Water Reuse

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Treatment of greywater using a non-aerated combined horizontal and vertical flow constructed wetland

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ABSTRACT

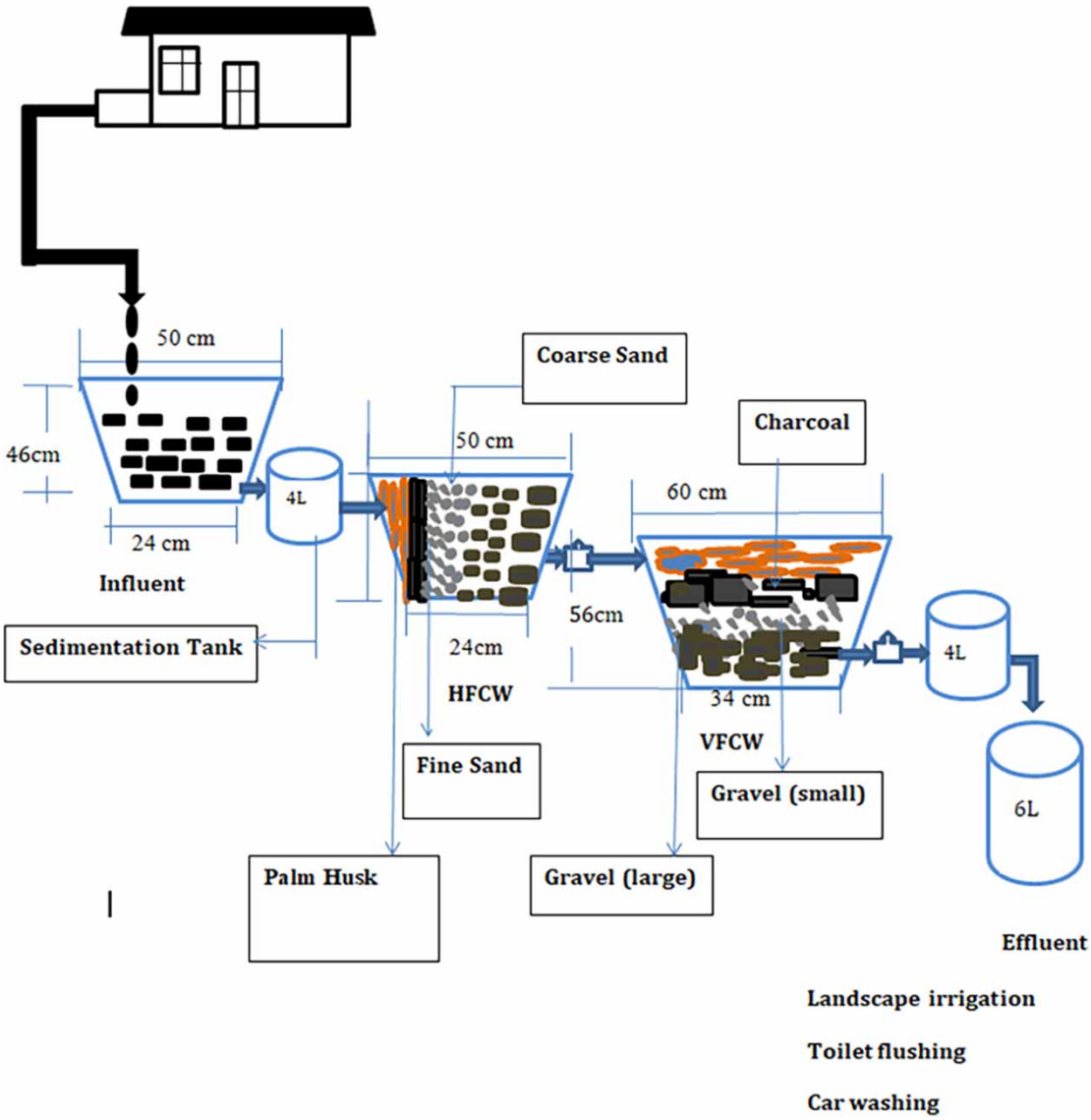
Human, animal, and plant health is universally paramount, yet the release of poorly treated wastewater into the environment poses a significant risk to all life forms. Hence the need to employ wastewater treatment technologies to curb these health risks. Due to the need to adopt sustainable wastewater treatment technologies, this study investigated the use of a non-aerated hybrid horizontal and vertical flow constructed wetland for the removal of heavy metals and microorganisms from greywater. This was done at six different hydraulic retention times. Results showed significant reductions ($p < 0.05$) in heavy metal (manganese, zinc, cadmium, magnesium, chromium, and iron) concentrations, with some showing compliance to Ghana's Environmental Protection Agency and the United Kingdom National Environment Regulation recommended discharge limits. Heavy metal concentrations in effluent samples ranged from as low as 0.00 ± 0.15–0.23 ± 0.06 mg/L. Furthermore, there were significant reductions in *Escherichia coli* and *Salmonella typhi* ($p < 0.05$), which also showed compliance to Ghana's Environmental Protection Agency effluent discharge standards. The effluents from the system at HRT 3 days showed high removal efficiency ranges of 82–90% of bacteria. It is recommended that hybrid constructed wetlands should be incorporated in the treatment of greywater.

