CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Greywater is domestic wastewater that includes wash water from the laundry, bathing, shower and hand wash or kitchen sink. As long as some necessary domestic activities contribute largely to human survival, greywater will always be generated. Improper disposal of greywater could have serious and devastating implications on public health; in other words, direct contact with greywater could be dangerous (MWI, 2007; Nabegu, 2010; Wolfgang *et al*., 2013). Despite the health implications of greywater however, empirical evidences suggest that available greywater treatment techniques are limited, particularly in-low income communities in Nigeria. People in low-income areas lack access to wastewater disposal facilities and consequently allow their greywater to flow on streets or get discharged into open ground and nearby vacant plots (Idris-Nda *et al.,* 2013; Kagu *et al.,* 2013). This is an indication that treatment and control of domestic effluent is inadequate, and it is necessary to explore reuse (recycling) option for a safer disaposal of greywater (Almuktar *et al*., 2018).

Greywater is categorized as low strength, high volume stream of wastewater from households (DHWA, 2002). It carries finite concentrations of microorganisms such as faecal coliforms, *Escherichia coli* and opportunistic pathogens (Eriksson *et al*., 2002; Zuma *et al*., 2009; Kulabakoa *et al*., 2011; Katukiza *et al*., 2014; Manzo *et al*., 2015). Also, easily degradable organic matter is present in greywater which could result in microbial re-growth (Merz *et al*., 2007). Re-growth and biodegradation of organic matters could lead to deterioration in dissolved oxygen concentration, evolution of odours and promotion of mosquito breeding (Finley *et al.,* 2008; Chidozie *et al*., 2016). Nonavailability of wastewater treatment system especially in densely populated areas could result into indiscriminate greywater disposal - often in an open ground - leading to ponding and surface run-off, thereby contaminating nearby streams and groundwater sources (Carden *et al*., 2007; Tandlich and Muller, 2008). Therefore, it is necessary to adopt greywater treatment in order to not only reduce surface and groundwater

contamination but also treat wastewater for re-use and prevent the spread of water-related diseases.

At present, wastewater treatment plants (WWTPs) commonly offer a treatment composed of several stages based on physical, chemical and biological methods (Al-Jayyousi, 2003; Carey and Migliaccio, 2009; Idris-Nda *et al*., 2013; Manzo *et a*l., 2015). However, the treatment systems frequently eliminate a fragment of the phosphorous and total nitrogen available in the effluents (Rawat *et al.,* 2011). Nevertheless, new treatment systems have been developed to improve wastewater quality with the use of algal biomass (Rawat *et al.,* 2011; Caporgno et al., 2015; Yang *et al*., 2015). Algae also play a significant role in meeting the demand for energy and serving as the primary feedstock for sustainable by-products: minerals, skin creams, medicines, chemicals, laxative, vaccines, foods, salad dressings, ice cream, puddings, animal feed, pigments and fertilizers (Edwards, 2008; Abinandan *et al*., 2013). Algae could also serve as feedstock in the production of biogas (Yen and Brune, 2007; Mehrabadi *et al*., 2016; Passos *et al*., 2016). Furthermore, algae production could utilize nutrients in municipal and industrial effluents thereby serving as treatment system (Kumar *et al.,* 2011).

Furthermore, microalgae biomass have been converted into tablet and/or powder form and marketed as food additives (Görs *et al*., 2010). Algae biomass are used in aquaculture mainly as feed for fish (Brown *et al*., 1997; Daniel *et al*., 2016) and inducement of essential biological activities in bred aquatic species (Muller-Feuga, 2000); stabilization and improvement of quality of culture medium ('green-water' technique) (Chuntapa *et al.,* 2003); and enhancement of the immune systems of fishes (Pulz and Gross, 2004). Nevertheless, wastewater must be adequately treated to reduce the nutrient load in the fresh water bodies, prevent breeding of pathogenic organisms and spread of water and sanitation-related diseases. However, majority of the households in Nigeria pay little or no attention to greywater treatment. Also, there is paucity of information on greywater treatment using algae-based technology and community-based related study has not been adequately documented. This study therefore evaluated the effectiveness of *Chlorella* sp. combined with Horizontal

Roughing Filter (CHRF) and *Scenedesmus* sp combined with Horizontal Roughing Filter (SHRF) in greywater treatment and production of useful biomass.

1.2 Statement of the Problem

In most developing countries, greywater does not receive adequate treatment before it is discharged into drains, streams, and wetlands, thus leading to pollution of the receiving water bodies (Awuah *et al*, 2002). Furthermore, greywater contains impurities and microorganisms that are capable of causing disease and illness if not adequately treated. Consequently, people are affected by sanitation-related infections which could result in high mortality and morbidity. The sustainable development goal 6 seeks to ensure availability and sustainable management of water and sanitation for all. However, target 6.3 of this goal is to improve water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse globally by 2030 (UN General Assembly, 2015). Regrettably, majority of the households from low- to middle-income communities in Nigeria pay little or no attention to greywater treatment. Most technologies, if they exist, could remove only a fraction of the supposedly major pollutant (total nitrogen and phosphorous) present in the effluent. Meanwhile, these pollutants are the main nutrient required by plants (including algae) to grow.

Without doubt, bioremediation with the use of algae has been considered as a viable technique for wastewater nutrient removal, and the resulting effluent can be reused to meet the demand for water and also to solve the problem of eutrophication (Abinandan *et al.,* 2013). Nutrients removal from wastewater improves quality of available water sources through reduction in eutrophication in the receiving watebodies (Foley *et al*., 2010). Greywater contains an easily biodegradable organic content and a relatively low pathogens content (Fittschen and Niemczynowicz, 1997) making it much easier to treat and safer to recycle for water uses that do not need potable water quality, such as toilet flushing, urban landscaping or road washing. In developing countries, reuse of greywater for irrigation, without any significant pre-treatment, is becoming

increasingly common, a practice mistakenly considered safe (CSBE, 2003). However, this form of application can damage soil health (Friedler and Hadari, 2006).

Low-cost technologies that have been used for greywater recycling ranged from simple 2-stage processes (coarse filtration and disinfection) to physical, chemical and biological processes (Jefferson *et al.,* 2004). For these technologies to be maximized however, more studies have to be carried out on the potential of producing single protein algae biomass from greywater treatment technology. This will be an advantage for human beings as the technology would not only reduce surface water pollution and sanitation-related diseases but also enhance production of single protein algae that can be used for other purposes. Empirical evidences on greywater treatment using algae-based technology at community level are scarce in the literature in Nigeria despite its potential to reduce water-related infections; contribute to clean environment and reduce water pollution. This is the literature gap this present study set out to fill.

1.3 Justification of the Study

Water scarcity and water pollution are some of the crucial issues globally. One of the ways to reduce the impact of water scarcity and pollution is to expand water and wastewater reuse (recycling). Greywater treatment and recycling of useful products (water, nutrients and organic matter) minimize both water shortages and environmental pollution. Treating greywater reduces the input of nutrients in nearby water bodies and prevents eutrophication. Meanwhile, some algae have been isolated to treat wastewater and their by-products used for production of animal feedstocks under laboratory experiments (He *et al*., 2002; Azaza *et a*l., 2008). There are several positive impacts of greywater management on public health and living condition, thus greywater could be considered as a resource of immense benefits.

Therefore, this study was designed with the hope that the outcome would be significant for three reasons:

i) It would preserve the fresh water primarily for drinking.
ii) Collecting and treating wastewater would protect existing sources of valuable fresh water, the environment in general, and public health. In fact, wastewater treatment and reuse (WWTR), not only protects valuable fresh water resources, can also supplement them, through aquifer recharge. If the enormous benefits

of environmental and public health protection are properly factored into economic analyses; wastewater collection, treatment and reuse will be one of the highest priorities for the public and development funds.

iii) Wastewater treatment using this approach would be able to produce algae biomass useful in the production of some by-products like animal feed (Brown *et al.,* 1997) and biofuel, valuable for biofuel industry (Nascimento *et al.,* 2012). It has already been used to improve quality of wastewater from different sources (Kumar *et al.,* 2011; Ruiz *et a*l., 2011). Therefore, this approach to greywater treatment has a big potential to bring about environmental, economic, and financial benefits.

1.4 Research Questions

The following questions were intended to be answered by this study:

1. What is the quantity of water consumed in Kube-Atenda community?

2. How much greywater is generated from all the sources in Kube-Atenda community?

3. What is the quality of greywater produced in Kube-Atenda community?

4. What is the optimal concentration of algae (*Chlorella* sp. and *Scenedesmus* sp.) inoculum during the laboratory experiment?

5. How much nutrients (Nitrogen, Phosphorous) can be removed from the greywater with the use of prototype out-door treatment unit?

6. What quantity of BOD can the prototype out-door treatment unit remove from the greywater?

7. What are the biochemical characteristics of the algae biomass resource produced?

8. What is the effect of the prototype out-door treatment unit on the greywater quality?

1.5 Objectives of the Study

1.5.1 Broad objective

The broad objective of the study was to evaluate effectiveness of *Chlorella* sp. combined with Horizontal Roughing Filter (CHRF) and *Scenedesmus* sp. combined with Horizontal Roughing Filter (SHRF) in greywater treatment and production of useful biomass.

1.5.2 Specific objectives

The specific objectives of this study were to:

1. Estimate the quantity of water consumed and the greywater generated in the selected households;
2. Characterize the greywater generated within the selected households;
3. Determine the optimal concentration of algae (*Chlorella* sp. and *Scenedesmus* sp.) inoculum in greywater used as a medium;
4. Assess the biochemical characteristics of the algae biomass resource produced for possible reuse;
5. Assess the effectiveness of a prototype out-door treatment unit on the greywater quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 Algae

'Algae' is one of the important concepts of this study, referred to as 'microalgae' in most of the literature. Algae are microspcopic organisms that have chlorophyll and are photosynthetic in nature. Priyadarshani *et al.* (2011) defines algae or microalgae as photosynthetic organisms usually present in marine as well as freshwater environments, whose photosynthetic mechanism resembles that of land-based plants. Algae (microalgae) cellular structure are simple and this increases their efficiency in the process of converting solar energy into biomass. The structure also allows algae to submerge in an aqueous environment and improve their efficiency in accessing carbondioxide, water and other nutrients. In another study, Rinanti *et al.* (2013) define algae as photosynthetic microorganisms with simple growing requirements (light, sugars, carbondioxide, nitrogen, phosphors, and kalium) that can produce lipids, proteins and carbohydrates in large amounts over short periods of time. All these definitions capture the fundamentals that describe a typical alga or microalga. However, the definition given by Rinanti *et al.* (2013) covers the major environmental condition required for algae growth as well as all the major components of the biomass produced by algae. Essentially, useful information about the basic requirements for algae cultivation and the expected valuable resources from the biomass is provided by this definition.

2.2 Greywater

The terms *greywater* and domestic *greywater* are sometimes used interchangeably in environmental literature. Greywater refers to wastewater that has emanated from household activities such as laundry, washing, and bathing, and which excludes any input from toilets. In essence, it is wastewater generated from the household, excluding toilet waste (Al-Jayyousi, 2003; Ahmed, 2007; Leonard *et al.,* 2016; Fowdar *et al*., 2017). Also, some authors (Al-Jayyousi, 2003; Kariuki *et al.,* 2011) define greywater

as wastewater discharging from laundry, showers, bathtubs and kitchen sinks; and it is about 50-80% of all residential wastewater. Greywater does not include the wastewater produced from toilet use, which is considered black water. Al-Mashaqbeh *et al.* (2012) concur with the above definition by defining greywater as wastewater from baths, showers, hand basins, washing machines and dishwashers, laundries, kitchen sinks and ablutions excluding wastewater from the toilet. While all the cited authors have provided similar definitions of greywater, Birks and Hills' (2007) is more precise in terms of classification based on its qualities. as According to them, greywater is a "combination of wastewater from bathroom sinks, baths and showers ('light grey') and more contaminated waste from laundry facilities, dishwashers and, in some instances, kitchen sinks ('dark grey')". This clearly characterises the types and quality of the greywater which might be generated at the household level. This however also describes the quantity of greywater at household level. This classification is as a result of high organic load and nutrient which might be present in the kitchen wastewater compared to other source at the household level.

2.3 Phytoremediation

Phytoremediation and bioremediation are interchangeably used terms to describe treatment of wastewater using plants including algae. It is a process of introducing algae into stream of wastewater in a confined container (bioreactor) over a period of time and at a specified environmental condition to remove excess nutrient load from wastewater and subsequently diminish the pollution load (Sharma and Khan, 2013). In one study, Dwivedi (2012) defines bioremediation as the process of using specific microorganisms to transform hazardous contaminants in soil/water to non-hazardous waste products. While most definitions of phytoremediation agree about its cleansing effect, only few of these definitions pinpoint the role of phytoremediation in the creation of useable feeds.

In another perspective, phytoremediation is defined as nutrient removal from municipal wastewater and effluents rich in organic matter and xenobiotic compounds, with the aid of alga-based biosorbents, carbondioxide sequestration and identification of toxic compounds using of alga-based biosensors (Ahmad *et al.,* 2013). This evidently reveals that phytoremediation could also reduce other toxic substances that might be

available in the effluent. Furthermore, algae have been shown to bio-transform the pollutants into their cells and produce valuable resources from the process Gani *et al.* (2016).

2.4 Concept Model

The process involving the use of algae for greywater treatment and conversion of its biomass into valuable byproducts is depicted in Figure 2.1. Households in high density and low-income community of cities in Nigeria discharge their greywater directly into the environment, often without treatment. Regrettably, little or no attention is paid to the greywater treatment and this has led to pollution problems of the water bodies and increasing public health hazards.

The organic and inorganic nutrient components of the greywater are powerful stimulants to algae growth, though they are the major pollutants and cause of eutrophication of the water body. These substances affect the water quality if allowed to run untreated into the water body. The nutrients are used up when the algae are introduced into greywater and consequently form biomass which is useful for production of valuable resources like animal feed, biodiesels, fertilizers etc (Rawat *et al.,* 2011; Abinandan *et al.,* 2013). Algae have been widely used for wastewater treatment because of their fast growth, potentials in absorbing the organic and inorganic substances in the wastewater that could cause pollution, and as well produce valuable by-products. The study proposed that algae is a photosynthetic microorganism with simple growing requirements (light, sugars, carbondioxide, nitrogen, phosphors, and kalium) that can produce lipids, proteins and carbohydrates in large amounts over short periods of time. Though algae is a photosynthetic organism, unlike other plants, it has no root, stem, leaf and vascular bundles but could be used in greywater treatment and produces valuable biomass (Ahmad *et al.,* 2013; Wang *et al.,* 2013). Also, conversion of algae biomass into valuable products like animal feedstock, biodiesel etc. has been documented by some researchers (Abou-Shanab *et al.,* 2013; Singh *et al.,* 2013; Houser *et al.,* 2014). While several advantages of algae in greywater treatment have been identified, the specific mechanisms by which the algae use up the nutrient (both organic and inorganic) are not known.

People have aversion for waste materials, particularly wastewater, probably because they are not aware of the benefits derivable from algae and potentials in greywater recycling and reuse. Many studies have suggested that wastewater should be used for an application that does not involve close personal contact such as irrigation, fire-fighting, and car washing (Robinson *et al.,* 2005; Alhumoud and Madzikanda, 2010; Ilemobade *et al.,* 2013). While algae-based treatment can be beneficial, beneficiaries also need to have knowledge which would assist in understanding the benefit derivable in the treatment of greywater with algae as pointed out in Figure 2.1.

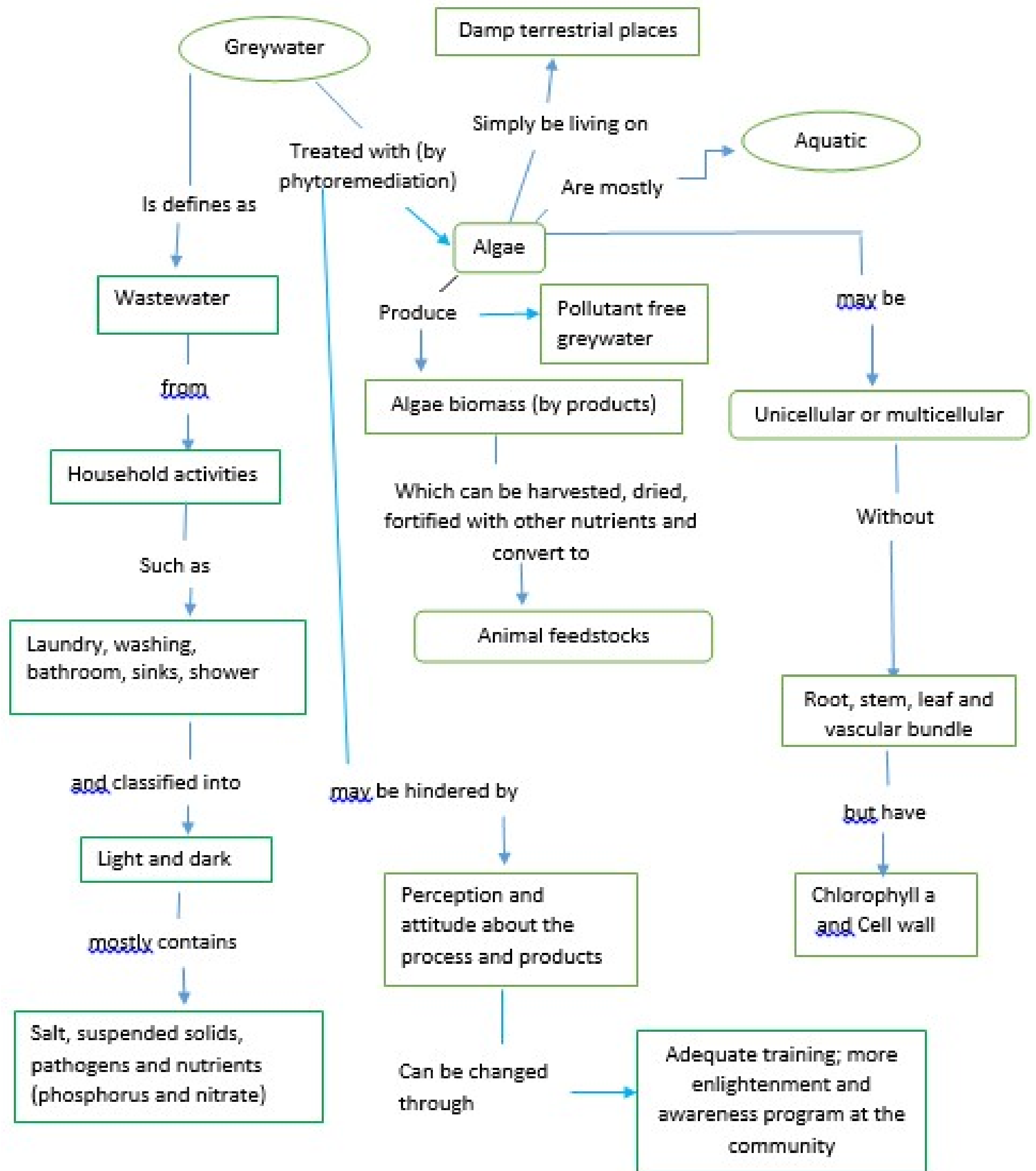


Figure 2.1: The Concept Diagram

Soruce: Author's data

2.5 Greywater Categories

Greywater could be categorised based on the levels of contamination; it is broadly categorized into light and dark greywater. Light greywater are the wastewater from the bathroom which include showers and tubs (Friedler and Hadari, 2006). However, the dark greywater is an exceedingly contaminated sources of wastewater from kitchen sinks laundry facilities and dishwashers (Birks and Hills, 2007). Figure 2.2 presents sources of some greywater and their constituents.

2.6 Quantity of Greywater

Water utilization at the household level ususally depends on several factors which include the population, socioeconomic status, and availability and functionality of the water sources. For instance, Ghaitidak and Yadav (2013) reported that the amount of greywater production depend on the overall household water consumption, standards of living, population composition (in terms of age and gender), household occupants' traditions, and water appliances available in a setting. Therefore, there is variations in the quantity (between 50% and 80%) of greywater generated by households (Flowers, 2004). Also, amount of greywater generation from an urban community could differ from that produced in a rural area. Studies have revealed that kitchen sink and dishwasher produce around 27% of greywater, wash basin, bathroom, and shower gererates 47% of greywater while laundry and the washing machine produce about 26% greywater (Jamrah *et al*., 2006; Ghaitidak and Yadav, 2013).

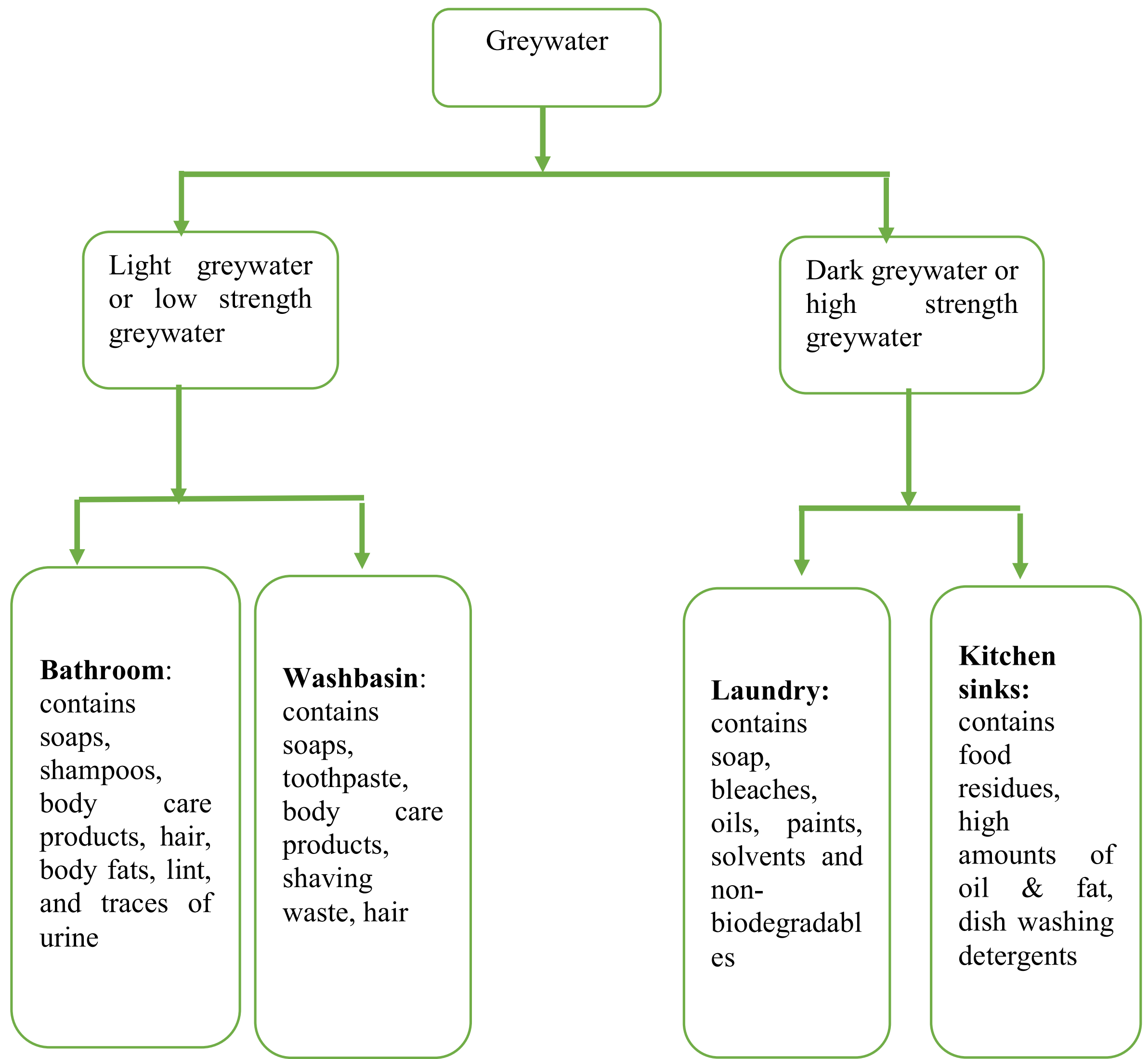


Figure 2.2: Sources and constituents of Greywater
Source: Ghaitidak and Yadav, 2013

2.7 Characteristics of Greywater

This section provides information on the characteristics of greywater. Characteristics of greywater are an essential variable to describe the components of greywater. There are significant variations in the composition of greywater depending on the sources and the location. Also, wastewater quality guidelines recommended by World Health Organization (WHO) and National Environmental Standards Regulation and Enforcement Agency (NESREA) are presented in Table 2.1.

