that mimicked a VSSFCW.

1.2 Statement of the problem

Discharge of large volumes of poorly treated wastewater into aquatic ecosystems not only impairs aquatic ecosystem processes and services but also leads to ecological and public health risk to downstream users. Wastewater Stabilisation Ponds and Constructed Wetlands present cost-effective treatment solutions to wastewater and are employed worldwide for removal of pathogens and organic matter. According to EPA standards, treated wastewater requires to attain <1000 CFUs/ 100 ml, which translates to 99.99 % reduction efficiency. Egerton University WSPs are important for treatment of wastewater from the campus community. Little information exists on the performance of this treatment plant, hence a need to enhance their efficiency.

1.3 General objective

To establish the characteristics of pathogen indicator organisms and organic matter in Egerton University WSPs and assess the substrate size efficiency in reduction of such pollutants of wastewater.

1.3.1 Specific objectives

1. To determine variations in concentrations of TC and *E. coli* along the treatment pathway in EU WSPs.
2. To determine Biochemical Oxygen Demand and Heterotrophic Plate Count levels as indicators of easily degradable organic matter in EU WSPs
3. To assess the effect of gravel aggregate sizes on reduction of PIO, BOD_5 and TSS using a mesocosm experimental set up.

1.4 Hypotheses

1. Variations in concentrations of TC and *E. coli* along the treatment pathway in EU WSPs are not significantly different.
2. Biochemical Oxygen Demand and Heterotrophic Plate Count levels as indicators of easily degradable organic matter in EU WSPs are not significantly different.
3. There is no significant difference in the reduction of PIO, BOD_5 and TSS by different gravel aggregate sizes.

1.5 Justification

The Kenyan 2010 constitution (Chapter 4, Part 1, section 42) entitles every citizen to clean and safe water and in adequate quantities (Kenya Constitution, 2010). According to the Kenya's National Environmental Management Authority (NEMA), discharge of wastewater into the environment is punishable by law unless a discharge permit is granted in conformity to pollutant set standards (EMCA, 1999). Wastewater stabilisation are commonly used for wastewater treatment in many sewered systems in Kenya, however their efficiency decreases if they are not routinely disludged. Constructed wetlands have been used to polish wastewater prior to discharge into aquatic receptacles. They are most preferred since no specific training is required in their operation, their operation and maintenance costs are lower, and they integrate well into the surrounding landscape.

The major contaminants in domestic wastewater are PIOs and organic matter. In Egerton University WSPs, concentration of these contaminants has not been extensively studied. In addition to that, use of CWs by most researchers has focussed on other types of CWs, documenting little information on potential use of VSSF CWs in removal of faecal contaminants. Moreover, substrate as a major component of a CW and its variability has a profound effect on the treatment efficiency though there is knowledge gap linking this to VSSF CWs. The current study provides suitable insights on treatment efficiency of WSPs at Egerton University and areas that need action on improvement of organic matter removal. Additionally, it further recommends the use of a VSSF CW filled with gravel substrate due to its ability to remove organic matter and faecal contaminants.

CHAPTER TWO

LITERATURE REVIEW

2.1 Wastewater characteristics

Wastewater is any water that has been contaminated by human use and includes any combination of domestic, industrial, commercial or agricultural activities, surface or storm water and any sewer inflow or sewer infiltration. Therefore, wastewater is a by-product of domestic, industrial, commercial or agricultural activities (Mara, 2004). The characteristics of wastewater vary depending on the source. Types of wastewater include: domestic wastewater from households, municipal wastewater from communities (also called sewage) or industrial wastewater from industrial activities. Wastewater can contain physical, chemical and biological pollutants. Households may produce wastewater from flush toilets, sinks, dishwashers, washing machines, bath tubs, and showers (New York Water Environment Association., 2014). Households that use dry toilets produce less wastewater than those that use flush toilets. After usage, it enters the wastewater stream where it flows to the wastewater treatment plant

2.2 Wastewater treatment systems

Wastewater treatment employs conventional and non- conventional methods of treatment.

2.2.1 Conventional wastewater treatment systems

They consist of preliminary, primary, secondary and tertiary treatments that are based on biological, physical and chemical processes (Vymazal, 2005). The most common biological processes of wastewater treatment are treatment with activated sludge, trickling filters, slow sand filtration and rotating biological contractors in which protozoa and microscopic metazoa use organic matter from wastewater as food and enhancement of biomass (Okoh et al., 2007). Biological treatment offers high quality removal of suspended solids, BOD and nutrients whereas waste sludge can be used in composting (Parać, 2015). Conventional biological treatment is highly efficient, uses less space compared to non-conventional treatments and their functioning is not dependent on outdoor conditions (Pena and Mara, 2004). The disadvantages of these convectional biological treatments are the constant high electrical energy requirements and the design, supervision, maintenance, and the general cost of construction that require highly skilled workers (Morsy et al., 2007). Moreover, tertiary treatment is required to for extra polishing of the wastewater to meet discharge standard requirements (Weber and Legge, 2008).

2.2.2 Non-conventional wastewater treatment systems

They are treatment systems with free surface and subsurface (horizontal and vertical) flow, including WSPs and CWs (Kadlec and Wallace, 2009). Non-conventional treatments, compared to conventional treatments, are easier to use, cost effective and are less complex in operation and design (Abdel-Raouf et al., 2012). However, non-conventional treatments have several limitations: treatment requires larger space, are sensitive to nutrients increase, toxic and heavy metal levels. Additionally, they require constant water supply, face competition from competing technologies and overloaded or badly maintained WSPs are prone to produce bad odour (Pena and Mara, 2004). They include wastewater stabilisation ponds (WSPs) and constructed wetlands.

Wastewater Stabilisation ponds are large, shallow basins in which raw wastewater is naturally treated with the use of aerobic and anaerobic bacteria (Abdullahi et al., 2014). Their use in wastewater treatment has been in place worldwide for the past 50 years (Fernando & Quiroga, 2011). Wastewater Stabilisation Ponds have proven to be effective for wastewater treatment especially in the tropics, where ambient temperature is less limiting. They are low energy consuming ecosystems that use natural processes in contrast to the complex and high energy consuming treatment system of conventional plants (Okoh et al., 2007). Kenya is one of the countries that have adopted WSPs for wastewater treatment, including other tropical countries like Tanzania, Zambia and Zimbabwe. However, their performance is limited by factors such as lack of proper maintenance and fluctuation in wastewater levels which in turn interferes with residence time (Kayombo, 2005). The ambient temperature and duration of sunlight in tropical countries offer an excellent opportunity for high efficiency and satisfactory performance for this type of water-cleaning system (Abdullahi et al., 2014). Wastewater Stabilisation Ponds comprise of a single string of anaerobic, facultative and maturation ponds connected in series, or parallel with respect to wastewater flow. A piping system ensures transfer of wastewater from the inlet of the anaerobic pond to the outlet of the final maturation pond.

(a) Anaerobic ponds

Anaerobic ponds receive raw wastewater and are designed for pre-treatment process. They are commonly 2-5 m deep and receive wastewater with high organic load (>100 g BOD/m^3 a day) (Abdullahi et al., 2014). They normally lack dissolved oxygen or algae (Okoh et al., 2007). The BOD is removed by means of sedimentation of solids and subsequent anaerobic digestion in the resulting sludge. The retention time in anaerobic ponds is usually 1.1-1.5

days, with anaerobic digestion temperatures usually above 15 °C (Kayombo, 2005). The anaerobic bacteria in these ponds are sensitive to pH >6.2, hence neutralization of acidic water is crucial if it is to be used in anaerobic ponds (Pena and Mara, 2004). A properly designed anaerobic pond will achieve about 40 % removal of BOD at 10 °C, 60 % at 20 °C and more than 75 % at 25 °C (Odjadjare, 2010).

(b) Facultative ponds

Facultative ponds receive effluent from anaerobic ponds. They are usually 1-2 m deep and are of two types, the primary facultative pond that receives raw wastewater and the secondary facultative pond that receives partially treated wastewater that is particle free (Okoh et al., 2007). Oxidation by aerobic bacteria is dominant in both primary and secondary facultative ponds. Facultative ponds are designed for BOD removal, and the oxygen used by the pond bacteria is generated by algal photosynthesis hence the need to have less suspended matter here (USEPA, 2000a). The ponds are usually dark green in colour since they contain algae, whose concentration depends on rate of mineralisation processes, temperature and sunlight. At peak algal activity, carbonate and bicarbonate ions react to provide more carbon dioxide for the algae, leaving an excess of hydroxyl ions, hence the pH of water rises above 9 a condition that eradicates faecal coliforms (Fernando & Quiroga, 2011; Abdullahi et al., 2014

(c) Maturation ponds.

Maturation ponds receive effluent from facultative ponds. They are usually 1-1.5 m deep, their primary function being pathogen removal with a small degree of BOD removal. They also contribute to removal of nutrients from wastewater (Fernando and Quiroga, 2011). The principal mechanisms for faecal bacterial removal in facultative and maturation ponds are attributed to residence time and temperature, high pH (>9), high light intensity, all these combined with high dissolved oxygen concentration (Kayombo, 2005; Abdullahi et al., 2014).

Wastewater stabilisation ponds seldom achieve the maximum effluent removal efficiency that meets the stipulated state standards. For example, studies indicate that raw wastewater may contain 10^6 to 10^9 coliforms/100 ml, while pond effluents proposed to be used for irrigation may need to be brought down in concentration to less than 1000 MPN/100 ml. Thus, removal efficiencies required are approximately 99.9 to 99.99 % or even higher (Tyagi et al., 2008). This study by Tyagi attained removal efficiencies for BOD, total coliforms and *E. coli* as 82.15 %, 99.42 % and 99.90 % respectively. In Kenya, Dandora WSP system treats industrial and domestic wastewater from Nairobi City, being the largest WSP in Africa. With a capacity to treat 80,000 m^3/day of wastewater, studies show 91 % BOD removal efficiency and >6 logs reduction in faecal coliforms, during dry weather (Pearson et al., 1996). This calls for the need to improve on the functioning of such systems through frequent monitoring of effluent quality to achieve even higher efficiencies.

Constructed Wetlands are an adopted green technology that has been in place since 1970's, engineered to mimic the processes that take place in a natural wetland to improve the quality of wastewater (Knight, 1999; Fernando and Quiroga, 2011). They utilize the physical, biological and chemical aspects of a natural system to remove organic matter, nutrients and pathogenic bacteria from pre-treated wastewater (Vymazal, 2005). The technology involves low maintenance cost, low energy requirements, making it suitable for use in remote areas, areas with poor infrastructure and more so in developing countries (Kivaisi, 2001; Morsy et al., 2007). Constructed wetlands are categorized differently, resulting to three designs: Hybrid Systems (HS), Free Water Surface (FWS) and Sub-Surface Flow (SSF) divided into Horizontal Sub Surface Flow (HSSF) and Vertical Sub-Surface Flow (VSSF) (Vymazal, 2005; Kadlec and Wallace, 2009; Sehar et al., 2014; Alexandros and Akratos, 2016). One of the key features of these systems is the substrate, which may vary depending on the design objective.

2.3 Substrate characteristics in constructed wetlands

Substrate is a key component of wetlands that provides surface area for attachment of macrophytes and microbial films (Sharma, 2013). The substrate used in CWs usually depends on desired flow patterns, nutrient and mechanical support for the chosen plant types, and as a nutrient source required for some biologically-mediated treatment processes (Weber and Legge, 2008). The media chosen as substrate needs to have a higher porosity as this helps to prevent clogging (Prochaska and Zouboulis, 2006). Grain size, media depth and pore size all contribute significantly to Hydraulic Retention Time (HRT), establishment of microbial communities and removal efficiencies of different pollutants. Long HRT positively correlates with any mechanism in removal of pollutants, increasing the removal efficiency (Toet et al., 2005). The substrate must be fine enough to retain organic matter yet rough enough to ensure no clogging while maintaining good oxygen penetration (Torrens et al., 2009). Multiple substrate types are employed in CWs, as documented by Weber and Legge (2008). They include: sand, gravel, soil, peat, compost, soil and crushed rocks. Furthermore, substrate type correlates with removal of faecal coliform (An et al., 2005 Loc cit; Morgan et al., 2008)

Moreover, multiple studies regarding removal of BOD using CWs have revealed varying results depending on the wetland type and substrate used. Use of stones in Sub Surface Flow (SSF) CW yielded 99 % removal efficiency (Kantawanichkul et al., 2009), shredded tyre chips yielded 92% removal efficiency in a HSSF CW (Garcia-Pérez et al., 2015), while volcanic rock yielded 74-78 % efficiency in a HSSF CW (Zurita et al., 2006). Performance of a CW is highly influenced by the substrate's hydraulic conductivity and this greatly influences HRT of the system (Kaseva, 2004). The main operational problem of SSF CWs is the progressive clogging of the substrate medium used. The development of clogging can be detected by the appearance of water on the surface of the granular medium near the inlet zone. When clogging becomes severe, water is seen overflowing onto the medium surface. Clogging results in various counter -productive situations: it decreases the hydraulic conductivity and porosity of the substrate media, it causes preferential water flows along the wetland, and it results in dead zones and/or short circuits. These processes diminish hydraulic performance, which consequently can affect the contaminant removal efficiency and life span of the system (Knowles et al., 2008).

Wetland systems with fine-and soil-based substrates have low hydraulic conductivity, while coarse sand-and gravel-based medium display higher conductivity (Sundaravadivel & Vigneswaran, 2012). Studies involving HSSF-CW in an arid area by Albalawneh et al. (2016) revealed that fine media (4-8mm) was more efficient in removal of faecal coliforms in comparison to course media (10-20mm). Vertical Sub-Surface Flow CWs have been observed to be superior to HSSF CW due to better Oxygen transport by multiple mechanisms and higher efficiencies in removal of PIO and organic matter (Kayombo, 2005). However, most studies focus on the use of gravel substrate in HSSF CW, but limited knowledge exists on the application of the same in VSSF CW.

The depth of the substrate media used ranges from 0.3 to 0.9 meters (1 to 3 feet) with 0.6 meters (2 feet) being most common. When gravel is used, the size ranges from fine gravel (≥0.6 centimetres) to large crushed rock (≥15.2 centimetres). The most typical size ranges from a combination of sizes from 1.2 centimetres to 3.8 centimetres. This gravel medium should be clean, hard, durable stone capable of retaining its shape and the permeability of the wetland bed over the long term (USEPA, 2000b). The choice of media is essential since efficiency of a CW is highly dependent on the substrate characteristics (Sharma, 2013).

