Khasisi D. Lukhabi et al. / Afr.J.Bio.Sc. 3(2) (2021) 16-26
https://doi.org/10.33472/AFJBS.3.2.2021.16-26
ISSN: 2663-2187

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper **Open Access**

Assessment of performance of wastewater stabilization ponds using faecal indicator bacteria reduction efficiency in a tropical environment

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Article Info

Volume 3, Issue 2, April 2021
Received : 14 July 2020
Accepted : 09 December 2020
Published : 05 April 2021
doi: 10.33472/AFJBS.3.2.2021.16-26

Abstract

Egerton University (EU) uses Wastewater Stabilization Ponds (WSPs) for wastewater treatment. An adjoining wetland with gravel bed substrate and free-surface flow meant to polish discharge before releasing into River Njoro is currently non-operational. The current study aimed at establishing the performance of WSPs in terms of concentration and reduction efficiencies of Faecal Indicator Bacteria (FIB); Total Coliforms (TC) and *Escherichia coli (E. coli)*. Wastewater samples were collected weekly for one month from mid-November to mid-December 2017. Total Coliforms and *E. coli* were isolated using selective and differential media following Membrane Filtration (MF) method. Colonies were cultured on Chromocult Coliform Agar (CCA) and enumerated using standard procedures for examination of water and wastewater. The results were expressed as Colony Forming Units (CFUs) per 100 ml of the original sample. The inlet showed highest concentration of FIB which reduced along the treatment pathway. Total Coliforms and *E. coli* ranged between 2.5×10^8 - 2.9×10^{11} and 5.9×10^5 - 1.8×10^{10} CFUs/100 ml respectively with cumulative reduction efficiencies between 2 to 4 log units for *E. coli* and 2 to 3 log units for TC in the two maturation ponds. Although concentration of FIB in EU WSPs reduces along the treatment pathway, the effluent quality is above recommended international standards for discharge into surface waters. The trend is attributed to lack of frequent monitoring, insufficient maintenance, together with short- circuiting effect due to by-passing of wastewater in the design of the new pond system.

Keywords: *Faecal Indicator Bacteria (FIB), Performance, Free-surface flow wetland, Tropics, Wastewater treatment*

1. Introduction

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). The most common technologies for wastewater treatment in developing countries are Wastewater Stabilization Ponds (WSPs) and Constructed Wetlands (CWs), Kivaisi (2001). Wastewater stabilization ponds are shallow basins that naturally treat wastewater using decomposition and autotrophic activities of aerobic and anaerobic micro-organisms. On the other hand, WSPs require large tracts of land for their construction, which was a promoting factor in EU that stands on about 1600 hectares of land, providing enough space for construction of a WSPs system.

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Lately, Universities, EU included, are being seen as 'small cities' due to their large size, population, and the various complex activities taking place in campuses, which may have serious direct and indirect impacts on the environment. A few studies on the EU WSPs exist; Kimani *et al*. (2009) studied the performance of the First Facultative Pond (FFP) and the two maturation ponds in reduction of faecal coliforms and helminth eggs. That study observed a reduction along the treatment pathway from facultative to maturation ponds. However, the ponds did not achieve the recommended WHO standards of wastewater discharge into surface waters. Mwanyika *et al*. (2016) focused on reduction of heavy metals by the WSPs. Being a dynamic system with scanty information regarding its performance, there is need to study how indicator parameters evolve with time.

In the tropical regions, WSPs have proven to be effective for wastewater treatment where ambient temperature is less limiting (Okoh *et al*., 2007). The ambient temperature and duration of sunlight within the tropics offer an excellent opportunity for high efficiency and satisfactory performance for this type of water-cleaning systems (Abdullahi *et al*., 2014). Nevertheless, changes in weather patterns including intense insolation increases evaporation rates from the WSPs hence influencing performance and functionality of these systems, EU WSPs being part of them. Moreover, the current study assessed the effects of physical-chemical parameters (temperature, dissolved oxygen, conductivity and pH) on reduction of FIB in the EU WSPs.

Normally, performance of WSPs is limited by factors such as; lack of proper maintenance and monitoring, fluctuation in wastewater levels, which in turn interferes with residence time (Kayombo, 2005). Overloaded or badly maintained WSPs are prone to produce bad odor (Peña and Mara, 2004). The EU WSPs system has been in existence for over three decades now. Due to increase in campus population and therefore wastewater discharge, the EU WSP system underwent modifications, involving addition of extra ponds, which changed its design as well as performance. There is no regular monitoring of these ponds and hence no data exists on how the modifications have affected their performance. The current study endeavored to establish whether the modifications in place had any influence on their performance in terms of reduction of FIB.