2.7.1 Physical characteristics

Physical parameters such as turbidity, colour and suspended solids are essential characteristics to describe the components of greywater. There are significant variations in the composition of these characteristics in greywater depending on the sources and the location. Differences in water consumption in connection with the amount of substances discharged could also lead to variations in the composition of greywater. Lending credence to these claims, Abedin and Rakib (2013) reported in their study that the values of the colour and turbidity of generated greywater from floor wash, laundry, and kitchen wastewater, bath and wash hand basin were higher than the standard permissible values for wastewater quality. Another study carried out in Debrecen (Hungary) observed that the turbidity value of greywater from kitchen and laundry was similar but exceeded the value obtained for wastewater sample from bath (Bodnar *et al.,* 2014). Greywater turbidity is often higher than the recommended limit by regulatory bodies, but there are variations in the composition of these parameters within the greywater stream. However, the highest turbidity level is typically found in kitchen and laundry greywater. Kulabakoa *et al.* (2011) found in their study that most of all the sources of greywater exhibited high turbidity while the kitchen wastewater samples had the highest in Kawaala, a peri-urban settlement, in Kampala city (Uganda).

Table 2.1: Wastewater quality guidelines

Parameters (Units)	Permissible limits	
	WHO	**NESREA**
Physicochemical		
pH	6.5-9.5	6.0-9.0
Temperature (^{0}C)	12-25	40.0
Turbidity (NTU)	5.0	5.0
Conductivity(µS/m)	400	NS
Total Dissolved Solids (mg/L)	50	500
Total Suspended Solids (mg/L)	NS	25
Oil and grease (mg/L)		10
Organics (mg/L)		
Chemical Oxygen Demand (COD)	80	60
Biochemical Oxygen Demand (BOD_5)	40	30-50
Nutrients (mg/L)		
Nitrate (NO_3-N)	NS	10
Phosphate as P	NS	10
Sulphate	NS	750
Chemicals (mg/L)		
Iron	NS	2
Lead	NS	0.05
Manganese	NS	0.2
Cadmium	NS	0.01
Bacteriological (CFU/100ml)		
Total Coliforms	$<10^3$	$<10^3$
E. coli	$<10^3$	$<10^3$

Source: WHO (2006) and NESREA (2009)

Note: NS=Not stated

2.7.2 Chemical characteristics

Quality of chemical parameters is important in determining the best treatment option suitable for the management of greywater. The major quality parameters are biochemical and chemical oxygen demand (BOD and COD), nutrient content (nitrogen, phosphorous), heavy metals, disinfectants, bleach, surfactants and organic pollutants in detergents (Oron *et al.,* 2014). The BOD and COD document the level of organic pollution in water. These parameters are usually dependent on the quantity of water and some of the products used (e.g detergents, soaps, oils and fats) within the household, and high quantity of organic solids mainly made up of leftover foods (Sally and Takahashi, 2006).

2.7.3 Nutrients in Greywater

All wastewater types contain nutrients, but the nutrients level in the greywater is usually low compared to wastewater from other streams like toilet wastewater. However, nitrogen and phosphorus have beneficial effects as nutrients which are important requirements for plant growth (Akponikpe *et al.,* 2011). The main source of nitrogen in greywater is kitchen wastewater (mainly from food residues) while bathroom and laundry greywater has been observed to have low concentration of nitrogen (do Couto *et al.,* 2013). The main sources of phosphorus in greywater are cleaning products which could be in form of dishwashing and laundry detergents. The average phosphorus level typically ranges from 4–14 mg/l in regions where non-phosphorous detergents are used (Eriksson *et al.,* 2002). However, the concentration can be as high as 45–280 mg/l in areas where households utilise phosphorous detergents (Friedler, 2004).

The Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) are also important parameters that determine the level of biodegradability and non-biodegradability of organic contaminants present in the effluent. This is also applicable to greywater. While BOD determines the biochemical oxidation through bacteria within a certain period of time (usually 5 days), COD determines the amount of oxygen needed to oxidise the organic matter present in wastewater. However, variability in the characteristics in greywater depends on quantity of water and some other factors. Jefferson *et al.* (2004) in a study conducted in Bedfordshire (United Kingdom) attributed changes in BOD of some samples of greywater to variability in lifestyles,

customs, installations, product preferences and washing habits of the population. Greywater sources such as shower, bath and hand basin sources have similar BOD contents but greater changes in COD concentrations (Jefferson *et al.,* 2004). Studies have reported a much higher COD concentration compared to BOD in samples of greywater collected from laundry compared to those from other sources such as bathing (Eriksson *et al.,* 2002; Katukiza *et al.,* 2014). However, Al-Jayyousi (2003) attributed high BOD in kitchen greywater to significant organic food remnants (such as rice, tomatoes and cooking fat) which might be present in the wastewater from low-income household in Tufileh (Jordan).

2.7.4 Heavy metals in greywater

Greywater could also contain heavy metals which might necessarily be as a result of certain groups of compounds that emanate from the materials and chemical products used in households. These materials include detergents, soaps, bleaches and perfumes. Greywater from different sources may have variation in heavy metal concentration. Jefferson *et al.* (2004) reported in their study that heavy metals such as lead, Manganese, Nickel, Copper, Iron and Chromium were deficient in greywater while Cobalt and Molybdenum were not present. Greywater from kitchen has lower load of these metals and is therefore more desirable in terms of treatment and reuse.

2.7.5 Microbiological characteristics

Pathogens present in greywater possibly expose humans to risks of infections either directly or indirectly. These risks could be influenced by some factors which include pathogen types and loads, potential for human exposure to the effluent, treatment quality and possible reuse of the greywater (Myers *et al.,* 1999). Oftentimes, effluents may contain greater number of microorganisms such as protozoa, viruses, intestinal parasites and bacteria. Most of these microorganisms emanate from faecal matter of infected individuals and sometimes find their way into the greywater through handwashing after visiting toilet and washing of babies' diaper. Al-Jayyousi (2003), in a study, observed that some households did not only bathe their babies in the sink but also wash diapers in the laundry. These practices could be a major source of faecal contamination to the greywater and are detrimental to those who might have contact with the greywater. There are other sources which can serve as entry points for some

pathogens into the greywater system especially in slum settelement (Katukiza *et al.,* 2014). This evidently reveals that other sources such as washing of vegetables and raw meat could also contribute to greywater contamination with high numbers of pathogens.

2.7.6 Organisms possibly present in greywater

Greywater has the potential to contain high level of disease-causing microorganisms as already concluded in some empirical studies. For instance, Katukiza *et al.* (2014) observed in a study that greywater contained nutrients which are necessary for plant growth and a high load of disease-causing microorganisms. These could pose serious health risk and render greywater not suitable for direct non-potable reuse. High numbers of total coliforms, *E.coli*, Salmonella sp. and faecal enterococci have been reported to be present in greywater from kitchen, washings, hand washings and shower (Katukiza *et al.,* 2014). However, Birks and Hills (2007) observed that the availability of high number of these pathogens indicates faecal contamination of the greywater.

Greywater from laundry was reported to contain high coliform content compared to other stream of greywater such as shower and hand-washing facility (Bodnar *et al.,* 2014). Pathogenic microorganisms such as faecal enterococci, Salmonella sp. and *E. coli* must not be available in greywater from a kitchen source. This apparently reveals that the coliform load in the greywater from kitchen is not from faecal origin. However, the main indicator organism commonly used for water and wastewater quality assessment was *Escherichia coli (E. coli)*. It is an indication that the greywater is highly polluted and contains pathogenic organisms of faecal origin. Kulabakoa *et al.* (2011) reported a high *E. coli* values in greywater from kitchen, bathroom and laundry, and attributed this to the presence of faecal contamination. High contamination of greywater with faecal matters could be present in households where there are more babies and young children than the elderly. However, the presence of *E. coli* in kitchen greywater could be a result of contaminated uncooked food and raw meat (Eriksson *et al.,* 2002).

2.8 Greywater Treatment Technologies

Treatment of greywater is a prerequisite to the reduction of health risks associated with its reuse. It is essential to ensure that the greywater quality satisfy the recommended limits before reuse in order to minimise the health risks related with the reuse of untreated effluent (Ghunmi *et al.*, 2011). Furthermore, greywater management would ensure certain challenges are overcome. For example, Ghunmi *et al*. (2011) reported the challenges of greywater management to include the problem causd by microorganism, organic matter, solids as well as aesthetic and health problems, and to meet standard for reuse. Also, there is inadequate empirical data on the cost-effective greywater and reuse methods suitable for a single household or small communities (Jabornig and Podmirseg, 2015). However, treatment principle was used to categorise technologies for the management of greywater. These comprise the physical, chemical, and biological technology, or a combined system involving either two or more (Boyjoo *et al.*, 2013).

2.8.1 Physical treatment systems

Filtration and sedimentation are the available physical greywater treatment options. Filtration is usually carried out before chemical or biological treatment (pre-treatment method) or before disinfection as a post-treatment method. Pre-treatment filtration method includes screen meshes, sand bed filtration, nylon sock type filtration, metal strainers, gravel filtration, and mulch tower system (Boyjoo *et al.*, 2013). However, it is insuffient to use only physical treatment as the main method of greywater treatment. Only physical treatment method does not ensure adequate reduction of pollutants in form of organics, phosphate and nitrates etc, except in greywater stream with extremely low organic strength (Ghunmi *et al.*, 2011; Ghaitidak and Yadav, 2013). Some factors such as greywater particle size distribution, pollutants and porosity filters'are responsible for the efficiency of the filtration techniques. For instance, Boyjoo *et al.*, (2013) observed that coarse filters could not efficiently remove the available pollutants in the household effluents. Furthermore, another study on the effectiveness of a sand bed filter in the treatment of bathroom greywater reported 30% Chemical Oxygen Demand removal and *E. coli* reduction of 2Log CFU/100 mL (Chaillou *et al.,* 2011).

Other physical treatment technologies such as membrane filtration (i.e. metal-made membranes), microfiltration (MF), ultrafiltration (UF) and nanofiltration (NF) generate high quality effluent which has similar proportion to the molecular weight cut-off (MWCO) of the membrane (Shin *et al.*, 1998; Ramona *et al.*, 2004; Kim *et al.,* 2007). UF membranes with 30-200 kDa pores sizes have been identified to filter between turbity and organic matter in the range of 92-97% and 45-70% respectively. Ramona *et al.* (2004) treated greywater from shower with NF and obtained better quality of the effluent and reduction of pollutant such as ionic species, organic matters and microorganism. This resulted to a better effluent quality which is suitable for all-purpose unrestricted reuse. Nevertheless, filters' efficiency could be restricted with a number of operational challenges including frequency of cleaning. However, the use of stabilization pond as a pre-treatment of raw greywater could partly reduce clogging of sand filters bed. However, Ghaitidak and Yadav, (2013) reported that post-treatment of greywater could be achieved with the use of membrane filtration (i.e., micro-, ultra-, and nano-filters) in order to attain the most stable quality.

2.8.2 Chemical treatment technologies

Several chemical treatment technologies are employed in greywater treatment. These treatment techniques are efficient to improve the quality of light greywater as well as laundry greywater. The chemical systems are also suitable to reduce pollutants in greywater in form of organic and turbidity to some level but not adequate enough for the treatment of high strength greywater (Boyjoo *et al.*, 2013). Another study utilized coagulation/flocculation treatment system for shower greywater and achieved BOD, COD, total N, TC and *E. coli* removal of 85 to 89%, 64 %, 13%, >99 %, and >99 % respectively (Pidou *et al*., 2008). Also, the process yielded better results in acidic pH that needs pH adjustment after treatment. However, Ghaitidak and Yadav, (2013) observed that pH adjustment pre- and post-treatment could contribute to the increase in the both the operational and mainteneance cost. In another study, the use of aluminium sulphate in a flocculation system (Kariuki *et al.*, 2011) was reported to have no effect on pH, electrical conductivity and salinity in greywater from both kitchen and laundry. Flocculated greywater met reuse standard for only the pH, but not other parameters.

Furthermore, Lin *et al.* (2005) has reported the effectiveness of electrocoagulation in the treatment of greywater from bathroom in a building in Taiwan. This system produces the required coagulant from aluminium ion (Al^{+3}) emanating from aluminium anodes. The Cathodes produces Hydrogen which create bubbles and allowed the particles to float, which were separated by skimming into a separate container. All the *E. coli* in the greywater could be removed through disinfection with sodium hypochlorate. Quality of the treated effluent obtained met the standard for non-potable reuse (Lin *et al.*, 2005). Moreover, the use of titanium dioxide (TiO_2) as a catalysts (photocatalysis) in post-treatment of greywater was found to be efficient particularly in biological treatment process (Gulyas *et al.*, 2007). Furthremore, Li *et al.* (2004) reported that photocatalysis can highly reduce pathogenic organisms in greywater, thus the disinfection process is not necessary. Previous study had utilized photocatalysis treatment with TiO_2 to successfully obtain 65% reduction in dissolved organic carbon concentration of light greywater from a hotel building (Sanchez *et al.,* 2010). However, the disinfection process is expensive and further treatment to remove the TiO_2 is essential (Ghunmi *et al.*, 2011). Treated greywater from the sand filtration process requires disinfection to satisfy the entire reuse quality (Ghaitidak and Yadav, 2013).

2.8.3 Biological treatment systems

There are various biological methods that have been used for the treatment of greywater. The treatment systems include Sequencing Batch Reactor (SBR), Rotating Biological Contactor (RBC), Membrane Bioreactors (MBR), Fluidized Bed Reactor (FBR) and Upflow Anaerobic Sludge Blanket (UASB). However, biological systems are either preceded or succeeded by one or more treatment techniques. For example, biological treatment method has been reported to be preceded by a coarse filtration followed by sludge removal through sedimentation and/or filtration, and succeded by disinfection through chlorination or UV for pathogens' removal (Boyjoo *et al.*, 2013). Excellent organic and turbidity removal rates could be achieved through aerobic biological processes. Greywater treatment, with aerobic biological technique, removed high loads of biodegradable substances and also eliminates re-growth of pathogens and odour challenges. Hence, the treated effluent would be more stable and stay longer period on storage. Thus, biological treatment techniques are suggested to be efficient for the treatment of medium to high strength greywater (Li *et al.*, 2009).

The MBR is the combination of biodegradation and membrane filtration and are efficient for solid liquid separation. The reported efficient removal rates of MBR were 98-99.9% for turbidity, around 100% for TSS, 93-97% for BOD, 86-99% for COD, 52-63% for total N, 10-40% for PO_4–P, 19% for total P, and 99.9% for FC (Ghaitidak and Yadav, 2013). The MBR-treated effluent has the qualities that satisfy several quality standards for reuse (Bani-Melhem *et al.*, 2015). A previous study, Lazarova *et al.* (2003) observed that MBR produce high quality and stable effluent with low sludge, and could be an atttractive greywater treatment option for a single residential building, especially in urban settings. However, the costs of MBR have been observed to be high and makes the technology less affordable for potential users in low income countries (Merz et al., 2007). The investment and operating costs reduction for a suitable payback period continues to be a problem for the application of MBR in single-household (Jabornig and Podmirseg, 2015).

Moreover, Nolde, (2000) observed the effectiveness of RBC and FBR in the treatment of light greywater. Application of RBC and FBR in the treatment of greywater from bath, washbasin and/or shower could achieve <5 mgL^{-1} BOD reduction from the initial concentrations of 50-250 (bath greywater) and 70-300 mgL^{-1} (washbasin and/or shower greywater) (Nolde, 2000; Friedler *et al.* 2006). However, low maintenance mechanism is essential to RBC in good shape, particularly for the increased number of phases while volume is not changed (Nolde 2000). However, RBC has been found to be more efficient for BOD removal compared to COD (Friedler *et al.*, 2006; Wu, 2019), and also efficient in the removal of micropollutants (Eriksson and Donner, 2009). Another biological technology is SBR, which represents a special form of activated sludge processing that uses reactor tank for all the treatment processes. SBR employs a time-controlled sequence in a single tank to carry out equalization, biological treatment as well as secondary clarification. Effluent from SBR-treated greywater satisfies wastewater reuse standard for NH_4–N, BOD, and COD, and removal of BOD varies from 80 to 98% while similar ranges was observed for COD (Lamine *et al.*, 2007).

Furthermore, Elmitwalli and Otterpohl, (2007) documented the UASB removal efficiency of 21.7-29.8% and 15.2-20.6% for TN and TP respectively. Nevertheless,

high quality treated effluent can be obtained from the use of anaerobic treatment combined with aerobic technology (Ghunmi *et al.*, 2011). While anaerobic treatment is simple and cost-effective (Halalsheh *et al.*, 2008), aerobic treatment produces higher quality treated effluent compared to anaerobic technology for the removal of pollutants in greywater (Leal *et al.*, 2011).

2.8.4 Natural greywater treatment systems

Natural greywater treatment systems involve the use natural media (e.g., soil and plants) in an extended unit for filtration and biological degradation. The technology could be efficient in the treatment of high strenght greywater but a disinfection is essential to produce effluent with minimal microorganism (Boyjoo *et al.*, 2013). The natural treatment systems include sand filter, horizontal-flow constructed wetland (HFCW), vertical-flow constructed wetland (VFCW), anaerobic filters and vertical-flow filter (VFF). These systems utilize the existing microorganisms in the set-up (e.g. earth-worms, plant roots, biofilm, slugs) in combination with physical methods (e.g. filtration) and biological technology. In addition, it is possible to combine the use of adsorption and chemical methods in the precipitation of greywater (Kivaisi, 2001).

The planted systems (i.e., VFCW, HFCW) usually remove nutrients such as phosphorus and nitrogen in wastewater through nutrient uptake. However, the constructed wetland has been found to be the eco-frienrdly and economical method for the treatment of greywater to satisfy reuse standard (Ghaitidak and Yadav, 2013). Constructed wetlands were observed to be a suitable greywater treatment option, due to their low cost, particularly in developing countries (Boyjoo *et al.*, 2013). Nevertheless, the technology need large surface area (about 0.5-3 m^2 per person) for construction (Paulo *et al.* 2009). Constructed wetland treatment technology was observed to achieved removal rates of 90-98%; >99 %; 81 to 82%; 26 to 82%; 0 to 63%; and 67% for TSS, BOD, COD, total N, B, and K. (Ghaitidak and Yadav, 2013). Manjate *et al.* (2015) reported good performance of the planted and unplanted VFCW systems while their simplicity makes them an attractive treatment system. Furthermore, Ghaitidak and Yadav, (2013) observed that it would take RVFCW technology about three years to yield good result, and unskilled operators can operate the system. Constructed wetlands could satisfy the reuse quality standard for BOD, TSS and pH, but post-treatment of

effluent is required for the removal of EC, As, Helminth eggs and *E. coli* to make greywater satisfy several standards for reuse (Gross *et al.*, 2007).

Moreover, small-scale wetland system has been observed to be efficient for the removal of contaminants and suitable for the treatment of different sources of greywater (Wurochekke *et al.*, 2015). Furthermore, Wallace and Knight (2006) reported that the operations and maintenance of SSWLs differentiate the system from large-scale constructed wetland systems. However, SSWL-treated effluents are held to similar quality as large-scale treated effluents. Thus, the effluent quality produced by SSWL system must be consistent despite variation in loading and flow rate and be constructed from local materials, particularly in low-income countries (Wallace and Knight, 2006). However, sustainable application of SSWLs requires a further exploration of designs which should consider application of hydrophyte, disinfection technology, and appraisal of the local climatic condition (e.g rainfall and temperature) and composition of greywater essential to improve effluent quality (Wurochekke *et al.*, 2014).

2.9 Reuse of Treated Greywater

The treated greywater can be reused for non-potable purposes such as agricultural irrigation, garden and landscape plant irrigation, toilet flushing, floor and car washing (Parjane and Sane, 2011; Karnapa, 2016). Also, it can be used for ground recharging (Karnapa, 2016) and mostly for cooling tower in industry (Asano et al., 2007). Non-potable application of greywater could reduce consumption of potable water and invariably preserving the existing water resources. For instance, about 10 – 20% in the demand for fresh water could be achieved by the reuse of greywater to flush toilet (Friedler, 2004). Agricultural irrigation is mostly favoured in tropical countries (Edwin *et al.*, 2014). There are concerns about the quality of the greywater particlarly for the use of irrigation porposes. For instance, Sridhar and Adejumo (2017), in a study, observed that the pH, lead, boron and chromium levels of some of the irrigation water samples were of concern which required immediate attention and appropriate treatment solution.

There are risk factors which may affect the quality of irrigation water and health of the users. The common risk factors are open defecation, discharge of municipal wastes and the leachates from solid wastes, washing of livestock, and other traditional activities including navigation. These activities enrich the waters with nutrients and also promote aquatic macrophyte growths and water-borne infections among the people living in the area (Sridhar and Adejumo, 2017). However, more research is required to understand the trends in the degradation of micro-pollutants in soil (Ternes and Joss, 2006; Hernandez Leal, 2010).

Greywater reuse must be environment friendly without causing any public health hazard. This is a source of serious concern as a result of nonexistence of regulations and laws that guide the treatment and reuse of graywater in several developing countries (Allen *et al*., 2010). Though the World Health Organization (WHO) published greywater reuse guidelines in 2006, microbial requirements are mainly taken into consideration in this guideline (WHO-guideline, 2006). However, all the users required full understanding of the challenges involved in greywater reuse (Albalawneh and Chang, 2015). In 2015, the WHO published sanitation safety planning methods covering the safe use and disposal of greywater and excreta as well as wastewater from various streams (WHO, 2015). However, in developing countries like Nigeria, it is highly essential to evaluate the quality of greywater before it is used for irrigation purposes. Also, the National Environmental Standards and Regulatory Agencies (NESREA), supervised by the Ministry of Environment, should educate the users and set out quality guidelines to prevent use of water sources that are untreated and/or contaminated for irrigation purposes.

2.9.1 Greywater reuse guidelines

There are four criteria required to be satisfied by the reclaimed greywater before reuse. These include aesthetics, environmental tolerance, hygienic safety and economic value (Nolde, 2000). However, different treatment techniques are required to meet different water specifications for different reuse applications. The treatment technique varies from basic to more sophisticated technology. Quality quidelines values for greywater monitoring differ by country while the development of most of the reuse standards did not consider greywater recycling (Li *et al*., 2009). Several countries (such as the

Australia, Jordan, Japan, UK and Germany) have developed quality guidelines to monitor the reuse of greywater. However, Pidou *et al.* (2007) observed that the existing variation in the reuse guideline shows varitation in application, needs and societal determinants.

In 2006, World Health Organization (WHO) publication on the greywater reuse guidelines was considered as a significant shift in perspective towards greywater and wastewater reuse. The guidelines consider some measures that are essential for appropriate health protection and could influence the accomplishment of health-based targets (WHO, 2006). However, Sinclair, (2010) observed that the use of these quality standards require stakeholders' involvement in the evaluation and management of possible hazards and risks.

2.10 Application of algae in wastewater management

Algae have been primarily cultivated on wastewater to absorb nutrient in form of nitrogen and phosphorous, thus improving its quality. For instance, wastewater discharged from household which contains many inorganic and organic pollutants has been treated with algae while nitrogen (i.e. ammonia and ammonium ion) and phosphorus were removed from the effluent (Kim *et al.,* 2010; Ahmad *et al.,* 2013). There is no restriction to the type of wastewater stream alga species could treat. Algae species have been observed to eliminate the available contaminants in the wastewater from domestic activities (Delgadillo-Mirquez *et al.,* 2016), local hotel sewage drain (Singh *et al.* 2013), piggery wastewater (Abou-Shanab *et al.* 2013) and domestic wastewater from different sources (Cabanelas *et al.*, 2013).

2.11 Process factors in alga-based wastewater management

2.11.1 Temperature

Changes in temperature of the harvesting medium are one of the process factors in the alga-based wastewater management. Lending credence to this claim is a study conducted in Busan (Korea) (Cho *et al.,* 2007) which showed that the optimal temperature for the growth of algae is a function of acclimated environment but varies with species. Generally, temperature of 35°C and above is harmful for several species of algae while temperature of 16°C and below have tendency to reduce the growth of

some algae except withdraw from harsh environments. However, for some algae in an environment with extreme weather condition, a particular adaptive technique is develoed to protect the algae from harsh weather and enhance their survival at the environment (Gomez *et al.,* 2009). Furtherrmore, all the species of various microalgae and bacteria that formed a consortium could influence their surrounding's temperature.

2.11.2 pH

Growth of alga species could differ as a result of increase or decrease in the pH range of the cultivating medium. Indeed, various microalga species could be cultivated at a varying optimal pH (Hoham *et al.,* 2007). For example, Martinez *et al. (*2011) in a study, reported an optimal pH of 7.2 for *Synechocystis* sp. In addition, pH is required during the process of abiotic nutrient removal. In a study conducted in Yongin (Republic of Korea) Zhang *et al.* (2012) reported that some of the inlet phosphorus and nitrogen were removed at a pH of 9 to 11. However, in alga-based cultivation technology, several factors may influence the pH. Another study has revealed that microalgal CO_2 and nitrate utilization could lead to an alkalinity concomitant (Perez-Garcia *et al.,* 2011). Moreover, according to Gonzalez *et al.* (2008) ammonium utilization and nitrification process could cause decrease in pH due to the release of hydrogen ion (H^+). Thus, the pH of algae growing medium has to be appropriately monitored to ensure optimal growth.