2.4 Mechanisms of pathogen and organic matter removal in constructed wetlands.

Pathogen and organic matter removal in CWs employs multiple host mechanisms, including: sedimentation, natural die-off, temperature, oxidation, predation, mechanical filtration, exposure to biocides and UV radiation (Vymazal, 2005; Morgan et al., 2008). The focus of this study was on those mechanisms that are influenced by substrate characteristics and loading regimes on contaminant removal, namely: sedimentation, natural die off, mechanical filtration and biofilm retention. However, all the mechanisms combined ensures higher removal efficiencies since none of them acts independently (Weber and Legge, 2008).

2.4.1 Sedimentation

Sedimentation has been cited as the basic pre-treatment mechanism of wastewater as it ensures high removal rates of particulate matter and suspended solids (Cronk, 1996). This process is highly affected by wetland configuration and the type of media used (Karim et al., 2004; Weber and Legge, 2008). Sediment density as well affects the rates of sedimentation and therefore higher sediment sizes ensures higher sedimentation rates for bacterial pathogens (Boutilier et al., 2009).

2.4.2 Natural die-off

Natural die- off of pathogens has been notably cited as a pathogen removal mechanism and it correlates well with HRT (Wand et al., 2007). The natural die off rates of bacterial pathogens varies between the water column and sediments. *Coliphage* survive longer in sediments and die naturally faster in the water column, while *Giardia* survives longest in the water column and dies faster in the sediments (Karim et al., 2004).

2.4.3 Mechanical filtration

This refers to adsorption of cells to filter bed solids mechanically in CW as studied by Wand et al. 2007. Mechanical filtration commonly removes indicators of faecal contamination such as *E. coli*, total coliforms, faecal S*treptococci* and *Enterococci* in VSSF CW (Arias et al., 2003). For sand media, average attachment 8.0×10^6 cells g^{-1} in VSSF constructed wetland has been recorded (Wand et al., 2007). It is therefore conclusive that media size has an effect on attachment of pathogens to bed media.

2.4.4 Biofilm Retention

Biofilm layers usually develop on the filter substrate grains and plant roots, which leads to bacterial attachment and protozoan grazing hence pathogen removal. Smaller substrate particles and suspended solids as well facilitate retention of other pathogens (Alexandros and Akratos, 2016). Pathogenic bacterial removal has been studied to enhance biofilm creation in the substrate layers (Kolari, 2003).

2.5 Bacterial indicators of faecal contamination and organic pollution of water sources

Most bacteria naturally inhabit animal and human digestive tract and are transferable into water bodies through faeces. Moreover, *E. coli*, *Enterococcus sp* and coliforms can be used to indicate levels of hygiene (Frahm & Obst, 2003). Environmental safety is questionable when high numbers of faecal contaminant indicators exist, as their presence indicates faecal sewage pollution (Poté et al., 2009), as well as assessment of food safety and water pollution (Jay, 1992). In general, detection, isolation and identification of pathogenic micro-organisms in wastewater of human origin is a complex process requiring technical expertise and the procedure is time consuming and expensive (Poté et al., 2009). Furthermore, many pathogens cannot grow in artificial culture media used in the laboratory. Thus, in water and wastewater bacteriological testing, indicators of faecal contamination are usually sought for in place of pathogens due to their co-existence with pathogens in the intestines of humans and warm-blooded animals and their release through faeces (WHO, 2001; Kavka et al., 2002; Alexandros and Akratos, 2016).

Coliform bacteria have been used as indicators of microbial contaminants as they are readily found in the environment, soil and vegetation. They are universally present in large numbers in faeces of warm blooded animals and moreover, easy to culture (USEPA, 2006). *Escherichia coli* is most preferred because in domestic wastewater its density is high ranging between 10^6-10^9 CFU/100 ml (George et al., 2002) and can easily be detected. Additionally, it does not survive for long in the environment thus its presence is a clear indicator of recent faecal pollution (Asano et al., 2007). The concentrations of heterotrophic bacteria correlate commonly to organic pollution and are used as indicators of pollution with easily degradable organic matter (Kavka et al., 2002; WHO, 2003). The occurrence of indicators and their existence is a clear indication of potential presence of pathogenic bacteria, viruses and parasites as well (USEPA, 2006; Morgan et al., 2008).

Water contamination by bacterial pathogens can either be as a result of faecal or non-faecal origin. For faecal contamination, the bacteria indicator organisms responsible must possess the following characteristics: they should be universally present in faeces of animals and humans in large numbers and not multiply in uncontaminated samples. They should be present in greater numbers than the pathogens and should be at least equally resistant as the pathogen to environmental factors and to disinfection in water and wastewater treatment plants. Moreover, they should respond to treatment processes in a similar fashion to faecal pathogens and should be detectable by means of easy, rapid, and inexpensive methods and above all, they should be non- pathogenic (Gadgil, 1998; Bitton, 2005).

2.5.1 Total Coliform bacteria

Total coliform bacteria include a wide range of aerobic and facultative anaerobic, Gram-negative, non-spore-forming bacilli (APHA, 2005). They can grow in presence of relatively high concentrations of bile salts and can ferment lactose at a temperature range of 35-37 ^{o}C with production of acid, gas and aldehyde within 24-28 hours (Weber and Legge, 2008, APHA, 2005). *Escherichia coli* and thermotolerant coliforms (faecal coliforms) are a sub set of the total coliform group that can ferment lactose at higher temperatures (USEPA, 2006). As part of lactose fermentation, total coliforms produce the enzyme β-galactosidase. Total coliforms group includes different faecal coliforms including *Escherichia coli* as the most common as well as members from the genera; *Enterobacter*, *Citrobacter* and *Klebsiella.* Total coliforms include a variety of bacteria that indicate both human and animal contamination, and their measurements indicate faecal contamination, but not necessarily of human origin because of ability of some coliforms to grow and proliferate in the environment (Weber and Legge, 2008). Due to this reason, they cannot be a useful as an index of human related faecal pathogens, but they can be used as an indicator of treatment effectiveness and to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms (Sueiro, 2001). Total coliform bacteria occur in both sewage and natural waters and some of these bacteria are excreted in the faeces of humans and animals, but many coliforms are heterotrophic and able to multiply in water and soil environments.

Total coliforms can also survive and grow in water distribution systems, particularly in the presence of biofilms. Their presence in distribution systems and stored water supplies can reveal regrowth and possible biofilm formation that is easily noticeable in water storage containers. Their presence also shows contamination through increase of foreign material, including soil or plants (Grabow, 1996; WHO, 2001 and Sueiro, 2001). Coliform group are bacteria that produce a red colony with a metallic (golden) sheen within 24 hours at 35°C on an endo-type media. Coliforms have long been used as an indicator for presence of microbes in drinking water because of their ease of detection. In raw domestic wastewater, faecal coliforms form 20-30% of the total coliforms while the rest is non-faecal in nature (Knight et al., 1999). Coliforms like *Klebsiella spp*, can grow in industrial and agricultural waste under specific environmental conditions, while others can reproduce in the environment (Alexandros and Akratos, 2016). Inadequate water treatment, post contamination or excessive nutrients will indicate presence of coliforms bacteria. Coliform test therefore helps to indicate treatment efficiency of the technology used (Weber and Legge, 2008).

Thermotolerant coliforms are coliform bacteria that ferment lactose at 44-45^{o}C. The most predominant genus in most waters is *Escherichi*a, though, other thermotolerant types include *Citrobacter*, *Klebsiella. E. coli* is the most common indicator bacterium used to detect faecal contamination (Sueiro, 2001). It originates from the intestines of both human and warm-blooded animals (Alexandros and Akratos, 2016). *Escherichia coli* grows at 44-45^{o}C on a complex media, ferments lactose and mannitol with the production of acid and gas, and produces indole from tryptophan (Weber and Legge, 2008). This analysis further notes that *E. coli* has various strains, and some of them can grow at 37 ^{o}C and some do not produce gas.

Typical concentration of *E. coli* in domestic wastewater varies between 10^{6} and 10^{9} CFU/100 ml (Asano et al., 2007) and in fresh human and animal faeces, it may attain concentrations of up to 10^{9} CFU per gram. It is found in wastewater, treated effluents, and all-natural waters and soils subject to recent faecal contamination, whether from humans, wild animals, or agricultural activity (Weber and Legge, 2008). *Escherichia coli* and their various strains are considered a public health concern, causing many waters related outbreaks worldwide both in developing and the developed world (Ashbolt, 2004). The presence of *E. coli* provides evidence of recent faecal contamination, and detection should lead to consideration of further action, which could include further sampling and investigation of potential sources such as inadequate treatment or breaches in distribution system integrity, (Grabow, 1996; WHO, 2001 and Sueiro, 2001).

2.5.2 Intestinal *enterococci*

Enterococci is a sub-group of faecal streptococci and differentiated from other streptococci by their ability to grow in 6.5% sodium chloride at pH 9.6 and a temperature of 10 ^{o}C - 45 ^{o}C. They are valuable bacterial indicators for determining the extent of faecal contamination in aquatic systems (APHA, 2005). Presence of faecal enterococci is an indicator of recent faecal pollution as well when *E. coli* is not detected.

2.5.3 *Clostridium perfringens*

Clostridium spp. are Gram-positive, anaerobic bacteria (APHA, 2005). They produce spores that are exceptionally resistant to unfavourable conditions in water environments, including UV irradiation, temperature and pH extremes, and disinfection processes, such as chlorination (WHO, 2001). The *C. perfringens* bacterium is a member of the normal intestinal flora of 13-35% of humans and other warm-blooded animals. They do not multiply in most water environments and are highly specific indicators of recent faecal pollution hence indicate sources liable to intermittent contamination. They are more often present in higher numbers in the faeces of some animals, such as dogs, than in the faeces of humans and many other warm-blooded animals (Ashbolt et al., 2001). The numbers excreted in faeces are normally substantially lower than those of *E. coli*. Vegetative cells and spores of *C. perfringens* are usually detected by MF technique in which membranes are incubated on selective media under strict anaerobic conditions (APHA 2005). They are used as indicators of remote pollution in a water source.

2.5.4 Heterotrophic bacteria

The numbers of CFUs of heterotrophic bacteria in water are indicators of pollution with easily degradable organic matter (Kavka et al., 2002). Colonies may arise from pairs, chains, clusters or single cells, all called colony forming units. The counts of heterotrophic bacteria before and after a treatment are useful in evaluating treatment effectiveness of removal and disinfection. The final count also depends on the interaction among the developing colonies (APHA, 2005). In surface water HPCs obtained are an indication that water is loaded with high concentration of Assimilable Organic Carbon (AOC) as it is normally the case with domestic wastewater pollution. High densities of HPCs in water may indicate high oxygen consumption, high heterotrophic activity, high BOD and low dissolved oxygen (APHA, 2005). They may also be used to assess the cleanliness and integrity of the distribution system and the suitability of the water for use in the manufacture of food and drink products, where high counts may lead to spoilage (Weber and Legge, 2008).

2.6 Bacteriological water quality analysis methods

The standard coliform methods for measurement in bacteriological identification of pathogen indicator organisms include Most Probable Number (MPN) method, Membrane Filtration Technique (MF) and Heterotrophic Plate Counts (HPC) determinations among others. Despite its simplicity, MPN method is labour intensive as it involves three tests: presumptive, confirmatory and completed test. It requires at least four days to get a result. Though use of expensive membranes, MF technique is the mostly employed as it is quicker, reproducible and convenient for testing large number of samples. It involves counting of bacterial CFUs which form after a short incubation period on selective and differential media for coliforms. HPCs are used to assess easily degradable organic pollutants. They are used as a basis to check proper water treatment with the objective of keeping the counts as low as possible (USEPA, 2006). Any procedure can be used for any of the indicator bacteria by varying such factors as growth media and incubation temperature.

2.6.1 Membrane Filtration Technique (MF)

Membrane Filtration technique (MF) is one of the most commonly used method of analysing indicators of faecal contamination in aquatic ecosystems because it is approved by EPA, it conforms to what many state laboratories use, it is long established and well recognized (USEPA, 2006). MF is highly reproducible, can be used to test relatively large sample volumes and usually yields numerical results than other methods. It also gives quantitative result and good precision if the numbers of colonies grow adequately and further cultivation steps are not always needed which lowers the costs and time needed for the analysis. However, the method is limited in terms of testing highly turbid water and large numbers of non-coliform bacteria (APHA, 2005).

2.6.2 Most Probable Number (MPN)

Most Probable Number involves using dilution test tubes and is a labour-intensive method, takes up significant incubator space and requires up to four days for result. This method does not yield direct count of bacteria, instead, the water sample is added to a series of tubes that contain a liquid medium. After incubation, each tube shows either a positive or negative reaction for the target organism (USEPA, 2006). Production of gas acid formation or abundant growth in the test tube after a certain incubation period at 35 ^{o}C constitutes a positive presumptive reaction. Both lactose and Laury Tryptose broths can be used as presumptive media. All tubes with positive presumptive reaction are subsequently subjected to a confirmatory test. The formation of gas in a Brilliant Green Lactose Bile (BGLB) broth fermentation tube at any time within 48 hours at 35 °C constitutes a positive confirmatory test. A test using an *E. coli* medium can be applied to determine TC that are FC and the production of gas after 24 hours of incubation at 44.5^{o}C in an *E. coli* broth medium is considered a positive result (Rompré et al., 2002).

2.6.3 Heterotrophic Plate Count (HPC)

The numbers of colony forming units of heterotrophic bacteria in water are indicators of pollution with easily degradable organic matter. Heterotrophic Plate Count also known as the standard plate count is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment, distribution or in swimming pools (Kavka et al., 2002).