Surface waters are contaminated with organic matter, total suspended solids, heavy metals, nutrients and micro-organisms, Mbwele (2006). Release of poorly treated wastewater to the natural environment can result in contamination of aquatic ecosystems with total and faecal coliforms in the order of 10^8-10^{10} and 10^7-10^9 CFU per liter respectively, George *et al*. (2002). 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 as shown by Alexandros and Akratos (2016). This study, furthermore, focused on concentration of FIB in each pond, presenting the counts as CFU per 100 ml.

2. Methods

2.1. Study site

The study was carried out in WSPs located within EU, Njoro Campus, Kenya. Egerton University is approximately 25 km South-West of Nakuru town, Njoro Sub-county in Nakuru County. The University area lies within latitude 0˚ 15'S and between longitudes 35° 50' and 35° 05'E standing on about 1600 hectares of land within the River Njoro watershed at an altitude of 1890-2190 m above sea level as shown in Figure 1.

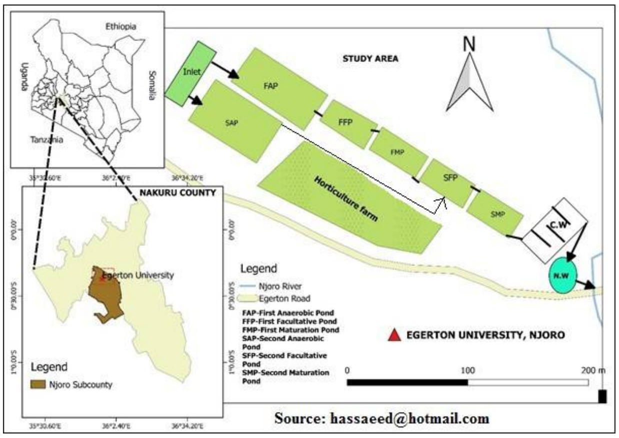

Figure 1: Map of Egerton University, Njoro showing the layout of the wastewater stabilization ponds

Source: hassaeed@hotmail.com

The two anaerobic ponds measure 160 × 100 × 1.5 m for length, width and depth respectively while the rest of the ponds are equal in size, with a uniform length of 110 m and width of 50 m. The pond input discharge is 800 m^3 of wastewater per day with 37 m^3 flowing into FAP as 680 m^3 flows into SAP while the output discharge for individual pollutants is calculated as: Mass removal rate (g/m^3/day) = $Q \times (C_i - C_{out})$ where Q is the discharge rate,(m^3 per day), C_i and C_{out} are influent and effluent concentration respectively.

2.2. Structure of the WSPs

The structure comprises of six WSPs incorporating an old system and a new system. The old system was constructed in early 1980s and it comprises of three ponds, the First Anaerobic Pond (FAP), FFP and First Maturation Pond (FMP) arranged in series. Two decades later, renovations were carried out and a new system added. The new system as well consists of three ponds, Second Anaerobic Pond (SAP), connected in parallel to FAP, (of the old system), Second Facultative Pond (SFP) and Second Maturation Pond (SMP) connected in series, preceding the old system. The SMP is connected to a Hybrid 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 River Njoro. Wastewater flows from the inlet to the outlet along the treatment pathway. There is a by-pass that allows flow of wastewater from SAP, joining the SFP along the wastewater treatment pathway (Figure 2).