Key words: enteric bacteria, greywater, heavy metals, horizontal flow, hybrid constructed wetlands, vertical flow

HIGHLIGHTS

- Greywater is a viable option for reuse when treated efficiently.
- Hybrid constructed wetlands show remarkable removal of pollutants.
- Heavy metal concentrations found in greywater were reduced after treatment.
- There were significant reductions in *Escherichia coli* and *Salmonella typhi* found in treated samples.
- *E. coli* counts in some effluents were within the Ghana EPA discharge limit, while most of the *S. typhi* counts were within the Ghana EPA discharge limits.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Water is essential for various uses in areas such as municipal, industrial, and agricultural sectors, among others (Govt 2013). Greywater is any household wastewater which contains little or no toilet input. It includes wastewater from showers, hand wash sinks, laundry discharges, and kitchen sinks, that are produced in homes, offices, and institutions (Boyjoo *et al.* 2013). Contaminants that can be found in greywater include hazardous metals and substances, as well as microorganisms, and radioactive substances. These contaminants can be detrimental to life upon exposure to the environment (Amouei *et al.* 2015).

It has been estimated that the total fraction of greywater by volume in combined residential sewage is around 75% (Ghaitidak & Yadav 2014). Special emphasis has been placed on the potential for reuse of greywater due to its quantity and quality. Greywater contains little or no fecal matter and is less polluted (Ghaitidak & Yadav 2014; Arden & Ma 2018). When assessing the prospects for the reuse of wastewater, including the need for treatment before use, the characteristics are of significance. It is important to consider health implications, especially pathogenic organisms, and environmental views, such as the accretion of chemicals or substances that are foreign to organisms as well as their biological systems. Some of the possible uses of treated greywater include crop irrigation and toilet flushing. It has been estimated that by reusing greywater for these activities, about 30% of the overall household potable water usage could be laid aside for such purposes (Abraham *et al.* 2018). The presence of

trace elements and heavy metals in any source of greywater determines its suitability for reuse and its capability to support life. For the physiological functions of living tissue, certain heavy metals such as mercury (Hg), manganese (Mn), zinc (Zn), cadmium (Cd), magnesium (Mg), chromium (Cr), and iron (Fe), present in trace amounts, are essential as they control many chemical processes in living tissues. However, these metals when released into water bodies in higher amounts may adversely affect humans and aquatic organisms (Tchounwou *et al.* 2012). In greywater, the virulent nature of these metals is contingent on the level of disintegration of the metal in question, and how the metal occurs. Human beings are prone to these heavy metals because of the increase in domestic, industrial, agricultural as well as commercial applications. Toxicity is of concern if the concentrations of these metals exceed their minimal concentrations (Bai *et al.* 2010).

Greywater contains a variety of bacteria, viruses, and protozoa. Most are harmless and can be used in biological processes. However, wastewater often includes microorganisms that are pathogenic. Total coliforms, *Salmonella* or *Escherichia coli* are indicator species currently employed in tracking the performance of treatment systems (Abdel-Raouf *et al.* 2012). The term 'total coliforms' comprises a large category of Gram bacteria which are rod-shaped and are found in soils, water, and in human intestines as well as in warm-blooded animals. They can produce gas at a temperature range of 35–37 °C after 48 h through lactose fermentation (Cabral 2010). The presence of total coliforms in wastewater gives a resounding indication of water contamination but not enough to conclude on fecal contamination (Gibson *et al.* 2012). Fecal coliforms, especially *E. coli*, are commonly used in aquatic bodies as indicators of fecal contamination. Their existence means that there could also be pathogenic microorganisms, leading to potential threats to human and animal health. *E. coli* is a microorganism that can also pass on genes horizontally to other optional or obligatory pathogenic bacteria (Maal-Bared *et al.* 2013). The species *S. typhi* is one of the pathogenic bacteria that could be spread by sewage to aquatic ecosystems. It is imperative to evaluate the prevalence, levels, and resistance of *S. typhi*. Greywater is perceived to be a typical medium for the delivery and transfer of *S. typhi*, capable of contaminating the environment and adversely interfering with human and animal life. Landscape irrigation using untreated greywater has been known to be the cause of contamination and spread of infections by *S. typhi*, and through soil percolation to groundwater or surface runoff into water bodies (Khadija *et al.* 2016). The source of *S. typhi* may be through animal or human fecal remains and sewage contamination. *S. typhi* can survive longer in the environment and therefore poses high risk of contamination.

