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Investigating the capacity of hydroponic system using lettuce (*Lactuca sativa* L.) in the removal of pollutants from greywater while ensuring food security

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Abstract—The study aims to investigate the removal of nutrients, organic pollutants and fecal bacteria from greywater while evaluating the growth and yields of lettuce using hydroponic system. Lettuces grow in hydroponic systems irrigated with untreated greywater, and fresh well water, separately. Nutrients, organic pollutants, and fecal bacteria of dishwashing greywater were monitored. Agronomical and microbiological parameters of lettuce were investigated. The results showed that removal efficiencies forammonia (NH_4^+), nitrate (NO_3^-), and total phosphorus (TP) were 78 %, 87 % and 56 %, respectively. The organic pollutants removal efficiencies were higher than 50% for Total Suspended Solids (TSS), 5days Biochemical Oxygen Demand (BOD_5) and Chemical Oxygen Demand (COD). The removal efficiencies are 88.68 % and 81.94 % for E. coli, and fecal coliforms, respectively. The fecal bacteria were not observed in the lettuce leaves. Besides, the lettuces irrigated with greywater produced higher biomass than the ones irrigated with fresh well water. The hydroponic system could be an alternative for greywater treatment and ensuring food security.

Keywords—Dishwashing greywater, Fecal bacteria, Hydroponic systems, Lettuce, Removal efficiencies.

I. INTRODUCTION

Wastewater reuse for irrigation is a long-standing practice and has many advantages especially in those areas where water resources are limited (Jaramillo and Restrepo, 2017). Treated wastewater contains nutrients that are useful for plant growth and help to reduce fertilizers needs (Sangare et al., 2017; Marzougui et al., 2018), thus ensuring a closed and environmentally favourable nutrient cycle that avoids the indirect return of nutrients, such are nitrogen and phosphorus to water bodies. Therefore, eutrophication conditions in water bodies would be reduced, as would the expenses for agrochemicals used by farmers (Candela et al., 2007). Greywater, which represents more than 75% of total global wastewater, and include wastewater from baths, showers, kitchen, hand wash basins and laundry, is the most popular fraction used for agriculture, as it contains low levels of pathogens (Morel and Diener, 2006; Hernandez et al., 2011). Based on such properties, greywater reuse for agricultural irrigation is receiving more and more attention (Li et al., 2009; Rodda et al., 2001). However, direct recycling of greywater is not recommended. Greywater may contain various microbial pathogens and hazardous chemicals depending on the nature of the raw greywater and the treatment efficiency (Maiga et al., 2018). There are several methods for the treatment of these generated domestic wastewater like Aerated Lagoons (Grady et al., 2011), High Rate Algal Ponds process (Sangare et al., 2017), Electrocoagulation Process (Moosavirad, 2017). However, these methods are very expensive, complex and energy intensive for use semi-urban, and rural areas of developing countries. To address these gaps, it is imperative to design green alternative domestic wastewater treatment technologies linked to crop production to control the long-term effects on water resources, and to minimize the pressures of population growth on food stock. Indeed, wastewater reuse in agriculture through hydroponic systems can be a viable technology to avoid environmental, public health impacts, and food security (Magwaza et al., 2020). The hydroponic systems are agricultural systems in which the development and growth of plants occur without the use of soil, and their roots are immersed directly in the nutrient solution (Martinez-Mate et al., 2018). A literature review conducted by the FAO (2010) showed that the application of the system has improved city environment, food security, and cities economy by providing wide variety of fresh fruits and vegetables through the year. In fact, the systems are widely used for the treatment of nutrient rich wastewaters (Prazereset al., 2017; Bawiec, 2019). For instance, removal efficiencies ranging from 47 to 91% for both nitrogen and phosphorus was reported when using hydroponic system with different plant species (López-Chuken, 2012; Haddad and Mizyed, 2011). The nutrient removal efficiency was found to be dependent on the life interactions of various species of bacteria, plant roots, gravel, water and sunlight. Indeed, the plant root system modifies the living conditions for the microorganisms present in municipal wastewater by releasing various metabolites, and thus enhancing the biological treatment of wastewater through nitrification and denitrification processes (Tsao, 2003). Moreover, by using hydroponic system with Romaine Lettuce, the removal efficiency of COD, BOD and TSS were 37.36%, 82.31 % and 89.42 %, respectively according to study conducted by Cui et al. (2003). Besides, hydroponic systems have been reported to enhance the removal efficiency of pathogens, in wastewater treatment system (Ndulini et al., 2018). These researchers showed a removal efficiency of 92.77% for faecal coliforms removal, using of Bidenspilosa L. and Amaranthushybridus L. In their study, Ottoson et al. (2005) have also been shown that hydroponics can be an alternative to conventional wastewater treatment through the virus and (oo)cyst removal. On the other hand, hydroponic systems can help in biomass production for