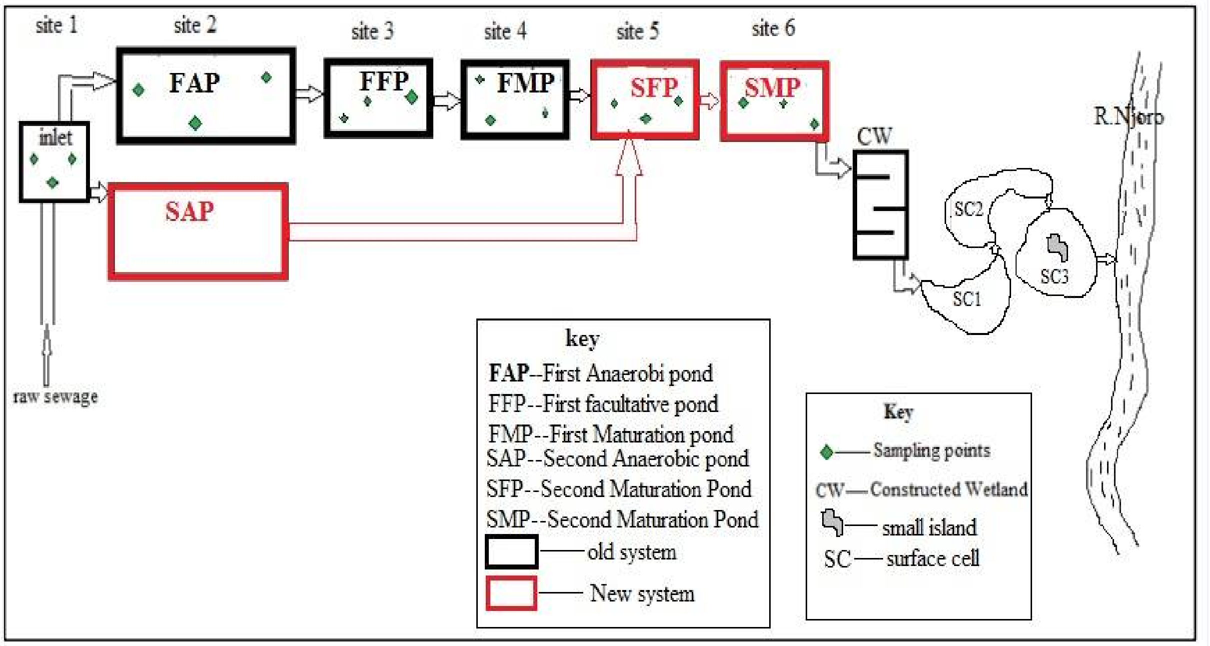

Figure 2: Schematic diagram showing design of the WSPs at EU

2.3. Egerton University population structure

The total population of Egerton University is 18,000 people according to Mwanyika *et al*. (2016). However, current sources indicate that the number of resident students is about 6,000, basing on number of rooms in individual hostels. The rest of the students, about 6,000 are accommodated outside campus premises. Inside the University are about 70 staff houses, which house averagely four individuals, hence 280 people. Among the students and staff are those who commute to campus daily and use toilet facilities, about 2,000 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 fluctuation in population, the WSPs system becomes so dynamic, with the wetland drying completely during low population season. 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, 2018).

2.4. Sample collection

Wastewater samples were collected using sterilized 500 ml glass bottles. During each sampling session, physical-chemical parameters including temperature, Dissolved Oxygen (DO), pH and conductivity were also measured *in situ* using a calibrated HQ 40d (HACH) multi-meter probe and results recorded in the field. Water samples were collected in triplicates from each of the six sampling sites on weekly basis for one month. Immediately after collection, the samples were kept in a cool box before being transported to the Biological Department laboratory within six hours of collecting the first sample where they were analyzed for *E. coli* and TC, APHA (2005).

2.5. Sample analysis

Once in the laboratory, TC and *E. coli* were isolated using selective and differential media following MF methods and colonies enumerated on CCA (APHA, 2005). Ten times 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 milliliters of the sample diluents were 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 CCA plates on a Petri dish gridded side up and incubated at 37 °C for 18-24 h to allow growth of FIB. Visually identifiable typical colonies appearing dark-blue to violet were counted as *E. coli* while those appearing pink to red were counted as other coliforms. The addition of both *E. coli* and other coliforms resulted into TC. Counting of colonies was done using a Fisher Accu-Lite Colony Counter Model 133-8002a and results expressed in numbers of CFU / 100 ml of the original sample.

2.6. Data analysis

Data was analyzed using Sigma plot version11. Descriptive statistics were carried out to summarize the data and results presented graphically. The variation in mean / median concentration of TC and *E. coli* along the pathway in WSPs was tested. Prior to parametric statistical tests, data was checked for normality using Shapiro-Wilk-W Test. One-Way ANOVA was used for normally distributed data and Kruskal-Wallis test for data that did not pass normality tests. Tukey's HSD post hoc test was used to separate the means. Pearson Product moment was used to establish temporal correlation between physical-chemical parameters and FIB concentration. In the statistical tests, the significance threshold was set at $\alpha = 0.05$ and significance level at 95%, i.e., $p < 0.05$.

3. Results

3.1. Spatial variation in physical-chemical parameters in WSPs

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, there was no significant variation in temperature along the treatment pathway (One-way ANOVA; $F_{(5, 66)} = 0.635$; $p = 0.674$). Dissolved Oxygen was very low at the inlet, 1.8mg / l while super saturation was observed in the FMP and FFP, with measured values of 10.9 mg / l and 14.6 mg / l respectively. The DO concentration varied significantly among the sampling sites (One-Way ANOVA; $F_{(5,66)} = 9.825$; $p < 0.05$), where Tukey's HSD test revealed a significant difference between the inlet and the mid ponds ($p < 0.05$) with no significant variation between inlet and SMP ($p > 0.05$). The highest electrical conductivity (1102.292 μS / cm) was recorded at the inlet with no significant variation among the sites (One-Way ANOVA; $F(5, 66) = 0.305$; $p = 0.908$). The pH values showed a significant variation among the sampling 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 ($p > 0.05$). A summary of physical chemical characteristics is shown in Table 1.