2.11.3 Light

Algae cultivation is totally photosynthetic and completely light powered, hence light could be described as a crucial component in algal growth. Munoz *et al.* (2006) in a study reported that the algal growth rate declined as the light intensity increases due to photo inhibition, though the additional light was not utilized. Furtrhemore, there is variation in the light intensity requirement among algae species. However, Cheirsilp and Torpee, (2012) observed that there is a uniques light saturation point variation for each microalgae but normally within the range of 200-400 $\mu E/m^2/s$. In addition, Guieysse *et al., (*2002) documented a decrease in saturation light intensity at high microalga concentration, since increase in algal cell densities reduced the growth of algae as a reslt of mutual shading within the cells. Indeed, the intensity of sunlight is prone to fluctuations with the seasonal changes and during day time.

2.11.4 The Concentration and ratio of nitrogen and phosphorus

Cultivation of algae requires nutrients. The most important nutrients required for algae biomass production are nitrogen (N) and phosphorous (P). However, in wastewater treatment using algae and adequate amount of nitrogen and phosphorous is essential for optimal algae biomass production. Christenson and Sims (2011) observed that the concentration of Nitrogen (20-85 mg N/L) and Phosphorous (4-16 mg P/L) in muncicipal wastewater could provide the required nutrient (N and P) for algae growth. However, certain factors could hinder the photosynthetic properties of some algae. For example, free ammonia has been reported to hinder photosynthetic process of several stains of algae due to the tendency of ammonia to fragment on photosynthesis in isolated chloroplasts (Yuan *et al.,* 2011). Adequate concentration of nitrogen and phosphorus is essential and should be one of the major factors to be considered during algae cultivation.

2.11.5 Microalgae species selection

Several algae species have been isolated and their performance investigated for their effectiveness in the treatment of wastewater, production of biomass, lipid and protein using different experiamental techniques. For instance in a study conducted in Valladolid (Spain), Godos *et al.* (2009) identified *Nitzschia, Chlorella*, *Chlamydomonas* as preferred algae species in the treatment of piggery wastewater due to their good tolerance of different environmental conditions among the different algae. However, Oswald (2003) revealed that *Micractinium, Chlorella*, and *Scenedesmus* species are the most available algae in algae ponds used for the treatment of wastewater, but *Euglena species* such as *Oscillatoria* and *Chlamydomonas* may be found in ponds with long residence times or excessive loadings. This is an indication that the six algae species mentioned may play crucial roles during wastewater treatment. In another study conducted in Valladolid (Spain) de Godos *et al.* (2010) observed that *Euglena viridis* and *Chlorella sorokiniana* were efficient in the treatment of piggery wastewater while *Chlorella sorokiniana* had high tolerance for ammonia.

2.11.6 Mixing

Mixing is a technique used to increase the algae contacts with the light in order to maximise the available light for biomass production. Mixing is necessary during algae cultivation. There are other benefits of mixing in the algae cultivation technology. For example, Munoz (2005) reported that this is an important factor that may improve the relationsip between the nutrients and cells of algae, hence reducing the potential decline in nutritional composition of the cultivating medium. Furthermore, mixing does not allow suspension of algae cells, and reduces anaerobic decomposition and the development of anaerobic zones (Munoz and Guieysse, 2006). However, different categories have been developed and applied as mixing tools in alga-based system for effluent treatment. Paddle wheels have been frequently used in an open pond system as low-cost mixing devices (Oswald and Gotaas, 1957), magnetic stirrers or rotary-shaker have been commonly employed in laboratory scale experiment (Guieysse *et al*., 2002). This technology requires much energy in form of electrical current. However, algae ponds that are drived by paddle wheel require high energe and this could be reduced by 80% with the use of the technologies that are drived by airlift (Ketheesan and Nirmalakhandan, 2011). Furthermore, it has been reported that there whould be an increase of about 75% in algae yield when airlift systems replaces paddle whell as a mixing device (Munoz and Guieysse, 2006).

2.11.7 Microalgae cultivation

There are two different ways to cultivate microalgae, an open and closed photobioreactor. Photobioreactors ensure huge amount of biomass production, light transfer, and transmission (Junying *et al*., 2013). Open ponds are set up outdoors, and microalgae use CO_2 and sunlight from outside. However, significant problems about open ponds are contamination and the fact that microalgae in this method are not generally grown with controlled properties such as pH, light and temperature. On the other hand, open photobioreactors can be used outdoor or indoor. When they are used indoor, light can be maintained from lamps, while sunlight is utilized outdoor (Bahadar, 2013). In the raceway ponds there should be a paddle wheel, which would ensure the supply of maximum light intensity for adequate penetration and mixing of microalgae. This process prevents microalgae precipitation in pond. Raceway ponds are less costly and do not require any energy inputs as well as cooling systems.

Microalgae are cultivated on attached surfaces using closed photobioreactors. This technology produces higher microalgae biomass yield and light can penetrate more easily while algae contact with contaminants could be highly controlled (Katarzyna *et al.*, 2015).

2.11.8 Microalgae harvesting

Harvesting step is important for high biomass production. However, the process is expensive due to energy input for discarding of water as it usually accounts for about 30% of the costs of production (Rawat *et al*., 2011; Barros *et al*., 2015). Harvesting of algae could be accomplished by filtration, flocculation, sedimentation and centrifugation. Each stage can be used according to isolated microalgae. Sedimentation cannot be used routinely for harvesting of microalgae. Some chemical coagulants such as ferric sulphate, ferric chloride and aluminium sulphate can be used for flocculation. Other coagulants include polymer flocculants which are suitable for some algae species like *Chlorella*.

Flocculation is a speedy and simple technique that reduces energy consumption and dewatering (Ndikubwimana *et al*., 2014). Also, filitration after the dosing of chemical coagulants needs pressure for filitrating of microalgae. Generally, microalgae cells of small sizes are filtered using chemical flocculation technique (Barros *et al*., 2015). High concentration of algae biomass is achievable with the use of chemical flocullation. However, membranes are expensive and require regular cleaning. Centrifugation process is quick but expensive technique because it requires more input of energy. Almost all algae can be harvested using centrifugation. However, setting of centrifuges are to ensure increase in capture efficiency. However, achieving cost-effective harvesting of algae is not a function of the maximum capture efficiency (Dassey and Theegala, 2013). Due to the small size of algae cells, it is essential to ensure longer retention times in order to promote effective sedimentation and achieve high harvesting efficiencies (Vandamme *et al*., 2013; Barros *et al*., 2015). However, combination of filtration and centrifugation processes could be efficient for harvesting algae that are cultivated in an enclosed bioreactors (Gong, 2011).

2.12 Compositional Parameters of Algae

Plastids is present in all the species of algae and are bodies with chlorophyll that enhance photosynthesis. However, different species of algae have unique pigment of chlorophyll content. Futhremore, all microalgae have other composition of nutrients such as proteins, fats, carbohydrates, nucleic acids and minerals, but in different proportions.

2.12.1 Proteins

Algae protein contents asssessement showed that the maximun values exist at an elevated stage of development. For example, Fernández-Reiriz *et al*. (1989) observed similar trends during cultivation of prasinophyte *Tetraselmis suecica*; however, the protein content of *Chaetoceros calcitrans* and *Phaeodactylum tricornutum* did not change during development. Furthermore, Silva *et al*. (2009) observed higher protein contents for cells during exponential development period of the prasinophyte *T. gracilisand*. The diatom *Cyclotella cassia*, contain an extensive high protein rate (62 and 55%, dew.). For sugars, distinct patterns were discovered, compared to protein. For all species, aside from *Chaetoceros sp*., expanding convergences of starches were found during all development phases. These findings were highlighted in some studies (Fernandez-Reiriz *et al.*, 1989; Laurence *et al*, 1997; Knuckey *et al.*, 2002). As indicated by Enrich *et al*. (1986), when the rate of cell division in microalgae is limited by nutrient depletion, cells adjust their metabolic system. Protein and chlorophyll diminish in nutrient limitation conditions and the concentration of lipid and/or carbohydrates increases (Gouveia, 2011).

2.12.2 Lipids

One of the major composition of several algae is lipids which could serve as an excellent fuel stock. For instance, Goncalves *et al*., (2016) in a study reported that microalgal cells are usually characterized by high photosynthetic efficiency and rapid cell division, and are an excellent source of lipids as potential fuel stocks. Also, Demirbas and Demirbas, (2011) observed that microalgae contain lipid contents of 20–50% per cell dry weigh. Other studies have however observed that microalgae can reach higher lipid content (Scott *et al*., 2010; Markou and Nerantzis, 2013; Medipally *et al*., 2015). However, there are variations in the lipid contents of microalgae. For

example, studies have reported that freshwater and marine algae respond to stress conditions by altering their metabolism and accumulating high amounts of neutral lipids and other compounds, such as carbohydrates and secondary metabolites (Griffiths and Harrison, 2009; Markou and Nerantzis, 2013). In addition, *Chlorella sp*. has been observed to produce low grade Polyunsaturated Fatty Acids (PUFA) that shows little content in the stationary development stage on the three essential fatty acids (Knuckey, 2002).

2.12.3 Pigments

Pigments are colorful chemical compounds which absorb and reflect certain wavelengths of visible light. Pigments act as light energy absorber in the photosynthetic system of microalgae. Chlorophylls, carotenoids and phycobilins has the main pigments. Chlorophylls are present in all higher plants and photosynthetic algae, only cyanobacteria and some red algae contains phycobilins while most algae contains carotenoids. Cultivation conditions control the pigment content in biomass. Studies have reported that higher amount of various secondary pigments assemble under stress condition, however, chlorophylls decompose under stress and experience significant decrease in biomass concentration (Spolaore *et al*., 2006; Alanis, 2013).

2.12.4 Vitamins and minerals

Algae biomass have been observed to contains most of the essential vitamins, pantothenic acid, folic acid, nicotinate, biotin and a substancial contents of mineral such as Ca, Mg, Na, K, Zn, Fe and trace minerals. High concentration of Iron and vitamin B12 are present in some microalgae and these make them essential source of nutritional supplements especially for vegetarian. The concentration of vitamins in algae can be influenced by changing some factors like the light intensity, the genotype, the nutritional condition of the alga and the stage in the growth cycle (Gouveia, 2008).

2.13 Animal Feed from Microalgae

The composition and quality of feeds are important external parameters that affects animal health. However, provision of better quality feed ingredients is essential in food safety and security, and it is possible to utilize algae biomass as feed supplement or feedstock. Algae has a diverse biochemical composition and this is one essential value

of algae (Gouveia, 2011). Biofuel production from microalgae was the major interest of attraction, but recently, more emphasis was shifted towards the use of microalgae in feed, food, as well as pharmaceutical and chemical sector (Wijffels and Barbosa, 2010). For instance, *Spirulina* is used as food supplements as a result of its digestibility and good nutritional component (Kumar *et al.*, 2005). Furthermore, Thajuddin and Subramanian, (2005) reported that *Spirulina* has high protein concentration (60–70 wt%) and rich in vitamins such as vitamin B12, minerals and b-carotene.

Chlorella is another microalga that is rich in nutrients and possesses adequate characteristics as a potential feed supplement or feedstock (Spolaore *et al*., 2006). Moreover, microalgae are crucial in providing high quality nutititonal food for animal such as aquaculture and farm animals. Some species of microalgae such as *Cryptonemia crenulata and Hypnea cervicornis* have high protein content and their effectiveness have been investigated in the feeding of shrimp (da Silva and Barbosa, 2008). Furthermore, Spolaore, *et al.* (2006) has identified protein content of about 5–10% in algae which can be used as protein source in poultry feed. Also, He *et al*., (2002) suggested that feed supplemented by *Laminaria digitata* alga-increase the weight of the pig for up to 10% daily.

2.14 Problems Facing Nigeria with Greywater Management

Most of the settlements in Nigeria are either not effectively planned or not served with effective and adequate wastewater disposal facilities. This is particularly so in most of the main cities and towns in Nigeria. For instance, Kagu *et al.*, (2013) pointed out that effective wastewater management facilities were not available and as a result, people allowed wastewater to flow freely into open space or into poorly constructed drainage network in a study conducted in Maiduguri Metropolis, Borno State (Nigeria). Indeed, greywater treatment and disposal method varies from one place to another depending on the available option and its affordability. In high-income settings, septic tanks and a soak away pits could serve the purpose of greywater disposal. However, people in low-income areas of Maduguri Metropolis usually allow their greywater to flow on their street or get discharged into open ground and nearby vacant plots Kagu *et al.*, (2013). This practice allows greywater to create ponds of foul-smelling stagnant water, often becoming a playing ground for children and domestic animals (Nabegu, 2010). In

another study conducted in Minna, Niger state (Nigeria), Idris-Nda *et al.* (2013) reported that inhabitants of some areas used unplanned and partially planned manually dug drainage to channel away domestic wastewater. This usually ends up forming pools of polluted water at the terminal end since there are no sewers in place to collect the wastewater.

Treatment and disposal of greywater in low-income settings has been showing a similar trend in most of the literature. Studies have documented that methods of greywater disposal include directly dumping it on the ground within the compound, throwing it on plants in the compound, throwing it over the fence, dumping it into a hole also used for rubbish, pouring it down a drain connected to a septic tank, or dumping it into the pit latrine (Armitage *et al.,* 2009; Alexander and Godrej, 2015). This is because there are no drainage systems, or where they exist, they are poorly constructed, particularly in least-developed areas.

Drainage of good capacity is required to overcome the problem of frequent greywater generation. The drainage must be free of any sort of hindrance which could deter free flow of polluted water. Any drainage that is blocked or poorly constructed has so many negative effects on the residents. As a result, people are exposed to certain amount of contaminants and vector-borne organisms such as disease-causing bacteria, infections, viruses, parasitic organisms, other pathogens as well as toxic metals, household chemicals, and excess nutrients such as nitrates (Short *et al.,* 2011). These contaminants have negative effects on the environment, drinking water and people's health. Carden *et al.,* (2007) reported that the quality of greywater in non-sewered areas differed significantly to the greywater that was generated in higher income areas. In another study, Moelants *et al.,* (2008) documented that many installed individual systems did not perform well. This might be as a result of the number of people that used the treatment facilities, the quantity of greywater the facility was designed to treat and the quality of the greywater.

There are other factors, apart from the state of drainage system that could influence greywater disposal. These include population and availability of water supply. In areas without enough water supplies, having high population with less disposal facilities,

greywater is disposed of indiscriminately. Flushing away household greywater wastes scarce potable water resources. It also adds pressure to non-existing or poorly constructed drainage infrastructure. Overpopulation increases pressure on drainage systems most especially where basic facilities are not well provided. For example, Manzo *et al.* (2015) demonstrate that the population of a setting is partly responsible for unhealthy situation because of the adoption of malpractices in the management of greywater.

Furthermore, there is possibility that lack of awareness affects appropriate greywater disposal. For instance Armitage *et al.* (2009) reported that residents of low-income settings hardly considered greywater as a serious problem. Their interest is on other much more pressing concerns while disposing their greywater to the detriment of downstream neighbours. This could increase the health risks owing to the fact that stagnated greywater may combine with the groundwater of the neighbourhoods. The groundwater becomes contaminated with excreta and other bacteria from decomposed solid waste that mixes with the greywater (Nabegu, 2010; Ojo and Adeniyi, 2012; Nwakanma and Okechukwu, 2016).

2.15 Environmental and Health Risks related to Greywater Reuse

2.15.1 Soil properties

The risk of soil receiving water contamination is the major problem related to infiltration of untreated greywater. This is essentially so due to the relatively high content of different types of pollutants such as chemical compounds and microorganisms present in greywater. Indeed, studies have evaluated the effects of wastewater on the physical and chemical properties of the irrigation soil. Tabari *et al.* (2008) reported that the use of wastewater for irrigation could increase the addition of heavy metals into the soil to the extent of posing potential risk to the environment as well as human health. In another study, Tabatabaei *et al.,* (2007) observed that soil infilteration characterisitics could be affected by the continuous application of wastewater to the soil. These practices could increase other soil properties such as electrical conductivity, organic matter, total Nitrogen, Sodium and heavy metals as claimed by Mojiri *et al.* (2013). Furthermore, changes in the pH of soil irrigated with

effluents were attributed to the organic matter decomposition and organic acids production in soils (Abegunrin, 2013; Singh *et al.* 2013).

2.15.2 Fate of pollutants

Occurrence, reaction and transport mechanism of actions are crucial in understanding the effects of pollutants - both the immediate and long term - in the environment. Indiscriminate disposal of greywater releases chemicals and microorganisms which can be detrimental to the environment. For instance, Gross *et al.* (2005) reported that high concentration of some chemicals, which may not be biodegradable such as boron, sodium or surfactants, could cause damage to the soil, crops and ground water. Also, eutrophication has been associated with the use of many sodium tripolyphosphate-containing detergents as the active ingredient (Kohler, 2006). Accumulated micro pollutants and heavy metals in the environment are potential toxic element in the food chain and consequently cause distortion in the ecological balance (Taghipour *et al.,* 2013). There is also a tendency that highly severed and long-term exposure to diseases caused by pathogenic organism in greywater might result into either morbidity or mortality (Eriksson *et al.,* 2002; Birks and Hills, 2007).

2.15.3 Mosquito breeding

Unsatisfactory discharges of greywater and poorly maintained disposal facilities are some of the factors that cause ponding of greywater. Surface ponds of greywater provide favourable breeding sites for mosquitoes. Furthermore, temporary and semi-permanent ponds that develop through indiscriminate wastewater disposal practices provide suitable breeding sites for some mosquito species. For examples, studies conducted in Nigeria have attributed indiscriminate disposal of greywater to the formation of stagnant pools in the neighbourhood, creating breeding sites for mosquitoes and causing malaria disease (Idris-Nda *et al.,* 2013; Chidozie *et al.,* 2016). This incidence is mostly common in neighbourhoods where majority of drainage channels are either lacked or blocked particularly as was the case in Minna, Niger state (Nigeria) during Idris-Nda *et al.'s* (2013) study. While indiscriminate wastewater disposal is encouraged due to lack of good drainage network, it leads to stagnant pool of wastewater which could serve as the breeding ground for disease-borne vectors such as mosquitoes, flies and other organisms (Kagu *et al.* 2013). There is possibility for

mosquitoes to also breed in any greywater treatment plant where water is allowed to stagnate for a few days. This may also result in potential health risks particularly water and sanitation-related diseases, especially among children under-five years in urban poor settings (Govender *et al.* 2011).

2.16 Common Treatment System for Greywater at Household/Community Level

Proper household wastewater management involves the determination of its quantity and quality; threat to the environment; collection; treatment and final disposal or reuse. Several low-cost technologies have been used for greywater recycling ranging from simple 2-stage processes (coarse filtration and disinfection) to physical, physico-chemical and biological processes (Al-Jayyousi, 2003; Prathaper *et al.,* 2005). However, high initial investment costs have been identified as a limiting factor against the use of these technologies especially in developing countries. However, domestic wastewater management in the developing countries consists of the use of septic tanks, and unplanned and partially planned open drainage systems (Idris-Nda *et al.,* 2013). Other technologies are constructed wetland and tricking filter (Al-Jayyousi, 2003).

Septic tanks are the most common small-scale domestic greywater treatment plants. Majority of the on-site greywater treatment systems were septic systems which had been in use for long (Levett *et al.,* 2010). Septic tank was also reported as the second most common greywater treatment facility aside from the disposal of greywater into an unplanned open drainage system (Idris-Nda *et al.* 2013). However, disposal of greywater in either traditional toilet or septic tanks generates effluents that are rich in faecal coliforms, helminths, viruses, protozoa, and other various chemical and physical pollutants (Manzo *et al.,* 2015). Consequently, infiltration of these microorganisms and other pollutants in aquifers or water distribution system can be a source of contamination and cause water-related disease such as diarrhoea in communities.

2.17 Factors Affecting Adoption of Alga-based Greywater Treatment System at Household/Community Level

2.17.1 Knowledge of household about environmental and health hazards associated with mismanagement of greywater

Knowledge about the potential health effects of inadequate greywater disposal is essential to the proper management of greywater. Regrettably, ignorance is very common especially among the most affected group. Consequently, a reputable source of information is required in order to encourage more people to understand greywater as an alternative water resource (Bakopoulou *et al.* 2008). However, most of the users of wastewater in the cited study had mixed knowledge about wastewater. In a study carried out among farmers in a low-income community of Bangladesh, some of the farmers had strong awareness of the fertility value of wastewater, while others were highly concerned with its negative impacts on health without considering any other benefit (Mojid *et al.,* 2010). Other studies reported that most of the people who reused wastewater had good knowledge about its reclamation, reuse and the benefit involved in appropriate management of wastewater (Robinson *et al.,* 2005; Chen *et al.,* 2015).

Incomes, education and age have been reported as some of those factors that significantly influence knowledge of wastewater management. For instance, people with low-income and less education were less likely to have good knowledge of wastewater management compared with high-income earners with higher education (Robinson *et al.,* 2005). Evidently, people who are in contact with wastewater are at risk particularly when appropriate practices are not considered at wastewater treatment plant. No doubt, people may be aware of risks that may arise from the reclaimed wastewater treatment resulting from operation errors, equipment failures, and accidental spills, and also from the utilization phase due to inadequate connections and poor practices among uses (Chen *et al.,* 2015).

2.17.2 Perception of households about environmental and health hazards associated with mismanagement of greywater

Perception of people involved in the greywater management and reuse could influence the adoption of wastewater as an alternative water source. Several authors perceived

wastewater as water without any other beneficial use except in application that does not engage people having close contact like irrigation, firefighting, car washing (Robinson *et al.,* 2005; Alhomoud and Madzikanda, 2010). Also, Vadachalam and Mancl (2010) in a study conducted on the campus of the Ohio State University, Columbus, reported that respondents were supportive of wastewater reuse, but really showed a strong concern about health safety. Their concern is noteworthy as safe practices should be ensured among those involved in the management and reuse of greywater in order to safeguard their health as well as that of the environment. This would enhance reuse among different categories of people while the number of beneficiaries that would accept to reuse greywater will increase (Hemobade *et al.,* 2013).

2.17.3 Economic (Cost-benefit) analysis of the alga-based greywater treatment system at household/community level

Cost-benefit analysis of developing alga-based greywater treatment considerations is essential to understand the extent of reusing greywater for algal cultivation rather than other household treatment options. The feasibility and economical benefit of this type of treatment is expected to be positive and outweigh the cost involved. For instance, Fan *et al.* (2013) observed a higher overall benefit of about 1.7 times compared to the whole cost incurred on wastewater reclamation and reuse programme in Beijing (China). Hence, this could motivate people to adopt reuse of treated wastewater. Furthermore, Godfrey *et al.* (2009) observed a high benefit of greywater reuse in residential schools in Madhya Pradesh (India). These benefits might assist the users to generate more revenue from selling the treated wastewater and improvement in the environment resulting from not allowing greywater to form ponds. There are also substantial benefits of converting algal biomass into valuable products such as animal feeds stocks, as well as raw materials for biodiesel and biogas production (Houser *et al.,* 2014).

2.18 Research Gap

One of the major gaps in most literature is the paucity of information on the use of algae for greywater treatment at household or community level. Despite the fact that algae can be used to improve the quality of wastewater and they produce useful by-

products from their biomass, a lot of people lack awareness, thus allowing their greywater to flow indiscriminately without harnessing its potential. Indeed, studies have reported the effectiveness of algae in improving effluent quality of different wastewater streams (Abou-Shanab *et al.,* 2013; Asmare *et al.,* 2014). Some studies (Idris-Nda *et al.,* 2013; Rana *et al.,* 2014) have identified septic tank, constructed wetland and intermittent sand filter as the common domestic greywater treatment methods.

However, these treatment designs have no facility to produce any useful by-products. Without doubt, the use of algal biomass to treat greywater could improve the quality of greywater, and yield primary feedstock sustainable for the production of valuable by-products like animal feed and fertilizers (Abinandan *et al.,* 2013). The foregoing has presented an evidence that greywater produced from household provides an essential nutrient adequate to cultivate algae, thereby improving the quality for reuse. In addition, harvested algal biomass could be converted to useful resources such as biodiesel, animal feedstock, fertilizers and biogas. This is one of the major focuses of this study.

In addition, there is an important gap in the literature regarding perception, beliefs, and the cost-benefit involved in the use of algae for greywater treatment. Despite the fact that most benefits of alga-based wastewater treatment have been documented (Wang *et al.,* 2013; Ahmad *et al.,* 2013), adoption of this technology at household level or community level could be hindered by social factors such as perception and belief about the process and products. For example, Ilemobade *et al.* (2013) in a study carried out in South Africa, pointed out that more beneficiaries agreed to reuse greywater when they perceived that the possibility of having contact with it is low. In other studies, most respondents opined that wastewater should be used for an application that does not involve close personal contacts such as irrigation, fire-fighting and car washing (Robinson *et al.,* 2005; Alhumoud and Madzikanda, 2010). These examples clearly reveal inadequate information about social factors that might influence use of algae for greywater treatment.