2.7 Biochemical Oxygen Demand (BOD)

A few studies have been carried out in Egerton University WSPs, and concentration of BOD in each of the ponds has not been extensively studied. Moreover, studies in CWs have focussed on use of macrophytes as they are known to increase removal efficiency for BOD (Kivaisi, 2001). This creates knowledge gap on the potential use of CWs in removal of BOD, employing different substrate sizes and without using any form of macrophytes. Biochemical Oxygen Demand is the amount of oxygen required by micro-organisms to break down all organic compounds in wastewater (Lee et al., 2014) an indirect measure of organic matter. Biochemical Oxygen Demand is specified as O_2 in mg/l which is consumed in a certain period, that is BOD_3, BOD_5 or BOD_7 for 3, 5 or 7 days of incubation respectively. For domestic wastewater, sample dilution is necessary since the amount of DO consumed by microorganisms is greater than the amount of DO available in the air-saturated BOD_5 sample (APHA, 2005). Biochemical Oxygen Demand in wastewater differ in strength depending on the source of wastewater, as shown in Table 2.1;

Table 2.1: Qualitative and quantitative ranges of Ranges of BOD and COD in Domestic Wastewater

Concentrations of Organics in Untreated Wastewater (Domestic)			
Contaminant	**Concentrations (mg/l)**		
	Weak	Medium	Strong
BOD	<100	~200-250	>300
COD	<250	~430	>800

Adapted from (Metcalf & Eddy, Inc., 2003)

Biochemical Oxygen Demand tests measure the BOD over a five-day period and this five-day test, called a BOD_5, is about 70 % of the waste systems total BOD. Five - day Biochemical Oxygen Demand is used out of convenience because it could take a very long time to determine the total amount of BOD (Lee et al., 2014; Tilak et al., 2016). Biochemical Oxygen Demand measurements are conducted in dark bottles to prevent the incidence of light to inhibit primary production by phytoplankton which generate oxygen. A well operated treatment system is expected to reduce up to 95% of BOD after five days. In the present study, the concentration of BOD_5 was determined in both WSPs and in the mesocosms units.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out at the wastewater stabilization ponds located within Egerton University, Njoro Campus, Kenya. Egerton University is located approximately 25 km south-west of Nakuru town in Njoro Sub-county in Nakuru County. Egerton University area lies within latitude 0° 15′S and between longitudes 35° 50′ and 35° 05′E (Figure 3.1), standing on about 1,580 hectares of land within the River Njoro watershed at an altitude of 1890-2190 m above sea level.

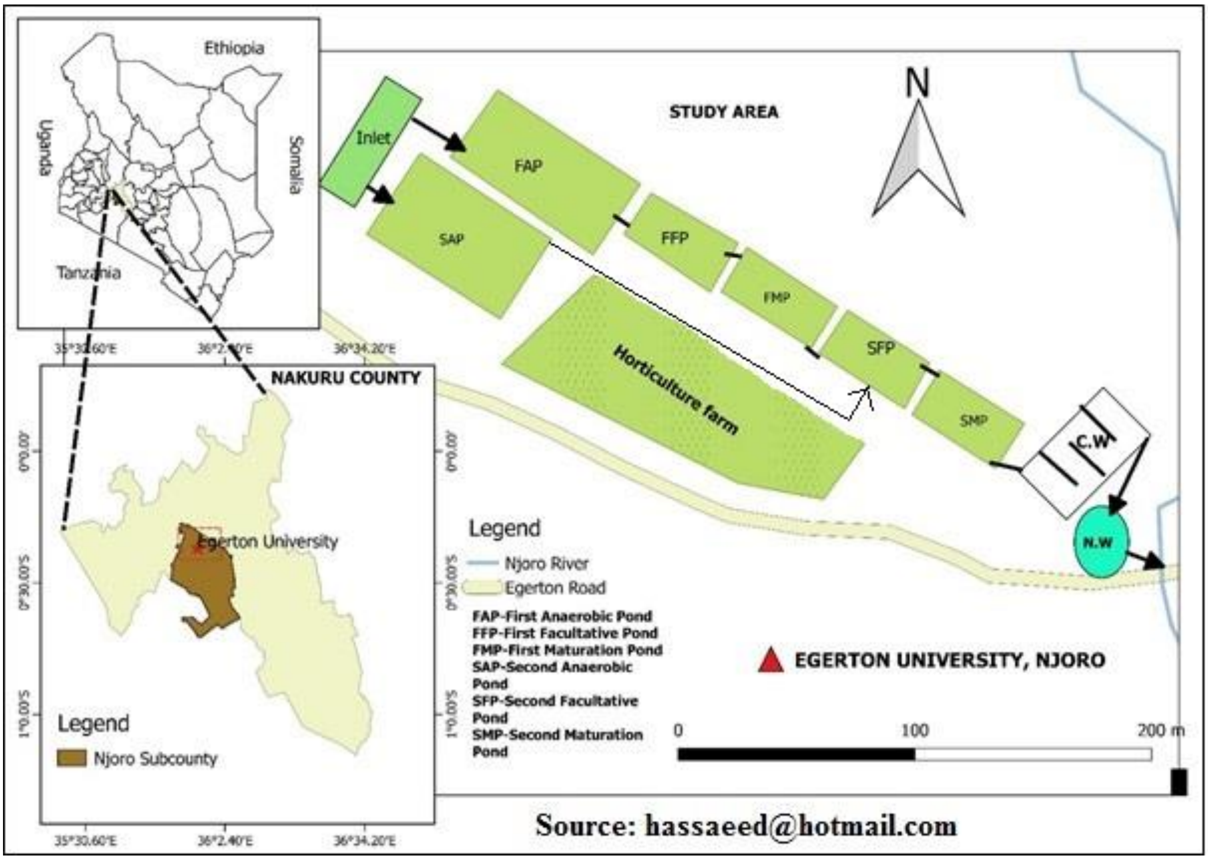


Figure 3.1: Map of Kenya showing Nakuru County and Egerton University, Njoro showing the study area.

3.2 Weather patterns

Some parts of the year, especially from July to August, the area is extremely cold with night temperatures going as low as 7 °C. Over the last century, the area has been receiving an average of 1145 mm of rainfall annually, with bimodal rainfall pattern. Long rains are experienced between March and May, ranging between 15.2 - 285.8 mm, averagely 124.12 mm. On the other hand, short rains appear between July and September, ranging between 25.2 - 220.3 mm, with an average of 116.6 mm. The average temperature is 20.8 °C, ranging between 15.3 - 20.8 °C (Personal communication, DCEEN- Egerton University, 2018). The area is located in an agriculturally endowed area and the produce from the farms in this area are sold in Nakuru town.

3.3 Staff and student population dynamics

The current total population of Egerton University, Njoro campus is 18,981 people (Admissions Department, Egerton University, 2018). However, at the time of sampling, the number of resident students was about 6,000, basing on number of rooms in individual hostels. The rest of the students, about 6,000 were accommodated outside campus premises. Inside the University are about 70 staff houses, which house averagely 4 individuals, hence 280 people. Among the students and staff are those who commute to campus daily and use toilet facilities, about 2000 people. These figures are not static due to continuous shifting of students and resident staff, leading to approximately 8,300 people (Personal communication, Estates Department, Egerton University, 2018). Due to this temporal variability in population, the wastewater system becomes dynamic, with the wetland drying completely during low population season. Wastewater generated depends on the source and amount of precipitation, as this influences the wastewater's residence time. Intense insolation increases evaporation rates from the WSPs, similarly affecting its functionality. Fifty percent of wastewater from residential houses within the campus are connected to septic tanks, while the other half is connected to sewer lines that transfer the wastewater generated to the WSPs (Personal communication, DWSS, Egerton University, 2017).

3.4 Design of the WSPs.

Egerton University has six WSPs incorporating an old system and a new system (Figure 3.2). The old system as presented in the diagram comprises of three ponds, the First Anaerobic Pond (FAP), First Facultative Pond (FFP) and First Maturation Pond (FMP) arranged in series. The second (new system) as well consists of three ponds, one anaerobic pond (SAP) connected in parallel to the anaerobic pond of the old system and connected directly to one facultative pond (Second Facultative Pond) and finally to one maturation pond (Second Maturation Pond) connected right after the old system, in a series. This direct connection of Second Anaerobic Pond to Second Facultative Pond is due to presence of horticultural farm, which limits space. The SMP is connected to a Hybrid wetland System (HS) with a baffled gravel bed and three surface cells to polish the pre-treated wastewater effluent from the WSPs before releasing into several few small natural wetlands which finally discharges into R. Njoro that flows into L. Nakuru as the only permanent river, alongside other seasonal small rivers, R. Makalia, Endeit, Naishi, Larmudiac and Ngosur (Gichuhi, 2008).

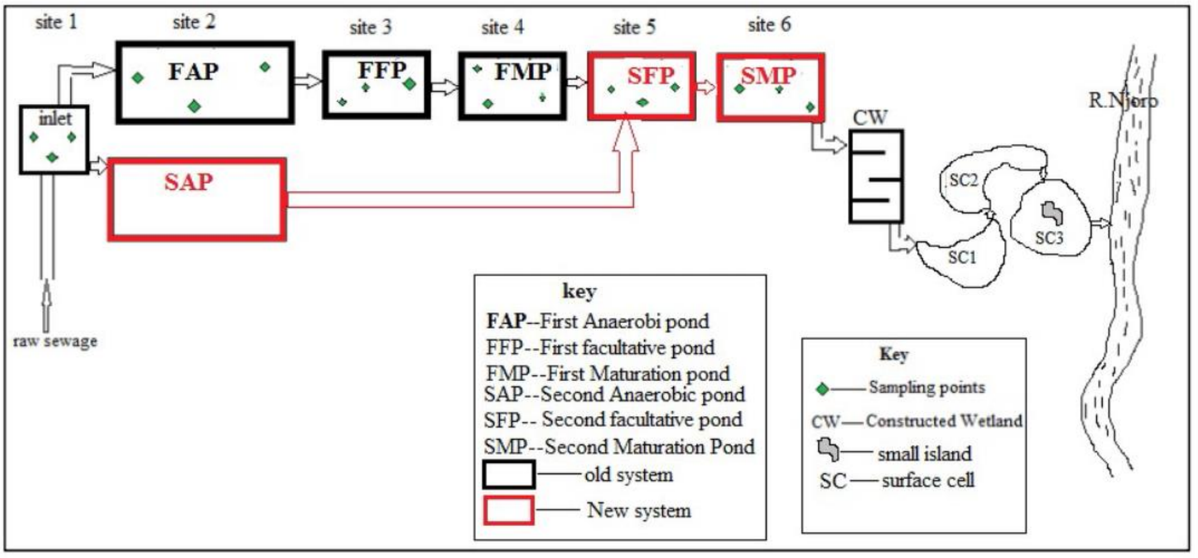


Figure 3.2: Schematic diagram showing design of WSPs ©Author

The first two anaerobic ponds are arranged in parallel and close to each other. They are large and wide, measuring (160 x 100) m^3 for length and width respectively. The FAP receives about 37 m^3day^{-1} while SAP receives an average of 680 m^3day^{-1}. The rest of the ponds are equal in size, with a uniform length of 110 m and width of 50 m and. The pond inlet receives up to 800 m^3 of wastewater generated from parts of the university on a daily basis. Pre-treatment takes place in the inlet with screening and grit removal taking place. The altitude of the ponds ranges between 2,210 m to 2,230 m above sea level with co-ordinates for each pond tabulated (Table 3.1)

Table 3.1: Position of the sampling points in terms of latitudes and longitudes

Site	Latitude	Longitude
Inlet	0°22'7.68"S	35°56'9.282"E
FAP	0°22'8.502"S	35°56'12.312"E
FFP	0°22'10.542"S	35°56'17.232"E
FMP	0°22'12.174"S	35°56'19.794"E
SFP	0°22'13.572"S	35°56'22.224"E
FMP	0°22'15.70"S	35°56'24"E

3.5 Overall study approach

The study approach consisted of two experiments. Experiment one involved an observational study where physio-chemical parameters were measured in the WSPs for a period of four weeks. This was done con-currently with characterisation of the ponds whereby the concentration of PIO and organic matter in each pond were determined. In the second experiment removal of contaminants by mesocosm experiment was conducted. The mesocosm experiment consisted of substrate preparation stage which involved sieving and grading to obtain the right aggregate for filling the respective mesocosms. This was followed by setting up the mesocosms outdoor and subjecting them to wastewater for six weeks to enable establishment and stabilization of microbial community. Sampling was then carried out in the mesocosm units on weekly basis for eight weeks to determine the efficiency of different substrate sizes in removal of PIO, organic matter and suspended solids.

Gravel sieving was carried out at the Egerton University Biological Science department. The sizes were in three categories, graded using standard wire mesh sieves with US sieve standards: 12. 5 mm (½''), 19 mm (3/4'') and 25 mm (1'') for small (<12.5 mm), mid-sized (12.5 - 18 mm) and large sized gravel aggregates (19-24 mm) respectively. The rationale for gravel sizes and depth used was adopted from USEPA (2000b), where the most commonly used gravel sizes for CWs ranges from 1.2 centimetres to 3.8 centimetres, while the depth ranges from 0.3 m to 0.9 m.

3.5.1 Mesocosm experimental set up

Graded gravel substrate was used in the mesocosm set up. The substrate was washed to reduce silt and other organic impurities and dried. Each substrate was filled into the respective mesocosm at a depth of 60 cm. The mesocosms consisted of three cylindrical metal tanks of 30 cm diameter each and 100 cm height replicated three times totalling to 9 tanks. These were labelled as: A1, A2, and A3 (small-sized gravel aggregate), B1, B2, B3 (Mid-sized gravel aggregate) and C1, C2, C3 (coarse gravel aggregate). The control was as well replicated three times and labelled T1, T2 and T3. Each mesocosm was fitted with a tap ½ inch, 10 cm from the bottom to act as an outlet. A 20 - litre bucket was placed 20 cm below each mesocosm to act as the effluent collection chamber.

The mesocosms were randomly arranged outdoor to account for potential variability in micro environmental conditions such as exposure to sunshine, shading and rainfall. Wastewater was sourced from the outlet of SMP and filled in the mesocosms, allowing a period of six weeks for establishment and stabilisation of microbial community. The system employed a batch reactor, with both influent and effluent samples being taken for laboratory analysis.

After each sampling occasion, refilling of the mesocosm units was done in preparation for the next sampling session, which was on a seven-day interval. The control experiment was subjected to same treatment, however, the mesocosms were not filled with substrate.

(a)

(b)

(c)

(d)

(e)

(f)

(g)

(h)

Source: Author

Plate 1: Materials used in the mesocosm study; (a) Assorted gravel sizes aggregates (b) Clean and washed gravel substrate (c) Small size sieve (12.5mm) (d) Mid-sized sieve (19 mm) (e)

Large size sieve (25 mm) (f) Small size gravel aggregate (<12.5mm) (g) Mid-sized gravel aggregate (12.5-18mm) (h) Large size gravel aggregate (19-24mm.

(a) **(b)** **(c)**

Source; Author

Plate 2: Mesocosm experimental set up; (a) mesocosm container (b) Experimental set up (c) Mesocosm sampling

3.6 Water sample collection scheme

Sampling was carried out in both the WSPs (n = 12) and the mesocosm units (n = 21). *In situ* measurements were taken for both the influent and effluent samples.

3.6.1 *In situ* field measurements

During each sampling session, physical- chemical parameters namely; temperature, DO, pH and conductivity were measured *in situ* using a calibrated HQ 40d (HACH) multi-meter probe and results recorded in the field. This was done before sample collection from both the WSPs and the mesocosms.