value-added crops thereby improving food security and income generation among poor communities (Eregno et al., 2017; Jesse et al., 2019). Further, this system is particularly suitable for agricultural reuse of domestic wastewater because it minimizes the health risks to workers, harvested crop and consumer that occur through contact with wastewater (Qadiret al., 2010). In addition, hydroponic greenhouses use 13 fold less water to produce comparable lettuce yields compared to field operations, according to Barbosa et al. (2015). Thus, wastewater reuse in hydroponic system represents a choice of production that can be adapted to the demand of high quality, high production and higher recovery of water and nutrients. However, limited studies have been conducted on potential cultivation of lettuce (Lactuca sativa L.) with greywater using hydroponic system, still need some investigations. Lettuce is among vegetables commonly used for hydroponic production due to their short growth cycle allowing better control and standardization of the cultivation process (Magwaza et al., 2020). Moreover, lettuce is popular edible leaf vegetable in tropical countries with a relatively high market value (Palada et al., 1999). In view of all the above, the aim of this study was to explore the treatment of dishwashing raw greywater by hydroponic system cultivated with lettuce (Lactuca sativa L.). It will focus on (i) the characteristics of raw and treated dishwashing greywater, (ii) the removal efficiency of nutrients, pollutant loads, and fecal bacteria, and (iii) the effect on the yield and microbiological quality of lettuce in hydroponic systems.

II. MATERIAL AND METHODS

2.1Experiment design and plant material

Experiments were conducted at the University of Nangui Abrogoua (5.38 °N, 4.01 °W) in Abidjan, Côte d'Ivoire. A schematic view of the experimental setup is presented in Fig. 1. It consists of four vertical 25 liters tanks and two horizontal bins as hydroponic reactors where the lettuce were grown. Two of the vertical tanks were used to receive raw dishwashing greywater (RGW), whereas the other two tanks received fresh well water (FWW). RGW was collected from the University of Nangui Abrogoua restaurant and FWW from a well nearby the university which is used by local population for gardening. These water sources were used as an irrigation/fertigation source for the production of lettuce. Each hydroponic reactor was made up of six (6) racks. The hydroponic reactors were fed in batch every two days. 50 liters of RGW and FWW were first poured into the receiving tanks, and then transferred into the hydroponic reactors at a controlled flowrate of 50 mL/min. The theoretical hydraulic residence time in the

hydroponic reactors was estimated at 225 min. To determine the effectiveness of the treatment, greywater samples were collected from the entry and exit of each reactor, equipped with an outlet valve.

After 14 days of seeding, lettuces were transferred to the hydroponic reactors. Each reactor accommodated 6 lettuce plants. The lettuce plants measurements were taken at 49 days after planting (DAP). Plant height was measured as the vertical distance between the top of the reactor and the highest living part of the plant. The number of leaves was measured by counting all the leaves on each plant and the mean of the six plants assumed as the number of leaves. Thereafter, the dry weights were obtained by drying the shoot and root for 48 h at 65°C.

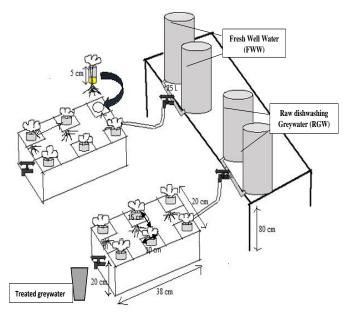


Fig 1. Greywater treatment and lettuce production using hydroponic system

2.2 Characteristics of greywaters

During one month of the experiment, raw greywater (RGW) and treated greywater (TGW) samples were collected every two days, to give a total of 15 samples. The samples were collected in 2.5 L bottles and stored at 4 °C in a thermal box before being transported to the laboratory for analysis. The analyses of pH and Electrical Conductivity (EC) were carried out in situ using a portable device (WTW Multi 350i). Physico-chemical characteristics were evaluated through assessment of ammonia (NH_4^+) , nitrate (NO_3^-) , and total phosphorus (TP) in raw and treated greywater using spectrophotometer UV visible (DR 6000). Total Suspended Solids (TSS), Chemical Oxygen Demand (COD) and 5-days Biochemical Oxygen Demand (BOD₅) were determined from homogenized samples to assess the removal

gravimetric method using glass microfiber filters Whatman (porosity $0.45 \ \mu$ m). All analyses were performed according to the Standard APHA methods (APHA, 2012).