Several studies report on the presence of *Salmonella* in untreated and treated greywater using various treatment methods and processes (Khadija *et al.* 2016). It is important to note that while constructed wetlands are commonly used to reduce microbial counts, they are not designed specifically to achieve complete elimination of pathogenic bacteria. High-level wastewater treatment is required for the effective elimination of *S. typhi*. To have successful and effective removal of pollutants in greywater, hybrid constructed wetlands could be built to take advantage of the separate systems. Vertical flow (VF) and horizontal flow (HF) phases are the common hybrid wetlands utilized (Zhang *et al.* 2018).

In addition to sewage, several other wastewaters have been managed by combined-built wetlands, such as landfill leachate (Wojciechowska 2017). Depending on the kinds of wastewater, constructed wetlands can be designed together for their treatment. Conversely, combined systems of built wetlands are most often VF and HF systems that are arranged sequentially.

The combined constructed wetlands take advantage of the benefits of either HF or VF systems to produce treated wastewater with low biological oxygen demand (BOD_5) (Vymazal 2017). In Ghana, there is scarce literature on greywater treatment using a hybrid unplanted constructed wetland to specifically target the removal of heavy metals and bacteria. This study aimed to assess the levels of heavy metals (Pb, Cd, Fe, Zn, Mg, Mn, and Hg) in both untreated and treated greywater, as well as to examine the presence of enteric bacteria (*E. coli* and *S. typhi*) in greywater before and after treatment.

2. MATERIALS AND METHODS

2.1. Research design

Samples of greywater were collected from the various outlets of the Valco Hall of Residence at the University of Cape Coast campus in the morning and evening. These samples were homogenized. Sampling was done thrice a month for each hydraulic retention time (HRT) used in the combined HFCW and VFCW.

2.1.1. Construction of combined HFCW and VFCW

The treatment system consisted of an influent tank connected to a sedimentation tank which was connected to the HF tank using polyvinyl chloride (PVC) pipe. The configuration (from left to right) of the HF tank was made of 5 mm diameter aggregate gravel (7.0 kg), followed by 3 mm diameter aggregate gravel (7.0 kg), followed by 0.3–1.18 mm coarse sand (6.0 kg),

followed by 0.075–0.20 mm fine sand (6.0 kg), Charcoal (1.5 kg), and Palm husk (0.5 kg). The HF tank was also connected to the VF tank with a configuration (from bottom to top) of 5 mm diameter aggregate gravel (7.0 kg), 3 mm diameter aggregate gravel (7.0 kg), 0.3–1.18 mm coarse sand (6.0 kg), 0.075–0.20 mm fine sand (6.0 kg), charcoal (1.5 kg), and palm husk (0.5 kg), which was connected to another sedimentation tank through an outlet valve. Water regulation was done using stop corks (Figure 1).

2.1.2. Design calculations

The following calculations were made for the design and construction of the treatment system.

Hydraulic flow rate (HFR) was established from the following equation (Reed *et al.* 1995).

$$\text{HFR} = \frac{\text{Volume of greywater}}{\text{HRT}} (\text{L/d or ml/min}) \quad (1)$$

Here, HRT is the hydraulic retention time and HFR is the hydraulic flow rate.

2.1.3. Data collection

Greywater samples were retrieved from the influent tank prior to treatment and from the effluent tank following treatment for each HRT. This process was repeated three times for each HRT under varying flow rates. The untreated and treated greywater samples were transported to the laboratory for analysis. The analysis focused on measuring heavy metals and bacteria levels in greywater before and after treatment.

2.1.4. Sampling procedure

Sampling of untreated greywater was conducted over a 6-month period, spanning from March to August 2021, for each HRT ranging from 0.5 to 3.0 days, and corresponding FRs ranging from 670 to 110 mL/min, respectively. All parameters were