3.6.2 Characterization of WSPs in terms of PIO and BOD

Three replicate samples were collected from the six sampling sites between 8 a.m. and 12 noon on weekly basis for one month (18 samples on each sampling episode). The anaerobic pond of the new system (SAP) was not sampled on assumption that it received equal load of wastewater with anaerobic pond of the old system (FAP), hence they functioned in a similar way. Also, the special interest was to find out concentration of pollutants along the wastewater treatment pathway, but SAP was not aligned in series with the rest of the ponds. At the time of sampling, the Constructed Wetland had dried up and therefore no samples were collected in R. Njoro. The samples were kept in a cool box before transportation to the Biological Sciences Department laboratory within 6 hours of collecting the first sample where they were analysed for *E coli*, TC, Heterotrophic bacteria and BOD (APHA, 2005). Wastewater for BOD_5 analysis was diluted in the ratio of 1:9 prior to incubation initial readings and incubation

3.6.3. Water sample collection in the mesocosm units

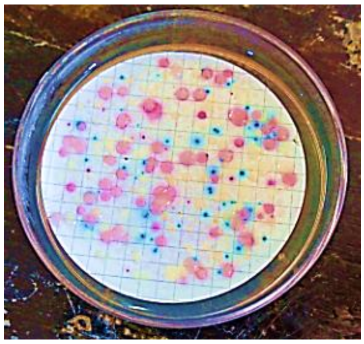
Sampling was done on weekly basis for eight weeks (3 influent samples and 12 effluent samples during each sampling episode). Physical-chemical parameters of influent samples were measured, and samples collected before allowing the wastewater to settle in the mesocosm units. Sampling was done using sterilized 500 ml glass bottles. The effluent was collected from the four sampling containers replicated three times with different substrate sizes including the control, after which they were immediately taken to the laboratory in the Biological Department for analysis of PIO, BOD and TSS. The mesocosms were immediately re-filled after each session in preparation for the next sampling session.

3.7 Wastewater sample processing

3.7.1 Membrane Filtration Technique for coliform bacteria

Once in the laboratory, 10x serial dilutions of wastewater were prepared using sterile 0.1 % bacteriological peptone solution to end dilution of either 10^7 or 10^8 depending on the site sample was taken from. Five millilitres of the sample diluent was introduced aseptically into a sterile stainless-steel filtration multi-channel apparatus containing a sterile membrane filter (47 mm diameter, 0.45 µm pore size) in each funnel. Filters were carefully taken by forceps and placed onto chromocult agar (Merck) plates on a petri dish gridded side up and incubated at 37 ^oC for 18-24 hours to allow growth of indicator organisms. Visually identifiable typical colonies appearing pink and dark blue were identified as coliforms and the blue colonies counted as *Escherichia coli*. Counting of colonies was done with the aid of a Fisher Accu-lite colony counter model 133-8002A. The results were expressed in numbers of “Colony Forming Units’’ (CFUs) per 100 ml of the original sample, as shown in Plate 3.0.

(a) **(b)**



(c) **(d)**

Source: Author

Plate 3: Membrane Filtration Technique (a) 10x serial dilutions (b) E. coli (blue) and Coliforms (pink) (c) Filtration unit (d) Colony Counter

3.7.2 Heterotrophic plate counts

An amount of 1 ml of each sample's dilution was placed onto 90 mm diameter plates and pour plated with molten heterotrophic plate count agar (Oxoid) held at 45 ºC in triplicates and incubated inverted at 37 ˚C for 48-72 hours. CFUs counted were expressed as cells per 1 ml. Both Membrane Filtration for coliform bacteria and Heterotrophic Plate Counts were conducted (APHA, 2005).

3.7.3 Biochemical Oxygen Demand Determination

Biochemical Oxygen Demand was determined according to Standard Methods for Examination of Water and Wastewater as stipulated in APHA (2005). Thirty millilitres of wastewater was added to BOD bottles with a capacity of 300mls and topped up with distilled water. Initial DO concentration was measured using a calibrated HQ 40D (HACH) multi-meter probe. The bottles were capped tightly and incubated in a cabinet whose room temperature ranged between 20 ˚C to 25 ˚C for five days. For this experiment five-day BOD was used and hence determined as BOD_5. After this incubation period, the final DO concentration was determined using the formula for unseeded water, (Pepper & Gerba, 2004).

$$BOD_5\ (mg/l) = \frac{(D_1 - D_2)}{P}$$

Where:

D_1 = initial dissolved oxygen (mg/l) in the diluted sample
D_2 = dissolved oxygen (mg/l) in the diluted sample after 5 days of incubation
P = the decimal volumetric fraction of sample used (10^1 dilution=$1/10^1$, or 0.1)

3.7.4 Total Suspended Solids Determination

The total suspended solids were estimated gravimetrically using glass micro-fibre filter paper method (Whatman GF/C filters with pore size 0.45µm) (APHA, 2005). A known volume of wastewater sample was filtered using pre-weighed Whatman GF/C filter and then dried at 95°C to a constant weight. The total suspended solids were estimated according to (APHA 2005) the formula:

$$TSS(mg/) = \frac{((Wf - Wc) \times 10^6)}{V}$$

Where:

TSS = Total Suspended Solids
Wc = Weight of pre-combusted filter in grams
Wf = Constant weight of filter + residue in grams
V = Volume of wastewater sample filtered in ml

3.8 Determination of substrate size efficiency in removal of PIOs and BOD

The overall removal efficiency of organic matter and PIO was calculated based on difference between the inlet and outlet mean concentration relative to the inlet mean concentration of measured parameters (*E. coli*, TC, HPCs, BOD_5 and TSS) using efficiency formula by Sperling, (2007):

$$E = \frac{(C_o - C_e)}{C_o} \times 100$$

Where:

E - Removal Efficiency (%)

C_0 - Raw sewage mean concentration (mg/l)

C_e - Final effluent mean concentration (mg/l)

3.9 Data Analysis

Sigma plot v.11 statistical package software was used for data analysis and prior to statistical tests, data from both WSPs and mesocosm study was checked for normality using Shapiro-Wilk W Test. Descriptive statistics were carried out to summarize the data and results presented graphically. The variation in mean / median concentration of total coliforms and *Escherichia coli* along the pathway in the Egerton University WSPs was tested using One-Way ANOVA for normally distributed data, Kruskal-Wallis test was used for data that did not pass normally tests. The same tests were used to determine the variation in the concentration of BOD_5 and HPCs as determinants of easily degradable organic matter in Egerton University WSPs. One Way ANOVA was used to test the significant differences in the removal of PIO, HPCs, BOD and TSS by different substrate sizes in the mesocosm experiment. In all the above cases, Tukeys' HSD post hoc test was used to separate the means. In all the statistical tests, the significance threshold was set at $\alpha = 0.05$ and significance level at 95 % i.e. $p < 0.05$.

CHAPTER FOUR

RESULTS

4.1 Physical chemical parameters in WSPs

4.1.1 Spatial variation

The mean values for physical - chemical parameters for specific ponds are shown in Table 4.1. Slightly higher temperature of 22.1 °C ± 0.5 was recorded in the SFP as compared to other ponds whose mean temperature ranged between 21.3-21.9 °C. However, no significant variation was seen in temperature along the pathway (One -way ANOVA; $F_{(5, 66)} = 0.635$; $p = 0.674$). Dissolved Oxygen was extremely low at the inlet, 1.8mg/ L while highest in the FMP and FFP, recording 10.9 mg/ L and 14.6 mg/ L respectively. The DO concentration varied significantly among the sites (One Way ANOVA; $F_{(5, 66)} = 9.825$; $p < 0.05$), where Tukeys' HSD test revealed a significant difference between the inlet and the mid ponds ($p < 0.05$) with no significant variation between inlet and SMP ($p > 0.05$).

Table 4.1: Physical-chemical parameters of wastewater in WSPs

Site	Temperature (°C)	DO (mg/l)	Conductivity (µS/ cm)	pH Range
Inlet	$(21.5 \pm 0.4)^{a}$	$(1.9 \pm 0.1)^{a}$	$(1102.3 \pm 157.4)^{a}$	6.9-9.0
	20.0 – 24.9	**0.1 – 7.7**	**632-2405**	
FAP	$(21.5 \pm 0.4)^{a}$	$(6.8 \pm 1.7)^{b}$	$(968.5 \pm 124.6)^{a}$	7.4-9.2
	19.4 – 24.3	**0.3 – 16.4**	**460.5 – 1607**	
FFP	$(21.3 \pm 0.3)^{a}$	$(14.6 \pm 0.2)^{b}$	$(949.7 \pm 107.2)^{a}$	7.9-10.0
	19.2 – 23.4	**2.7 – 20.0**	**589 – 1337**	
FMP	$(21.8 \pm 0.4)^{a}$	$(10.9 \pm 0.2)^{b}$	$(935.9 \pm 101.5)^{a}$	7.9-9.7
	19.3 – 24.3	**3.0 – 21.4**	**590.5 – 1330**	
SFP	$(22.1 \pm 0.5)^{a}$	$(9.5 \pm 0.2)^{ab}$	$(939.6 \pm 99.2)^{a}$	7.8-10.2
	19.1 – 24.3	**2.8 – 21.2**	**660.0 – 1322**	
SMP	$(21.9 \pm 0.4)^{a}$	$(6.5 \pm 0.1)^{a}$	$(941.5 \pm 99.4)^{a}$	7.9-9.5
	20.3 – 24.5	**3.1 – 10.4**	**592 – 1307**	

The figures in bold are values for mean and standard errors except pH, while those in parenthesis represent the range. The means with same superscript letter are **not** significantly different at p=0.05 level; n=12 while those with different letters indicate significant differences.

Electrical conductivity was highest at the inlet with no significant variation among the sites (One - ANOVA; $F (5, 66) = 0.305$; $p = 0.908$). The pH values showed a significant variation among the sites (One Way ANOVA; $F (5, 66) = 3.942$; $p = 0.003$) where a post hoc revealed a significance difference between the inlet and mid ponds (FFP and FMP) ($p<0.05$) with no variation between the inlet and other ponds ($p> 0.05$). At the inlet, high electrical conductivity negatively correlated with low pH.

4.1.2 Temporal variation

The first sampling period saw relatively high DO concentration as shown in Figure 4.1. The mean DO for sampling on 28/11/2017 was 11.5mg/l which went down in the subsequent sessions to 7.8 and 6.4 mg/l on 5/12/2017 and 12/12/2017 respectively, and finally a slight increase to 8.1 mg/l on 19/12/2017. Temporal variations in DO were not significant (One Way ANOVA; $F_{(3,\ 68)} = 2.376$; $p = 0.078$). The range of pH was 6.9-10.2 from sampling periods 1-4 with significant variation among the sampling sessions (One Way ANOVA; $F_{(3,\ 68)} = 19.706$; $p<0.05$). A post hoc analysis indicated a significant difference between sampling period 1 and periods 3 and 4 (Tukeys', HSD test, $p<0.05$) with no variation between sampling periods 1 and 2 ($p>0.05$).

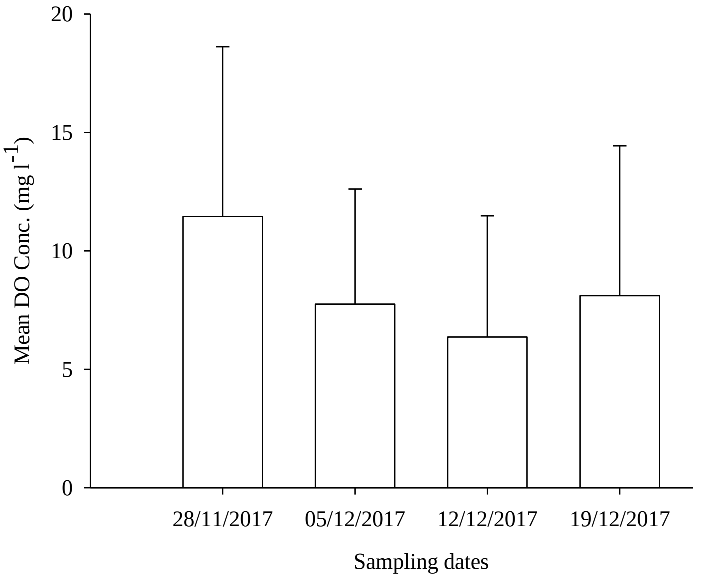


Figure 4.1: Temporal variation in DO in the WSPs (mean ± SD, n = 18)

Just like concentration of DO, similar trends were observed for temperature. During sampling the first sampling period, the mean temperature was 22.8 °C, and dropped slightly in the next two sampling periods to 22.5 °C and 20.2 °C respectively. The last sampling period saw a slight increase in mean temperature to 21.2 °C, however, there was no significant difference among the sampling periods (One Way ANOVA, $F_{(3, 68)} = 1.884$; $p = 0.140$).

Sampling periods 1 and 2 dominated in recording a high electrical conductivity mean of 1290 and 1374 µS/cm respectively, which was followed by a drop to 621 and 605 µS/cm for the last two sampling periods respectively as shown in Figure 4.2. A significant variation existed among the sampling periods (One Way ANOVA, $F_{(3, 68)} = 126.969$, $p<0.05$). A post hoc analysis revealed that sampling sessions were different from each other ($p<0.05$) except similarity that existed between sampling periods 1 and 2 ($p>0.05$).

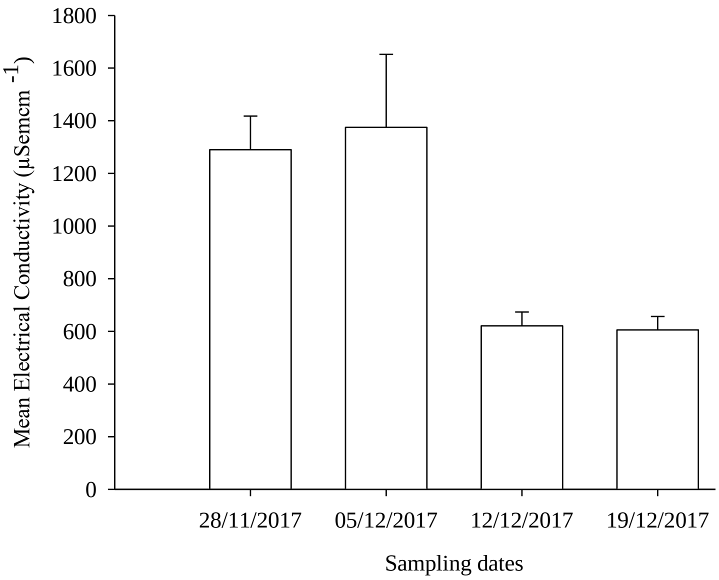


Figure 4.2: Temporal variation in Electrical conductivity in the WSPs (mean ± SD, n = 18)

4.2 Spatial variation in Pathogen indicator organisms in WPs

The highest concentration of both *E. coli* and TC was recorded in the inlet, just as it was expected in raw wastewater. Spatial variation indicated a reduction in concentration of both *E. coli* and TC along the wastewater treatment pathway from the inlet which slightly increased towards SMP. The inlet had highest mean concentration of $1.4 \times 10^{10} \pm 1.9 \times 11^{10}$ CFUs/100 ml for *E. coli* while the FFP had the lowest concentration of $3.0 \times 10^{6} \pm 8.4 \times 10^{6}$ CFUs/100 ml. The mean concentration of TC was highest as well at the inlet, $9.2 \times 10^{10} \pm 1.5 \times 10^{11}$ CFUs/100 ml while the lowest concentration was recorded in the SMP, $7.5 \times 10^{7} \pm 8.4 \times 10^{7}$ CFUs/100 ml.