Escherichia coli (*E. coli*) and fecal coliforms were monitored as indicator bacteria for microbiological pollution assessment. The spread plate method was used after an appropriate dilution of the samples in accordance with the procedure in Standard Methods (APHA, 2012). Chromocult Coliform Agar (Merck KGaA 64271, Darmstadt, Germany) was used as the culture medium for both *E. coli* and fecal coliforms.

efficiency of organic parameters. TSS were measured by a

2.3 Assessment of fecal bacteria on lettuce leaves

For fecal bacteria assay, 10 g of lettuce leaves sample was weighed into separate, sterile bottles (6). 1 N NaCl solution was added to each 10 g sample in each bottle and homogenized for 15 min. The homogenised supernatant of 10-1, and appropriate dilutions were made. A suspension of 0.1 mL of the stock solution and of each of the dilutions were inoculated. The Chromocult coliform agar plates were incubated at 37°C to 24 h, and E. coli and fecal coliforms colonies were counted(APHA, 2012).

2.4 Treatment Efficiency

The removal efficiencies of nutrient (NO_3^- , NH_4^+ , TP) and organic pollutants (TSS, BOD₅, COD) were determined. The removal rate of each parameters was calculated according to equation (1):

$$P(\%) = \frac{(C_{0-}C_i)}{C_0} \times 100 \qquad (1)$$

Where C_0 is the initial parameter concentration and C_i is the parameter concentration at the time i. All parameters will be measured using this method for all the tests.

2.5 Data Analysis

The data were processed using Excel and R software (3.0.1 version). Student's t-test was used to compare data sets. Lettuce growth and yield determined at each treatment were compared using one-way ANOVA followed by a post hoc comparison of means using Tukey's post hoc test (p < 0.05).

III. RESULTS AND DISCUSSION

3.1 Raw greywater and treated greywater characteristics

The characteristics of RGW and treated greywater (TGW), and fresh well water (FWW) are presented in Table 1. The mean pH was decreased to values from alkaline to neutral from raw greywater (8.14) to treated greywater (7.37). In their study, Maiga et al. (2014) showed that the dishwashing raw greywater exhibited the mean pH of 9. This finding is explained by the use of potassium hydroxide for cooking in study area. Furthermore, dishwashing activity are carried out using soaps and detergents such as OMO which show an alkaline pH when diluted in water (Ojo and Oso, 2008). Electrical conductivity (EC) decreased from an average of 2028.25 µS/cm in the raw greywater to 1154.67 µS/cm in the treated greywater. The nutrient analysis in raw dishwashing greywater showed that the mean concentration of ammonia (NH₄⁺), nitrate (NO₃⁻), and total phosphorus (TP) was 8.55, 14.90 and 8.33 mg/L, respectively, and was largely higher as compared to those of treated dishwashing greywater and fresh well water. Del Porto and Steinfeld(2000) have reported nitrogen in greywater originates from ammonia and ammoniacontaining cleansing products as well as from proteins in meats. vegetables, protein-containing. Further, dishwashing is the main source of nitrogen in domestic greywater. And, the primary source of phosphorus found in greywater comes from washing detergents, according to Eriksson et al. (2002). On the other hand, the results of pollutant load analysis in the raw greywater showed that the mean values of TSS, COD and BOD₅ was 416.67 mg/L, 346.33 mg/L and 216.47 mg/L, respectively, and was relatively higher as compared those of treated greywater. However, these pollutant loads were less polluted in terms of organic matter pollution than mixed laundry-dishwashing greywater (SS = 1230 mg/L, COD = 3916 mg/L and BOD5 = 1375 mg/L) reported by Maiga et al. (2014). BOD and COD concentrations in greywater strongly depend on the amount of water and products (especially detergents, soaps, oils and fats) used in the household (Morel and Diener, 2006).