Moreover, most literatures have not adequately examined the cost-benefit analysis of developing alga-based greywater treatment. Though Godfrey *et al.* (2009) reported a substantial high benefit of greywater reuse in residential schools in Madhya Pradesh (India), the study did not produce any sound evidence of cost and benefit of the process. Furthermore, studies have documented conversion of algal biomass into valuable products like animal feedstock, biodiesel etc. (Abou-Shanab *et al.,* 2013; Houser *et al.,* 2014). However, there still remains a gap in documenting the cost and the benefit of this process. In another study conducted in Chiang Mai (Thailand), Promya *et al.* (2008) documented the production variable cost, but information on the cost benefit analysis of alga-based technology for greywater treatment was inadequate.

Evidently, previous literatures provided little information on the knowledge about greywater treatment with the use of algae and production of valuable products from its biomass. For example, Chen *et al.* (2015) in a study conducted in Beijing (China) reported that most people had good knowledge of wastewater reclamation and reuse. The study focused only on people's knowledge about wastewater reuse without considering its beneficial effect in cultivation of algae. In another study, Robinson *et al.* (2005), majority of the respondents demonstrated good knowledge about the benefit of wastewater treatment. These examples, without doubt, show that there are gaps in people's knowledge about the benefit of wastewater. This might create a challenge for the sustainability of alga-based technology for greywater treatment and recovery of valuable product from harvested algal biomass at household level, hence the need to document people's knowledge.

Furthermore, there is gap in the literature about willingness of people to accept the use of algae for treatment of greywater and conversion of the biomass to useful products at household level. For instance, Al-Mashaqbeh *et al.* (2012) reported that more people revealed their willingness to accept the reuse of greywater and to adapt its treatment in order to secure their water needs for irrigation in Deir Alla (Jordan). Al-Mashaqbeh *et al.*'s study focused on the greywater treatment only without considering the recovery of any useful by-products from the process. Also, Bakopoulou *et al.* (2008), in a study among residents of Thessaly region of Greece, also documented that people's willingness to use agricultural products irrigated with recycled water. It is evident that

people's willingness to accept the use of any products from wastewater treatment processes is necessary for the sustainability of this treatment process at household level.

2.19 Summary

This study aimed at investigating the challenges of greywater disposal in communities and finding community-based strategies to manage greywater with benefits of resource recovery, and improved health of the populations. Appropriate greywater management comprises collection, treatment and reuse or disposal. This prevents human beings from having direct contact with it and as a result reduces pathogen transfer. A robust greywater treatment also limits the input of nutrients on the nearby surface water bodies and therefore prevents eutrophication (algal bloom). However, the use of algae in greywater management does not only assist in improving the wastewater quality but also has potentials for reuse. This literature survey has captured some of the salient pieces of information required to execute this study. The section has provided information on what researchers have done about this approach to greywater treatment. It has also highlighted some of the gaps in the previous studies which this present study is aimed to fill and the procedure to achieve it.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of the Study Area

This study was carried out in Kube-Atenda community, in Ibadan North Local Government Area, Ibadan (Nigeria). Ibadan City is located near the forest-grassland boundary on latitude 7^0 23′0″N, and longitude 3^0 56′0″E in Southwest area of Nigeria. Ibadan city has 11 local government administrative areas, 5 within the metropolis and 6 peripherals with a mix of urban, semi-urban and rural communities. Ibadan metropolis with its 5 Local Government Areas (LGAs) has an estimated population of 3,565,108 (NPC, 2018). The climate condition of Ibadan include the raining season which runs from March through October, while the dry season stretches from November to February. About thirty-five percent (34.9%) of the land area (approximately 36.25sq.km) were devoted for uses such as residential area, markets, industrial and commercial areas, public buildings and facilities, social amenities, open spaces, as well as educational institutions. Non-urban uses (such as farmlands, aquatic environment and forest reserves) claimed the remaining 63.75 sqkm (Areola, 1992).

3.2 Study Site

Greywater sampling was carried out at Kube-Atenda community (Figure 3.1). It is located behind D-Castle Inn, Ibadan North LGA. The community is located at 7^0 39′ 4″ N and 3^0 9′14″ E. The community has a population of about 10,000 people (based on field estimate). Major occupations of the inhabitants of this community are petty trading, artisanship and civil service. There are two protected springs located in the community. The first spring, which is funded by Sustainable Ibadan Project (SIP), is located at the inner core of the community while the second improved spring (Alagbafo spring), located at the entrance, is funded by an NGO (Sathya Sai International Organization) as part of its global community services for the benefit of humanity. The sanitary condition around the spring has improved compared to what was obtained in the past.

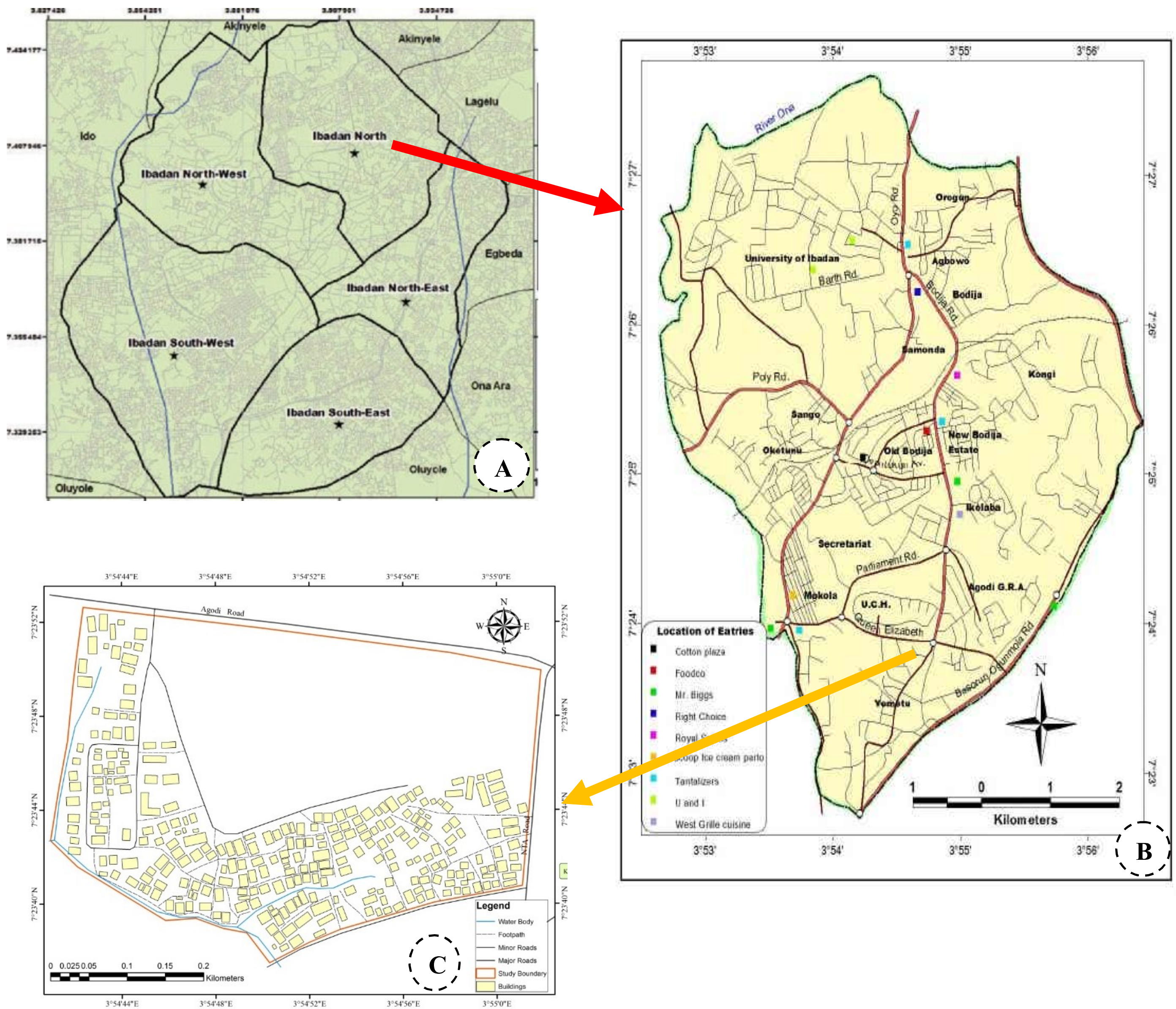


Figure 3.1. Map of the study Area (A=LGAs within Ibadan metropolis; B=Ibadan North LGA; C=Kube Attenda Community)

Sources: NPC 2018

3.3 Greywater Sample Collection

3.3.1 Sampling point selection

At the initial stage of the study, the chairman of the landlords' association within the community was contacted to seek permission to carry out the study and consent was granted. Thereafter, houses along one of the main drainages that connect the stream were selected to participate in ths study. Representatives of each of the selected houses were met to discuss the objectives and focus of the study and 8 representatives granted the consent to participate in the study. In each of the houses where consent was obtained, greywater samples were collected from one selected household. Sample was collected 4 times in a week over a period of 8 weeks.

3.3.2 Sample collection for physico-chemical analysis

Greywater samples were collected according to recommended standard methods described by the American Public Health Association (APHA, 2005). The sample containers were appropriately labeled before sample collection. Samples for the physico-chemical parameters-pH, Electrical conductivity (EC), Temperature, Total Dissolved Solids (TDS), Turbidity were collected in 2-liter capacity plastic kegs. Samples for Biochemical Oxygen Demand (BOD_5) analysis were collected using BOD bottles and analysis commenced within 6 hours of collection. The bottles were completely filled during collection in order to expel bubbles. Heavy metals samples were collected in 100 ml capacity plastic bottles and fixed with 0.2 ml of HNO_3. Eight (8) samples were collected from each of the selected households once a week for a period of 8 weeks. Also, samples from each of the treatment points and the final treated samples were collected to assess the effectiveness of the treatment plant. An airtight insulated container with ice packs was used to transport the samples to the laboratory.

3.3.3 Sample collection for bacteriological analysis

Glass sample bottles were pre-washed with distilled water and sterilized in an oven at 170^0C for 1 hour. Sterile capped bottles were used for collecting samples for the enumeration and isolation of total coliform, aerobic organism, and faecal coliform organism. The samples were stored in lightproof containers with ice packs, and taken to laboratory for immediate analysis.

3.4 Algae Strains Collection and Culture Maintenance

This study utilized pure cultures of two algae: *Scenedesmus* sp. and *Chlorella* sp.. These cultures were isolated from a private fish pond within Ibadan metropolis (Plate 3.1). Appropriate identification was done in accordance with the standard procedure of APHA, (2005). These microalgae strains were carefully scaled up into larger volumes more appropriate for inoculating the indoor laboratory batch scale greywater treatment plant. The pure culture was transferred into a clean 250 ml Erlenmeyer flask, and then plant nutrients composed of Macronutrients (N, P, K), trace elements, and vitamins (Anderson, 2005) were added from a prepared medium (Bold Basal medium) (APHA, 2005). The flask was then brought to volume with distilled water. Light is essential for microalgae growth, a dark period is also required for respiration. A balance between light and dark, similar to natural sun cycles, is required for cells to photosynthesize and metabolize carbon (Darley, 1982). The 250 ml culture flasks were stored indoor under artificial light using led bulbs.

Once the 250 ml culture was sufficiently dense (1-2 weeks), based on dry-mass and chlorophyll-a measurements, the culture was transferred to a sanitized 1L Erlenmeyer flask and distilled water was added to make the solution to volume. All cultures for the two algae strain (*Chlorella* sp. and *Scendesmus* sp.) were maintained indoor under artificial light using lead bulb. Cultures were maintained to reach sufficient density, 40% transmittance at 665 nm, prior to inoculation for the indoor greywater treatment experiments.

Plate 3.1: Collection of algae from a private fish pond in Ibadan

3.5 Experimental Setup

3.5.1 Sterilization of apparatus

All the glasswares (e.g enhelemeyer flask, testubes, and pipette) used in this study were thoroughly cleaned with detergent and rinsed with water. The centrifuge tubes were filled with 10% HCl to about one-tenth capacity and swirl to bathe the entire inner surface. The tubes were then rinsed thoroughly with water. All the cleaned glasswares were dried at 105 ^{o}C in an oven for one hour (APHA, 2005). Materials such as mouth of test tube, inoculating loop and inoculating needle were sterilized using flame lamp before and after inoculation to prevent contamination. All media used for isolation, cultivation and identification of micro algal isolates were sterilized by autoclaving at 121°C for 15 minutes under pressure.

3.5.2 Media formulations

This study made it essential to put into consideration the natural habitat and environment requirements of the two selected algae species *(Chlorella and Scenedesmus)* when choosing the culture media. Chlorella and Scedesmus algae species used are characterized by a high specific growth rate, autotrophic metabolism, and a wide environmental plasticity (Laura and Paolo, 2006). Therefore, two growth media, Bold Basal medium and Blue Green-11 Medium were used for the growth of microalgae species. The mineral salts media were autoclaved at 121°C for 15 minutes and allowed to cool. Appropriate controls of the two-growth media were also set up (Hi Media, 2011).

3.5.3 Media Composition

3.5.3.1 Bold basal medium

Composition of BBM (per L)

Potassiun nitrate (KNO_3), 1210 mg; KH_2PO_4, 230 mg; $ZnSO_4.7H_2O$, 0.882 mg; Co $(NO_3)_2.6H_2O$, 0.049 mg; EDTA, 50 mg; $CuSO_4.5H_2O$, 0.157 mg; MoO_3, 0.07 mg and $CaCl_2.2H_2O$, 35 mg; $MnCl_2 .4H_2O$, 0.144 mg; $MgSO_4.7H_2O$, 70 mg;. The solution was sterilised in an autoclave at 121°C for 15 mins after preparation.

3.5.3.2 Blue Green-11 medium

Composition of BG-11 (per L)

The concentrations of nutrients in this medium (mg/L of sterile water) were: 40 mg K_2HPO_4, 1500 mg $NaNO_3$, 6 mg Citric acid, 75 mg $MgSO_4 \cdot 7H_2O$, 6 mg Ferric ammonium citrate, and 20 mg Na_2CO_3 and 1 mg EDTA.

3.5.4 Culturing of the samples and growth conditions

The fishpond water samples were first examined microscopically to observe the presence or absence of the microalgae. Thereafter, the water samples from the pond were filtered using a sterile filter paper to recover a concentrated amount of the algae. A sterilized spatula was then used to transfer the algae from the filter paper to 20 ml of the sterile media. Algae were cultured in the growth media by inoculating different millilitre (vol /vol) aliquots of fish pond water to the synthetic medium in 250 ml Erlenmeyer flask to allow light penetration. Incubation was done at room temperature (25^0C) under an illumination provided by 18 W fluorescent lamp with a light and dark cycle of 16:8 hours. Sterile cotton wool was used to cork the 250 ml Erlenmeyer flask to allow proper aeration of the cultured medium (Ayodhya, 2013) and mixing is carried out manually by shaking the bottle at 2-hour interval, for 5 times daily to enhance the growth. These experiments were restarted every 15 days to always have fresh cultures available. Laboratory culture of the selected algae was carried out in a transparent plastic material with a dimension of 820 mm x 600 mm x 480 mm (LxBxH). The constructed greenhouse was used for the cultivation of the two algae (*Chlorella* and *Scenedesmus)* in the media. Illumination was provided in the green house using 18 W fluorescent lamp.

3.6 Identification and Isolation of the Culture of Algae

Isolation of each of the microalgae strain (*Chlorella* sp. and *Scenedesmus* sp.) in the growth media was carried out using single cell isolation technique (Burris, 1977). An aliquot of the water sample was collected from the private fish pond with the aid of 1ml of sterile pipette, after which it was placed on a clean glass slide and viewed under X40 objective of the Binocular microscope (Uniscope Brand). A micropore Pasteur pipette fitted with a rubber bulb was used to suck out cells of the microalgae, transferred into a flask containing 5-10ml sterile liquid medium. Growth was then allowed for 7 days and mixing was carried out manually 5 times daily at 2-hour

interval. The method was carried out repeatedly until a mono specific pure colony was observed. The cells were sub-cultured once a week to keep them healthy (Andersen, 2005). The microalgae were properly identified by their morphological characteristics with the use of the Biology of the Algae (Round, 1973; APHA, 2005). The microscopic pictures of the algae are shown in Plate 3.2.

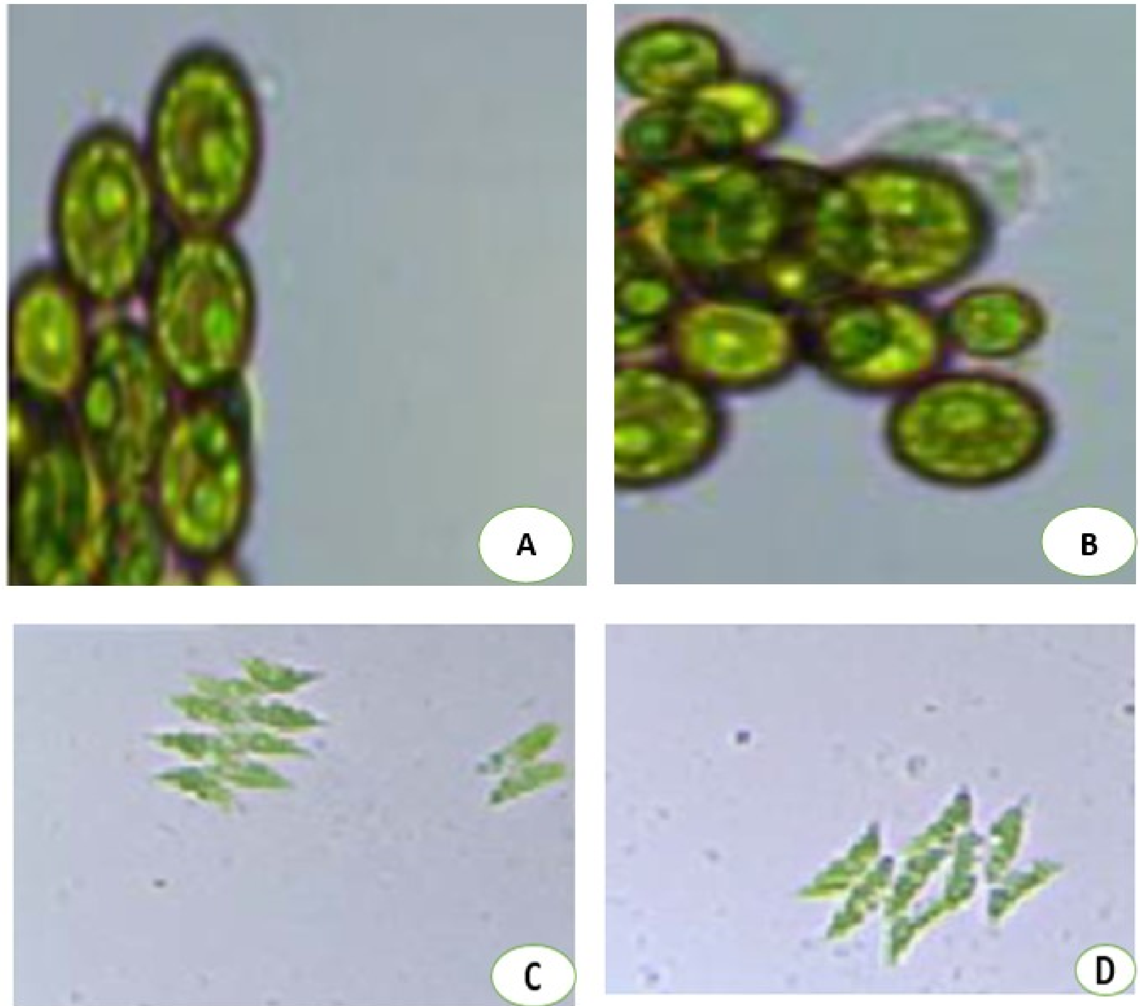


Plate 3.2: Microscopic pictures of *Chlorela sp.* (A and B) and *Scenedesmus sp.* (C and D)

3.7 Algae Growth Estimation

Biomass concentration and chlorophyll-a content was used to estimate algae growth according to APHA, 2005.

3.7.1 Determination of biomass concentration (Optical density) of the algae

From the microalgae species contained in the Erlenmeyer flask, 25 ml (10%), 37.5 ml (15%), 50 ml (20%), 62.5 ml (25%) and 75 ml (30%) aliquots were removed and inoculated into fresh greywater (in 250 Erlenmeyer flask) using a sterile pipette. The algae growth was monitored for 12 days and mixing was carried out manually 5 times daily at 2-hour interval. The cell density was estimated with the aid of a spectrophotometer (Spectronic 721 model) at 600 nm. The spectrophotometer was set at 600 nm and 1 ml of the blank (sterile, uninoculated media) was transferred into a labeled cuvette to determine the optical density of the blank. Then each culture was transferred (using sterile pipette) into labeled cuvettes and the optical density was read from the spectrophotometer.

3.7.2 Biomass Wet Weight (WW) measurement

At 24-hour interval during the experiments, 200 ml of the algal culture was centrifuged at 1000 rpm for 15 minutes, filtered through dried pre-weighed 0.45 μṁ cellulose acetate filter paper and weighed (WW).

3.7.3 Biomass Dry Weight (DW) measurement

At 24-hour interval during the experiments, 200 ml of the algal culture was centrifuged at 1000 rpm for 15 minutes, filtered through dried pre-weighed 0.45 μṁ cellulose acetate filter paper, oven dried for 6 hours at 75 ^{0}C. The difference between the initial and final weight of the filter paper was taken as the dry weight (after drying to constant weight) of the algae (DW) (Plate 3.3).

3.7.4 Determination of specific growth rate of algae

The Maximum specific growth rate of the algae (*Chlorella sp. and Scenedesmus sp.)* was evaluated (Levasseur *et al*., 1993; Madkour *et al.,* 2012) using Equation 3.1:

$\mu_m(div/day) = ln(X_2/X_1)/t_2 - t_1$ ……………………….. (Equation 3.1)

where: X_1 = cell concentration at time t_1,

X_2 = cell concentration at the time t_2.

Plate 3.3. ***Chlorella*** **sp. on the filter paper after drying**

3.7.5 Chlorophyll-a Determination

Algae sample was isolated from the water through filtration. Two hundred milliliters (200 ml) of the culture was collected and filtered through 0.45 µṁ cellulose acetate filter paper. The isolated biomass was separated for extraction of chlorophyll. The chlorophyll extraction was carried out by solvent extraction with an aqueous acetone solution. After isolating the biomass from water and extracting the chlorophyll, chlorophyll-a was estimated using spectrophotometer.

Procedure

Preparation of Aqueous Acetone Solution:

One (1.0) gramme of finely powdered $MgCO_3$ was added to a small volume of distilled water, mixed thoroughly and diluted to 100 mL. Then 90 parts (900 mL) reagent grade acetone was mixed with 10 parts (100 mL) saturated magnesium carbonate solution and the solution was mixed thoroughly.

Sample Concentration and Extraction:

Fifteen milliliters (15 ml) of well-mixed sample was measured into screw-capped graduated centrifuge tubes and centrifuged at 1000 rpm for 15 minutes. The supernatant water was removed using a pipette and the extracted slurry rinsed with acetone solution into clean labeled 15 mL centrifuge tubes with acetone solution. The volume in centrifuge tube was filled with acetone solution to a final volume of 15 mL. Then the sample was stored for 24 hours in dark refrigerator (4 ^{0}C).

The samples were removed from refrigerator and centrifuged at 500 rpm for 20 minutes. Then, 3 mL of clarified extract (supernatant) was transferred into a 1 cm cuvette. The optical density (OD) was read at 750 and 664 nm. Then, the extract was acidified with 0.1 mL of 0.1 N HCl and allowed to stand for 90 seconds. Optical Density was read at 750 and 665 nm (APHA, 2005).

Calculations:

The OD value at 750 nm was subtracted from the 665 nm and 664 nm values respectively. The chlorophyll-a content was calculated (Equation 3.2) and converted to appropriate units.

$$Chlorophyll - a, mg/m^3 = \frac{26.7\ (664b - 665a) \times V1}{V2 \times L}$$ …(Equation 3.2)

Where,

V1 = volume of extract, L

V2 = volume of sample, m^3

L = path length of cuvette, cm

664b, 665a = optical densities before and after acidification, respectively.