There existed a significant variation in both *E. coli* (Kruskal-Wallis test; H=28.517; df =5; $p < 0.05$) and TC (Kruskal-Wallis test; H=37.711; df=5, $p<0.05$). For both *E. coli* and TC, a post hoc analysis revealed no significant variation between the inlet and FAP (Tukeys', HSD test, p>0.05), while a significant variation existed between the inlet and the rest of the ponds (p<0.05), as shown in Figure 4.3.

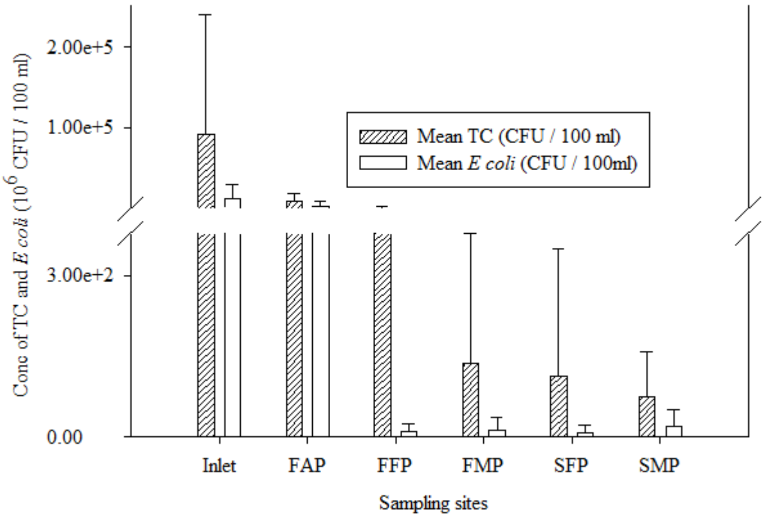


Figure 4.3: Spatial concentration of TC and *E. coli* in WSPs

4.3 Temporal variation in pathogen indicator organisms in WSPs

4.3.1 Total Coliforms

A general trend indicated an increase in concentration of TC from initial to final sampling period as shown in Figure 4.4. A significant variation existed among sampling periods in the inlet (Kruskal- Wallis; H=8.897; df =3, p=0.03). Total coliform concentration obtained during sampling on 19/12/2017 was significantly different from sampling done on 12/12/2017, (Tukeys', HSD test; p=0.031) with no significant variation among the first two sampling periods on 28/11/2017 and 05/12/2017 respectively (p>0.05). In the FAP, statistical analysis indicated a significant difference among sampling periods (Kruskal-Wallis; $F_{(3, 80)}$ =5.439, p=0.025. Sampling period 4 differed significantly from sampling periods 2 and 3 (Tukeys', HSD test; p=0.025), while no significant variation occurred between sampling periods 4 and 1 (p>0.05). Total coliform concentration did not vary significantly in the FFP among the sampling periods (One Way ANOVA; $F_{(3, 8)}$ =1.052; P=0.421). In the FMP, TC coliform concentration differed significantly among the sampling periods (One Way ANOVA; $F_{(3, 8)}$ =5.545, p =0.024). A post hoc analysis indicated that sampling on 19/12/2017 differed significantly from sampling done on other sampling periods: 28/11/201/, 05/12/20/1/2017 and 12/12/2017 (Tukeys', HSD test; p = 0.024). However, among the first three sampling periods, no significant variation occurred (p>0.05). There was no significant variation among sampling periods in TC concentration at the SFP (One Way ANOVA; $F_{(3,\ 8)} = 0.979$; $P=0.0449$). Similarly, there was no significant variation among sampling periods in the SMP in TC concentration (One Way ANOVA; $F_{(3, 8)} = 3.625$; $p = 0.064$).

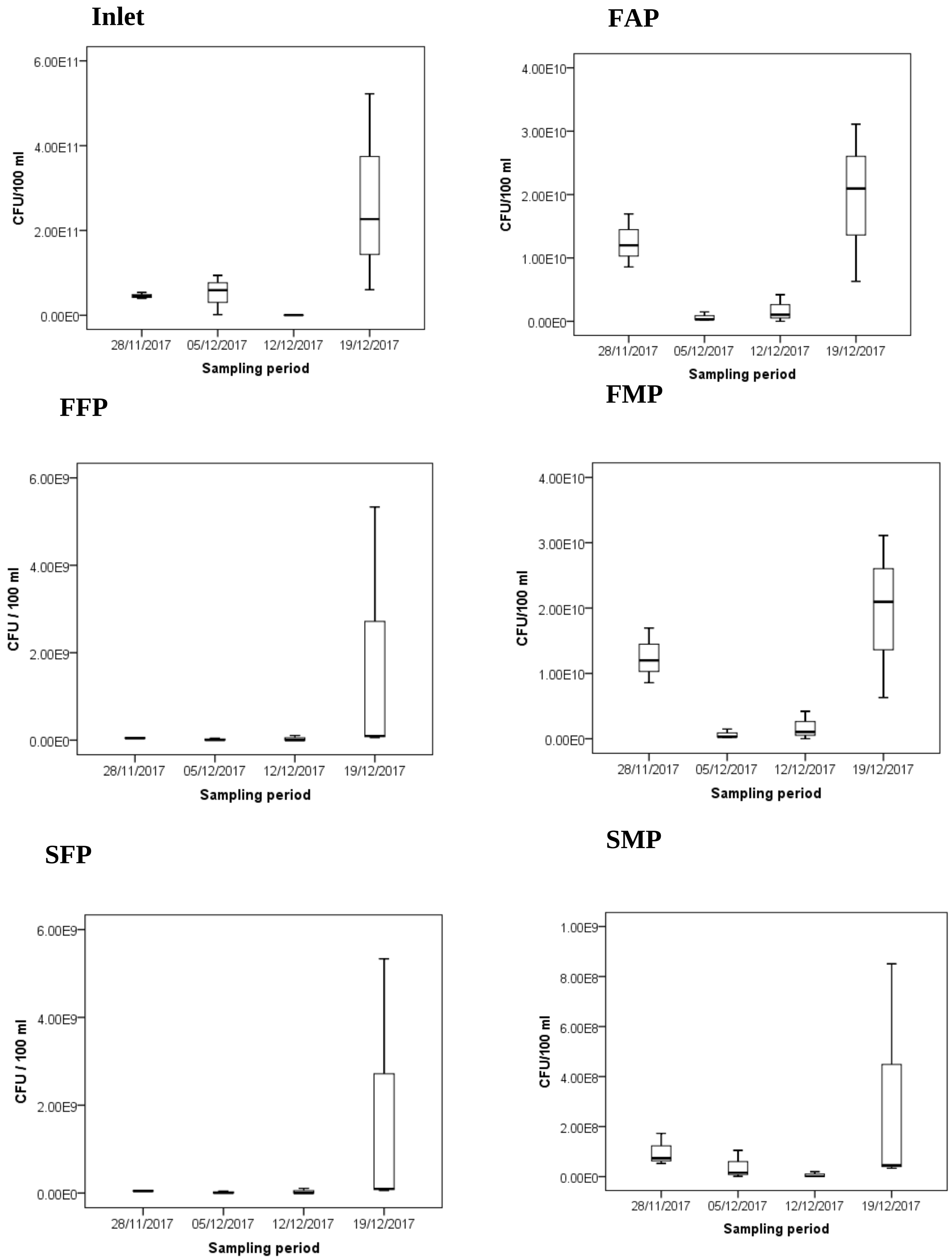


Figure 4.4: Temporal variation in TC concentration in WSPs presented as CFUs/ 100 ml. Box and whisker plots of median (25-75 percentiles) are shown; n= 12.

4.3.2 *Escherichia coli* concentration

All the ponds harboured high concentration of *E. coli* during the last sampling period (19/12/2017) apart from SFP. However, *E. coli* concentration among various ponds did not show significant variations at different sampling periods as shown by One Way ANOVA results in table 4.2

Table 4.2: One Way ANOVA results for temporal variation in *E. coli* concentration among the ponds.

Site	F	P-value
Inlet	0.824	0.517
FAP	1.110	0.400
FFP	2.062	0.184
FMP	2.976	0.097
SFP	1.172	0.379
SMP	2.811	0.108

The table shows One -way Anova results for temporal variation in *E. coli* concentration among the WSPs. The F values and P-values are shown. Degrees of freedom between and within groups are 3 and 8 respectively for both the inlet and the rest of the ponds

Temporal trends for *E. coli* concentration are in all ponds are presented in the figure 4.5.

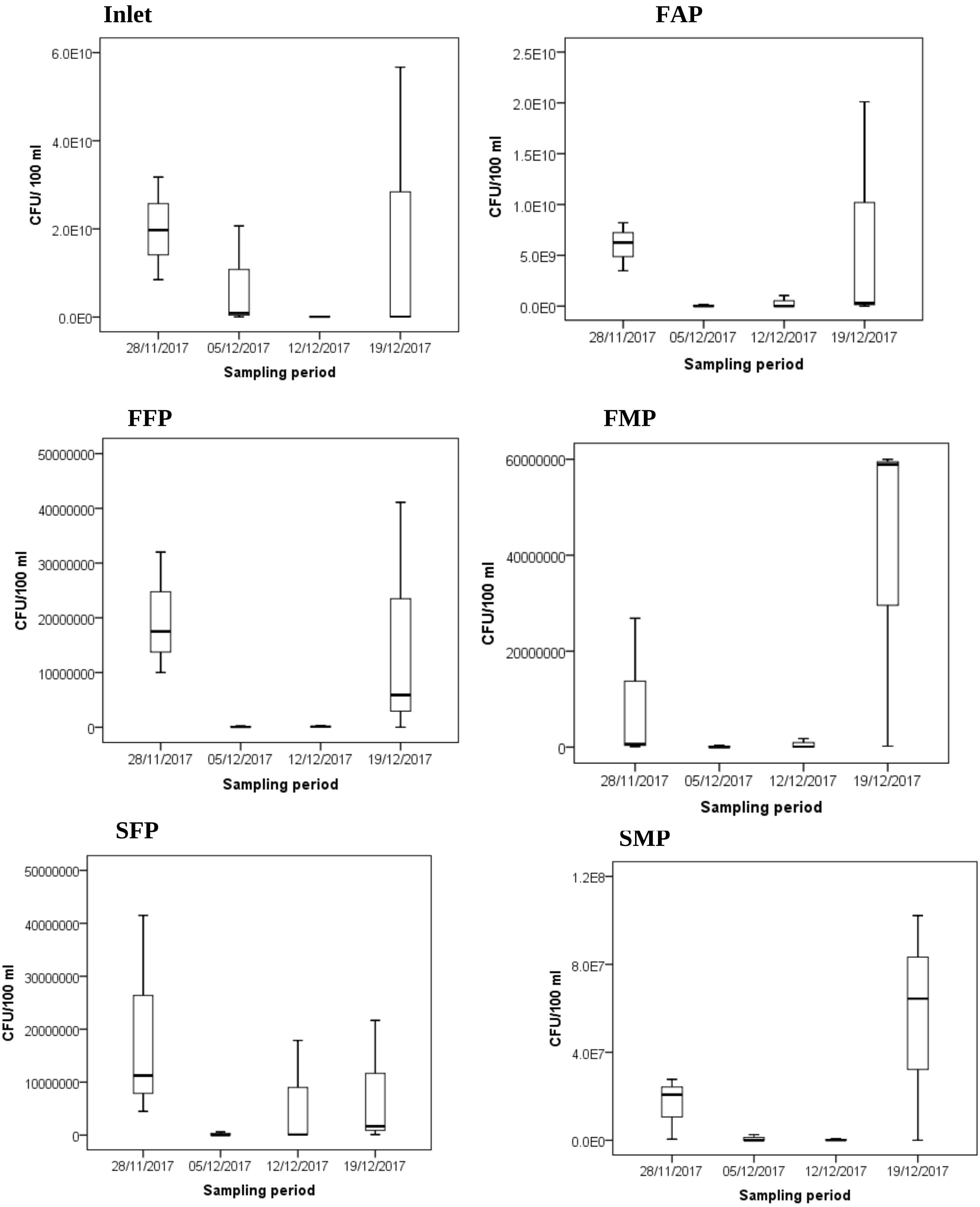


Figure 4.5: Temporal variation in *E. coli* concentration in WSPs presented as CFUs/ 100 ml. Box and whisker plots of median (25-75 percentiles) are shown; n= 12.

4.4 BOD as indicator of easily degradable organic matter in WSPs

4.3.1 Spatial variation

The mean BOD_5 for each pond was 163.55 ± 71.9 mg/l, 162.83 ± 47, 142.75 ± 80.6, 145.5 ± 96.3, 148.03 ± 89.5 and 152.88 ± 63.85 mg/l for the inlet, FAP, FFP, FMP, SFP and SMP respectively. The inlet had the highest mean BOD_5, while FFP recorded the least mean BOD_5 (Figure 4.6). The range of BOD_5 was between 3-242 mg/l, however, no significant variation existed in mean BOD_5 among the sites (One Way ANOVA; $F_{(5, 66)} = 0.161$; $p = 0.976$).