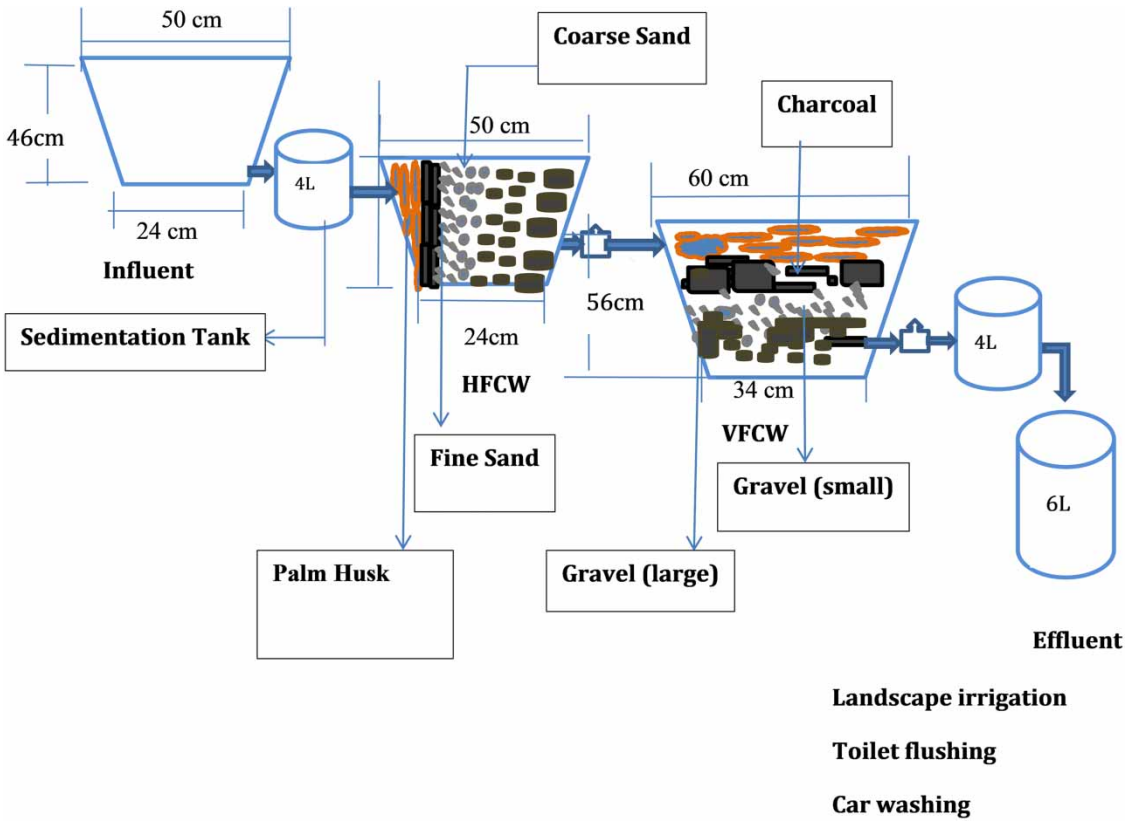


Figure 1 | Schematic diagram of the hybrid constructed wetland.

analyzed both before and after treatment. Greywater samples were collected using pre-treated plastic containers and promptly transported to the laboratory for analysis. A total of 36 samples from 18 composites were evaluated.

2.1.5. Test for heavy metals: digestion according to USEPA method 3010 (acid digestion of extracts)

The acid digestion process was used to examine heavy metal residues. This approach was accomplished by subjecting a sample to a strong acid at a reasonable temperature, which allows the sample to thermally disintegrate and permits analytical techniques to measure the sample due to the solubility of heavy metal ions in the solution.

In a 100 mL borosilicate beaker, 40 g of greywater sample was placed. In a fume chamber, 5 mL aqua regia was added to the sample. The glassware was placed on a hot plate and digested for 3 h at 450 °C, capped with a thin cling film. The sample was placed in a 100 mL graduated cylinder after digestion. Deionized water was added till it reached the 30 mL mark. Digested samples were kept in 15 mL polyethylene tubes in a 40 °C cool environment. The AA-7000 UV-6705 UV/VIS SHIMADZU Atomic Absorption Spectrophotometer and Inductively Coupled-Atomic Emission Spectrophotometer (SHIMADZU Corporation, Kyoto Japan) were used to analyze heavy metals in the samples (APHA 2012).

2.1.6. Test for microbial parameters: pour plate method

2.1.6.1. E. coli and S. typhi. All test tubes, petri dishes and pipette tips were sterilized in an autoclave. Greywater samples were collected and brought to the Environmental Science Department Laboratory in sterilized plastic bottles. The samples were tested for *E. coli a*nd *S. typhi* following standards set by the Ghana Standards Authority.

2.1.6.2. Preparation of Endo Agar. Endo Agar (EA) was prepared based on the manufacturer's instructions. An amount of 12.45 g of EA was weighed and poured into 300 mL of distilled water and whirled to achieve a uniform mixture. The pH was allowed to be at 7.5 ± 0.2 at room temperature. It was then autoclaved at 121 °C for 15 min. The media was allowed to cool to 50 °C. A 1.0 mL of the greywater sample was serially diluted using 10^8 dilutions. Afterwards, 0.1 mL of the diluted sample was aseptically introduced onto sterilized petri dishes, which had been labeled. The media was then dispensed into the various petri dishes and swirled under sterile conditions. The media in the petri dishes were allowed to solidify and placed in an incubator at 37 °C for 24 h. Both positive and negative controls were prepared for better comparison. The positive control was prepared using Nutrient Broth as instructed by the manufacturer. Each of the presumptive colonies of *E. coli* and *S. typhi* were counted. The microbial count was computed using the formula below.

$$\mathrm{CFU/ml} = \frac{number\ of\ counts \times df}{volume\ of\ sample} \tag{2}$$

where, *CFU* is coliform unit and *df* is dilution factor.