As possible indicators for fecal contamination, E. coli and fecal coliforms were enumerated. The mean values in the raw dishwashing greywater were from 2.78×10^5 CFU/100 mL, 1.15×10^5 CFU/100 mL for *E. coli* and fecal coliforms, respectively (Table 1). The highest values of up to 2.3×10^7 CFU/100 mL for *E. coli* and up to 3×10^7 CFU/100 mL for fecal coliforms have been reported from dishwashing of household in rural area (Maiga et al., 2014). Fecal contamination in dishwashing greywater is due to contaminated vegetables and raw meat used during the cooking process (Ottosson, 2003). Based on WHO (2006) guideline for greywater reuse for restricted (E. coli< 10⁵ CFU/100 mL) agricultural irrigation, the results from the present study demonstrated that dishwashing raw greywater and after hydroponics treatment should be microbiologically acceptable. However, for non-restricted (E. coli< 10³ CFU/100 mL) agricultural irrigation, our greywaters are not safe using for irrigation (WHO (2006).

	8		
Parameters	Raw Greywater	Treated Greywater	Fresh Well Water
	n=15	n=15	<i>n</i> = 15
pH	8.14±0.44	7.37±0.34	6.89 ± 0.78
Temperature	25.6±0.65	26.48±1.25	27.18±1.38
EC (µS/cm)	2028.27±926.43	1154.67±435.18	2974±586.79
NH_4^+ (mg/L)	8.55 ± 2.68	1.88 ± 0.47	3.16±1.98
NO_3 (mg/L)	14.90 3.60	1.79±1.03	8.05±2.13
TP (mg/L)	8.33±2.04	3.63±1.59	5.98 ± 3.48
TSS (mg/L)	416.67±112.58	115.25±50.26	878.15±128.64
COD (mg/L)	346.33±178.16	164.33±88.28	428.12±125.43
$BOD_5 (mg/L)$	216.47±124.44	77.76±22.35	398.27±107.85
<i>E. coli</i> (CFU/100mL)	$(2.78\pm2.1)\times10^{5}$	$(2.84 \pm 1.02) \times 10^4$	(3.46±0.45)×10 ⁵
Fecal coliforms (CFU/100mL)	$(1.15\pm1.01\times10^{5}$	$(1.05\pm0.88)\times10^4$	$(2.68 \pm .58) \times 10^4$

 Table 1. Characteristics of Raw greywater, Treated

 greywater and Fresh well water

Values are means \pm standard deviations, n: number of sample

3.2 Nutrients removal efficiencies of hydroponic system

The average removal efficiencies of nutrients of hydroponic systems are shown in Fig. 2. They are 78 % for ammonia (NH4⁺), 87 % for nitrate (NO3⁻) and 56 % for total phosphorus (TP) during 48 hours. According to the study conducted by Cui et al. (2003), a hydroponic system to treat wastewater with Romaine lettuce removed 66.76% of N and 47.62 % of P. Further, Haddad and Mizyed, 2011) have reported that total nitrogen removal was 34% for cut flowers and vegetables planted in hydroponic channels and 21% for trees planted in hydroponic barrels, and also the average removal efficiencies of total phosphorous was 37%. The different nutrient removal efficiencies could be explained by the combination of physical, chemical and biological processes may take place synchronously in hydroponic wastewater treatment systems (Magwaza et al., 2020). These processes include sedimentation, microbial biodegradation, adsorption, nitrification as well as plant uptake (Haddad and Mizyed, 2011). Regarding the phosphorus, their removal have greatly improved by the presence of bacterial in raw greywater. Indeed, the organic phosphorus is converted by the bacterial activity into mineral phosphorus that can be assimilated by the plants. It was also reported by Shelef et al. (2013), that biological removal of phosphorus was achieved by the combination of cell uptake for growth, macrophytes uptake as well as biofilm growth. On the other hand, the removal efficiency of NO3⁻ was higher than those NH4⁺ and TP in this present study. High removal rate of NO3⁻ could be attributed to microorganisms break down inorganic nitrogen mostly through the process of denitrification which converts nitrate to nitrogen gas, resulting so in the removal of nitrate (Gebeyehu et al., 2018). Furthermore, the average pH of raw greywater was 8, which is the optimum pH level, to contribute the nutrient removal through ammonification, nitrification and

denitrification in our treatment system. Moreover, the biological nitrogen removal is most efficient at temperatures between 20-25 °C (Lee et al., 2009). These ranges were similar to those found in our study, which support our findings that temperature to favour nutrients removal. However, the contradicts studies have shown that temperature and pH did not have much effect in the reduction of contaminants (nutrients and faecal coliforms) for treatment of wastewater using hydroponic system (Ndulini et al., 2018). According to these authors, the high removal efficiencies of nutrient were probably due to the increase in hydraulic retention time (Ndulini et al., 2018). Indeed, they have reported that the high removal of nutrient, such are ammonia, nitrate, and total phosphorus reached 87%, 99%, and 87% respectively, with raw wastewater using beggar's tick and green amaranth between 24-240 hours.