Table 1: Physical-chemical parameters of wastewater in WSPs

Site	Temperature (°C)	DO (mg/L)	Conductivity (μS/ cm)	pH range
Inlet	$(21.5 \pm 0.4)^a$ **20.0 – 24.9**	$(1.9 \pm 0.1)^a$ **0.1 – 7.7**	$(1102.3 \pm 157.4)^a$ **632 – 2405**	6.9-9.0
FAP	$(21.5 \pm 0.4)^a$ **19.4 – 24.3**	$(6.8 \pm 1.7)^b$ **0.3 – 16.4**	$(968.5 \pm 124.6)^a$ **460.5 – 1607**	7.4-9.2

Table 1 (Cont.)

Site	Temperature (°C)	DO (mg/L)	Conductivity (μS/ cm)	pH range
FFP	(21.3 ± 0.3) ^{a} **19.2 – 23.4**	(14.6 ± 0.2)^{b} **2.7 – 20.0**	(949.7 ± 107.2)^{a} **589 – 1337**	7.9-10.0
FMP	(21.8 ± 0.4)^{a} **19.3 – 24.3**	(10.9 ± 0.2)^{b} **3.0 – 21.4**	(935.9 ± 101.5)^{a} **590.5 – 1330**	7.9-9.7
SFP	(22.1 ± 0.5)^{a} **19.1 – 24.3**	(9.5 ± 0.2)^{ab} **2.8 – 21.2**	(939.6 ± 99.2)^{a} **660.0 – 1322**	7.8-10.2
SMP	(21.9 ± 0.4)^{a} **20.3 – 24.5**	(6.5 ± 0.1)^{a} **3.1 – 10.4**	(941.5 ± 99.4)^{a} **592 – 1307**	7.9-9.5

Note: The numbers in parenthesis are values for mean and standard errors except pH, while those in bold represent the range. The means with **same** superscript letter are **not** significantly different at $p = 0.05$ level; $n = 12$ while those with **different** superscript letters indicate significant differences. Key: FAP=First Anaerobic Pond, FFP=First Facultative Pond, FMP=First Maturation Pond, SFP=Second Facultative Pond, and SMP=Second Maturation Pond.

3.2. Spatial variation in concentration of Faecal Indicator Bacteria in WSPs

During the study period a high concentration of both *E. coli* and TC was observed in the wastewater inlet. Spatial variation indicated a reduction in concentration of both *E. coli* and TC along the wastewater treatment pathway from the inlet which increased slightly towards SMP. The wastewater inlet had a high mean concentration of $1.8 \times 10^{10} \pm 6.0 \times 11^{4}$ CFU / 100 ml for *E. coli* while the SFP had the lowest concentration of $5.9 \times 10^{5} \pm 3.2 \times 10^{2}$ CFU / 100 ml. The mean concentration of TC was also highest at the inlet, $2.9 \times 10^{11} \pm 1.5 \times 10^{5}$ CFU / 100 ml while the lowest concentration was recorded in the FMP, $2.5 \times 10^{8} \pm 6.3 \times 10^{3}$ CFU / 100 ml. During the study period, 3 and 4 log units cumulative reduction efficiencies were observed in FMP for TC and *E. coli* respectively, while SMP reached cumulative reduction efficiencies of 2 log units for both the former and later respectively. There was 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 (Tukey's 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 3.