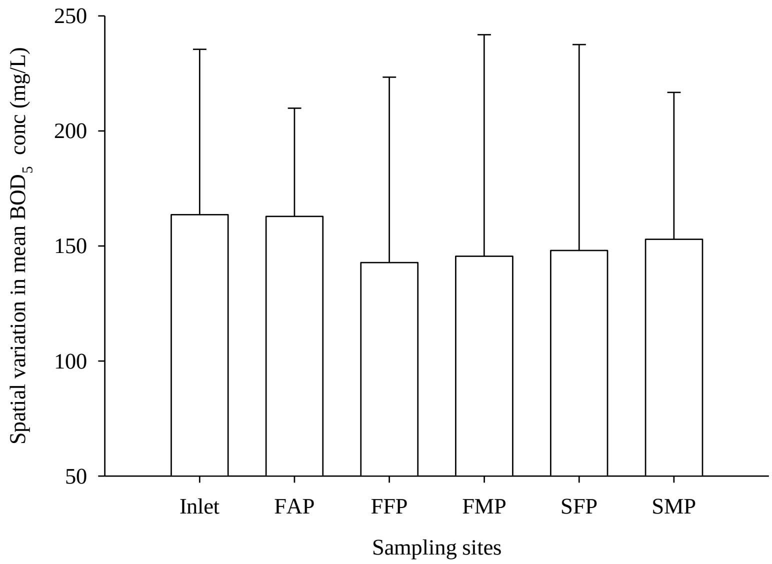


Figure 4.6: Spatial variation in BOD5 at the WSPs (values are mean ± SD, n = 12)

4.4.2 Temporal variation

Among all the sites, only the Inlet showed no significant variation in BOD_5 concentration throughout the sampling period (One Way ANOVA; $F_{(3, 8)} = 3.386$; $p=0.075$). There was a significant variation in the FAP among the sampling dates (One Way ANOVA; $F_{(3, 8)} = 65.415$; $p<0.05$. Sampling date 12/12/2017 differed from two other dates; 5th and 19th/12/2017 (Tukeys', HSD test; $p<0.05$) with no variation between sampling date 12/12/2017 and 28/11/2017 ($p>0.05$). The FFP, FMP, SFP and SMP indicated a significant variation during the sampling period (One Way ANOVA; $F_{(3, 8)} = 58.912$, $F_{(3, 8)} = 158.639$, $F_{(3, 8)} = 27.454$, $F_{(3, 8)} = 44.825$, $p<0.05$ respectively). In all these ponds, sampling session 1 and 2 were similar in BOD_5 concentration (Tukeys', HSD test; $p>0.05$) while a significant difference existed among the rest of sampling sessions ($p<0.05$) as shown in Figure 4.7.

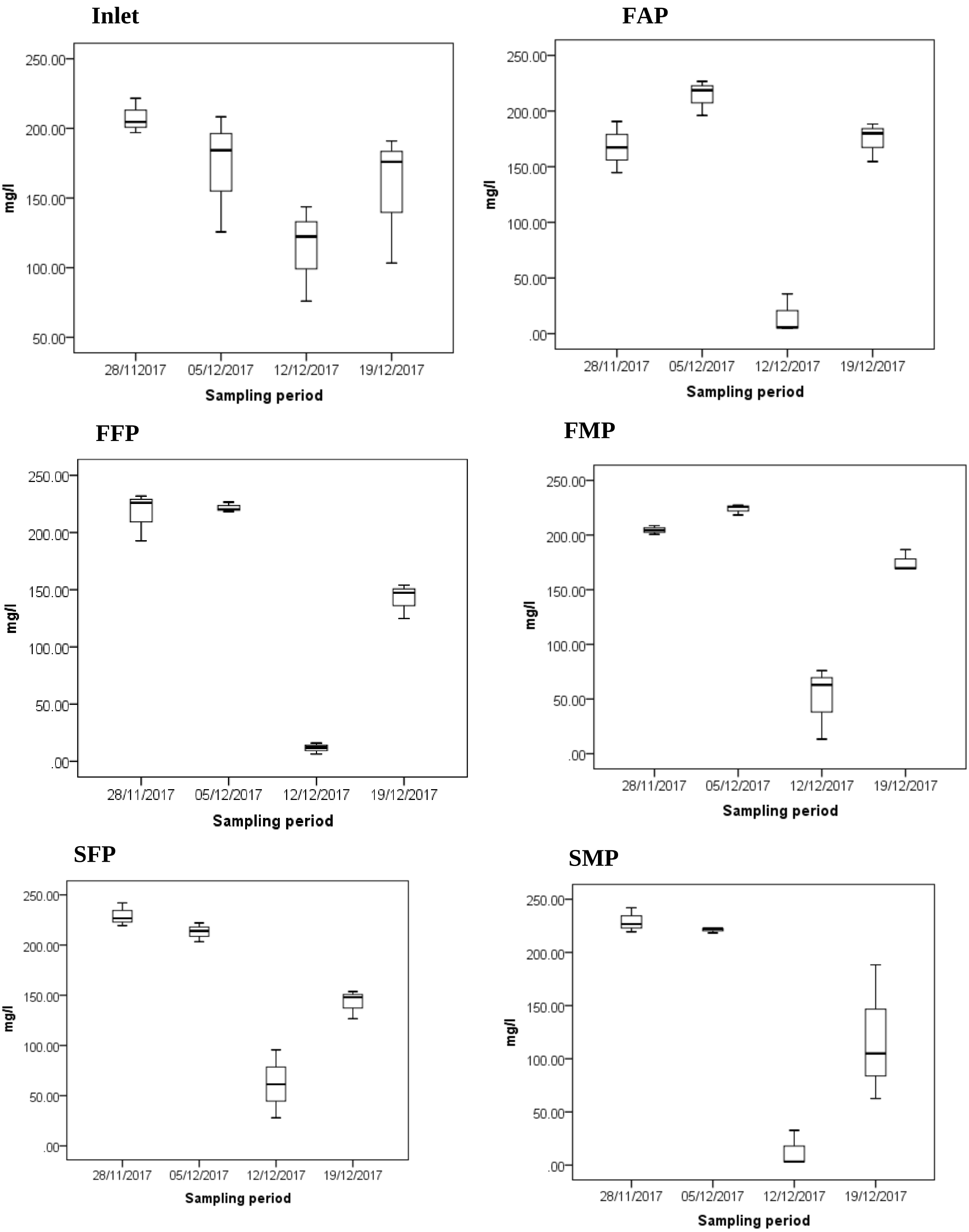


Figure 4.7: Temporal variation in BOD_5 concentration in WSPs presented as mg/l. Box and whisker plots of median (25-75 percentiles) are shown.

4.5 Heterotrophic Plate Counts as indicators of easily degradable organic matter

4.5.1 Spatial variation

The mean concentration of HPCs in each pond is presented in Figure 4.8. The highest concentration of HPCs was recorded in the FAP ($5.0 \times 10^9 \pm 4.1 \times 10^{10}$) while the lowest was in SFP ($4.6 \times 10^6 \pm 7.1 \times 10^6$). A slight increase in HPC concentration was observed in the last pond (SMP). A significant variation existed among the sites (Kruskal-Wallis test; H=29.443, df =5; $p<0.05$). Tukeys', HSD test showed that the inlet was significantly different from all other sites ($p<0.05$) with no significant variation from FAP ($p>0.05$).

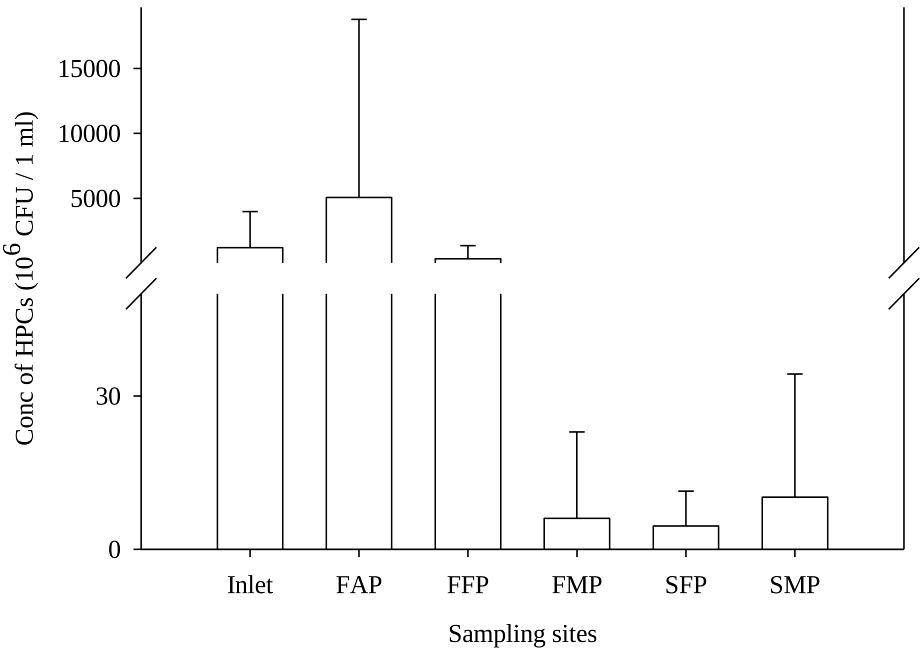


Figure 4.8: Spatial variation in mean HPCs concentration in WSPs (mean ± SD, n = 12)

4.5.2 Temporal variation

The last sampling period saw a relatively high concentration of HPCs in all the ponds except the SFP. However, there was no significant variation among sampling periods in all ponds in HPCs concentration, as shown by the One -Way ANOVA results in Table 4.3:

Table 4.3: One Way ANOVA results for temporal variation in HPCs concentration among the ponds.

Site	F	P value
Inlet	0.824	0.517
FAP	1.110	0.400
FFP	2.062	0.184
FMP	2.976	0.097
SFP	1.172	0.379
SMP	2.811	0.108

The table shows One -way Anova results for temporal variation in HPC levels among the WSPs. The F values and P-values are shown. Degrees of freedom between and within groups are 3 and 8 respectively for both the inlet and the rest of the ponds

Temporal variation in concentration of HPCs among different ponds is shown in Figure 4.9.

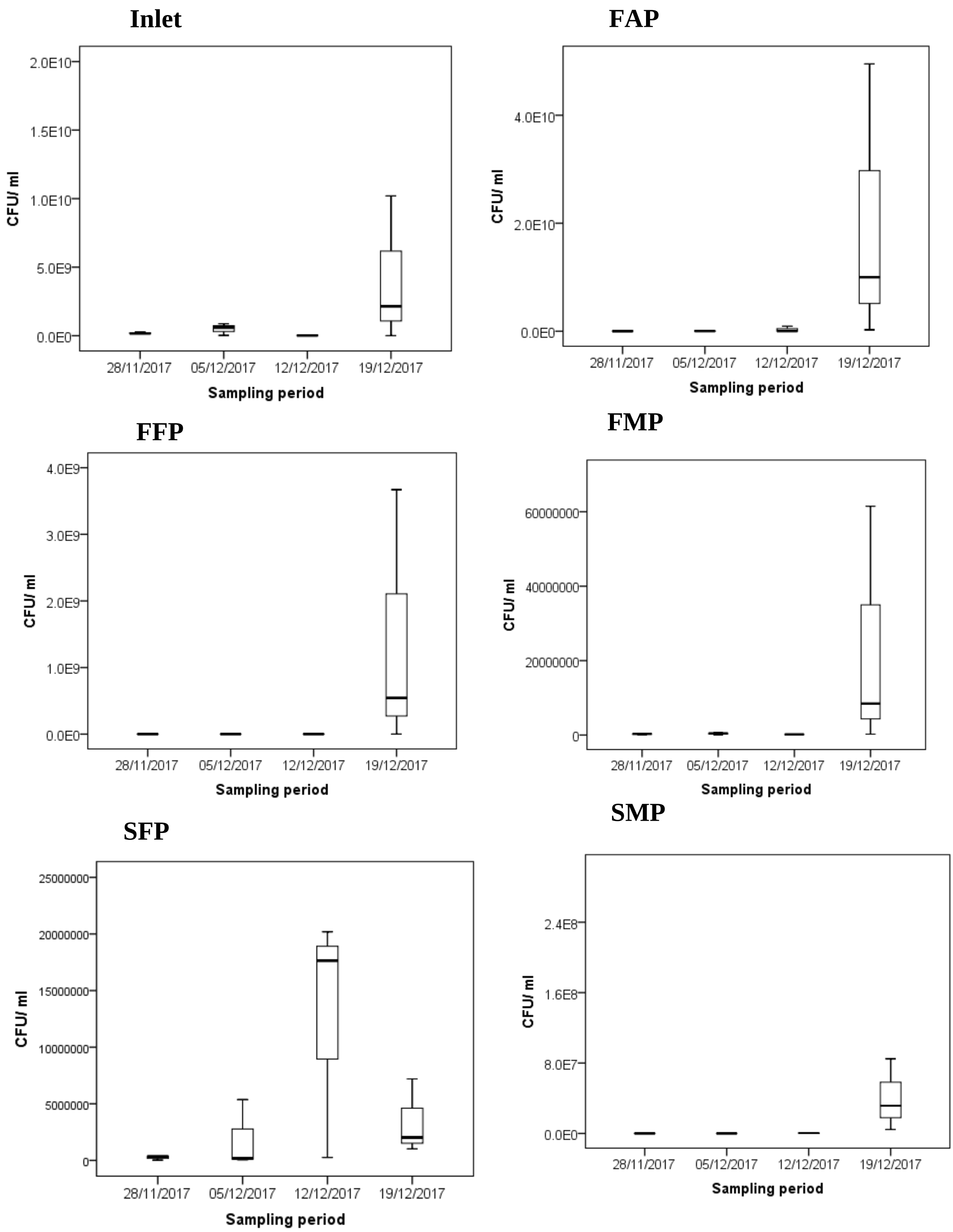


Figure 4.9: Temporal variation in HPCs in WSPs presented as CFUs/ ml. Box and whisker plots of median (25-75 percentiles) are shown; n= 12.

4.6 Physical chemical parameters of the mesocosm study

Temperature values and DO concentrations were high in the influent as compared to the effluent. Influent water temperature was 17.7 °C, but this decreased in all the mesocosm units to averagely 14 °C. Temperature varied significantly in all the mesocosms (One Way ANOVA, $F_{(4, 100)} = 46.209$, $p< 0.05$). Tukeys', HSD test revealed a significant variation in temperature between the influent and all the effluent mesocosms ($p< 0.05$), while no significant variation occurred among different gravel aggregate sizes including the control ($p>0.05$). Dissolved Oxygen concentration was 3.1 mg/l in the influent, which reduced to 1.3 mg/l and 1.8 mg/l in small and mid-sized gravel aggregate respectively. This slightly increased to 2.0 mg/l in large sized gravel aggregate and 2.7 mg/l in the control. There was a significant difference in DO concentration among the mesocosm units (Kruskal=Wallis; H=20.04; df=4; $p< 0.05$). Post hoc analysis showed that the influent was significantly different from small sized gravel aggregate (Tukeys', HSD test; $p< 0.05$) but did not vary significantly with mid-sized, large sized gravel aggregate and control ($p>0.05$). There was no significant variation amongst small, mid-sized and large gravel aggregates in DO concentration ($p>0.05$). High mean electrical conductivity of averagely 800 µS/ cm was recorded in all the effluent mesocosms in comparison to the influent (583.8 µS/ cm). Electrical conductivity in the mesocosm effluents showed a significant difference (One Way ANOVA, F (4, 100) = 14.611; $p<0.05$). The influent varied significantly from all the effluent units (Tukeys', HSD test; $p<0.05$), however, electrical conductivity did not vary in small, mid and large sized gravel aggregate ($p>0.05$). The range of pH was 5.6 - 10.7 with in the influent while all the mesocosms containing the effluent ranged between pH of 7.2-11.5, implying an increase in pH across all the gravel aggregate sizes (Table 4.4).