E. coli on EA appeared golden green and *S. typhi* appeared light pink or pinkish white (APHA 2012).

2.1.6.3. Preparation of Brilliant Green Agar. Brilliant green agar (BGA) was prepared based on the manufacturer's instructions. 15.81 g of BGA was weighed and poured into 300 mL of distilled water and whirled to achieve a uniform mixture. The pH was allowed to be around 6.9 ± 0.2 at room temperature. It was heated in a water bath at 100 °C for 45 min and ensured complete dissolution of the powder. After dissolution, it was autoclaved at 121 °C for 15 min. The media was allowed to cool to 50 °C. Following this, 0.1 mL of the diluted sample was carefully introduced onto sterilized petri dishes which had been labeled. The media was poured into the various petri dishes within a fume chamber under sterile conditions and allowed to solidify. The petri dishes were placed in an incubator at 37 °C for 24 h. Both positive and negative controls were prepared for better comparison. The positive control was prepared using Nutrient Broth as instructed by the manufacturer. Each of the presumptive colonies of *E. coli* and *S. typhi* were counted.

S. typhi appeared pink-white and *E. coli* appeared golden green (APHA 2012).

2.1.7. Data analyses

Microsoft Excel (2016) and SPSS version 21 were used to present tables and analyze the data obtained from the laboratory analysis. The greywater parameters were compared to the Ghana Environment Protection Agency (EPA 2012) and UK National Environment Regulation (NER 1995) standards. A one-way analysis of variance (ANOVA) was done to determine the differences in parameters between the untreated and treated greywater samples. Pearson correlation was used to assess

the relationships that exist between the greywater parameters. In all the statistical tests, the significance threshold was set at $\alpha = 0.05$ and the significance level at 95%, i.e., $p < 0.05$.

3. RESULTS AND DISCUSSION

The average heavy metal concentrations in influent and effluent samples of greywater at different HRTs were presented in Table 1. The least influent Pb concentration was 0.08 ± 0.03 mg/L which entered the treatment system at HRT 0.5 day and the highest influent Pb concentration was 0.23 ± 0.13 mg/L which was introduced to the system at HRT 2.0 days. The lowest mean Pb effluent concentration was 0.02 ± 0.01 mg/L released from the system at HRT 3.0 days while HRT 2.0 days recorded the highest mean Pb effluent concentration of 0.13 ± 0.06 mg/L. The one-way ANOVA test showed no statistically significant distinction in the mean influent and effluent concentrations of Pb ($p > 0.05$). Except for the effluent that was released from the system at HRT 2.0 days, all effluent concentrations of Pb were within the Ghana EPA and UK NER discharge standards.

The mean Cd influent concentration of 0.18 ± 0.02 mg/L which entered the system at HRT 0.5 day was the lowest and the highest influent Cd concentration was 0.24 ± 0.07 mg/L which was introduced into the system at HRT 1.5 days. The lowest mean cadmium effluent value was 0.10 ± 0.04 mg/L and 0.10 ± 0.00 mg/L and was recorded from the system at HRT 1.0 day and 3.0 days. The system with the highest Cd effluent (0.16 ± 0.05 mg/L and 0.16 ± 0.04 mg/L) were from the system at HRT 0.5 day and 1.5 days, respectively. Mean Cd influent and effluent values showed no significant difference ($p > 0.05$). The mean effluent values were a little above the Ghana EPA discharge standard while some were within the UK NER standard.

The mean Fe influent value of 0.04 ± 0.01 mg/L was the lowest and was introduced into the system at HRT 2.0 days while the highest mean Fe influent value of 0.24 mg/L was introduced into the system at HRT 0.5 day. The least mean Fe effluent value was 0.02 ± 0.00 mg/L, and this was released from the system at HRT 3.0 days whereas the highest mean Fe effluent was 0.13 ± 0.10 mg/L which was released from the system at HRT 0.5 day. There were no significant distinctions between influent and effluent Fe concentrations ($p > 0.05$). The mean effluent concentrations were within the Ghana EPA and UK NER effluent discharge standards.

The lowest mean Zn influent concentration of 0.04 ± 0.01 mg/L entered the system at HRT 1.5 days and the highest concentration of 0.23 ± 0.15 mg/L entered the system at HRT 2.5 days. The lowest Zn effluent concentration was 0.02 ± 0.01 mg/L which was released from the system at HRT 1.5 days while the highest Zn effluent concentration was 0.15 ± 0.12 mg/L which was released from the system at HRT 2.0 days. There were no notable differences in influent and effluent Zn concentrations ($p > 0.05$). The mean effluents were within the Ghana EPA and UK NER discharge standards.

The Cr influent concentration ranged from 0.19 ± 0.10 mg/L to 0.36 ± 0.03 mg/L, with the lowest concentration at HRT 1.0 days and the highest at HRT 2.5 days. HRTs 1.0 day and 3.0 days recorded the lowest mean chromium effluent concentrations of 0.18 ± 0.10 mg/L while HRTs 0.5, 1.5, 2.0 and 2.5 days released the highest effluent concentration of 0.23 ± 0.06 mg/L. There was no significant distinction in Cr concentrations among the mean influents and effluents ($p > 0.05$). The effluent values were above the Ghana EPA discharge standard but within the UK NER standards (Table 2).