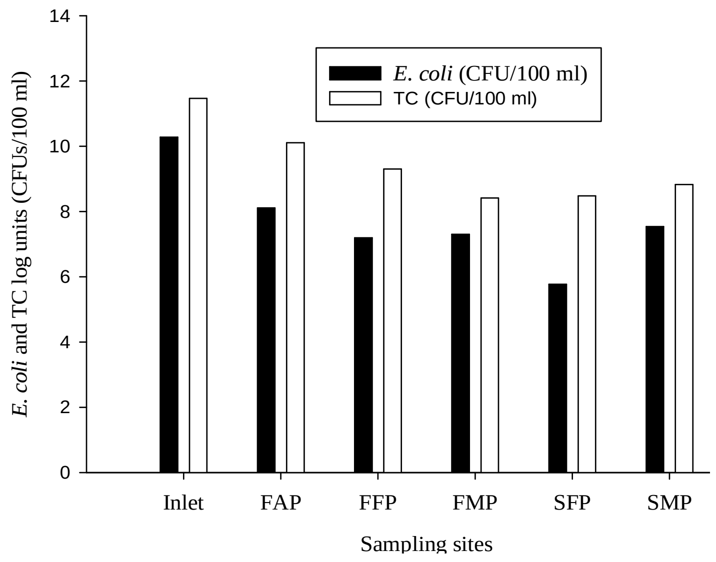

Figure 3: Spatial variation in concentration of *E. coli* and TC and in WSPs. Bars represent log units for TC and *E. coli* counts. Key: FAP = First Anaerobic Pond, FFP = First Facultative Pond, FMP = First Maturation Pond, SFP = Second Facultative Pond, and SMP = Second Maturation Pond.

3.3. Temporal variation of temperature with FIB

Temperature varied considerably with concentration of *E. coli* and TC across the ponds. At the inlet as well as in all the other WSPs, temperature ranged between 21 - 23 °C during week 1 and week 3. However, in week 3, temperature dropped sharply to 20 °C in all the ponds, which later on slowly rose above 20 °C with a peak of 22 °C in the SMP. A slight increase in temperature saw an increase in the concentration of both *E. coli* and TC. Similarly, a slight drop in temperature saw a decrease in FIB concentration as well. Significant positive correlations (r-Spearman, $p < 0.05$) were found in FAP between temperature and *E. coli* suggesting an increase in temperature with *E. coli* concentration. Moreover, no other significant correlations were observed for any of the studied sampling sites. The temporal variations in temperature with FIB are shown in Figure 4.