Table 4.4: Physical - chemical characteristics of wastewater in the mesocosm over the study period

Parameter	Influent	Small size	Mid-size	Large size	Control
Temp (°C)	17.7 ± 1.0	14.1 ± 0.7	14.4 ± 0.7	14.8 ± 0.6	15.8 ± 1.3
DO (mg/l)	3.1 ± 2.3	1.3 ± 0.9	1.8 ± 1.1	2.0 ± 0.6	2.7 ± 0.7
Cond (µS/ cm)	583.8 ± 246	866 ± 120	870 ± 94	859.8 ± 100	763 ± 94
pH Range	5.6 - 10.7	7.2 -10.1	7.9 -11.5	7.8 -10.1	7.9 - 9.5

Physical- chemical parameters were taken on weekly basis for 8 weeks and presented are averages ± SD with exception of pH presented as range; n=21.

4.7 Pathogen indicator organisms and reduction efficiency in mesocosm study

The performance of mesocosms was assessed over the eight weeks period and results showed a difference in concentration of pathogen indicator organisms (*E. coli*, TC and HPCs) between the influent and effluent with different gravel aggregate sizes. Influent mean concentration was $3.3 \times 10^{9} \pm 1.2 \times 10^{10}$ CFUs / 100 ml, $5.3 \times 10^{10} \pm 6.7 \times 10^{10}$ CFUs / 100 ml and $2.8 \times 10^{11} \pm 1.3 \times 10^{12}$ CFUs / 1 ml for *E. coli*, TC and HPCs respectively. Lower values for effluent means were recorded as shown in Table 4.5. *Escherichia coli* percentage reduction efficiency were computed as 95.2%, 94.2%, 88.4% and 29.3% for small, mid, large sized gravel aggregate and control respectively. Though the computation revealed the highest removal efficiencies for *E. coli* to be in mesocosm unit with small sized gravel aggregate, there was no significance difference among the treatments (One Way ANOVA, $F_{(4, 100)}$ =1.098, p =0.362).

Total Coliform removal revealed a variation in the percentage reduction from the influent which included, 95.3 %, 90.4 %, 88.8 % and 32.1 % for small, mid, large gravel aggregate sizes and control respectively. Just like in *E. coli* reduction, small sized gravel aggregate performed better. A significant variation occurred among the treatments (Kruskal-Wallis, H=16.772, df=4, p=0.002). A post hoc analysis revealed that the influent varied significantly with all the gravel aggregate sizes (Tukeys', HSD test; p=0.002), with no significant difference among the individual gravel aggregate sizes (p>0.05). This implied equal performance amongst the different gravel aggregate sizes in TC removal.

High percentage reduction in HPCs was recorded in comparison to other PIOs, implying better performance of mesocosms in HPCs reduction. Reduction efficiency for small, mid, large gravel aggregate sizes and control were recorded as 99.8 %, 99.7 %, 99.5 % and 21.4 % with the largest range of 0.3 % existing between small and large sized gravel aggregate. The reduction of HPCs varied significantly among the treatments (Kruskal- Wallis; H=15.984; df= 4; p=0.003). The influent indicated a significant variation with all the sizes of gravel aggregate (Tukeys', HSD test; p=0.003), while no significant variation was found in the effluent amongst different sizes of gravel aggregates (p>0.05). Conclusively, results from this experiment showed a high reduction efficiency of HPCs but little difference among all the treatments.

Table 4.5: Concentration of pathogen indicator organisms in the mesocosm study

PIOs	Influent	Effluent **Small size**	Effluent **Mid-size**	Effluent **Large size**	Effluent **Control**
E. coli (CFUs/100 ml	3.3×10^{9} $\pm 1.2 \times 10^{10}$	1.6×10^{8} $\pm 2.6 \times 10^{8}$	1.9×10^{8} $\pm 3.6 \times 10^{8}$	3.8×10^{8} $\pm 1.4 \times 10^{9}$	2.3×10^{9} $\pm 6 \times 10^{9}$
% Removal		**95.2**	**94.3**	**88.4**	**29.3**
TC (CFUs/ 100ml	5.3×10^{10} $\pm 6.7 \times 10^{10}$	2.5×10^{9} $\pm 3.6 \times 10^{9}$	5×10^{9} $\pm 1 \times 10^{10}$	5.9×10^{9} $\pm 1.6 \times 10^{10}$	3.6×10^{10} $\pm 1.8 \times 10^{10}$
% Removal		**95.3**	**90.4**	**88.8**	**32.1**
HPCs (CFUs/1 ml)	2.8×10^{11} $\pm 1.3 \times 10^{12}$	3.8×10^{8} $\pm 8.5 \times 10^{8}$	6.6×10^{8} $\pm 2.5 \times 10^{9}$	1.1×10^{9} $\pm 2.6 \times 10^{9}$	2.2×10^{11} $\pm 9.5 \times 10^{12}$
% Removal		**99.8**	**99.7**	**99.5**	**21.4**

Comparison of means ± SD of *E. coli*, TC and HPCs at each gravel aggregate size and control within the mesocosm study. Percentage reduction are given, n=21.

4.8 Comparison of influent and effluent mean values of BOD and TSS in the mesocosm study

During the entire study period, BOD and TSS were recorded for both influent and effluent samples. The mean BOD and TSS for the entire study period was higher in the influent and this reduced in all effluent mesocosms. For BOD_5 concentration, influent concentration of BOD_5 was 148.5 ± 37.6 mg/ L. Small sized gravel aggregate had the lowest BOD_5 concentration of 124.9 ± 37.2 mg/l, while this value increased with increase in gravel sizes. Mid-sized gravel recorded a mean of 133.7 ± 35.3 mg/ L while large sized gravel aggregate displayed the highest BOD_5 concentration of 135.9 ± 33.9 mg/l. Despite the differences in BOD_5 concentration in effluent of different sizes of gravel aggregates, there was no significant variation amongst all the mesocosm units (One Way ANOVA; $F_{(4, 100)} = 1.4$; $p = 0.241$). Just like in BOD_5, TSS in small sized gravel aggregate had the lowest effluent mean of 92.8 ± 84.9 mg/l while mid-sized and large sized gravel aggregate recorded a mean of 147.6 ± 84.3 mg/ L and 161.9 ± 135.3 mg/l respectively. The influent, on the other hand, had the highest TSS mean concentration of 340.4 ± 216.4 mg/ L hence a significant variation in the mesocosm units (Kruskal-Wallis; H=22.640; d.f=4, $p < 0.05$). The influent was significantly different from both small and mid - sized gravel aggregate (Tukeys', HSD test; $p<0.05$), however, it did not significantly differ from large sized gravel aggregate ($p>0.05$). Additionally, there was no significant variation in the effluent in all the three gravel aggregate sizes ($p>0.05$). None of the gravel substrate sizes was therefore found to perform better in both BOD and TSS removal, as shown in Figure 4.10.

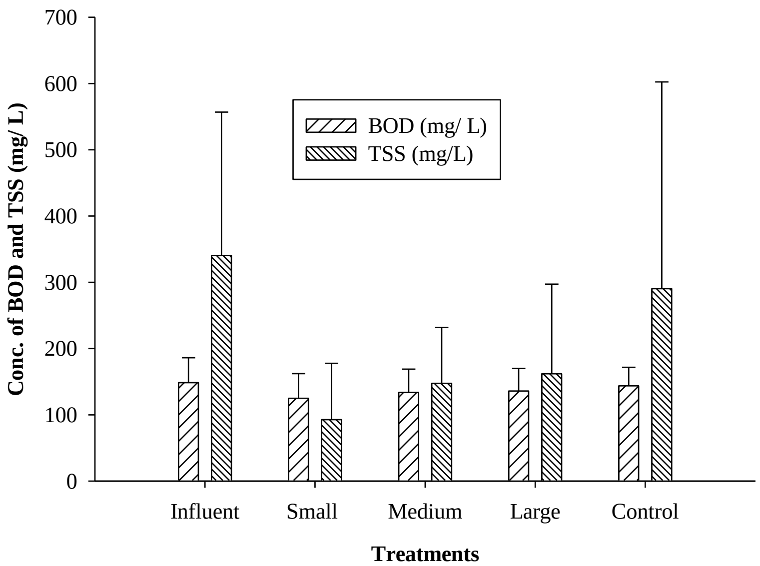


Figure 4.10: Concentration of BOD and TSS in the mesocosm study in mg/l. The bars represent mean ± SD; n=21.

4.9 Comparison of effluent reduction efficiency of BOD and TSS in the mesocosm study

Reduction efficiency was computed based on difference between the inlet and outlet mean concentration relative to the inlet mean concentration for both BOD and TSS. In BOD reduction, low reduction efficiency (less than 20 %) were recorded for all gravel aggregate sizes; small= 15.9 %, medium = 9.9 %, and large =8.5 %. Despite the low removal efficiencies, the trend signified an improvement in BOD_5 removal from large sized gravel aggregate towards small-sized gravel aggregate. In TSS, similar trend was observed where higher reduction efficiency was recorded in the small sized gravel aggregate, followed by mid- sized and finally lowest in the large sized gravel. Reduction efficiency was recorded as 72.7%, 56.6%, 52.4% and 14.6% for small, mid, large sized gravel aggregates and control respectively. In this set up, the mesocosms performed better in removal of TSS in comparison to BOD as shown in Figure 4.11.

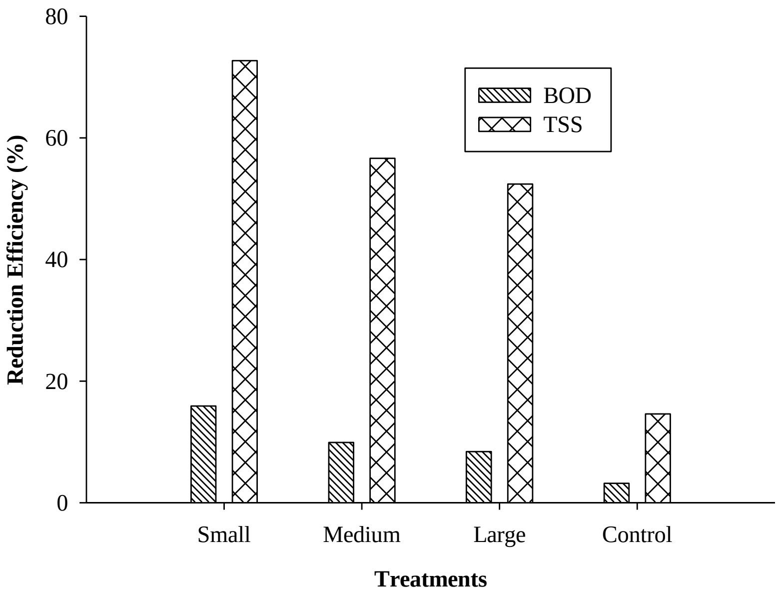


Figure 4.11: Reduction Efficiency (%) for BOD and TSS concentration in the mesocosm study; n=21.

CHAPTER FIVE

DISCUSSION

5.1 Physical-chemical parameters of Wastewater Stabilization Ponds

Physical- chemical parameters are an important aspect of wastewater treatment. Temperature, Dissolved Oxygen and pH immensely contribute to bacterial pathogen decay in wastewater (Mairi et al., 2004). Studies have found out that higher temperatures contribute to higher removal efficiencies for both organic matter and pathogenic bacteria. Anaerobic ponds, for instance, will achieve about 40 % removal of BOD at 10 °C, 60 % at 20 °C and more than 75 % at 25 °C (Odjadjare, 2010). The anaerobic bacteria in these ponds are sensitive to pH >6.2, and therefore the higher the pH the higher the efficiency in the anaerobic ponds. In facultative and maturation ponds, the principle mechanism for organic matter and pathogen removal are time, temperature pH >9 and high solar radiation (Ultraviolet), which are conditions best suited for WSPs in tropical regions (Abdullahi et al., 2014).

In the current study, insignificant variation in temperature in the sampling sites could depict uniform insolation, wind mixing due to low depths in the ponds raising water temperatures to uniformity. Besides, in the tropical environment, the effect of direct solar irradiation during the day affects temperature too. This phenomenon may significantly overshadow increased temperatures from bioenergetics arising from breakdown and transformations of organic matter in different compartments of WSPs. The ultimate result is uniformity of temperature in the WSPs. Additionally, wastewater temperature seemed not to have been affected by sampling time since it did not change significantly. A slight decrease in temperature in the last two sampling periods could be related to changes in the ambient temperature, which decreased from averagely 17 °C to 16 °C from November to December 2017. During the entire sampling period, there were no rainfall episodes, hence no great variations in the wastewater temperature.

The low DO at the inlet could be attributed to high demand by micro-organisms to degrade organic matter and some inorganic compounds, as evidenced by high BOD at the inlet. Biological nitrification could as well have played a role as unstable species of nitrogen such as ammonium created oxygen demand in the processes of conversion into nitrites or nitrates. Furthermore, aerobic biotransformation process must have led to consumption of DO during mineralization of organic matter into inorganic matter. Increase in DO concentration gradient along the treatment series is consistent with what is expected in WSPs (Pena and Mara, 2004). oxygen in the maturation ponds produced by photosynthetic community is important for bacterial aerobic breakdown of organic matter hence reduction of easily degradable particulate and dissolved organic matter in the ponds. The unexpected low DO in the SMP could be attributed to reduced light penetration following the blooming of algal mats in the pond. Additionally, probable increase in oxygen consumption to deal with added dissolved organic matter from dead algal biomass could have reduced DO concentration. Finally, faecal wastes from the avian community in the ponds could have increased oxygen consumption of the effluents, as indicated in the study by Murray and Hamilton, (2010). Super-saturation of DO in the FFP could be explained by photosynthetic oxygenation because of the algal mat that was seen floating in this pond. In the early afternoons, there was high solar insolation and consequently high that led to photosynthesis reaching its peak. Similar observations were made by Tadesse et al., (2004).