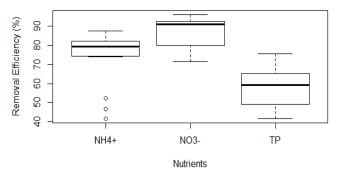


Fig 2. Average removal efficiency of nutrient from raw greywater in a hydroponic system

3.3 Organic pollutants removal efficiencies of hydroponic system

In the present study, the removal efficiencies of the organic pollutants are presented in Fig. 3. Globally, the average removal efficiency of the pollutant organics was higher than 50% during 48 hours (2 days). The removal efficiency were of 67, 53 and 72% for biochemical oxygen demand (BOD₅), chemical oxygen demand (COD), and total suspended solids (TSS), respectively. The results of this study corroborates with the results of Cui et al. (2003), in which a hydroponic system using Romaine lettuce for treating domestic wastewater, and the resulting removal efficiencies were 82.31 % for BOD₅ and 89.42 % for TSS. COD was also reduced, nevertheless, this organic pollutant only presented a percentage of removal around 37.36%. In addition, these authors were also used the water spinach for treating wastewater, and they concluded that hydroponic systems with romaine lettuce showed a good performance in removing organic pollutant compared to the water spinach. Vaillant et al. (2003) achieved depletions of organic matter (BOD₅ = 91%%; COD=82% and TSS =98%), when treating municipal wastewater using hydroponic system for the growth of Daturainnoxia plants. The organic pollutants removal could be attributed by the filtration and adsorption, thus the solids trapped in the root systems are then decomposed and mineralized by bacteria. On the other hand, COD removal were low compared to that of the BOD₅ and TSS, which exhibited very high removal efficiency, likely due to the ability of microorganisms to decompose BOD₅ and TSS. The low COD removal efficiency compared those COD and BOD₅, could be explained by short hydraulic retention time. In that way, the study of Keeratiurai(2013) have shown that the COD removal efficiency was correlated by the hydraulics retention times. The authors reported that the long hydraulics retention times could be increased COD removal efficiency.

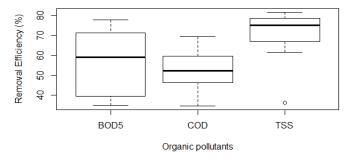


Fig 3. Average removal efficiency of organic pollutants from raw greywater in a hydroponic system.

3.4 Fecal bacteria removal efficiencies of hydroponic system

The average removal efficiencies for the hydroponic system are 81.94 % and 88.68 %, showing 1.00 log units and 1.06 log units for fecal coliforms and E. coli, respectively (Fig. 4). The results obtained by Eregno et al. (2017), showed that the lettuce can successfully be produced with treated greywater without posing a substantial health risk for pathogens, while also providing a 5.1 log units. reduction of E. coli in the final effluent. The Hydroponic Bidenspilosa L and Amaranthushybridus L. production using domestic wastewater has been investigated and found a removal efficiency of 92.77% for faecal coliforms (Ndulini et al., 2018). As constructed wetlands, hydroponic systems are generally considered to feature a combination of biological and physical factors, including mechanical filtration and sedimentation in terms of bacterial removal (Morató et al., 2014). Indeed, plant uptake and absorption to the root could be contributed microbial removal hydroponic cultivation (Ottoson et al, 2005). This could be due the exudation of oxygen through roots increases the concentration of dissolved oxygen in the system, and the elevated level of dissolved oxygen is

reported to have a direct correlation with pathogen reductions (Wu et al., 2016). On the other hand, Moriarty et al. (2019) demonstrated that UV light successfully reduced natural levels of total coliforms by 3 log CFU/mL in aquaponics system. In this way, there is a direct relationship present between the intensity of sunlight that reaches a pathogen and its inactivation rate (Nguyen et al., 2015).