The Mn influent concentrations ranged from 0.00 ± 0.17 mg/L to 0.27 ± 0.03 mg/L. The least influent concentration was introduced to the system at HRT 1.5 days while the entered the system at HRT 2.5 days. The lowest mean effluent concentration of 0.00 ± 0.15 mg/L was released from the system at HRT 1.5 days while HRT 2.0 days released the highest effluent concentration of 0.19 ± 0.04 mg/L. The influent and effluent concentrations showed no significant differences ($p > 0.05$). The effluent values were within the Ghana EPA and UK NER effluent discharge standards.

HRT 2.5 days received the lowest Hg influent concentration of 0.04 ± 0.01 mg/L and HRT 1.5 days received the highest mean Hg influent concentration of 0.19 ± 0.04 mg/L. The least Hg effluent concentration of 0.01 ± 0.06 mg/L was released from the system at HRT 3.0 days and the highest concentration of 0.17 ± 0.04 mg/L was released from the system at HRT 1.5 days was the highest. There were no noteworthy distinctions between influent and effluent concentrations ($p > 0.05$). The effluent values were above the Ghana EPA discharge standard while others were within the UK NER discharge standard.

The study revealed that the effluent concentrations of Pb, Fe, Zn, and Mn were within the recommended discharge limits set by the Ghana EPA and UK NER. This shows that the effluent will not pose any toxic effect to users and to any water bodies it finds its way into. Cd, Cr, and Hg had effluent values above the recommended threshold by the Ghana EPA but

Table 1 | Average heavy metal concentrations in influent and effluent samples of greywater at different HRTs

	HRT 0.5 (days)		HRT 1.0 (days)		HRT 1.5 (days)		HRT 2.0 (days)		HRT 2.5 (days)		HRT 3.0 (days)	
	Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE		Mean ± SE	
PARAMETER	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Pb (mg/L)	0.08 ± 0.03	0.06 ± 0.04	0.14 ± 0.07	0.04 ± 0.00	0.18 ± 0.10	0.08 ± 0.03	0.23 ± 0.13	0.13 ± 0.06	0.18 ± 0.09	0.07 ± 0.03	0.09 ± 0.03	0.02 ± 0.01
Cd (mg/L)	0.18 ± 0.02	0.16 ± 0.05	0.19 ± 0.10	0.10 ± 0.04	0.24 ± 0.07	0.16 ± 0.04	0.23 ± 0.07	0.11 ± 0.02	0.21 ± 0.05	0.12 ± 0.02	0.22 ± 0.05	0.10 ± 0.00
Fe (mg/L)	0.24 ± 0.07	0.13 ± 0.10	0.12 ± 0.07	0.08 ± 0.04	0.14 ± 90.08	0.12 ± 0.07	0.04 ± 0.01	0.03 ± 0.00	0.20 ± 0.12	0.12 ± 0.07	0.10 ± 0.06	0.02 ± 0.00
Zn (mg/L)	0.14 ± 0.08	0.09 ± 0.07	0.16 ± 0.12	0.07 ± 0.06	0.04 ± 0.01	0.02 ± 0.01	0.19 ± 0.16	0.15 ± 0.12	0.23 ± 0.15	0.11 ± 0.07	0.16 ± 0.11	0.06 ± 0.03
Cr (mg/L)	0.24 ± 0.07	0.23 ± 0.10	0.19 ± 0.10	0.18 ± 0.10	0.27 ± 0.03	0.23 ± 0.06	0.35 ± 0.01	0.23 ± 0.06	0.36 ± 0.03	0.23 ± 0.06	0.34 ± 0.01	0.18 ± 0.10
Mn (mg/L)	0.14 ± 0.07	0.07 ± 0.10	0.06 ± 0.14	0.02 ± 0.06	0.00 ± 0.17	0.00 ± 0.15	0.23 ± 0.01	0.19 ± 0.04	0.27 ± 0.03	0.13 ± 0.01	0.23 ± 0.01	0.16 ± 0.04
Hg (mg/L)	0.08 ± 0.03	0.02 ± 0.01	0.15 ± 0.13	0.06 ± 0.05	0.19 ± 0.04	0.17 ± 0.04	0.10 ± 0.11	0.10 ± 0.09	0.04 ± 0.01	0.02 ± 0.01	0.10 ± 0.11	0.01 ± 0.06

Table 2 | Recommended heavy metal discharge limits from Ghana EPA and UK NER

	Pb (mg/L)	Cd (mg/L)	Fe (mg/L)	Zn (mg/L)	Cr (mg/L)	Mn (mg/L)	Hg (mg/L)
Ghana EPA (2012)	0.1	<0.1	2	2	0.005	0.2	0.005
UK NER (1995)	0.1	0.1	5	20	0.1	0.2	0.01

some within the UK NER (1995). The mean influent and effluent concentrations were not statistically significant. Overall, the discharge concentrations were commendable. Improving system removal would require the addition of macrophytes, as reported by other authors (Karunarathna *et al.* 2007).