3.8 Design and construction of the outdoor pilot scale treatment technology

The outdoor treatment unit design has three different component. Figure 3.2 revealed the detailing using AutoCAD 2010 to produce the drawings. The first container is the balancing tank with a capacity of 165litres. The size of the unit was designed to be 1250 mm length, 620 mm width by 480 mm height. The second units housed the filter bed (the Horizontal Roughing Filter) with a dimension of 630 mm by 620 mm by 480 mm. The unit is partitioned into three component, each with a dimension of 210 mm by 620 mm by 480 mm to house gravel, coarse sand and fine sand respectively. The third container serves as the bioreactor for algae cultivation. It was designed to be 930 mm length, 620 mm width by 480 mm height. It has a rotating paddle which is powered by electricity.

The first container which served as a balancing tank was fitted with screen material for the removal of suspended solids. First container served as stabilization tank for the removal of suspended solids, and oil and grease. The second container housed the filter bed (Roughing filter), made of gravel (4 mm grain size), Coarse sand (1-2.0 mm grain size) and fine sand (0.3-0.8 mm grain size) for effective removal of organics and microbes (Plate 3.4). Sand filters can remove particles that are smaller than the spaces between sand grains. The gravel and sharp river sand were obtained locally. All these materials (sand and gravel) were carefully washed and rinsed repeatedly in clean water and dried. Effluent from the filter bed was collected into the third container which served as the bioreactor for the algae cultivation. Effluents at each point of the treatment setting were collected in replicates (4 times) and analysed.

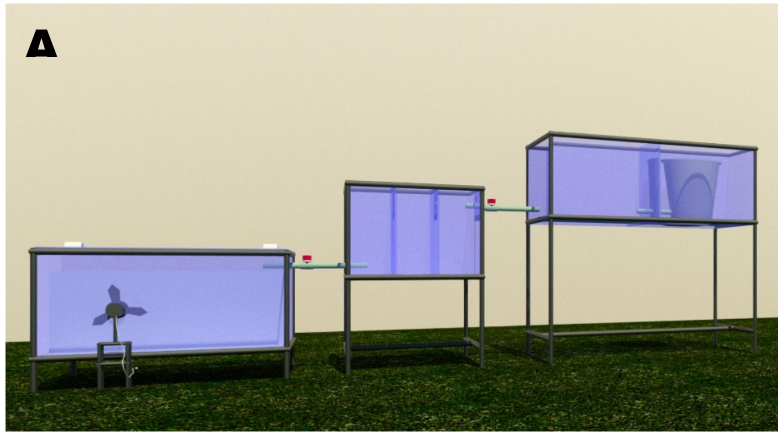
3.8.1 Lighting system

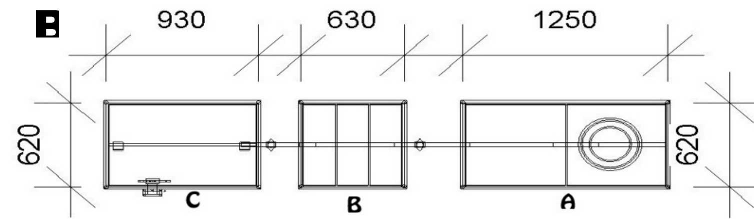
The lighting system depended on sunlight in outdoor since the overall goal was to scale the microalgae cultivation into large scale cultures which would be easy to maintain by the people in the community. The laboratory scale experiments used led bulbs which are known to emit required wavelength for algal growth. The temperature was maintained at 17 ^{0}C -22 ^{0}C. A balance between light and dark, similar to natural sun cycles, was maintained indoor using 18 watt fluorescent tube. The culture was grown at a temperature between 17 ^{0}C-22 ^{0}C at a distance of 15-20 cm from the light source (led bulbs). At the outdoor, sunlight was used as the light source.

Plate 3.4: The horizontal roughing filter

3.8.2 The outdoor treatment unit

The algae pond in the outdoor design of the treatment technology was designed to be 930 mm length by 620 mm width by 480 mm height as shown in Figure 3.3. This unit can hold between 85 and 175 liters of greywater in one batch. The cost of construction of the prototype unit was estimated at NGN175,750.00 (572.48USD at ₦307/USD exchange rate) with the Bill of Engineering Measurement and Evaluation (Table 3.1).





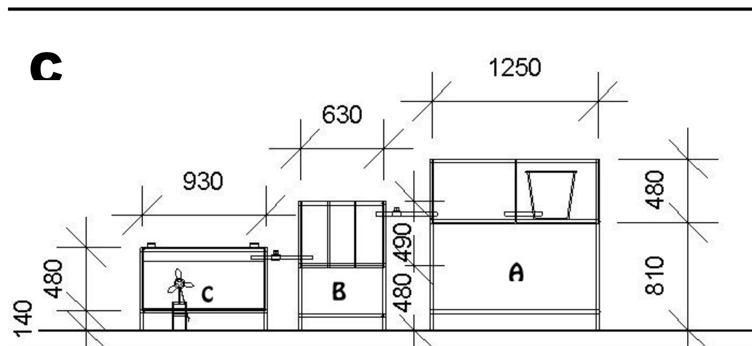


Figure 3.2. Outdoor treatment unit (A. Orthographic projection; B. Plan; C. Side Elevation; D. Picture of the whole set-up)

Table 3.1 The Bill of Engineering Measurement and Evaluation (BEME) for the plastic alga-based greywater treatment system as at May 28, 2019

ITEM	DESCRIPTION	QTY	UNIT	RATE	AMOUNT
A	Procurement of plates of borosilicate plastics	8	mm^3	15000	120,000.00
B	Iron to construct stands	6	mm	1750	10,500.00
C	Taps	3		750	2,250.00
D	pipe	1		1500	1,500.00
E	Metal work	Sum			8,500.00
F	Plumbing work	Sum			3,000.00
G	Transportation	Sum			5,000.00
H	Workmanship (coupling)	Sum			25,000.00
	TOTAL				**₦175,750.00**

3.9 Operation

3.9.1 Inoculation of the alga into the greywater in the out-door treatment plant

About 75 litres of greywater sample collected from the community was used immediately after characterisation. Twenty per cent (20%) of the alga culture was aseptically transferred into the out-door scale greywater treatment system. The pH, temperature, BOD, nitrate and phosphorous were monitored *in situ* for 10 days. Samples of treated greywater were collected at an interval of 24 hours for quality analysis.

3.9.2 Harvesting and drying processes of the algae

Filtration method using filter cloth was adopted to harvest the algae biomass as shown in Plate 3.5. Both *Chlorella* sp. and *Scenedesmus* sp. used in this study were grown on a cotton cloth substrate; when the algal cells achieved a high density on the substrate, the cloth was removed and allowed to drip, then air-dried. The cotton cloth was then returned into the bioreactor, and re-inoculated with algae to begin another cycle. This harvesting method is economical and easy to maintain. Sun drying process was used to dehydrate the biomass produced.

Plate 3.5: Filter cloth used to harvest algae biomass (A - before algae inoculation; B - during harvesting)

3.10 Characterization of Algae

Sampling and Preparation for Analysis

The harvested algae biomass (*Chlorella* sp. and *Scenedesmus* sp.) were dried and finely ground.

3.10.1 Analytical methods

Algal biomass was assessed daily by measurig the optical density (600 nm), while algal dry weight was determined (daily) gravimetrically according to standard method by APHA, (2005).

3.10.2 Analysis of dried biomass of algae

Samples from each of the dried *Chlorella* sp. and *Scenedesmus sp.* were subjected to proximate analysis such as ash, moisture content, crude lipids, crude protein, Nitrogen Free Extract and crude fibre according to Association of Official Analytical Chemists, International procedure (AOAC, 2000).

3.10.2.1 Moisture content

Moisture content in the harvested algae biomass (*Chlorella sp.* and *Scenedesmus sp.*) must be monitored to avoid any conducive environment for insect proliferation and spoilage. Moisture content determination method was based on drying a sample in an oven and determining moisture content by the weight difference between dry and wet material.

Procedure

Samples (10 g) of harvested algae biomass (each of *Chlorella sp.* and *Scenedesmus sp.*) were weighed and dried in an oven at 105 °C to a constat weight. The samples were allowed to cool and kept in desicator. Then, the dried samples were weighed and the differences in wet and dry weight were recorded while the moisture content was calclulated using Equation 3.3.

Calculation

$$Moisture\ content(\%) = \frac{(B-A)-(C-A)}{(B-A)} X\ 100 \qquad \text{(Equation 3.3)}$$

Where:

A = weight of clean, dry filter paper (g)
B = weight of pre-weighed filter paper + wet sample (g)
C = weight of pre-weighed filter paper + dry sample (g)

3.10.2.2 Crude protein

Determination of crude protein in the harvested algae biomass samples (*Chlorella* and *Scenedesmus sp.)* was carried out by Kjeldahl's method. Algae biomass samples were digested and the total nitrogen contents of the samples were determined after digestion.

Reagents

i. Potassium sulphate or anhydrous sodium sulphate,
ii. Mercuric oxide,
iii. 40% solution of sodium hydroxide: 400 g of sodium hydroxide was dissolved in distilled water and diluted to 1,000 ml
iv. Paraffin wax
v. Sulphuric acid (98%), nitrogen free
vi. Standard solution of 0.1 N chlorhydric acid
vii. Boric acid indicator solution: 5 ml of a solution with 0.1% methyl red and 0.2% bromocresyl green was added to a saturated boric acid solution
viii. 4% sodium sulphate solution

Material and equipment

i. 500 ml Kjeldahl flasks
ii. Glass beads
iii. 250 ml Erlenmeyer flasks
iv. Kjeldahl digestion and distillation apparatus

Procedure

Digestion: One gram (1 g) each of *Chlorella* and *Scenedesmus* sp. algae biomass was weighed and placed in the Kjeldahl flask, then 10 g potassium sulphate, 0.7 g mercuric oxide and 20 ml concentrated sulphuric acid were added. The Kjeldahl flask, with its content, was placed in a Kjeldahl digestion unit for about 1 hour until the content was

brought to boiling point. The boiling continued for about 30 minutes until the digest cleared. The solution was allowed to cool, and 90 ml distilled water was added. When cold, 25 ml sodium sulphate solution was added, and the solution was stirred. Then, one glass bead and 80 ml of 40% NaOH solution was added to form two layers.

Distillation: The distillation unit was steamed up and 10 mls of the digest was addeded into the apparatus through a funnel and the content was allowed to boil. Then, 10 mls of sodium hydroxide was added from the measuring cylinder so that ammonia was not lost. The distillate was collected into 50 mls of boric acid indicator solution.

Titration: The alkaline ammonium borate formed was titrated directly with 0.1N HCl. The titre value (the volume of acid used) was recorded. Nitrogen in sample was calculated using Equation 3.4 while Crude protein was computed using Equation 3.5.

Calculations

$$Nitrogen\ in\ sample\ (\%) = 100\left[\frac{A \times B}{C}\ 0.014\right]$$ …………………….. (Equation 3.4)

Crude protein (%) = nitrogen in sample × 6.25 ……..………………….. (Equation 3.5)

Where:

A = chlorhydric acid used in titration (ml)

B = normality of standard acid

C = weight of sample (g)

3.10.2.3 Crude lipids

Petroleum ether was used to extract fat from the algae samples and evaluation was performed prior the evaporation of the solvent.

Reagents, materials, and equipment

i. Laboratory kiln set at 105 °C
ii. Petroleum ether, boiling point 40–60 °C
iii. Soxhlet extraction apparatus
iv. Extraction thimbles

v. Dryer

Procedure

One hundred and fifty milliliters (150 ml) of an anhydrous diethyl ether (petroleum ether) of boiling point of 40-60 ^{0}C was placed in the flask of Soxhlet extraction apparatus. Then 5 g each of dry samples of *Chlorella* and *Scenedesmus* algae sp. was weighed into an extraction thimble and the thimble was plugged with cotton wool. The thimble with content was placed into the extractor and the ether in the flask was heated. The solution was boiled, and the extraction continued for 4 hours. Then the thimble was removed, and ether was evaporated by distillation. The flask was disconnected and placed in an oven at 65 ^{0}C for 4 hours, then cooled in a desiccator and weighed thereafter while crude lipid is calculated using Equation 3.6.

Calculations

$$\textbf{\textit{Crude lipid content}}\ (\%) = \frac{B-A}{C} \text{ X } 100 \qquad \text{……………………. (Equation 3.6)}$$

Where:

A = weight of clean dry flask (g)

B = weight of flask and the extract (g)

C = weight of sample (initial sample) (g)

3.10.2.4 Crude fibre

In this method, samples were digested in sulphuric acid and sodium hydroxide solutions and the residue was calcined. And the crude fibre content of the sample was computed by findings the difference in weight after calcination.

Reagents

i. Ethyl alcohol at 95% (v/v)
ii. Sulphuric acid solution 0.255 N
iii. Petroleum ether
iv. Antifoam (e.g. octyl alcohol or silicone)
v. Sodium hydroxide solution 0.313N, free of sodium carbonate
vi. Chlorhydric acid solution at 1% (v/v)

Material and equipment

i. 600 ml flat-bottomed balloon flask with roughened neck
ii. Condensation unit for flask
iii. 11 Kitazato flask
iv. Buchner funnel
v. Filtration crucible
vi. Rubber cones
vii. Whatman No 54 filter paper
viii. 500 ml retort
ix. Dryer
x. Laboratory kiln
xi. Crucible furnace

Procedure

Three grams (3g) each of *Chlorella* sp. and *Scenedesmus* sp. sample was weighed and placed in the flask and 200 ml of pre-heated 1.25% H_2SO_4 was added. The solution was then gently boiled for about 30mins while the constant volume of acid was maintained by the addition of hot water. The Buckner flask funnel fitted with whatman filter was pre-heated by pouring hot water into the funnel. The boiled acid sample mixture was filtered hot through the funnel under sufficient suction. The residue was then washed several times with boiling water (until the residue was neutral to litmus paper) and transferred back into the beaker. Then 200 ml of pre-heated 1.25% Na_2SO_4 was added and boiled for another 30 mins. The residue was filtered under suction and washed thoroughly with hot water and twice with ethanol. The residue was dried at 65^0C for 24 hours and weighed. The residue was transferred into a crucible and placed in muffle furnace (400-600 ^{0}C) and ashed for 4 hours, then cooled in desiccator and weighed while the crude fibre was calculalted using Equation 3.7.

Calculations

$$\boldsymbol{Crude\ fibre\ content}\ (\%) = \mathbf{100}\ \frac{\boldsymbol{A-B}}{\boldsymbol{C}} \qquad \text{(Equation 3.7)}$$

Where:

A = weight of crucible with dry residue (g)

B = weight of crucible with ash (g)

C = weight of sample (g)

3.10.2.5 Ash

This technique used calcination principle to determine ash content in the algae samples. Ash is considered as the total mineral or inorganic content of the sample.

Material and equipment

i. Dryer
ii. Crucible furnace
iii. Porcelain crucibles

Procedure

Five grams (5 g) of dry sample was placed in a crucible and brought to constant weight. The crucible was placed in a furnace at 550 °C for 12 hours (to obtain whitish-grey ash). Then the crucible was placed in the desiccator and weighed while Ash content was computed using Equation 3.8.

Calculations

$$\textit{Ash content}\,(\%) = 100\,\frac{A-B}{C} \quad \text{……………………………………} \quad \text{(Equation 3.8)}$$

Where:

A = weight of crucible with sample (g)
B = weight of crucible with ash (g)
C = weight of sample (g)

3.10.2.6 Nitrogen-free extract (NFE)

Nitrogen-free-extract composed mainly of other non-nitrogen soluble organic compounds, digestible carbohydrates and vitamins. And it is usually computed as seen in Equation 3.9.

Calculations

Nitrogen-free extract (%) = 100 - (A + B + C + D + E) ………….. (Equation 3.9)

Where:

A = moisture content (%)

B = crude protein content (%)

C = crude lipid content (%)

D = crude fibre content (%)

E = ash content (%)

3.11 Greywater Sample Analysis

Samples from untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF) were analysed for physico-chemical, bacteriological, and heavy metals characteristics using standard procedures (APHA, 2005). The harvested algal biomass for each of *scenedesmus* sp. and *chlorella* sp. was analysed for proximate composition such as moisture content, crude protein, crude lipids, crude starch, ash and Nitrogen-Free Extract (proximate analysis) using Association of Official Analytical Chemists (AOAC, 2000) procedures.

3.11.1 Physico-chemical analysis

Samples from untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF) were analysed for physico-chemical characteristics such as pH, Electrical Conductivity, Total Dissolved Solids, Total Suspended Solids, Turbidity, Biochemical Oxygen Demands (BOD_5), Nitrate, Phosphorous, Sulphate as well as selected heavy metals (Fe, Mn, Cd) using standard methods described by the American Public Health Association (2005).

3.11.1.1 pH

The pH of the samples was determined using a calibrated pH meter. Two hundred milliliters (200 ml) each of the samples obtained from the untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF) was measured into a beaker. The pH meter was calibrated using buffer solutions of pH 4.0 and 7.0 at a temperature of 25 ^{0}C before use. The pH meter probe and temperature probe were inserted making sure they did not touch the beaker. The pH reading was taken from the LCD display after it had stabilised.

3.11.1.2 Temperature

Temperature was measured with a glass thermometer with 0.1 °C graduations. The probe of the thermometer was rinsed with a portion of the sample and the rinse water was discarded. Then the measurements were taken by immersing the probe of the thermometer in the sample. The thermometer was allowed to stay in the sample for approximately one (1) minute or longer when the reading had become stabilised.

3.11.1.3 Electrical conductivity

Electrical Conductivity test was carried out using a Jenway 470 (England) Conductivity meter. This equipment was calibrated with 0.01 mol/l KCl at 25 ^{0}C before use. A hundred milliliters (100 ml) each of untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF) were measured into beaker. Then the meter probe of the device to determine conductivity was inserted and it was ensured that it did not touch the bottom of the beaker. The LCD display the reading and it was allowed to stabilize before the value was recorded.

3.11.1.4 Total Dissolved Solids (TDS)

Total Dissolved Solids was measured with the use of Jenway 470 (England) electrical conductivity meter. The mode of the meter was switched to measure TDS; 50 ml of the samples was measured into beaker and the probe of conductivity meter was inserted into it until the TDS was displayed on the screen and the reading was recorded.

3.11.1.5 Total Suspended Solids (TSS)

Total Suspended Solids (TSS) is the non-filterable residue retained on a standard filter paper after filtration of a well-mixed sample of water or wastewater. A filter paper of pore size 125 mm after weighed until constant weight was recorded (W_1). The filter paper was folded inside a funnel and placed on a conical flask. A thoroughly mixed 100 ml samples was obtained from the untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF) were passed through the filter paper. After filtration, the filter paper was removed and oven dried at 105^{0}C for one hour. It was removed from oven and cooled in a desiccator. Thereafter it was dried until a constant

weight was obtained. The weight of the filter paper was measured and recorded as W_2. TSS was calculated using Equation 3.10.

Calculation:

$$mg/l\ TSS = \frac{(W_2 - W_1) \times 1000}{Volume\ of\ sample\ (ml)} \quad \text{.................................. (Equation 3.10)}$$

Where; W_1 = weight of filter paper before filtration

W_2 = weight of filter paper and residue

3.11.1.6 Turbidity

This was measured using Nephelometric Method. It was carried out on the untreated greywater (control) and treated effluents: Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF using a HACH DR/2000 spectrophotometer. It was configured to read turbidity at the wavelength of 450 nm specified for measuring turbidity. Distilled water (turbidity-free water) was first poured into a 25 ml cuvette and inserted into the turbidity meter. The calibration button was pressed and the instrument was calibrated. Each of the samples to be read was poured into a 25 ml cuvette and inserted into the turbidity meter. The turbidity of the samples was displayed on the LCD panel of the instrument in Nephelometric Turbidity Units (NTU). After each reading, the turbidity meter was calibrated again with the distilled water before being used on the next sample.

3.11.1.7 Oil and grease

Oil and grease was analysed using Soxhlet extraction method.

Apparatus and equipment

i. Soxhlet extraction assembly

ii. Electric heating mantle

iii. Buchner funnel, 120mm dia

iv. Vacuum pump or other source of vacuum

v. Whatman No. 2 Filter paper, 110 mm diameter

vi. Extraction thimble, paper

vii. Muslin cloth discs, 110 mm diameter

viii. Desiccators

ix. Water bath, capable of maintaining 70°C

Reagents
i. HCl (1+1)

ii. Diatomaceous-silica filter aid suspension, 10 g/L distilled water

iii. Freon (BP 47 °C)

Procedure
Samples (1 liter) obtained from untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF) were measured into a wide-mouth glass bottle and the water meniscus was marked. Each sample was acidified to lower pH (pH $\leq$2) with HCl (1+1). The pH values were determined using a calibrated pH meter. Thereafter, a filter paper was placed in the Buchner funnel and the filter was wet with distilled water, then the edges of the assembled filter were pressed down to secure a seal. With vacuum on, 100 mL of the filter aid suspension was passed through the filter and washed with 1L distilled water. Vacuum was applied until no more water passed through filter. Acidified sample was filtered through the preapared filter pad under vacuum and the vacuum was continued until no more water passed through the filter. The filter paper was transferred into a watch glass using forceps.

Then, the inside and cap of the sample bottle, and the inside of the Buchner funnel were wiped with pieces of filter paper soaked in Freon to remove all oil. The pieces of the filter paper were folded into an extraction thimble. Then the filter paper, and all soild matters were added to the thimble. The thimble was filled with glass wool and dried in an oven at 103 ^{o}C for 30 minutes. Freon was added to the distilling flask (pre-dried in an oven at 103 ^{o}C for 30 minutes and stored in dessicator) and connected to the soxhlet apparatus in which the extraction thimble had been placed. The extraction was carried out and the solvent was evaporated from the extraction flask in a water bath at 70 ^{o}C. Then the flask was placed on a covered water bath at 80°C for 15 minutes. Air was drawn through the flask by means of applied vacuum for one minute. The flask was cooled in desicator for 30 minutes and weighed while oil and grease was calculated using Equation 3.11.

Calculations

Total gain in weight A, of tared flask and less calculated residue B, from solvent blank is the amount of oil and grease in the sample.

Mg/L, Oil and grease n= $\frac{(A-B)}{V}$ ……………………….. (Equation 3.11)

Where: A = Residue, gross weight of extraction flask minus the tare weight, in milligrams

B= blank determination, residue of equivalent volume of extraction solvent, in milligrams

V = volume of sample, determined by refilling sample bottle to calibration line and correcting for acid addition, if necessary, in liters.

3.11.1.8 Biochemical Oxygen Demands (BOD_5)

Samples of untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF) were diluted with distilled water (25 ml of the untreated greywater to 275 ml of the distilled water, and 50 ml of the treated greywater to 250 ml of the distilled water) and the dilution factor recorded (APHA, 2005). Fifty milliliters (50 ml) of distilled water was poured into a beaker and the DO meter probe was inserted into the beaker. The calibration button was pressed for the instrument to be calibrated. The DO of the samples was measured using the DO meter and recorded as D_1. The diluted samples were kept in an incubator at 20 ^{0}C for five days. On the fifth day, the DO content was measured again, recorded as D_2 while the BOD_5 was calculated using Equation 3.12.

Calculation

$BOD\ (mg/L) = \frac{(D_1 - D_2)\times 100}{\%\ dilution\ factor}$ ……………………………………….. (Equation 3.12)

where D_1 = Initial Dissolved Oxygen (DO of the diluted sample)

D_2 = Final Dissolved Oxygen (DO of diluted sample after 5-day incubation)

3.11.1.9 Nitrate

Nitrate was measured using phenol disulphonic acid (PDA) method.

Apparatus

i. UV/Visible Spectrophotometer (at wavelength 410 nm)
ii. Nesler tube, capacity 100 mL
iii. Beakers, capacity 100 mL
iv. Water bath

Reagent preparation

i. **Phenol disulphonic acid:** Twenty-five grams (25 g) of phenol was dissolved in 150 ml of concentrated sulphuric acid. Eighty-five milliliters (85 ml) of sulphuric acid was further added and heated on water bath for about 90 minutes. The solution was subjected to cooling and stored in black bottle.
ii. **Sodium hydroxide (NaOH):** Fifty grams (50 g) of NaOH was added into 150 ml distilled water and then allowed to cool.
iii. **Stock nitrate solution:** Seven hundred and twenty-two milligrams, 721.8 mg, (0.722 g) of potassium nitrate (KNO_3) was dissolved in little distilled water and then diluted up to 1000 mL with distilled water.
iv. **Standard nitrate solution:** Standard potassium nitrate (KNO_3) solution was prepared by evaporating 50 mL of the stock solution to dryness in the water bath. The obtained residue was dissolved in 2 mL of phenol disulphonic acid and diluted to 500 mL, to give 1 mL (10 µg N). The solution of various strengths, ranging from 0.0 (blank) to 50.0 mg/L at the interval of 10 mg/L, was prepared by diluting stock solution with distilled water.
v. **Ammonium hydroxide:** NH_4OH conc.