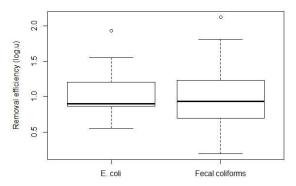


Fig 4. Average removal efficiency of Fecal bacteria from raw greywater in a hydroponic system

3.5 Indicators of fecal bacteria on lettuce leaves

The result shows that no E. coli and fecal coliforms were observed in the lettuce leaves samples collected. This indicates that the contamination of the lettuce leaves from the dishwashing greywater is very limited due to the hydroponic irrigation system. This is in accordance with previous reports that there was no detectable E. coli and fecal coliforms in any of the hydroponic lettuce production using treated effluents from a compact WWTP (Keller et al., 2005), and treated post-hydrothermal liquefaction wastewater (Jesse et al., 2019). Same results were obtained by Eregno et al. (2017), who are no E. coli found in the lettuce biomass with treated greywater using hydroponic system. This is probable due for the fact that hydroponic cultivation allows different forms of technique such as water culture, drip irrigation technique, which are capable at reducing risks compared to field applications (Oyama et al., 2008). Contrary to leaves, the plant roots with the organic matter provide a large surface for these bacteria (Keller et al., 2005). This indicate that great care must be taken at the moment of harvesting to avoid contamination of leaves with the effluent.Globally, the hydroponic system is particularly suitable for agricultural reuse of wastewater because it minimizes the health risks to workers, harvested crop and consumer that occur through contact with wastewater (Qadir et al., 2010).

3.6 Growth and yield of lettuce from hydroponic systems

The growth (number of leaves and height) and yield (dry matter) of lettuce plant are shown in Table 2. The lettuce irrigated with raw greywater (RGW) possessed higher number of leaves (22), dry matter (97.58 g) and taller (15.50 cm) than the ones irrigated with the fresh well water (FWW). Indeed, the number of leaves and dry yield from RGW were significantly higher than those FWW. There was not a significant difference in lettuce plant height between RGW (15.50 cm) and FWW (12.32 cm). The highest lettuce plant yield with raw greywater using hydroponic system could be attributed to the nutrients solution sourced from greywater/wastewater are considered quick release fertilizers because the nutrients are present as dissolved ionic form and thus directly available for plant uptake (Magwaza et al., 2020). Nevertheless, Tomasi et al. (2015) showed that several important factors (i.e. pH, nitrogen forms) are considered when using treated wastewater as nutrient solutions in wastewater hydroponic system. Contrary to our study, wastewater using hydroponic system showed a significant difference reduction in fresh and dry weight weights compared to those treated with water supply and mineral fertilizers, and effluent treated + mineral fertilizers or /and Hoagland nutrient solution (Carvalho et al., 2018). Globally, several studies reported that wastewaters are used in hydroponic systems as nutrient solutions, provides nutrients needed for the development and growth of fruitproducing plant and vegetables that have commercial value in the market (Jin et al., 2020).

 Table 2: Effects of RGW and FWW irrigations on the growth and yield of lettuce

Water irrigation	Number of leaves	Plant height (cm)	Dry matter (g)
RGW	22a	15.50a	97.58a
FWW	13b	12.32a	63.17b

IV. CONCLUSION

The results obtained in this research has shown suitability of lettuce plant (*Lactuca sativa* L.) for removal of nutrients, pollutant organics, and fecal bacteria in the untreated dishwashing greywater hydroponic system, in addition to production of valuable biomass. Indeed, the average removal efficiencies for ammonia (NH₄⁺), nitrate (NO₃⁻), and total phosphorus (TP) were 78 %, 87 % and 56 %, respectively. The removal efficiency of the pollutant organics was higher than 50% for TSS, COD and BOD₅. Further, the average removal efficiencies for the hydroponic system are 88.68 % and 81.94 % for *E. coli*, and fecal coliforms, respectively. The results also indicated that no E. coli and fecal coliforms were observed in the lettuce leaves samples collected. On the other hand, the lettuce irrigated with dishwashing raw greywater possessed higher number of leaves (22), shoot dry matter (97.58 g) and taller (15.50 cm) than the ones irrigated at the fresh well water. The effluent after passing through this hydroponic system met the guidelines for open irrigation/ disposal or further use. The hydroponic system proved to be a method of greywater treatment and offer an useful for plants growth and help to reduce fertilizer needs. The process requires little technical knowledge to operate and can be utilized easily, and it is also very cost-effective for use semi-urban, and rural areas of developing countries.

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