The concentrations of *E. coli* were estimated for both Endo agar (EA) and BGA and the results are presented in Table 3. The lowest mean influent count of *E. coli* using EA was $1.3 \times 10^9 \times 1.96$ CFU/mL which entered the system at HRT 2.5 days and the highest mean influent count was $19.4 \times 10^9 \pm 9.98$ CFU/ mL which was introduced into the system at HRT 1.5 days. The lowest mean effluent count of *E. coli* was $2 \times 10^8 \pm 0.94$ CFU/ mL released from the system at HRT 3.0 days while the highest mean effluent count was $6.1 \times 10^9 \pm 9.49$ CFU/ mL released from the system at HRT 1.5 days. Using BGA, the lowest *E. coli* influent count recorded was $1.3 \times 10^9 \pm 3.93$ CFU/mL which was introduced into the system at HRT 2.5 days while the mean influent concentration of $22.3 \times 10^9 \pm 42.21$ CFU/mL was the highest and entered the system at HRT 1.5 days. The lowest mean effluent count of *E. coli* was $3 \times 10^8 \pm 0.27$ CFU/mL released from the system at HRT 3.0 days and the highest mean effluent concentration value was $5.9 \times 10^9 \pm 23.97$ CFU/mL released from the system at HRT 1.5 days (Figure 2). There were significant variations in influents and effluents *E. coli* counts ($p < 0.05$). The effluent concentrations were within Ghana EPA standards (Table 4).

Using EA, the lowest mean influent count of *S. typhi* recorded were $1.1 \times 10^9 \pm 3.31$ CFU/mL and $1.1 \times 10^9 \pm 2.37$ CFU/mL at HRTs 2.5 days and 3.0 days, respectively, with HRT 0.5 day recording the highest mean influent count of $19.5 \times 10^9 \pm$ CFU/mL. The lowest mean effluent counts of *S. typhi* were $2 \times 10^8 \pm 1.63$ CFU/mL and $2 \times 10^8 \pm 0.94$ CFU/mL at both HRT 1.0 day and HRT 3.0 days, respectively, while the highest mean effluent counts of *S. typhi* was $6.3 \times 10^9 \pm$ 17.44 CFU/mL at HRT 0.5 day. For BGA, the lowest mean influent count of *S. typhi* recorded was $2.5 \times 10^9 \pm$ 1.44 CFU/mL at HRT 3.0 days and the highest mean *S. typhi* influent count was $15.9 \times 10^9 \pm 40.72$ CFU/mL released from the system at HRT 0.5 day. The smallest mean effluent count of *S. typhi* was $4 \times 10^8 \pm 0.82$ CFU/mL at HRT 3.0 days and the highest count was $4.7 \times 10^9 \pm 16.98$ CFU/mL at HRT 0.5 day. There were significant variations in mean influent and effluent counts of *S. typhi* ($p < 0.05$). Some of the effluent counts were within Ghana EPA standard such as HRTs 1.0, 2.0, 2.5 and 3.0 days while others were above the standard as in HRTs 0.5 and 1.5 days. When the analyses of *E. coli* and *S. typhi* were done for influent and effluent greywater samples, the results revealed that there were considerable reductions in the count of these microbes in the effluents. Most of the mean effluent concentrations were within the Ghana EPA standards, while others were above the standard. The mean values were significant across the various HRTs. If effluent concentrations surpass the recommended thresholds and are utilized for irrigation, farmers may face potential exposure to pathogens (Faruqui *et al.* 2004). It is recommended that further subjection of the effluent to ultraviolet light would be beneficial. The factors that contribute to microbial removal in constructed wetlands are HRT, temperature, and light intensity (Hodgson 2007). pH values above 10.7, would lead to no bacterial counts in effluents (Hodgson & Larmie 1998). It has been reported that temperature above 37 °C should be maintained for 15 days to kill bacteria in wastewater (Larney *et al.* 2003). According to Khadija *et al.* (2016), wastewater treatment plants are usually constructed to reduce the numerical value of microbes but are not conceived specifically to provide total termination of the disease-causing bacteria. High-level wastewater treatment is required for effective removal.

4. CONCLUSION

The study investigated the presence of heavy metals (Pb, Cd, Fe, Zn, Mg, Mn, and Hg) and enteric bacteria (*E. coli* and *S. typhi*) in untreated and treated greywater using a combined HF and VF constructed wetland system. This was done by evaluating the treatment performance of the combined system based on effluent quality across six HRTs. The combined treatment system showed commendable reductions of contaminants in effluents. There were no significant decrements in influents and effluents heavy metal concentrations ($p > 0.05$), however, Pb, Fe, Zn, and Mn effluents concentrations showed compliance

Table 3 | Average counts of *E. coli* and *S. typhi* found in influent and effluent greywater samples at different HRTs