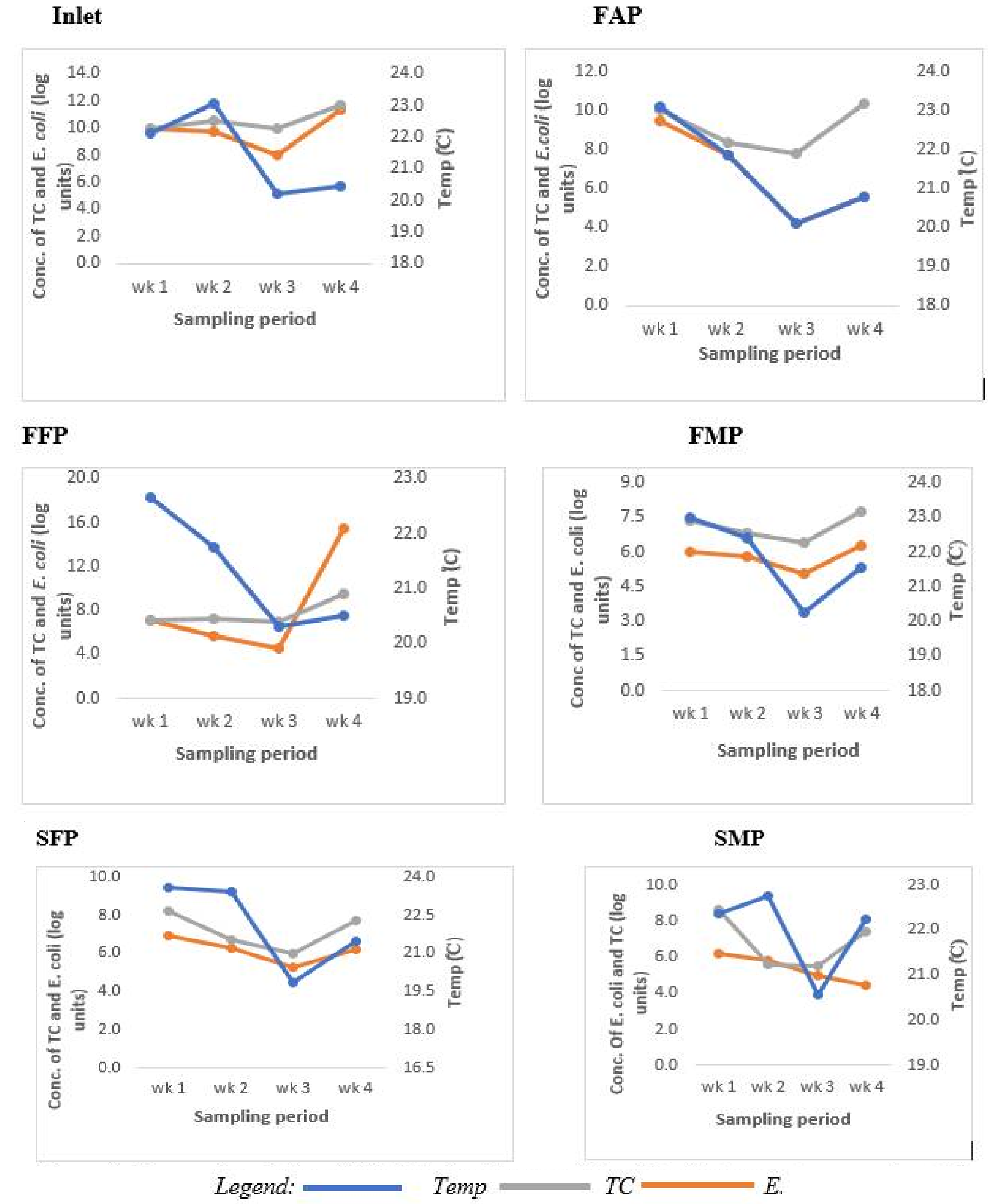

Figure 4: Temporal variation of FIB (Total coliforms and *E. coli*) with temperature over the study period in the wastewater stabilization ponds. Faecal Indicator Bacteria counts are represented as log units, temperature (°C) and sampling period in weeks (weeks 1 to 4). Key: FAP = First Anaerobic Pond, FFP = First Facultative Pond, FMP = First Maturation Pond, SFP = Second Facultative Pond, and SMP = Second Maturation Pond.

3.4. Temporal variation of DO and with FIB

Dissolved oxygen varied considerably with concentration of FIB within the four weeks in some of the sampling sites. Significant correlations (r-Spearman, $p < 0.05$) were found in FFP between DO and both *E. coli* and TC. The perfect positive correlation suggested that an increase in DO lead to an increase in FIB concentration. However, no other significant correlations were observed for any of the studied sampling sites. On the other hand, significant statistical correlations (r-Spearman, $p < 0.05$) were reported between week 1 and all the other weeks apart from week 4. A perfect positive correlation was observed between week 2 and 3 (r-Spearman, $p < 0.05$) as far as DO concentration was concerned. No significant correlations were observed for any of the weeks in terms of concentration of *E. coli.* For TC concentration, significant correlations (r-Spearman, $p < 0.05$) were seen between week 2 and both week 3. The temporal variations in FIB with DO and Conductivity are shown in Table 2.

Table 2: Temporal variations of FIB with DO and conductivity

Sampling site	Parameter	Week 1	Week 2	Week 3	Week 4
Inlet	DO	3.1	0.2	0.1	2.2
	Cond	1208	1806	715	680
	E. coli	9.9	9.7	7.9	11.3
	TC	10.1	10.5	9.9	11.6
FAP	DO	4.8	6.8	5.6	10.1
	Cond	1430	1317	578	549
	E. coli	9.5	7.7	4.2	5.6
	TC	10.1	8.4	7.8	10.4
FFP	DO	15.4	13.9	11.9	19.1
	Cond	1326	1283	599	591
	E. coli	7.0	5.7	4.5	15.4
	TC	7.1	7.3	7.0	9.5
FMP	DO	17.9	8.9	7.6	8.8
	Cond	1278	1265	601	599
	E. coli	6.0	5.8	5.1	6.3
	TC	7.4	6.9	6.4	6.3
SFP	DO	18.4	10.3	8.2	8.9
	Cond	1233	1303	614	609
	E. coli	6.9	6.3	5.3	6.2
	TC	8.2	7.0	6.0	7.7
SMP	DO	9.0	6.5	5.1	5.8
	Cond	1266	1275	618	607
	E. coli	6.2	5.8	4.9	5.4
	TC	8.6	5.6	5.5	7.4

Note: The variables under investigation are FIB (*E. coli* and TC) and physical-chemical parameters (DO and Conductivity). The units for measurements are: (*E.coli* and TC (CFU/ 100 ml), DO (mg/l), Conductivity (μS/cm). Key: FAP=First Anaerobic Pond, FFP=First Facultative Pond, FMP=First Maturation Pond, SFP=Second Facultative Pond, SMP=Second Maturation Pond.