Procedure

Fifty milliliters (50 ml) each of the samples from untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella sp.* combined with HRF and *Scenedesmus sp.* combined with HRF) was measured into a porcelain dish and evaporated to dryness on a hot water bath. Two milliliters (2 ml) of phenol disulphonic acid was added to dissolve the residue by constant stirring with a glass rod. Concentrated solution of ammonium hydroxide (10 mL) and distilled water was added with stirring to make it alkaline. This was filtered into a Nessler's tube and made up to 50 ml with distilled water. A blank sample was prepared in a similar way using fifty mililitre (50 ml) of distilled water. The absorbance was read at 410 nm using a

spectrophotometer (Jenway 6405 UV/Visible Spectrophotometer) after the development of colour; the same procedure was followed for standards. Absorbance readings were recorded. A standard graph was prepared (Appendix I) with concentration along X-axis and the absorbance readings along the Y-axis. The concentration of nitrate in unkown sample was found by comparing absorbance of the sample with the standard curve and expressed in mg/L. Nitrate was calculated using Equation 3.13.

$$Nitrate\ (mg/L) = \frac{Absorbance\ of\ sample \times Conc.of\ standard \times 1000}{Absorbance\ of Standard \times Sample\ taken}$$ ....(Equation 3.13)

3.11.1.10 Phosphate

Phosphate was measured using stannous chloride method.

Apparatus

UV/Visible Spectrophotometer was used at wavelength 690 nm.

Reagent preparation

i. Ammonium molybdate reagent: Twenty-five grams (25 g) ammonium molybdate was dissolved in 175 ml distilled water and 280 ml concentrated sulphuric acid was added to 400 ml distilled water and cooled. Molybdate solution was added and the mixture diluted to 1000 ml with distilled water.

ii. Stannous chloride reagent: Three grams (3 g) fresh stannous chloride was dissolved in 100 ml glycerol, heated on water bath and stirred with the glass rod to hasten dissolution.

iii. Standard phosphate solution: Two hundred and twenty milligrams (219.5 mg) of dried AR potassium hydrogen phosphate was dissolved in distilled water and diluted to 1 Litre. A standard graph was prepared (Appendix II) with concentration along X-axis and the absorbance readings along the Y-axis. The concentration of phosphate in unkown sample was found by comparing absorbance of the sample with the standard curve and expressed in mg/L.

Procedure

Four (4) mL of ammonium molybdate reagent and about 4-5 drops of stannous chloride reagent were added to 50 mL of each of the filtered sample from untreated greywater (control) and treated effluents Horizontal Roughing Filter (HRF), *Chlorella*

sp. combined with HRF and *Scenedesmus sp.* combined with HRF. After about 10 minutes, the colour developed was measured using a UV/Visible spectrophotometer at 690 nm. The calibration curve was prepared using standard solution of various concentrations while the value of phosphate was obtained as mg/L using equation 3.14.

$$\text{Phosphate (mg/L)} = \frac{\text{Absorbance of sample} \times \text{Conc.of standard} \times 1000}{\text{Absorbance ofStandard} \times \text{Sample taken}} \text{ ....(Equation 3.14)}$$

3.11.1.11 Sulphate

Gravimetric method was used to determine sulphate. One (1:1 HCl) was added in drops to 100 ml of each of the samples from untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella sp.* combined with HRF and *Scenedesmus sp.* combined with HRF until it turns acidic (through pH determination with pH meter)) after which three drops were added in excess. The solution was evaporated to 50 ml. Barium chloride was added to the solution after boiling until all the sulphate was precipitated. It was digested in a water bath until the precipitate was settled. A glass crucible was dried to constant weight at 105°C and the sample precipitate was filtered using sintered glass crucible. The crucible with the precipitate was dried in an oven at 105^{0}C to constant weight. The weight of the precipitate (mg of $BaSO_4$) was calculated by finding the difference in the weight of the dried crucible only and dried crucible with precipitate. Sulphate was calculated using Equation 3.15.

$$Sulphate(mg/L) = \frac{Mg\ of\ BaSO_4 \times 1000}{ml\ of\ Sample} \text{............................ (Equation 3.15)}$$

3.11.2 Heavy metal analysis

Samples digestion

Apparatus and Materials

i. Beakers of assorted sizes or equivalent
ii. Watch glasses or equivalent
iii. Whatman No. 1 filter paper and filter funnels
iv. Measuring cylinder
v. Electric hot plate

Reagents

i. Concentrated Nitric acid
ii. Concentrated Hydrochloric acid
iii. Distilled water

Procedure

One hundred (100) mL of well mixed samples from each of untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF) was measured into a beaker. Then 5 mL of concentrated HCl and 2 mL of concentrated HNO_3 were added. The sample was covered and heated on a steam bath at 90 to 95 ^{o}C until the volume had been reduced to 15-20 mL. The samples were prevented from boiling and the beakers were removed and allowed to cool. The beaker wall was washed down with distilled water, and the samples were filtered to remove insoluble material that could clog the nebulizer. The final volume of the samples was adjusted to 100 mL with distilled water.

Determination of heavy metals

Determination of Iron (Fe), Manganese (Mn), Lead (Pb) and Cadmium (Cd) was carried out by direct reading from Atomic Absorption Spectrophotometer.

Apparatus:

Atomic Absorption Spectrophotometer (AAS) with specific cathode lamp for each of the metals to be analysed

Reagents

The analysis was carried out using stock standard reagents (1000 mg/L), supplied along with atomic absorption spectrophotometer. The following shows the preparation of the working standard solution and optimum range for each element.

i. Iron (Fe) standards: 0, 1, 2, 3, 4, 5 mg/L
ii. Lead (Pb) standards: 0, 5, 10, 15, 20, 25 mg/L
iii. Manganese (Mn) standards: 0, 5, 10, 15, 20, 25 mg/L
iv. Cadmium (Cd) Standards: 0, 0.5, 1.0, 1.5, 2.0, 2.5 mg/L

Procedure

Stock standard solutions (1000 mg/l) was used to prepare the working standards of each of the elements. The hollow cathode lamp for each of the required heavymetal was fixed and the needed wavelength was set at each time. The wavelengths were 510 nm, 520 nm, 525 nm and 535 nm for Iron, Lead, Manganese and Cadmium respectively. The instrument was put on for about 15 minutes to allow it stabilise, while the compressor was set on to supply air. The fuel acetylene was set on and regulated. The ignition control knob was pressed for flame to light. Blank solution was introduced and aspirated into the flame. The blank control was adjusted to set zero absorbance. Working standards was introduced differently and adjusted until agreeable reading was obtained. The reading of absorbance of standards against concentration was taken in milligram per Liter (mg/L) and standard curves were prepared (Appendices III to IV). Then digested samples from untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella* sp. combined with HRF and *Scenedesmus* sp. combined with HRF) were fed into the AAS and the reading of absorbances were taken. Concentration of the Fe, Pb, Mn and Cd were obtained by interpolating the absorbance values in the standard curve.

3.11.3 Bacteriological analysis of greywater

The study used two methods of bacteriological analysis. They were Multipe tube fermentation technique for the enumeration of coliform and *Escherichia coli* count, and the pour plate technique for heterotrophic plate count of bacteria in the greywater samples.

Preparation of culture media

Nutrient agar: Twenty-eight grams (28 g) of nutrient agar powder was weighed and added to 1 litre of distilled water. It was allowed to soak for 10 minutes and swirled to mix. It was then autoclaved at 121 ^oC for 15 minutes. The medium was cooled to 47 ^oC after autoclaving before pouring into plates and used for the bacterial plating.

MacConkey agar: Fifty-two grams (52 g) of MacConkey agar was homogenized in 1liter of distilled water using water bath at 100 ^oC. This was then autoclaved at 121 ^oC

for 15 minutes. The medium was cooled at 45 ^{o}C after autoclaving before pouring into plates and used for the bacterial plating.

MacConkey broth (MB): Two different concentration (the single strength and the double strength) of culture media were prepared. Thirty-five (35) grams of the powdered media was dissolve in 1L distilled water to prepare the single strength. Seventy (70) grams of the powdered MB was dissolved in 1L distilled water to prepare the double stength. For each sample, 20 fermentation tubes with Durham tubes were prepared. Then the media were dispensed into the tubes and sterilised using an autoclave at 121 ^{0}C for 15 minutes.

3.11.3.1 Multiple Tube Fermentation Technique (MPN)

Multiple tube fermentation technique was used to determine the most probable number (MPN) of coliforms available in the samples of greywater (raw and treated). MPN is an estimate based on certain probability formula. The presumptive test for coliform was used (APHA, 2005).

Water sample preparation

Four (4) serial dilution samples from untreated greywater (control) and treated effluents (Horizontal Roughing Filter (HRF), *Chlorella sp.* combined with HRF and *Scenedesmus sp.* combined with HRF) were prepared: undiluted sample; 1:10 sample, 1:100 sample, 1:1000 sample and 1:10000 sample because of the greywater contamination level. The undiluted sample is the sample without any dilution, 1ml of the undiluted sample was added to 9 ml of sterile distilled water to prepare 1:10 dilution series while 1ml of 1:10 serial dilution was added to 9 ml of sterile distilled water to preprare 1:100 dilution series. The 1:1000 dilution series was prepared by adding 1 ml of 1:100 serial dilution to 9 ml of sterile distilled water. The diluent (distilled water) used was sterilised in an autoclave at temperature of 121 ^{0}C for 15 minutes.

Inoculation and incubation

Ten milliliters (10 ml) of the undiluted sample was dispensed into each of the five (5) tubes containing 10 ml of the double strength medium already prepared. One milliliter

(1 ml) of the original sample was pipetted into each of the five tubes containing 5ml of the prepared single strength medium. One milliliter (1 ml) of the 1:10 sample already prepared was measured into each of the five tubes containing 5 mls of the prepared single strength medium while 1 ml of 1:100 sample prepared was measured into each of the five tubes containing 5 ml of the single strength media already prepared. Similarly, 1 ml of 1:1000 sample prepared was dispensed into each of the five tubes containing 5 ml of the single strength media while 1 ml of 1:10000 sample prepared was measured into each of the five tubes containing 5 ml of the single strength medium already prepared. Different pipette was used for each of the measurements during inoculation. Then the tubes were incubated for a period of 24 to 48 hours at 37 ^{0}C.

Detection and Enumeration of the Coliform Organisms

At any time within the period of 24 to 48 hours of incubation, tubes that shows positive results were recorded and then the result was estimated with the McGrandy's statistical table.

Confirmed Test (*Eschericia coli*)

Procedure: The confirmed test was performed on all the presumptive fermentation tubes showing positive result (gas formation and colour change) within 24 to 48 hours of incubation.

The culture medium and preparation

Brilliant green lactose bile broth produced by OXOID Limited, England, was used as culture medium for the confirmed test. Twenty eight grams (28 g) of the powdered Brilliant green lactose bile broth was carefully weighed and dissolved in 1 litre of distilled water. Fermentation cylinder, with inverted vials, equal in number to the tubes that were detected positive in the presumptive test were prepared. For each sample, the vials were appropriately washed and labeled with the reference number of the sample. Five (5) ml of the Brilliant green lactose bile broth prepared was dispensed into each of the prepared tubes.

Media inoculation

The tubes that shows positive results at the end of presumptive fermentation were appropriately shaken and the inoculation was carried out with a sterile wire loop which was usually sterilised between consecutive transfers. Culture of one (1) loopful was placed in the tubes containing the media and were incubated at 44 ^{o}C for 24 to 48 hours.

Detection and Enumeration

Tubes that shows positive results within 24-48 hours of incubation were recorded and the most probable number of *Eschericia coli* was estimated with the McGrandy's statistical table.

3.11.3.2 Hetertrophic plate count

This was done using pour plate method. A sterile pipette was used to measure 1ml of the 1:10 diluted sample into a sterile petri-dish and molten nutrient agar at 45 ^{o}C was poured on it aseptically. This was repeated three times and the petri dishes were swirled gently for even distribution of the inoculums in the agar. After solidification, the plates were inverted and incubated at 37 ^{o}C in an incubator for 24 hours. This was also done for MacConkey agar which is a different medium.

Media

The following media were used

i. Nutrient agar: This serves as the basic microbiological medium. It consists of nutrient broth to which 1.5% agar was added.

ii. MacConkey agar: This is a medium based on MacConkey‘s broth but with neutral red as indicator. It is the selective and differential medium for coliforms (members of *Enterobacteriaceae*).

iii. Pseudomonas agar: This is a selective and differential medium for pseudomonas.

Procedure

One (1) ml of greywater sample was measured into sterile Petri-dishes with the aid of a sterile pipette. Ten ml of the molten nutrient agar and MacConkey agar (cooled to 45

^{o}C) was poured on the sample; one (1) medium for each Petri-dish. The dishes containing the media and the innoculum were swirled gently. After solidification, the dishes were inverted and incubated at 37 ^{o}C for 24 hours. The different colonies were picked from the dishes and streaked on corresponding fresh agar plates to obtain the pure cultures. They were gram stained and observed under the microscope.

Gram staining process

A smear of the organism was made on a slide by using a wire loop. It was emulsified into sterile water and allowed to dry. Crystal violet was used as a stain for 60 seconds and the solution was poured off (primary staining). It was then rinsed with water and the mordant, lugols iodine was added for 60 seconds, thus, allowing the fixing of the stain on the cells for visibility under the microscope. It was rinsed with water, decolourized with ethanol for 30 seconds and rinsed again immediately with water. It was counter stained with safarinin for 60 seconds, rinsed with water and allowed to dry. The slide was then observed under the microscope.

Identification of isolates

Identification of isolates was done on nutrient and MacConkey agar after examining the culture, morphological and microscopic examination of the various isolate on the plate using the shapes, size, elevation, edges, colour and pigmentation. Organisms such as *Flavobacterium sp., Bacillus sp., Proteus sp., Micrococcus sp.* and *Pseudomonas sp. w*ere identified on the nutrient agar plate while *Enterobacter sp., Aeromonas, Salmonella* and *Eschericia* coli were identified on MacConkey agar plates.

3.12 Review of Meteorological Data from Ibadan City

Meteorological data of Ibadan city was collected from data management unit, Nigeria Meteorological Agency, Abuja. Information collected was daily rainfall, relative humidity, sun cycles and temperature (maximum and minimum). The data were collected from April 2017 to June 2019 (27 months). The metrological data were able to provide information on the actual weather condition in the study area during the period of field data collection. This data were used to ascertain the variability in weather condition of Ibadan, particularly the annual air temperature, rainfall and sun cycle which might likely affect the cultivation of algae.

3.13 Quality Control and Quality Assurance

All the greywater and algae biomass samples were analysed in the laboratory while the standard analytical methods were used (APHA, 2005). The steps involved in ensuring quality of the analysis were as follows:

- New sampling containers were used for all samples collected. The containers were thoroughly washed and rinsed with distilled water before use.
- Sample identity was preserved through proper labelling (name, time, date and place) of each of the samples collected.
- Analytical determination was done with the use of analytical grade reagents and appropriate standidisaton was carried out on each of the chemical.
- Manufacturers' recommended protocol was strictly followed to oprerate all the equipment items.

3.14 Data Management and analysis

Data were properly recorded in a prepared data sheet daily, checked for completeness and possible error during recording in each day. A data entry clerk was given adequate training that enabled him identify problems with data quality during data entry. A logic check was developed to minimize data entry errors and all the variables were checked for outliers, odd values and skewed data after data entry. The EPI-Info statistical package (3.5 window version) was used for data entry while statistical analysis was carried out using STATA. Data were summarise using the mean and the corresponding standard deviation. Statistical analysis was carried out as stated in Table 3.2 to 3.4. However, Linear Regression Model, at $\alpha_{0.05}$, was used to predict other parameters that contribute to the rapid growth and production of algae biomass.

Table 3.2: Statistical analysis to answer objective 1

Objectives	Variables	Data manipulation: Recoding, Computation of composite variables	Analysis Methods
1. Characterize and quantify generation pattern of greywater from various sources within the study community.	1. **Characteristics:** i. Physico-chemical parameters (pH, turbidity, TDS, BOD, Nitrate, Phosphate, Sulphate); ii. Heavy metals (Pb, Fe, Mn, Cd); iii. Bacteriological parameters (Total coliform count, *Eschericia Coli* count) iv. Bacterial isolates 2. **Generation pattern:** i. Quantity of water used in each house over a period of 8 weeks expressed in Liter per capital per day (Lpcd). ii. Quantity of greywater generated per houses over a period of 8 weeks expressed in Lcpd	Some data (total coliform count, Eschericia Coliform count, BOD, Nitrate, Phosphate, Sulphate, Pb, Fe, Mn, Cd) were skewed. Log-transformation was computed to improve normality before the commencement of the analysis. There were multiple isolates per sample of greywater. This was treated as multiple response. Log-transformation was computed for the skewed data before the commencement of the analysis.	1. Mean and standard deviation; minimum and maximum values 2. Compare the mean using t-test and ANOVA for un-skewed data. However, equivalent non-parametric test (e.g Krussal Wallis test) was used for comparison of skewed data. 3. Only the isolate names were presented. 4. Mean and standard deviation; minimum and maximum values.

Table 3.3: Statistical analysis to answer objectives 2 and 3

Objectives	Variables	Data manipulation: Recoding, Computation of composite variables	Analysis Methods
2. Determine optimal conditions for the growth of selected algal species (*Chlorella sp.*) in greywater	1. Parameters that determine algae growth condition: Temperature, pH, Nitrate concentration, Phosphate concentration).	Log transformations were computed for the skewed data. Unskewed data were used as they were.	Mean and standard deviation; minimum and maximum values.
3. Determine the biomass yield and biochemical characteristics of algae biomass produced for possible resource reuse	1. Biomas yield (Biomass wet weight, Biomass dry weight, Chlorophyll-a content) 2. Biochemical characteristics (Ash, moisture content, crude lipids, crude protein, Nitrogen Free Extract and crude fibre)	Log transformations were computed for the skewed data. Unskewed data were used as they were.	Mean and standard deviation; minimum and maximum values.
	3. specific biomass grow rate (day^{-1})		μ=log (B1/B0) (T1-T0) –Asmare et *al.*, 2014. μ= specific growth rate B0 and B1=Initial and final biomass concentration (g/L) respectively, T0 and T1 Initial and final times (day) respectively

Table 3.4: Statistical analysis to answer objective 4

Objectives	Variables	Data manipulation: Recoding, Computation of composite variables	Analysis Methods
4. Assess the effectiveness of algal based greywater treatment technique on the greywater quality.	Quality of the effluent (greywater) after treatment with (i) horizontal roughing filter, and (ii) algae **Characteristics to be determined are:** i. Physico-chemical parameters (pH, turbidity, TDS, BOD, Nitrate, Phosphate, Sulphate); ii. Heavy metals (Pb, Fe, Mn, Cd); iii. Bacteriological parameters (Total coliform count, Eschericia Coliform count) iv. Bacterial isolates (Aerobic organisms, Total Coliforms and Coliform organisms of faecal origin)	1. Some of the values were skewed and log-transformation was computed before the commencement of the analysis. 2. Log Removal Value (LRV) was computed based on: This has been used to establish treatment efficiency of some wastewater treatment plant (Carducci *et al.*; 2008; Hendricks and Pool, 2012). An LRV of 1 is equivalent to 90% removal of a target pathogen (*E. coli*), an LRV of 2 is equivalent to 99% removal and an LRV of 3 is equivalent to 99.9% removal and so on.	1. The mean value at the end of the experiments was compared with National Environmental Standards Regulations and Enforcement Agency (NESREA) and WHO guideline limits for effluents disposal. 2. The mean of raw greywater was compared with: (i) effluent from horizontal roughing filter, (ii) effluent from the final treatment with algae using t-test and ANOVA for un-skewed data. However, equivalent non-parametric test (e.g Krussal Wallis test) was used for comparison of skewed data. 3. Isolate name from filter treated greywater and algae treated greywater were presented. 4. Log Removal of *E. Coli* in the treated greywater was computed to access treatment efficiency of the algal-based treatment method. 5. Removal efficiency (%) of all the parameter of interest = $(C_0-C_1/C_1)*100$ C_0 and C_1 = Initial and final concentration respectively

3.15 Ethical Consideration

The institutional ethical clearance was granted by the University of Ibadan/University College Hospital, UI/UCH Ethical review committee before the commencement of the field work (Appendix VII). In addition, community leaders gave permission and informed verbal consents were obtained from the household heads for greywater sample collection. There was no undue influence on the participants to volunteer their greywater.

CHAPTER FOUR

RESULTS AND DUSCUSSION

This chapter presents the findings and discussion of the study. The results of estimated quantity of greywater generated and its quality (Physico-chemical, nutrients, heavy metals and bacteriological) are presented first. Secondly the results of laboratory experiments to determine the optimal condition for the growth of the two algae species used are presented. Further, results of biomass yield and biochemical characteristics of the outdoor pilot scale are presented. Results of assessment of the effectiveness of alga-based treatment technology are also presented. Mean and standard deviation were used to present all the values. The chapter also revealed the statistical significant difference of some quality and effectiveness parameters at $\alpha_{0.05}$.

4.1 Household Characteristics, Estimated Quantity of Water Consumed, and Greywater Generated

Table 4.1 shows the results of house and household characteristics, estimated quantity of water consumed, and greywater generated per household during the sampling period. Eight houses were visited and the mean number of rooms in the buildings was 5.0±1.1 (range= 4-6), mean number of households was 3.8±0.7 (range=3-5) and the mean number people per house was 14.2±6.4 (range=3-22). This study revealed that the number of households ranged from 3 to 5 while people per household ranged 3 to 22. This figure (people per household) involved all categories of occupants such as children and adults irrespective of their gender or age. In Australia, Whitehead and Patterson (2007) conducted a study in a household comprising six people to determine the rate of greywater generation. The study reported that population density was among the factors that influence the quantity and quality of greywater produced in a household. This is an indication that high number of people living in a household would produce more greywater. Previous studies have reported similar findings (Eriksson *et al.,* 2002; Kariuki *et al.*, 2012).

The mean quantity of water used for laundry, bathing and kitchen in the morning were 20.9±6.9 Lpcd (range=10-35), 10.8±4.3 Lpcd (range = 3-20) and 5.9±2.5 Lpcd (range 2-15). Mean quantity of water consumed for the three activities (laundry, kitchen and bathing) was 72.9±21.7 Lpcd (range = 22-112.0) in the morning and 33.3±15.4 Lpcd (range = 4-60.0) in the evening. The mean water consumed for all the three activities in both morning and evening was 53.5±27.4 Lpcd (range = 4.0-112.0). Overall, laundry activity consumed more water compared to bathing and kitchen. This also influenced the quantity of greywater generated. Several studies reported similar findings (Kulabakoa *et al.,* 2011; Katukiza *et al.*, 2015). However, a study conducted in Malaysia revealed that higher percentages of household greywater were generated from kitchens and hand-washing basins while the low percentages were produced from bathrooms and wash machines (Al-Mughalles *et al.*, 2012).

The mean quantity of greywater generated for the three activities (laundry, kitchen and bathing) was 62.0±18.5 Lpcd (range = 18.7-95.2) in the morning and 28.3±13.1 Lpcd (range = 3.4-51.0) in the evening. The mean amount of greywater produced from all the three activities both in the morning and evening was 45.2±19.6 Lpcd (range = 3.4-95.2). High quantity of greywater from laundry and variation in the greywater quantities across the households could be as a result of differences in lifestyle and possibly the season during which data collection was conducted. For instance, in Nigeria, much dust and excessive sweating characterise dry season and this could necessitate constant cloth washing and more water use. In this study, it was found that highest quantity of water was used on Saturday morning for bathing, laundry and kitchen activities. Moreover, the lowest quantity of water was consumed on Monday for bathing, kitchen, and laundry during the morning period. This could be responsible for the production of little quantity of greywater at the stated period compared to other days of the week. This is in consonance with the finding of Abedin and Rakib (2013) that household with restricted access to water tends to use little quantity and this could be responsible for such household to produce small amount of greywater.

Findings on quantity of water consumed and greywater generated across days of the week are presented in Figure 4.1. It was revealed that highest quantity of water was used (for

bathing, kitchen and laundry) on Saturday morning whereas highest amount of water was consumed (for bathing, kitchen and laundry) on Wednesday evening. However, during the morning hours, the highest quantity of greywater generated was on Saturday morning while highest quantity obtained during the evening period was on Wednesday evening.