	HRT 0.5 (days)		HRT 1.0 (days)		HRT 1.5 (days)		HRT 2.0 (days)		HRT 2.5 (days)		HRT 3.0 (days)	
	Mean ± SE (CFU/ mL)		Mean ± SE (CFU/ mL)		Mean ± SE (CFU/ mL)		Mean ± SE (CFU/ mL)		Mean ± SE (CFU/ mL)		Mean ± SE (CFU/mL)	
MICROORGANISM	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
E. coli (Endo agar)	$17.3 \times 10^9 \pm 21.19$	$4.6 \times 10^9 \pm 19.07$	$13.1 \times 10^9 \pm 22.12$	$3.7 \times 10^9 \pm 8.02$	$19.4 \times 10^9 \pm 9.98$	$6.1 \times 10^9 \pm 9.49$	$7.6 \times 10^9 \pm 30.21$	$5 \times 10^8 \pm 2.88$	$1.3 \times 10^9 \pm 1.96$	$6 \times 10^8 \pm 1.63$	$1.9 \times 10^9 \pm 1.63$	$2 \times 10^8 \pm 0.94$
E. coli (BGA)	$18.0 \times 10^9 \pm 25.16$	$4.5 \times 10^9 \pm 7.68$	$4.3 \times 10^9 \pm 11.52$	$1.1 \times 10^9 \pm 3.57$	$22.3 \times 10^9 \pm 42.21$	$5.9 \times 10^9 \pm 23.97$	$4.5 \times 10^9 \pm 33.20$	$5 \times 10^8 \pm 2.18$	$1.3 \times 10^9 \pm 3.93$	$5 \times 10^8 \pm 2.37$	$3.1 \times 10^9 \pm 2.06$	$3 \times 10^8 \pm 0.27$
S. (Endo agar)	$19.5 \times 10^9 \pm 21.85$	$6.3 \times 10^9 \pm 17.44$	$1.5 \times 10^9 \pm 2.88$	$2 \times 10^8 \pm 1.63$	$17.7 \times 10^9 \pm 62.37$	$3.9 \times 10^9 \pm 13.21$	$5.1 \times 10^9 \pm 37.29$	$3 \times 10^8 \pm 1.09$	$1.1 \times 10^9 \pm 3.31$	$3 \times 10^8 \pm 1.44$	$1.1 \times 10^9 \pm 2.37$	$2 \times 10^8 \pm 0.94$
S. (BGA)	$15.9 \times 10^9 \pm 40.72$	$4.7 \times 10^9 \pm 16.98$	$9.4 \times 10^9 \pm 29.39$	$2.2 \times 10^9 \pm 0.94$	$13.4 \times 10^9 \pm 21.60$	$2.5 \times 10^9 \pm 3.57$	$4.6 \times 10^9 \pm 21.60$	$7 \times 10^8 \pm 1.96$	$3.6 \times 10^9 \pm 10.71$	$1.3 \times 10^9 \pm 4.25$	$2.5 \times 10^9 \pm 1.44$	$4 \times 10^8 \pm 0.82$

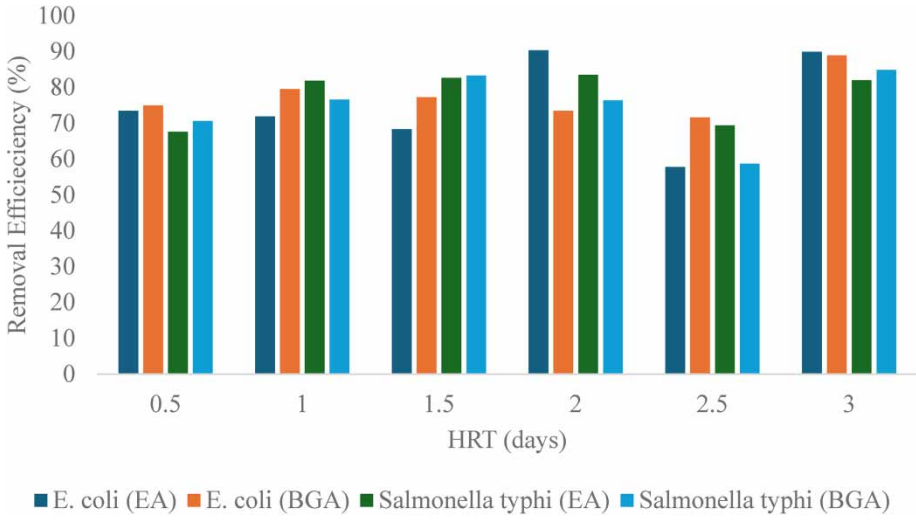


Figure 2 | Removal efficiencies of enteric bacteria.

Table 4 | Ghana EPA discharge limits

Microorganisms	Ghana EPA (CFU/mL)
E. coli (Endo agar)	10
E. coli (BGA)	10
Salmonella (Endo agar)	10
Salmonella (BGA)	10

with the Ghana EPA and the UK NER discharge standards. The results also revealed noteworthy reductions in mean effluent counts of *E. coli* and *S. typhi* as compared to influents ($p < 0.05$). Some of the bacterial counts in effluents were below the effluent discharge standards prescribed by the Ghana EPA. The results suggest that the hybrid treatment system was a good and effective system for treating greywater.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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