4. Discussion

4.1. Physical-chemical parameters of WSPs

Previous studies on similar systems have shown that higher temperatures contribute to higher reduction efficiencies for both organic matter and FIB. Anaerobic ponds, for instance, achieve about 40 % reduction of Biochemical Oxygen Demand (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 hydraulic regime related mechanisms for organic matter and pathogen removal in absence of short circuiting are time, temperature, pH > 9 and high solar radiation (Ultraviolet), which are conditions best suited for WSPs in tropical regions, Kayombo (2005). In the current study, insignificant variation in temperature in the sampling along the wastewater treatment pathway sites could depict uniform insolation, wind induced mixing due to shallow depth in the ponds raising water temperatures to uniformity. This phenomenon may significantly overshadow increased temperatures from bioenergetics arising from breakdown and transformations of organic matter in different compartments of WSPs, which ultimately result in both spatial and lateral uniformity of temperature in the WSPs.

The low DO at the inlet could be attributed to high demand by micro-organisms to degrade organic matter and some inorganic compounds perhaps present in the inflow. Furthermore, aerobic biotransformation process must have led to consumption of DO during mineralization of organic matter into inorganic form. Increase in DO concentration gradient along the treatment pathway is in tandem with what is expected in WSPs (Pena and Mara, 2004; Butler *et al*., 2015). The unexpected low DO concentration in the SMP could probably be attributed to increase in oxygen consumption to deal with added dissolved organic matter from dead algal biomass. Furthermore, faecal wastes from the avian community in the ponds could have increased oxygen consumption of the effluent, a phenomenon described by Murray and Hamilton (2010). Finally, the wastewater by-passing from SAP could be contributing partially organic wastes with low DO. Super-saturation of DO in the FFP could be explained by photosynthetic oxygenation because of the algal mat that was seen floating in this specific pond. In the early afternoons, there was high insolation and consequently high solar intensity that led to photosynthesis reaching its peak. Similar effects have also been observed in other studies (Tadesse *et al*., 2004).

4.2. Concentration of total coliforms and E. coli in WSPs

In this study, there was a gradual reduction in concentration of *E. coli* and TC from the inlet along the treatment pathway to the last stabilization pond. This could probably have been accelerated by parameters such as high temperatures, high pH, high solar irradiation and elevated DO concentrations due to photosynthetic community additions as suggested by Sperling (2007). *E. coli* concentration figures remained high in the SMP contrary to the expectation that this ought to decline. This could be attributed to possibility of incomplete preliminary treatment of effluent by-passing from the SAP and joining the FFP before reaching the SMP. Additionally, there was evidence of avian community seen to be in contact with the SMP and faecal matter seen at the edges of this pond was probably washed into the pond during a storm. Kipkemboi *et al*. (2006) reported a significant presence of waterfowl in the same system. However, it is not clear if the avian densities are still appreciably high and this could be recommended in a further study.

Low TC concentration recorded in the FMP was expected since the primary function of maturation ponds is removal of pathogens according to Kayombo (2005), which was probably because of shallow depth (1.5 m) and high pH range (7.9-9.7). Shallow depth in maturation ponds leads to well oxygenation as there is less vertical biological and physical-chemical stratification throughout the day (Tadesse *et al*., 2004). Algal population density in maturation ponds is much more diverse than in the facultative and anaerobic ponds with the rapid photosynthesis in the maturation pond leading to elevation of pH, particularly in the afternoons (Pham *et al*., 2014). During photosynthesis, algae consumes CO_2, faster than it can be replenished by bacterial respiration in the pond, hence 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 contributing to their die-off (Tyagi *et al*., 2008).

Essentially, high reduction efficiency was expected in the SMP for both TC and *E. coli* basing on the fact that the effluent had moved all the way from the inlet along the treatment pathway. However, the highest reduction efficiency was exhibited by SFP for *E. coli* (5 log units) and FMP for TC (3 log units). The most probable reason for this trend could be the pond design where the SMP precedes the SFP and FMP. Similarly, the effect of by-passing wastewater directly from SAP to join the ponds connected in series could as well have contributed to this trend. Furthermore, the ponds have never been disludged and this negatively affects pond performance as suggested by Barjenbruch and Erler (2005) and Sperling (2007).

Intense insolation has destruction effects on coliforms (Bansah and Suglo, 2016). Solar radiation of a wavelength between 425-700 nm affects faecal coliform when absorbed by humic substances in wastewater. The UV-B portion of the solar spectrum is the most important in bacterial die off, causing direct photobiological damage. At wavelengths of > 329 nm, photochemical mechanisms (particularly photooxidation) become more important, acting through photosensitizers to damage organelles, principally the cytoplasmic membrane. Because irradiance at the earth's surface falls sharply below 400 nm, longer solar wavelengths may account for a significant proportion of bacterial inactivation (Moeller and Calkins, 1980; and Davies-Colley *et al*., 1993). The present study reached cumulative reduction efficiencies between 2 to 4 log units for *E. coli* and 2 to 3 log units for TC in the two maturation ponds. Similar studies in the tropical environment that achieved related results includes studies by Tyagi *et al*. (2008) and Bansah and Suglo (2016).