Establishment of alkaline pH sometimes recording pH values greater than 10 along the wastewater treatment pathway was a good indication that the microbial diversity of photosynthetic organisms is actively involved in the removal of pollutants in these ponds. Alkaline pH reduces pathogens and *E. coli* in waste WSPs and understanding the physical chemical parameters in which the wastewater treatment occurs is essential. The values reported here are within the ranges observed in other similar studies, (Pearson et al., 1996; El-Deeb Ghazy et al, 2008; Tyagi et al., 2008; Kang et al., 2014)

5.2 Variation in concentration of Total coliforms and *Escherichia coli* in WSPs.

Indicator microorganisms are used to evaluate the water quality and in wastewaters the most used indicator bacteria are the total coliforms and *E coli*. These organisms are often used to reflect possible pathogen level in wastewater, since detection of pathogens is costly and time consuming (Poet et al., 2009). The behaviour, population and presence of indicator organisms and the pathogens are usually assumed to be correlated (Bitton, 2005). The main source of pathogens in receiving natural waters is domestic wastewater and there is shear need to monitor indicator microorganisms to prevent outbreaks of enteric diseases.

In the current study, a reduction in concentration of *E. coli* and TC from the inlet along the wastewater treatment pathway is similar to results obtained in an earlier study (Kimani et al., 2009). The reduction in concentration of PIO along the pathway could have been catalysed by parameters such as higher hydraulic retention time, high temperatures, high pH, high solar irradiation and elevated DO concentrations due to photosynthetic community additions.

Despite reduction in concentration of PIO in the ponds along the wastewater treatment pathway the effluents cannot be recommended for direct use in irrigation. *E. coli* concentration figures remained high in the subsequent ponds contrary to the expectation and this could be attributed to avian presence in the ponds. However, it is not clear if the avian densities are appreciable and this could be recommended in a further study. *E. coli* does not survive for long in the environment thus when detected in wastewater it is a clear indicator of recent faecal contamination (Sueiro, 2001; Asano et al., 2007). The low TC concentration recorded in the FMP was expected since the primary function of maturation ponds is removal of pathogens (Kayombo, 2005) which was probably because of shallow depth (1-1.5m) and high pH range (7.9-9.7). Shallow depth in maturation ponds leads to well oxygenation as there is less vertical biological and physiochemical stratification throughout the day (Tadesse et al., 2004). Algal population in maturation ponds is much more diverse than in the facultative and anaerobic ponds with the rapid photosynthesis in the maturation pond leading to high pH. The high pH consumes CO_2 faster than it can be replenished by bacterial respiration in the pond, leading to dissociation of carbonate and bicarbonate ions. The resulting CO_2 is fixed by algae and the hydroxyl ions dissociate, raising pH to values above 9, which pathogens cannot withstand and therefore they die immediately (Pearson et al., 1987c Loc cit; Kayombo, (2005) and Tyagi et al., 2008).

Intense solar insolation has destruction effects on coliforms (Bansah et al., 2016). Light wavelength between 425-700 nm affect faecal coliform when absorbed by humic substances in wastewater. Bacterial die- offs due to light also depend on DO levels, coupled by by high pH. The sun is therefore an important factor in removal of PIO, as it plays three-fold role; i.e directly promoting removal of faecal bacteria in WSPs, increasing pond temperature and providing energy needed for rapid algal photosynthesis leading to increased pH and photo-oxidative damage (Kayombo, 2005; Abdullahi et al., 2014).

Many studies carried out on WSPs worldwide are comparable to this study, eg Bansah et al., 2016). They show effluent reach recommended EPA standards, with removal efficiencies as high as 99.9 %. The FMP in the current study registered 99.8 % (2 log units) and 99.9 % (3 log units) removal efficiencies for TC and *E. coli* respectively, while SMP reached removal efficiencies of 99.9 % (3 log units) and 99.8 % 2 log units) for the former and later respectively.

In Ghana, a study on sewage treatment using WSPs recorded reduction efficiencies of 99.3 % and 95.6 % in the FMP for TC and *E. coli* respectively while the SMP recorded 99.7 % and 98.9 % in the SMP for TC and *E. coli* respectively (Bansah et al., 2016). Other studies in warm tropical climates that established similar reduction efficiencies in the maturation ponds, comparable to current study include; Performance evaluation of a WSP in a rural area in Egypt, (99.9% reduction efficiency for both TC and *E. coli*), (El-Deeb Ghazy et al., 2008) and removal of faecal indicators and pathogens in a WSP in India, (99.5% and 99.9% reduction efficiency for TC and *E. coli* respectively), as reported by Tyagi et al (2008).

5.3 BOD and HPC levels as indicators of easily degradable organic matter in WSPs.

The main ecological implication of organic pollution in wastewater is a decrease in levels of dissolved oxygen. Wastewater, treatment using aerobic processes requires adequate supply of oxygen so that stabilisation of organic matter can be achieved through metabolic processes of the micro-organisms. Therefore, indirect quantification of the wastewater potential to generate an impact is obtained by the measurement of oxygen consumption which determines strength of wastewater. The BOD_5 values obtained in each pond (Table 2.1) can be classified as medium strength wastewater (Metcalf and Eddy, 2003).Other studies have documented even higher values (above 300 mg/ L) which is classified as high strength wastewater (Sunder and Satyanarayan, 2013).

The inlet was medium strength in BOD. That could be attributed to several reasons. For instance, the micro - organisms responsible for decomposition possibly were not adapted to the waste, or rather they were inhibited or killed by possible presence of heavy metals, ions and other toxic substances at conductivity above 900 µS/ cm. According to (Abdullahi et al., 2014), 2014), anaerobic and facultative ponds are designed for removal of (BOD) and not toxic substances. Unexpected high range of BOD_5 recorded in the FMP at the same point (3-242 mg/ L) but sampled at different sampling periods, could be due to increased algal photosynthesis that saw oversaturation in DO concentration hence the minimum BOD_5 concentration of 3 mg/ L (SFP, sampling period 3; 12/12/2017). On the other hand, the sampling period when the BOD_5 maximum concentration of 242 mg/ L was reached (SFP, sampling period 1; 28/11/2017) dead birds were seen floating on the pond surface at that site, which could indicate highest oxygen consumption as shown in appendix 2.

Another aspect that was observed was an increase in BOD in the SMP, which could be attributed to perhaps dead algal biomass and faecal dissolved organic content from avian community in the pond. Less residence time could also be related to wastewater treatment in the SMP, hence BOD remains high. Similar trend where BOD_5 was high in the inlet and reduced substantially is documented (Abira, 2008 ; Tyagi et al., 2008; Olutiola et al., 2010).

Lack of statistical difference among all other ponds in BOD_5 concentration could be due to other sources of pollution that substantially increased in BOD_5 concentration, coupled with excessive growth of *Lemnar spp* and algae, which die and decompose increasing organic matter content. The high content of organic matter from these plant species and other possible sources might not be easily degradable.

High concentration of heterotrophic bacteria in water is may be an indication of pollution with easily degradable organic matter. Microorganisms recovered through HPC tests generally include those that are part of the natural microbiota of water; in some instances, they may also include organisms derived from diverse pollutant sources (WHO, 2003). In the current study, high concentration of HPCs was realized in anaerobic pond (FAP) probably due to more labile nutrients coming in with wastewater load. A shorter residence time in the FAP could explain why there was the insignificant difference between the inlet and FAP.

There is a possibility that the two anaerobic ponds; FAP and SAP, have different loading rates, SAP receiving more influent than FAP which drains into SFP. There is little information on bacterial removal within WSPs and even in the few studies reported, most studies focus attention on removal of coliforms due to health implications but seldom on HPCs. As a result, there is limited knowledge existing on figures captured for removal efficiency in WSPs. Heterotrophic plate count measurements are used as a measure of effectiveness of water treatment process, thus an indication of pathogen removal (WHO, 2003). In the current study, concentration of HPCs in the WSPs ranged between 4.5×10^6 and 5.0×10^9 CFU/1 ml. There is need to study concentration of other bacteria to arrive at a conclusion on bacterial removal.

5.4 Effect of substrate size on removal of PIO, HPCs, BOD_5 and TSS using a mesocosm experimental set up.

Substrate characteristics have a profound effect on pollutant removal in a CW. Many studies have documented different pollutant removal efficiencies by employing various types and sizes of substrate Coarse rock, gravel, sand and other soils have been used but gravel substrate is the most commonly used media in CW e.g, (Rompré et al., 2002). According to Olsson et al, (2011), general factors that contribute to higher pollutant removal efficiencies in a CW include: smaller grain size that ensures a larger surface area, better physical filtration of solids, more biofilm growth, higher oxygen levels and longer HRT.

In the current study, small sized gravel aggregate performed better than all other substrate sizes in removal of *E. coli*, TC and HPCs, BOD and TSS, followed by mid- size and finally large sized gravel aggregate. This is probably due to microbial attachment to the gravel surface forming biofilms which had capacity for filtration and adsorption of pathogens (USEPA, 2000b ; Prochaska and Zouboulis, 2006 ; Morsy et al., 2007). In a different study, it was argued that greater filtration efficiency of a CW bed media overtime was attributed to acclimation phase where biofilm growth occurred on the bed media (Richardson & Rusch, 2005). Another possibilty to explain the scenerial could be the 7-day HRT, which was long enough to promote contact time between pathogens and biofilm, and short enough to prevent low hydraulic conductivity that could lead to clogging (Sehar et al., 2014).

Fine media provides a large surface area for attachment of organic matter and other particulates hence high removal efficiency (Albalawneh et al., 2016). Despite good performance by small sized gravel aggregate in BOD removal in the current study, the removal efficiencies for all the gravel aggregates were generally low (less than 20%). The unexpected low removal efficiencies for BOD over the study period might have been influenced by physical-chemical characteristics (Temperature and Dissolved Oxygen) that have been documented to affect BOD removal. During the study period, temperature in the mesocosm units ranged between 14.1 and 15.8 °C (possibly due to sampling time; 0800hours), while DO ranged between 1.8-2.7mg/ L. These are the two parameters expected to play a significant role in BOD_5 removal, however, they were not in the optimal range for functioning of the mesocosm units. The low BOD_5 removal efficiencies could as well be related to existence of non-biodegradable compounds which need HRT >8 days to be degraded (Akratos & Tsihrintzis, 2007; Abira, 2008).

The results of the current study are in agreement with a a two-stage vertical flow CW in Denmark, where removal efficiency increased from small sized to large sized gravel aggregate (Arias et al., 2003). It has been documented that media size plays a significant role in pathogen inactivation. Fine gravel (2-13 mm) has a higher efficiency compared to coarse gravel (5-25 mm) (Gracia et al., 2003). Reduction efficiency in the control experiment was lowest for PIO, BOD and TSS, suggesting it was behaving more or less like a sedimentation tank. Other studies (Marika et al., 2009) have documented higher removal efficiencies in CW contributed by presence of macrophytes, but the current study attributes the high removal efficiencies to other mechanisms rather than macrophytes. A study whose *E. coli* results agree with the current study investigated removal of faecal pathogens by both planted and non-planted sand beds. It pointed out mechanical filtration, natural die offs and predation as the major removal mechanisms in non-planted cells (Wand et al., 2007).

High removal efficiency (72 %) for TSS was observed in small sized gravel aggregate while large sized gravel recorded the lowest efficiency (52 %). This must have been through a higher sedimentation rate in small and medium sized gravel aggregate that retained suspended matter as compared to the large sized gravel aggregate. In comparison to BOD_5 removal, TSS removal efficiency was higher probably because the main removal mechanism for TSS is through physical processes (sedimentation and filtration) which take place despite the prevailing conditions unlike BOD_5 which is biological and depends on other factors (Vymazal, 1998).

From this study variation in substrate sizes did not seem have an effect on removal of both TSS and BOD_5 as there existed no significant variation between the effluent units. Similarly, both TSS and BOD_5 concentration did not meet the stipulated state standards of <30 mg/l into receiving surface water by a magnitude of (28%) for the former and (>80 %) for the later.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

With respect to variations in concentration of TC and *E. coli* in Egerton University WSPs, there was progressive reduction from the wastewater along the pathway from the inlet towards the outlet. Biochemical Oxygen demand conducted in 5 days in these ponds may not be an indicator of easily degradable organic matter. Biochemical Oxygen demand did not vary among the ponds with reduction occurring only in the FFP and substantially increasing in the SMP. The presence of potential non-biodegradable organic matter due to faecal pollution from birds and dead algal biomass led to the effluent being medium strength class, but not within the recommended standards. Concentrations of HPCs reduced along the pathway, indicating good pond performance in removal of easily degradable organic matter. The mesocosm study indicated the effect of different gravel aggregate sizes on reduction of PIOs, HPCs, BOD_5 and TSS, which had significant variation between the influent and effluent samples. Gravel substrate sizes chosen showed no influence on removal efficiency of PIOs and organic matter.

6.2 Recommendations

1. Long term sampling in Egerton University wastewater stabilization ponds should be carried out to capture demographic dynamics and hence volumetric flows and different weather patterns. This will provide clarity on the actual status of the effluent hence a recommendation can be made regarding the effluent suitability downstream users in accordance to NEMA standards (Appendix 1). Existence of avian population in the WSPs could have influenced concentration of *E. coli* in the WSPs, but their effect was not studied since it would be out of the scope of the current study. Future studies could find out if the population is appreciable and their influence in the WSPs.
2. Future designs and expansion should consider the potential impacts associated with performance of WSPs more so, in BOD_5 reduction. Additionally, performance of WSPs is tied to their management and therefore continuous disludging will improve in removal of organic matter.
3. In the constructed wetland, gravel bed needs to be filled with small sized gravel aggregate to ensure high pollutant reduction rates. Furthermore, incorporation of

small, medium and large - sized gravel aggregate and the effect of increasing HRT could be employed in a future study.

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APPENDICES

Appendix 1: Kenya's guideline values of different parameters for discharge into public water

Parameter	**Unit**	**NEMA Standards**
Temperature, max	°C	±3 of ambient water body temperature
pH	pH Units	6.5-8.5
TDS	mgl^{-1}	1500
TSS	mgl^{-1}	<30
BOD	mgl^{-1}	<30
COD	mgl^{-1}	50
Total Coliforms	CFU (Counts)	1000/100 ml
E. coli	CFU (Counts)	1000/100 ml

Source: The EMCA (Water Quality) Regulations, 2006

Appendix 2: Dead birds floating on the surface of the SFP, an indication of highest Oxygen consumption at the site.