Table 4.1: Household characteristics and Estimated Quantity of water consumed and greywater generated

Description (Units)	Mean±SD (Range)
Mean Number of rooms (room)	5.0±1.1 (4-6)
Number of households (household)	3.8±0.7 (3-5)
Number of people in the house (people)	14.2±6.4 (3-22)
Number of children in the household (children)	7.1±4.1 (2-13)
Number of adult in the household (adult)	8.0±2.7 (3-12)
Reported quantity of water used in the morning (7-10 am)	
Laundry (Lpcd)	20.9±6.9 (10-35)
Kitchen (Lpcd)	5.9±2.5 (2-15)
Bath (Lpcd)	10.8±4.3 (3-20)
Reported total quantity of water used (for Laundry, kitchen and bath)	
Morning (7-10 am) (Lpcd)	72.9±21.7 (22-112.0)
Evening (4-7 pm) (Lpcd)	33.3±15.4 (4-60.0)
From all activities, morning and evening (Lpcd)	53.5±27.4 (4-112.0)
Total quantity of greywater generated (from Laundry, Kitchen and bath)	
Morning (7-10 am) (Lpcd)	62.0±18.5 (18.7-95.2)
Evening (4-7 pm) (Lpcd)	28.3±13.1 (3.4-51.0)
From all activities, morning and evening (Lpcd)	45.2±19.6 (3.4-95.2)

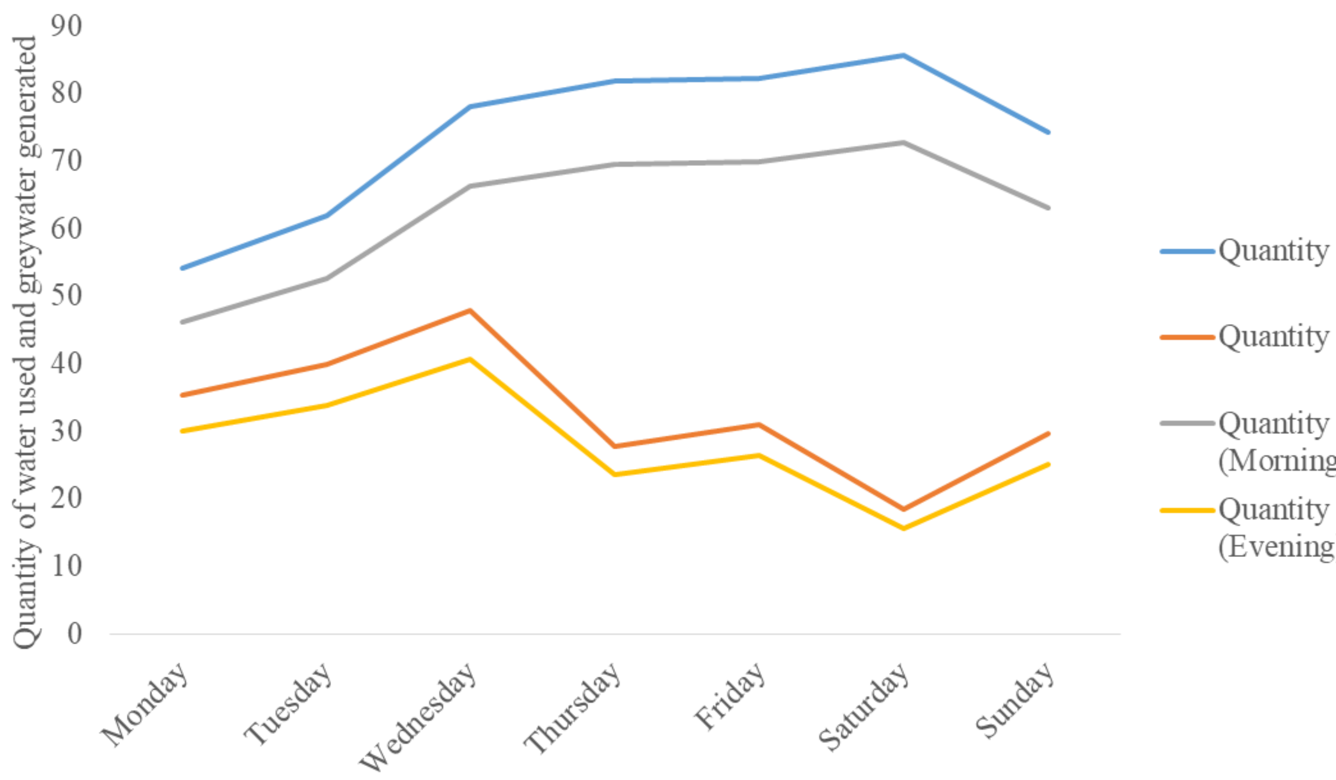


Figure 4.1: Trends in Morning and Evening Water Consumption and Greywater Generation across Days of the Week

4.2 Characteristics of Greywater before treatment

Characteristics of the raw greywater sample are presented in Table 4.2. The Table also shows the permissible limits specified by Nigeria's National Environmental Standards and Regulations Enforcement Agency (NESREA), (2009) and World Health Organisation guideline for greywater (WHO, 2006). The table shows that level of physico-chemical parameters determined fell within the stipulated limits except for turbidity, BOD_5 and TSS. The mean Turbidity, BOD and TSS were 59.7±6.6 NTU (range = 47.76-69.96 NTU), 125.7±6.4 mg/L (range = 113.0-138.4 mg/L) and 31.5±6.1 mg/L (range= 23.8-39.6mg/L) respectively. The observed concentrations exceeded the recommended limit for wastewater by NESREA and WHO as seen in Table 4.2. The mean concentration of nitrate and phosphorous in the raw greywater were 42.2±2.9 mg/L (range = 34.80-48.88 mg/L) and 16.8±3.9 mg/L (range = 10.16-26.51 mg/L) respectively. This study revealed that the pH values of the greywater were within the permissible limits recommended by National Environmental Standards and Regulations Enforcement Agency (NESREA, 2009) and World Health Organisation (WHO, 2006). Furthermore, temperature and conductivity values were below the recommended limit by NESREA.

The greywater sample had a mean Turbidity value of 59.7±6.6 NTU. The value was above the NESREA and WHO recommended limits of 5 NTU (WHO, 2006; NESREA, 2009). The mean TDS was below the recommended limits by NESREA and WHO. However, TSS, Biochemical Oxygen Demand, Nitrate, Phosphate, and Sulphate were found to be higher in the greywater. These findings are similar to the report of some previous studies (Finley *et al*., 2008; Bodnar, *et al*., 2014). However, there was high concentration of Biochemical Oxygen Demand; Nitrate, Phosphate, and Sulphate were some of the pollutants in the greywater. This could cause pollution problem in the receiving water bodies or soils, thus increasing public health hazards, if disposed indiscriminatley or without aequate treatment.

Iron (Fe), Lead (Pb) and Manganese (Mn) were detected in the raw greywater samples while cadmium was not detected. Iron, manganese, and lead were found in varying concentrations in the greywater while cadmium was not detected. This might be attributed to the use of some chemical products and materials within the households particularly for

washing and cleaning. The finding is similar to that of Jefferson *et al.* (2004) which reported that heavy metals such as Lead, Manganese, Nickel, Copper, Iron and Chromium were deficient in greywater while Cobalt and Molybdenum were not present. Indeed, increase in the level of toxic materials in the food chain is associated with accumulation of micro-pollutants and heavy metals in the environment and this could cause distortion in ecological balance (Schwarzenbach *et al.*, 2006; Taghipour *et al.* 2013).

Bacteriological analysis revealed that the mean total Bacterial Count in the raw greywater was (2.87±0.5) x10^7 CFU/100ml, total coliform count was (8.3±2.1) x10^3 CFU/100ml while faecal coliform count was 129.1±32.3 CFU/100ml as presented in Table 4.2. Correlation between the physico-chemical and bacteriological parameter of the raw greywater are shown on Table 4.3. Significantly, a positive correlation existed between oil/grease and turbidity of the raw greywater ($r=0.559$, $p<0.001$). This result indicates that oil/grease increases the turbidity of the raw greywater. However, a significantly negative correlation existed between the Nitrate concentration and turbidity of the raw greywater ($r=-0.351$, $p=0.026$). Correlation between turbidity, BOD, phosphate, TBC, TCC and FCC was not significant. Likewise, correlation between oil/grease and BOD, phosphate, TBC, TCC and FCC were not significant.

Data from the study revealed that bacteria and coliforms were present in the greywater. This study observed high Total Bacterial Count (CFU/100ml), Total coliforms (CFU/100ml) and Faecal coliform counts (CFU/100ml). These findings indicate high contamination of the greywater with several pathogens, and these could constitute a major component of the greywater. Higher numbers of microorganisms like total coliforms, *E.coli, Salmonella* sp. *and Faecal enterococci* have been revealed to be present in different streams of greywater such as washing, kitchen, shower and hand-washing basins (Abedin and Rakib, 2013). However, contaminated uncooked food and raw meat could contribute to the presence of *Eschericia coli* in kitchen greywater (Eriksson *et al.,* 2002). Furthermore, a significat positive correlation was observed between oil and grease, and turbidity of the greywater, indicating that increase in oil and grease increases the turbidity of greywater. However, there was no significant correlation between turbidity and Bichemical Oxygen Demand, phosphate, Total Bacteria Count, Total Coliform Count, Feacal Coliform Count.

The bacterial isolates (aerobic and coliforms) that were identified in the raw greywater samples are presented in Tables 4.4. Aerobic organisms isolated in the raw greywater were *Bacillus, Pseudomonas, Proteus, Flavobacterium* and *Micrococcus.* Coliform organisms identified in the raw greywater were *Enterobacter, Aeromonas, Salmonella* and *Eschericia* coli. However, *Salmonella* and *Eschericia* coli were the major organisms from faecal origin detected in the greywater.

According to Katikiza *et al.* (2014), greywater in drains may be contaminated with many types of pathogens from different points and diffuse sources in the slum areas. In this study, *Bacillus, Pseudomonas, Proteus, Flavobacterium and Micrococcus* were detected from the greywater sample. Furthermore, Coliform organisms such as *Enterobacter, Aeromonas, and faecal indicator organisms-Salmonella* and *Eschericia coli* - were isolated. This indicated that the untreated greywater contained some disease-causing bacteria. These disease-causing organisms could transmit diseases which could leads into either severe disease outbreak or death based on the exposure period and gravity (Eriksson *et al.*, 2002; Birks and Hills, 2007).

Table 4.2: Characteristics of greywater before treatment

Parameter (Units)	Mean±SD (Range)	Permissible limits	
		NESREA	WHO
Physicochemical			
pH	6.6±0.5 (6.03-7.40)	6-9	6.5-9.5
Temperature (^{0}C)	27.8±1.7 (25.0-29.3)	40	12-25
Conductivity (μS/cm)	97.9±15.6 (74.4-139.80)	NS	400
Turbidity (NTU)	59.7±6.6 (47.76-69.96)	5	5
Oil and Grease (mg/L)	46.8±6.6 (37.78-59.10)	10-100	
TDS (mg/L)	60.3±10.5 (44.8-83.6)	500	50
TSS (mg/L)	31.5±6.1 (23.8-39.6)	25	-
BOD_5 (mg/L)	125.7±6.4 (113.0-138.4)	30-50	40
COD	213.1±10.9 (191.53-234.58)	50-250	-
Sulphate (mg/L)	5.4±2.4 (0.96-10.52)	300	-
Nutrient			
NO_3-N (mg/L)	42.2±2.9 (34.80-48.88)	10-15	-
Phosphate_Phosphorus (mg/L)	16.8±3.9 (10.16-26.51)	2-5	-
Heavy metal			
Fe (mg/L)	2.5±1.2 (0.63-4.87)	3	-
Pb (mg/L)	0.03±.01 (0.00-0.507)	0.1	-
Mn (mg/L)	0.4±0.1 (0.098-0.634)		-
Cd (mg/L)	ND	0.1	-
Bacteriological			
Total bacteria count	$[2.87±0.5]x10^7$ ($[0.086-9.6]x10^7$)	$<10^3$	-
Total coliforms	$[8.3±2.1]x10^3$ ($[0.2-66]x10^3$)	$<10^3$	-
Faecal coliforms	129.1±32.3 (10-770)	$<10^3$	

ND= Not Detected [Detection limit of the instrument: Fe=0.05; Pb=0.08; Mn=0.03; Cd=0.0]

Table 4.3: Correlation matrix between turbidity, BOD, Nitrate, phosphate and microbial load

Variables	Turbidity	Oil and grease	BOD_5	Nitrate	Phosphate	Total bacteria count	Total coliform count	Faecal coliform count	Streptococcus count
Turbidity	1								
Oil and grease	0.559**	1							
BOD_5	0.015	0.273	1						
Nitrate	-0.351*	-0.213	0.015	1					
Phosphate	-0.064	0.221	0.001	-0.189	1				
Total bacteria count	0.136	0.338*	-0.028	-0.060	-0.100	1			
Total coliform count	-0.153	-0.068	-0.024	-0.053	-0.169	0.572**	1		
Faecal coliform count	0.184	-0.141	-0.254	-0.130	-0.330	0.364*	0.317	1	
Streptococcus count	-0.189	0.192	-0.023	-0.330	0.569	-0.502	-0.432	-0.309	1

Table 4.4: Isolated bacteria from the greywater

Days of the week	Aerobic organisms	Total Coliforms	Faecal Coliforms
Monday	*Pseudomonas*, *Bacillus, proteus*	*Enterobacter, Aeromonas, Salmonella* and *Eschericia* coli	*Salmonella* and *Eschericia* coli
Tuesday	*Bacillus, Micrococcus, Pseudomonas* and *Flavobacterium*	*Enterobacter, Aeromonas, Salmonella* and *Eschericia* coli	*Salmonella* and *Eschericia* coli
Wednesday	*Bacillus, Pseudomonas, Proteus* and *Flavobacterium*	*Enterobacter, Aeromonas, Salmonella* and *Eschericia* coli	*Salmonella* and *Eschericia* coli
Thursday	*Bacillus, Pseudomonas, Proteus* and *Flavobacterium*	*Enterobacter, Aeromonas, Salmonella* and *Eschericia* coli	*Salmonella*
Friday	*Bacillus, Pseudomonas, Proteus* and *Flavobacterium*	*Enterobacter, Aeromonas, Salmonella* and *Eschericia* coli	*Salmonella* and *Eschericia* coli
Saturday	*Bacillus, Pseudomonas, Proteus* and *Flavobacterium*	*Enterobacter, Aeromonas, Salmonella* and *Eschericia* coli	*Salmonella* and *Eschericia* coli
Sunday	*Bacillus, Pseudomonas, Proteus* and *Flavobacterium*	*Enterobacter, Aeromonas, Salmonella* and *Eschericia* coli	*Salmonella* and *Eschericia* coli

4.3 Optimal Quantity of Algae

Laboratory experiment was performed to determine the optimal inoculum of *chlorella sp. and scenedesmus sp.* algae for the treatment of the greywater. The results for the different quantity of the inoculum are presented in Table 4.5. It was revealed that the pH of the BBM culture was 7.6±1.2 and 7.3±1.4 after inoculation with both the *Chlorella* and *Scenedesmus* algae respectively. However, pH increased compared to the BBM culture pH of 7.6±1.2 as against 8.5±0.2, 8.7±0.7, 9.0±0.6, 8.6±2.7, and 8.5±0.8 recorded for 10%, 15%, 20%, 25% and 30% of the chlorella algae inoculation in greywater respectively. Likewise, there was an increase in the pH of scenedesmus algae inoculation in greywater as seen in Table 4.5.

Data from the study revealed that variations occured among the optimal parameters of different quantities of algae (Chlorella and Scenedesmus) inoculum during the laboratory experiment. Five different quantities (10%, 15%, 20%, 25% and 30%) of algae (Chlorella and Scenedesmus) inoculum were studied to determine the optimal quanity of both algae. The mean pH of the Bold Basal medium (BBM) culture was 7.6±1.2 and 7.3±1.4 after inoculation with both the Chlorella and Scenedesmus algae respectively. However, increases in pH of 8.5±0.2, 8.7±0.7, 9.0±0.6, 8.6±2.7, and 8.5±0.8 were recorded for 10%, 15%, 20%, 25% and 30% after inoculation with chlorella algae and scenedesmus algae. The pH range obtained in this study were within the optimal pH values of between 7.5 and 11 for most of the microalgae species such as *Scenedesmus* sp. and *Chlorella vulgaris* (Sengar et al., 2011; Gong et al., 2014; Jais et al., 2017).

The highest growth of the cultured algae was observed on the 10th day of culture in both (BBM and greywater) media. Among the different concentrations of the inoculum 20 % Chlorella and Scenedesum showed better growth performance than other concentrations in the 250 ml greywater (Table 4.5). The Chlorohyll-a value at 20% *chlorella* inoculum was 4.9±1.4 mg/L compared to 2.5±0.8 mg/L, 3.4±1.5 mg/L, 4.4±2.9 mg/L and 4.6±1.3 mg/L at 10%, 15%, 25% and 30% inoculum. In addition, the cell weight mg/L was higher 186.1±7.9 mg/L at 20% Chlorella inoculum compared to other concentration. This indicates that among the five concentration of Chlorella

inoculum, 20% showed the best growth performance for optimal growth. Similarly, the chlorophyll-a value at 20% *scenedesmus* inoculum was 5.6±0.9 mg/L. This value was higher compared to the value at 10%, 15%, 25% and 30% inoculum. It can be deduced from Table 4.5 that 20% algae inocula (Chlorella and Scenedesmus) produced the highest Chlorophyll-a and cell weight and was therefore selected for the final treatment of the greywater.

The highest growth of the cultured algae was observed on the 10th day of culture in both (BBM and greywater) media. Among the different concentrations of the inoculum, 20% Chlorella and Scenedesum showed better growth performance than other concentrations in the 250 ml greywater. The Chlorohyll-a value at 20% *chlorella* inoculum was higher (4.9±1.4 mg/L) compared to 10%, 15%, 25% and 30% inoculum. In addition, the cell weight was higher (186.1±7.9 mg/L) at 20% Chlorella inoculum compared to other concentrations. This indicated that among the five concentrations of Chlorella inoculum, 20% showed the best growth performance for optimal growth. Similarly, the chlorophyll-a value at 20% scedesmus inoculum was 5.6±0.9 mg/L. This value was higher compared to the value at 10%, 15%, 25% and 30% inoculum. These findings show that 20% algae inoculum (Chlorella and Scenedesmus) produced the highest Chlorophyll-a and cell weight, and was therefore selected for the out-door greywater treatment.

Table 4.5: Growth Performance of *Chlorella sp. and Scenedesmus sp.* for Optimal Concentration for Inoculation in Different Media at 10 Days of Culture in the Laboratory

Algae Species	Parameters (Units)	BBM	Algae inoculum in greywater				
			10%	15%	20%	25%	30%
Chlorella sp.	pH	7.6±1.2	8.5±0.2	8.7±0.7	9.0±0.6	8.6±2.7	8.5±0.8
	Temperature (^{0}C)	26.9±0.4	27.2±0.6	25.8±1.9	26.6±0.5	27.1±1.8	27.8±1.5
	Chlorophyll_a (mg/L)	5.9±1.3	2.5±0.8	3.4±1.5	4.9±1.4	4.4±2.9	4.6±1.3
	Cell weight (mg/L)	198.5±5.6	119.73±3.1	145.3±3.9	186.1±7.9	161.4±5.8	139.7±6.6
Scenedesmus sp.	pH	7.3±1.4	8.6±0.9	8.7±0.7	8.9±1.1	8.4±0.5	8.1±1.1
	Temperature (^{0}C)	26.4±0.4	27.9±1.4	26.5±1.9	27.6±2.1	28.2±1.8	26.9±1.6
	Chlorophyll_a (mg/L)	7.5±2.8	4.1±1.2	3.9±1.6	5.6±0.9	4.8±2.1	3.7±1.3
	Cell weight (mg/L)	305.3±8.7	207.3±3.2	238.3±4.1	282.2±3.9	267.7±5.2	230.9±4.2

4.4 Nitrate and Phosphorous Level of Greywater after Treatment with Algae at the Laboratory

During the Laboratory experiments, nitrate and phosphorus levels of the greywater sample after treatment with the algae (*Chlorella and Scendesmus*) were determined as presented in Table 4.6. The phosphorus values of greywater treated with *Chlorella* sp. was reduced by 71.3% from 16.4±0.8 mg/L to 4.7±1.9mg/L while nitrate was reduced by 73.1%. Similarly, the phosphorus value of greywater treated with *Scenedesmu*s sp. was reduced by 76.2% while the nitrate was reduced by 75.6%. Figure 4.2 presents the finding on phosphate concentration of the raw greywater at different days of the treatement with both *Chlorella and Scenedesmus sp.*. It was revealed that the nitrate concentration of the *chlorella* and *Scenedesmus* sp. treated greywater reduced appreciably at the day 10 of the treatment. Similarly, nitrate concentration of the *Chlorella and Scenedesmus*-treated greywater was reduced at the day 10 of the treatement (Figure 4.3).

The mean nitrate and phosphorus values recorded at 20% algae (Chlorella and Scenedesmus) inoculum were minimal. The phosphorous value of greywater treated with chlorella sp. was reduced by 37.2% while nitrate was reduced by 73.1%. Similarly, phosphorus value of greywater treated with *Scenedesmu* sp. was reduced by 41.5% while the nitrate was reduced by 75.6%. The reduction could be attributed to the fact that alga sp. used up the nitrate and phosphorous content in the greywater during cultivation period as a source of nutrients. The phosphorus removal efficiencies achieved in this study were within the previously reported values (8 to 88.5 %) for other *Chlorella* sp. grown in diluted piggery effluents (Wang *et al*., 2012).

Table 4.6: Nitrate and Phosphorous of Greywater after Inoculation with 20% Algae (*Chlorella and Scenedesmus species*) in the Laboratory

Algae sp.	Parameters (Units)	Greywater		
		Day 1	Day 10*	
Chlorella sp.	pH	6.7±0.7	9.0±0.6	
	Temperature (^{0}C)	25.8±1.9	29.3±0.5	
	Phosphorous (mg/L)	16.4±0.8	4.7±1.9	(71.3)
	Nitrate (mg/L)	42.7±2.1	11.5±0.4	(73.1)
Scenedesmus sp.	pH	6.7±0.7	8.8±1.1	
	Temperature (^{0}C)	25.8±1.9	28.6±2.1	
	Phosphorous (mg/L)	16.4±0.8	3.9±1.7	(76.2)
	Nitrate (mg/L)	41.9±1.1	10.2±1.6	(75.6)

***Values in parenthesis are % reduction**

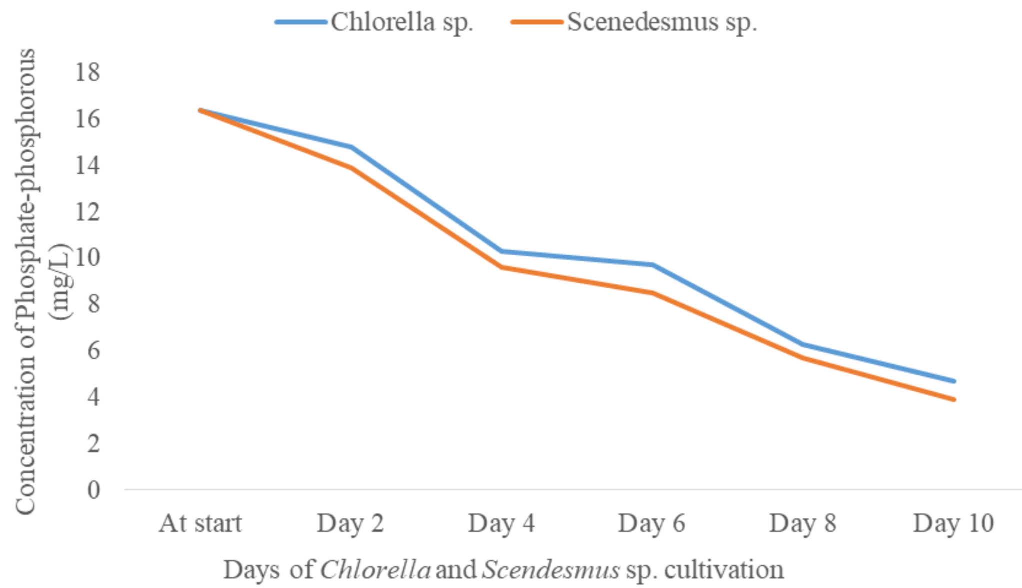


Figure 4.2: Variation in Phosphate Concentration at the Beginning through Day 10 of *Chlorella* and *Scendesmus* sp. Cultivation

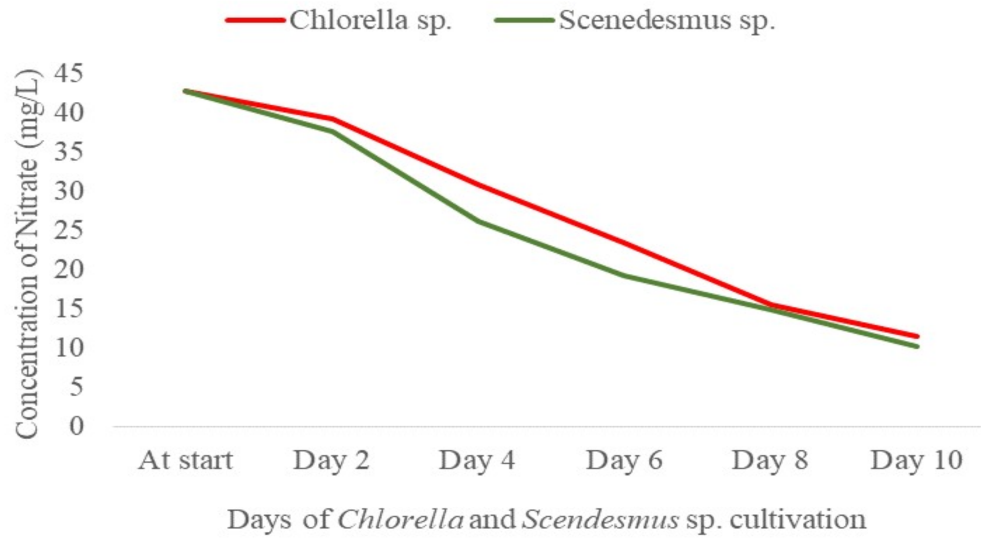


Figure 4.3: Variation in Nitrate Concentration at the Beginning through Day 10 of *Chlorella* and *Scendesmus* sp. Cultivation

4.5 Biomass Yield of the Algae at the Outdoor Pilot Scale

Table 4.7 presents the results of biomass yield and biochemical characteristics of the algae-*Chlorella* and *Scenedesmus* species. The mean chlorophyll-a content of the chlorella sp and scenedesmus sp. were 3.8±2.7 mg/L and 4.2±2.8 mg/L respectively. Mean wet and dry weight of *Chlorella* and *Scenedesmus* species were 1388.1±102.6 g and 576.2±95.7 g; and 1588.4±101.8 g and 612.9±93.1 g respectively. Data from this study revealed that the chlorophyll-a values obtained for both the *Scenedesmus sp.* (4.2±2.8 mg/L) and *Chlorella* sp. (3.8±2.7 mg/L) algae were similar. These chlorophyll-a values were slightly lower than those reported in a study conducted in a laboratory to cultivate *Scenedesmus obliquus* using sweetmeat factory waste media and Bold Basal medium prepared in the Laboratory (Toyub *et al*., 2008). However, other studies reported lower Chlorophyll-a values from algae cultivation. In one study, Khan (2003) found chlorophyll-a values of 0.37 to 0.41 respectively, when cultured *Chlorella vulgaris* in BBM and sugar mill effluent media. In another study, Habib (1998) reported 0.40 mg/L chlorophyll-a value of *Chlorella vulgaris* when cultured in Nitrogen Phosphorous and Potassium (NPK) fertilizer and different concentrations of standard Malaysian rubber effluent media.