4.3. Temporal variation of physiochemical parameters (temperature and dissolved oxygen) with FIB

In WSPs, temperature exhibits marked seasonal and daily variations. It influences photosynthesis, the growth of micro-organisms, and the bio-decomposition of organic carbon in the system (Kayombo, 2005). Normally, pond temperature directly depends on sunlight intensity at the pond surface and the time the pond remains exposed to sunlight (Peña and Mara, 2004). During week 3, sampling occurred in the middle of a heavy storm for the entire week that lasted from mid-morning to later in the afternoon. This affected sunlight intensity on the pond surface hence temperature of wastewater in the ponds. Since bacterial growth rates increase with temperature, a drop in temperature indicated a reduction in concentration of FIB.

Absence of a storm during week 4 saw a slight increase in temperature, as well as a slight increase in FIB concentration as the pond surfaces finally became exposed for longer hours to sunlight intensity. Anaerobic and Facultative ponds are designed for BOD removal while maturation ponds are designed for removal of faecal bacteria. However, in some cases, removal of faecal bacteria occurs in anaerobic ponds as seen in the current study. The strong correlation between temperature and *E. coli* concentration in the FAP could be attributed to uniform wind mixing of wastewater in the pond, which ensured uniform temperature. The mixing was due to shallow depth of the pond (1.5 m), which ensured no thermal stratification. In the FAP, intense anaerobic digestion occurred since temperature remained above 20 °C, hence directly influencing *E. coli* concentration.

The process of photosynthesis occurs in both facultative and maturation ponds. A mutual relationship exists between pond micro-algae that grow naturally on the ponds and pond bacteria. Micro-algae provide the oxygen required by the pond bacteria to oxidize the wastewater BOD while pond bacteria provide carbon dioxide needed by the algae, from the bacterial metabolism end-product (Peña and Mara, 2004). The algae on wastewater surfaces makes the ponds appear green in color. In the current study, the FFP appeared greenish and it is in this pond that notably high Dissolved Oxygen (DO) values were recorded in comparison to all other sampling sites. Dissolved Oxygen varied diurnally due to photosynthetic activities of the FFP, hence the algal mats were responsible for creating conditions within the pond that facilitated bacterial growth and die-offs. The absence of correlation could be due to the different rates of survival of pond bacteria in each pond. For instance, the last pond recorded lower DO values in comparison to preceding ponds, indication increased contamination as a result of by-passing effect of the new system.

5. Conclusion and Recommendations

The study provides information on concentration and reduction efficiencies of FIB in EU WSPs. Total coliforms and *E. coli* show progressive reduction in FIB in wastewater along the treatment pathway from the inlet towards the outlet. Total Coliforms and *E. coli* ranged between $2.5 \times 10^{8} – 2.9 \times 10^{11}$ and $5.9 \times 10^{5} – 1.8 \times 10^{10}$ CFU / 100 ml respectively with cumulative reduction efficiencies between 2 to 4 log units for *E. coli* and 2 to 3 log units for TC in the two maturation ponds. The effluent from the final discharge point cannot be recommended for irrigation downstream as it is above the permissible World Health Organization (WHO) standard of ≤ 105 and ≤ 1000 CFU / 100 ml for restricted and unrestricted irrigation respectively. Furthermore, the effluent is above Kenya's guideline value for discharge into surface water, which is ≤ 30 and NIL CFU / 100 ml for TC and *E. coli* respectively.

To ensure higher reduction efficiencies for FIB and other contaminants in WSPs, proper operation and maintenance guidelines need to be implemented. The wetland preceding WSPs need to be rehabilitated in order to polish the wastewater prior to discharge into the recipient ecosystem. In case of expansion of a WSP, there is need for consultation between the designing engineer and the ecologist to ensure the design does not interfere with proper functioning of the pond system.

Acknowledgment

The authors are grateful to the Austrian Development Cooperation for financing the project.

Conflict of interest

The authors declare no conflict of interest

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Cite this article as: Khasisi D. Lukhabi, Muia A. Wairimu and Kipkemboi K. Julius (2021). Assessment of performance of wastewater stabilization ponds using faecal indicator bacteria reduction efficiency in a tropical environment. *African Journal of Biological Sciences*. 3(2), 16-26. doi: 10.33472 / AFJBS.3.2.2021.16-26.