The wet weight of *Chlorella sp.* was lower than that of *Scenedesmus sp.* while both the Chlorella and scenedesmus sp. had similar dry weight. The greywater used as the medium supported the cultivation of both species of algae - *Chlorella* and *Scenedesmus*. This study found a higher biomass growth rate for both the chlorella and scnedesmus over the period of the cultivation. This study revealed that growth rate of the *chlorella sp.* (2.37 g/day) was slightly higher than that of *scenedesmus sp.* (1.83 g/day). These values were higher than the values obtained in the previous studies (Obata *et al*., 2009; Ong *et al*., 2010; Chia *et al*., 2013). The increase might be attributed to the concentration of the nitrate and phosphorous in the greywater that served as the cultivation medium.

Data on the biomass growth rate across days of the week revealed that there was an increase in the concentration of algae biomass for both the *Chlorella* and *Scenedesmus* over the period of cultivation (Figure 4.4). The figure also shows that *Chlorella* sp. had higher growth rate (2.37 g/day) compared to *Scenedesmus* sp. (1.83 g/day).

Biochemical characteristics of the algae revealed that *Chlorella* and *Scenedesmus* species had the mean moisture content (%) of 2.1±0.12 and 2.3±0.3 respectively. The mean crude protein (%) was 12.7±1.4 (*Chlorella* app) and 14.6±1.8 (*Scenedesmus* sp.). Crude fat (%) and crude fibre (%) were (0.8±0.1 vs 10.9±0.2) for *Chlorella* sp. and (0.9±0.1 vs 12.9±0.5) for *Scenedesmus* sp.. The mean ash (%) content for the *Chlorella* sp. and *Scenedesmus* was 50.3±0.4 and 50.1±1.6 respectively. The mean totals carbohydrate by difference (%) were 34.1±1.6 for *Chlorella* sp. and 35.2±1.9 for *Scenedesmus* sp.. The mean biomass concentration (Unit) was 179.7±119.6 for *Chlorella* sp. and 185.7±123.6 for *Scenedesmus* sp..

Comparison of compositional parameters of the *Chlorella* and *Scenedesmus* sp. cultivated on the greywater across the period of observation is presented in Table 4.8. Crude protein (%) of the *Scenedesmus* sp. (14.6±1.8) was significantly higher compared to that of *Chlorella* sp. (12.7±1.4) at the end of the outdoor experiment. Significantly, the crude fibre (%) of *Scenedesmus* sp. (12.9±0.5) was higher compared to the *Chlorella* sp. (10.9±0.2). Also, a significant difference existed between the crude fat (%) value of *Scenedesmus* sp. (0.9±0.1) and *Chlorella* sp. (0.8±0.1).

This study found that the moisture content (%) of dry *Chlorella* and *Scenedemus* algae were 2.1±0.12 and 2.3±0.3 respectively. The *Chlorella* and *Scenedesmus* algae produced a protein content (%) of 12.7±1.4 and 14.6±1.8 respectively. This study found a lower protein content than the value recorded in previous studies where dry *Chlorella sp.* was reported to have a protein content in the range of 50-60% of dry weight, similar to yeast, soy flour and milk protein (Seyfabadi *et al*., 2011; Kovač *et al*., 2013). However, it has been reported that a significant variation in total protein content (8–50% dry weight) of algae (Fleurence, 1999) and numerous algae generally contain all the essential amino acids. (Ortiz *et al*., 2006; Dawczynski *et al*., 2007). The decrease in the protein content observed in this study might be attributed to the growth medium (greywater) used. A previous study has attributed the variation in the proximate composition of algae to the effect of growth medium and growth stage (Leonardos and Lucas, 2000).

Chlorella sp. had a mean crude fat (%) of 0.8±0.1, while *Scenedesmus* sp. had mean crude fat value of 0.9±0.1. The crude fat values obtained for both the *Chlorella* and *Scendesmus* were similar. In a study to determine the total lipid content of *Chlorella vugaris*, Yoo *et al*. (2010) reported about eleven percent lipid content. The value was higher than the value obtained in this study. This might be attributed to the medium of cultivation as described in Leonardos and Lucas (2000). Furthermore, crude fibers (%) were observed in both the *Chlorella* and *Scenedesmus*. In this study, the mean crude fibre obtained for *Chlorella* (10.9±0.2) was slightly lower than that obtained for *Scenedesmus* (12.9±0.5). The mean total carbohydrate by difference (%) was 34.1±1.6 for *Chlorella sp.* and 35.2±1.9 for *Scenedesmus sp.*. The carbohydrate composition obtained in this study was slightly higher compared to the values reported in a study by Elumalai *et al.*, (2014).

A comparison of proximate composition of the *Chlorella* and *Scenedesmus* sp. cultivated on the greywater showed that crude protein (%) of the *Scenedesmus* sp. (14.6±1.8) was significantly higher compared to the value obtained for *Chlorella* sp. (12.7±1.4) after the outdoor treatment. This is an indication that the *Scenedesmus* sp. produced biomass with slightly high protein content than the *Chlorella* sp.

Table 4.7: Biomass Yield and Compositional Parameters of Alga Species

Characteristics (Units)	Mean±SD	
	***Chlorella* sp.**	***Scenedesmus* sp.**
Biomass yield		
Chlorophyll_a (mg/L)	3.8±2.7	4.2±2.8
Wet weight (g)	1388.1±102.6	1588.4±101.8
Dry weight (g)	576.2±95.7	612.9±93.1
Compositional parameters		
Moisture content (%)	2.1±0.12	2.3±0.3
Crude protein (%)	12.7±1.4	14.6±1.8
Crude Fat (%)	0.8±0.1	0.9±0.1
Crude fibre (%)	10.9±0.2	12.9±0.5
Ash (%)	50.3±0.4	50.1±1.6
Total carbohydrate by difference	34.1±1.6	35.2±1.9
Cell weight (g/L)	179.7±11.9	185.7±12.3

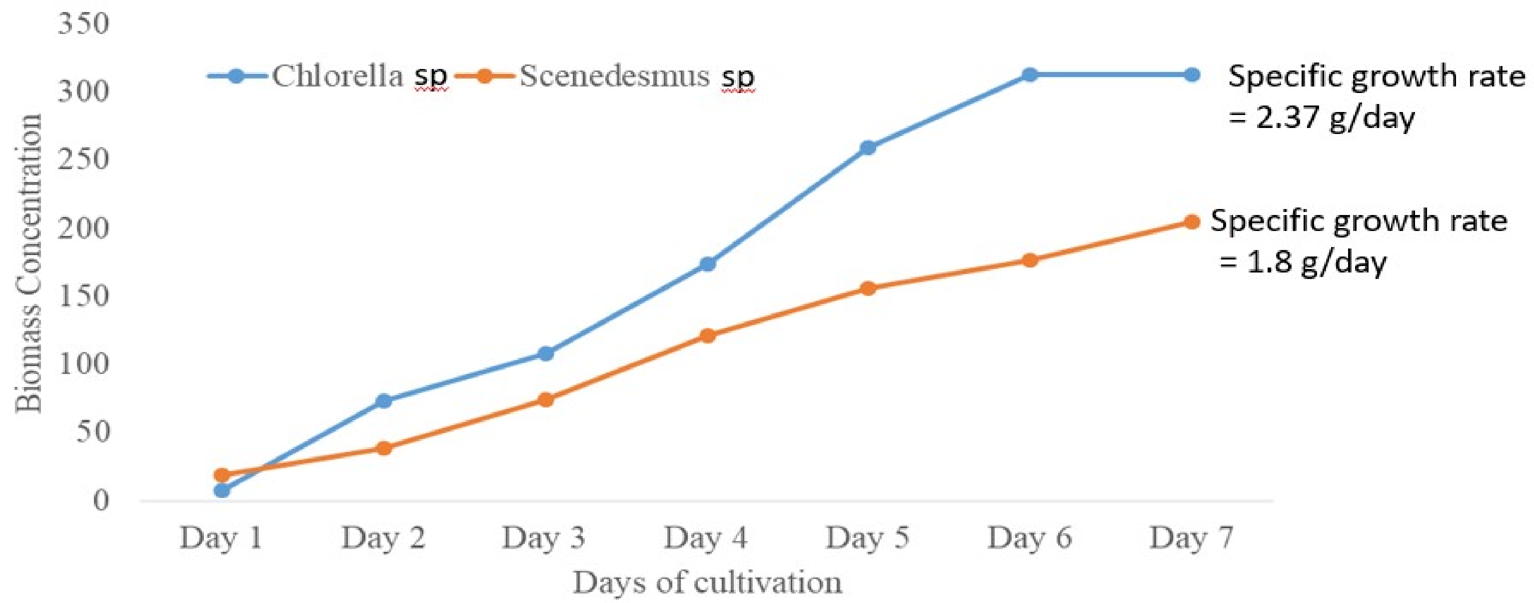


Figure 4.4: Biomass Grow Rate (per day)

Table 4.8: Comparison of Compositional Parameters of the *Chlorella sp.* and *Scenedesmus sp.*

Characteristics (Units)	Algae specie	N	Mean± SD	T-test	P-value
Moisture content (%)	*Chlorella*	15	2.1±0.1	-1.667	0.10
	Scenedesmus	15	2.3±0.3		
Crude Protein (%)	*Chlorella*	15	12.7±1.4	-3.163	0.004
	Scenedesmus	15	14.6±1.8		
Crude Fat (%)	*Chlorella*	15	0.8±0.1	-3.492	0.002
	Scenedesmus	15	0.9±0.1		
Crude Fibre (%)	*Chlorella*	15	10.9±0.2	-14.589	<0.001
	Scenedesmus	15	12.9±0.5		
Ash (%)	*Chlorella*	15	50.3±0.4	0.566	0.576
	Scenedesmus	15	50.1±1.6		
Total carbohydrate by difference	*Chlorella*	15	34.1±1.6	-1.891	0.069
	Scenedesmus	15	35.2±1.8		
Cell weight (mg/L)	*Chlorella*	15	179.7±119.6	-0.134	0.895
	Scenedesmus	15	185.7±123.6		

4.6 Effect of Roughing Filter, *Chlorella* sp. and *Scenedesmus* sp. on Greywtaer Quality

The qualities of raw greywater sample treated with roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp. are compared with guideline limits by the WHO as shown in Table 4.9. The findings. The mean pH of the roughing filter-treated greywater, RF+*Chlorella* sp. and RF+*Scenedesmus* Sp.-treated samples were 6.7±0.5, 9.1±0.7 and 8.8±0.5 respectively. The mean pH value for the roughing filter-treated greywater samples was within the WHO permissible limit. However, there was an increase in the mean pH values for RF+*Chlorella* sp. and RF+*Scenedesmus* sp. treated greywater. The mean temperature values (^{0}C) for roughing filter-treated greywater, RF+*Chlorella* sp. and RF+*Scenedesmus* sp. treated samples were 27.8±1.7, 28.1±1.8 and 28.7±1.3 respectively. Values for the temperature were similar and fell within the NESREA permissible limits, but slightly higher than the WHO (12-25 ^{0}C) recommended limit. Values for conductivity (µS/cm), oil/grase (mg/L) and sulphate (mg/L) were similar for roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater samples. Iron (Fe), Lead (Pb) and Manganese (Mn) were detected in the roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater samples were similar to the value obtained for the raw greywater samples, and cadmium was not detected.

However, the mean turbidity of 8.6±0.9 NTU, 8.9±0.9 NTU and 8.8±0.8 NTU were recorded for RF, RF+*Chlorella* sp. and RF+*Scenedesmus* sp. treated greywater samples respectively. The results showed a high reduction in the turbidity level of the raw greywater water after the treatments. Treatment with roughing filter recorded a turbidity reduction of 85.59% while that of RF+*Chlorella* sp. and RF+*Scenedesmus* sp. treated greywater produced 85.09% and 85.30% turbidity reduction respectively (Table 4.10). The TDS (mg/L) values of roughing filter (39.0±7.0), RF+*Chlorella* sp. (24.4±4.2) and RF+*Scenedesmus* sp. (25.1±4.9) treated greywater samples were reduced by 35.3%, 59.5% and 58.4% compared to the TDS values obtained from the raw greywater (60.3±10.5). The BOD_5 (mg/L) values obtained for roughing filter (49.1±2.6), RF+*Chlorella* sp. (43.4±2.1) and RF+*Scenedesmus* sp. (42.4±2.2) treated greywater samples were reduced compared to the values (125.7±6.4) obtained for the raw greywater. Treatment with

roughing filter alone produced 60.9% BOD_5 reduction while that of RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater recorded a BOD_5 reduction of 65.5% and 66.3% respectively. The BOD values for roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater were within the recommended limit (50 mg/L) by NESREA and WHO.

The mean nitrate values of roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater sample were 31.7±2.3 mg/L, 11.6±0.8 mg/L and 10.4±0.7 mg/L respectively. The nitrate level of the raw greywater was reduced after treatment by 24.9% with roughing filter, 72.5% with RF+*Chlorella* sp. and 75.4% with RF+*Scenedesmus* sp. as indicated in Table 4.10. Phosphorous level of the raw greywater was reduced by 12.5%, 35.7% and 42.3% with roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp. respectively. Reductions in the physico-chemical parameter and nutrient level were compared between RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater as presented in Table 4.11. There was no significant differences in level of Turbidity (NTU), TDS (mg/L) and BOD_5 (mg/L) between RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater. However, the mean nitrate concentration in RF+*Scenedesmus* sp. treated greywater (10.4±0.7 mg/L) was significantly lower compared to nitrate concentration in RF+*Chlorella* sp.-treated greywater (11.6±0.8 mg/L). There was no significant difference in the phosphorous concentration between RF+*Chlorella* sp.-treated (10.8±2.7 mg/L) and RF+*Scenedesmus* sp.-treated (9.7±2.3 mg/L) greywater.

Data from this study revealed that the pH values of the greywater treated with roughing filter were within the NESREA guideline limit (6.5-9.8) for wastewater discharge (NESREA, 2009). However, an increase in the pH values was observed for RF+*Chlorella* sp. (9.1±0.7) and RF+*Scenedesmus* Sp. (8.8±0.5) treated greywater. This is similar to the findings of Marín *et al*., (2018). High operational pH values have been reported to promote a productive microbial activity (Posadas *et al*., 2017a and b; Al-Gheethi *et al*., 2019). The ambient temperature (^{0}C) values at which both chlorella and scenedesmus were cultivated were below 30^{0}C. Different algae have different optimal growth temperatures. For example, *Chlorella vulgaris* have been reported to have a decreased growth rate if the

temperature of the medium is above 25 degrees Celsius, but they still grow in temperatures up to at least 40 degrees (Sayed and El-Shahed, 2000).

Furthemore, the temperature values were similar and fell within the NESREA permissible limits, but slightly higher than the WHO (12-25^{0}C) recommended limit. The mean conductivity (µS/cm), oil/grase (mg/L) and sulphate (mg/L) values were similar for roughing filter, RF+Chlorella sp. and RF+Scenedesmus sp.-treated greywater samples.

Data from the study revealed a high reduction in the turbidity level of the greywater after RF+*Chlorella* sp. and RF+*Scenedesmus* sp. treatments. Treatment with only the roughing filter recorded a turbidity reduction of 85.59% while that of RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater produced 85.09% and 85.30% turbidity reduction respectively. This study shows that the BOD_5 (mg/L) values obtained for roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater samples were reduced compared to the values obtained for the raw greywater. Treatment with roughing filter alone produced 60.9% BOD_5 reduction while that of RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater recorded a BOD_5 reduction of 65.5% and 66.3% respectively. The BOD values for RF+*Chlorella* sp. (43.4±2.1) and RF+*Scenedesmus* sp. (42.4±2.2) treated greywater were within the recommended limit (NESREA 2009).

The levels of Iron (Fe), Lead (Pb) and Manganese (Mn) detected in the roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp.-treated greywater samples were within the recommended limits (NESREA, 2009). In this study, the concentrations of heavy metals for the raw greywater, roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp. treated greywater were similar. This indicated that there was no bioaccumulation of these heavy metals by both the *Chlorella* and *Scenedesmus* species during greywater treatment. This contradicts the findings of a study which reported that *Chlorella, Chlamydomonas* and *Scenedesmus* genus of algae could support bioaccumulation of heavy metal like Zn, Pb and Cu from 30 to 200 mg metal/g microalgae (Maznah *et al.*, 2012).

Furthermore, the nitrate level of the raw greywater reduced after treatment by (24.9%) with roughing filter, (72.5%) with RF+*Chlorella* sp. and (75.4%) with RF+*Scenedesmus* sp. This supports the findings of a previous study which documented 53% removal efficiency of total nitrogen using 7.5 Litre closed tubular biofilm bioreactor fed with raw (undiluted) swine slurry (De Godos *et al*., 2009). In addition, Gonza´lez-Ferna´ndez *et al*. (2011) documented 95% removal efficiency of total nitrogen using a 3 Litre open pond fed with fresh slurry. Phosphorous level of the raw greywater was reduced by 12.5%, 35.7% and 42.3% with roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp. respectively. This finding concurs with the study of Wahyunanto *et al*., (2016) who reported that phosphate showed a removal efficiency of between 21% and 32% in the first eight days of cultivation in a secondary wastewater treated effluent. However, reduction in the phosphorus level of greywater treated with RF+*Chlorella* sp. and RF+*Scenedesmus* Sp. could be attributed to the utilization of phosphorus by microalgae for their growth. This finding is similar to the findings of previuous studies that microalgae utilize phosphorus in form of inorganic orthophosphate (PO_4^{-3}) for their growth (Lee and Lee, 2001; Travieso *et al*., 2006).

A comparison of the mean turbidity (NTU), TDS (mg/L) and BOD_5 (mg/L) between RF+*Chlorella* and RF+*Scenedesmus*-treated greywater showed that there was no significant difference. This indicates that treatment of the raw greywater with RF+*Chlorella* and RF+*Scenedesmus* had similar effect. Hence, the two treatment techniques gave similar results. However, the mean nitrate concentration in RF+*Scenedesmus*-treated greywater was significantly lower compared to nitrate concentration in RF+*Chlorella*-treated greywater. This is an indication that *Scenedesmus* sp. uptake more nitrate in the greywater compared to *Chlorella* specie.

Table 4.9: Quality of Greywater Sample Treated with Roughing Filter, *Chlorella sp.* and *Scenedesmus sp.*

Parameter (Units)	Control (Raw greywater)	Roughing filter treated	Algae pond		NESREA	WHO STD
			Chlorella sp.	*Scenedesmus sp.*		
Physicochemical						
pH	6.6±0.5	6.7±0.5	9.1±0.7	8.8±0.5	6-9	6.5-9.5
Temperature (^{0}C)	27.8±1.7	27.8±1.7	28.1±1.8	28.7±1.3	40	12-25
Conductivity (μS/cm)	97.9±15.6	88.4±15.0	88.3±14.2	89.0±14.5	NS	400
Turbidity (NTU)	59.7±6.6	8.6±0.9	8.9±0.9	8.8±0.8	5	5
Oil&Grease (mg/L)	46.8±6.6	25.1±3.5	18.9±2.7	18.7±2.6	10-100	
TDS (mg/L)	60.3±10.5	39.0±7.0	24.4±4.2	25.1±4.9	500	50
TSS (mg/L)	31.5±6.1	27.0±7.7	23.5±6.4	21.9±6.6	25	-
BOD_5 (mg/L)	125.7±6.4	49.1±2.6	43.4±2.1	42.4±2.2	30-50	40
Sulphate (mg/L)	5.4±2.4	5.1±2.2	5.7±2.5	5.9±2.6	300	-
Nutrient						
Nitrate (mg/L)	42.2±2.9	31.7±2.3	11.6±0.8	10.4±0.7	10-15	-
Phosphate (mg/L)	16.8±3.9	14.7±3.4	4.3±2.3	3.4±2.1	2-5	-
Heavy metal						
Fe (mg/L)	2.5±1.2	2.4±1.2	2.6±1.3	2.5±1.2	3	-
Pb (mg/L)	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.1	-
Mn (mg/L)	0.4±0.1	0.4±0.1	0.3±0.1	0.4±0.1		-
Cd (mg/L)	ND	ND	ND	ND	0.1	-
Bacteriological						
Total bacteria count (CFU/100ml)	(2.87±0.5) x10^7	(8.85±0.99)x10^6	(4.05±0.45)x10^6	(3.38±0.39)x10^6	<10^3	-
Total coliform (CFU/100ml)	(8.3±2.1)x 10^3	(1.91±0.30)x10^3	(9.01±0.14)x10^2	(7.86±0.12)x10^2	<10^3	-
Faecal coliform (CFU/100ml)	129.1±32.3	18.3±2.6	8.51±1.2	7.28±1.0	<10^3	-

Table 4.10: Percentage Reduction in Physiochemical and Nutrients Parameters

Parameter (Units)	Control (Raw greywater)	Percentage reduction		
		Roughing filter treated	RF+Chlorella sp.	RF+ Scenedesmus sp.
Physicochemical				
Turbidity (NTU)	59.7±6.6	8.6±0.9 [85.59]	8.9±0.9 [85.09]	8.8±0.8 [85.30]
TDS (mg/L)	60.3±10.5	39.0±7.0 [35.3]	24.4±4.2 [59.5]	25.1±4.9 [58.4]
BOD_5 (mg/L)	125.7±6.4	49.1±2.6 [60.9]	43.4±2.1 [65.5]	42.4±2.2 [66.3]
Nutrient				
Nitrate (mg/L)	42.2±2.9	31.7±2.3 [24.9]	11.6±0.8 [72.5]	10.4±0.7 [75.4]
Phosphate (mg/L)	16.8±3.9	14.7±3.4 [12.5]	4.3±2.3 [35.7]	3.4±2.1 [42.3]

Note: Values in parenthesis are % reduction.

Table 4.11: Comparison of Physicochemical and Nutrient Removal by *Chlorella sp.* and *Scenedesmus sp.*

Parameter (Units)	Algae pond		t-test	p-value
Physicochemical	***Chlorella sp.***	***Scenedesmus sp.***		
Turbidity (NTU)	8.9±0.9	8.8±0.8	0.492	0.624
TDS (mg/L)	24.4±4.3	25.1±4.9	-0.665	0.508
BOD_5 (mg/L)	43.4±2.1	42.4±2.2	1.917	0.059
Nutrient				
Nitrate (mg/L)	11.6±0.8	10.4±0.7	6.093	<0.001
Phosphate (mg/L)	4.3±2.3	3.4±2.1	1.786	0.079

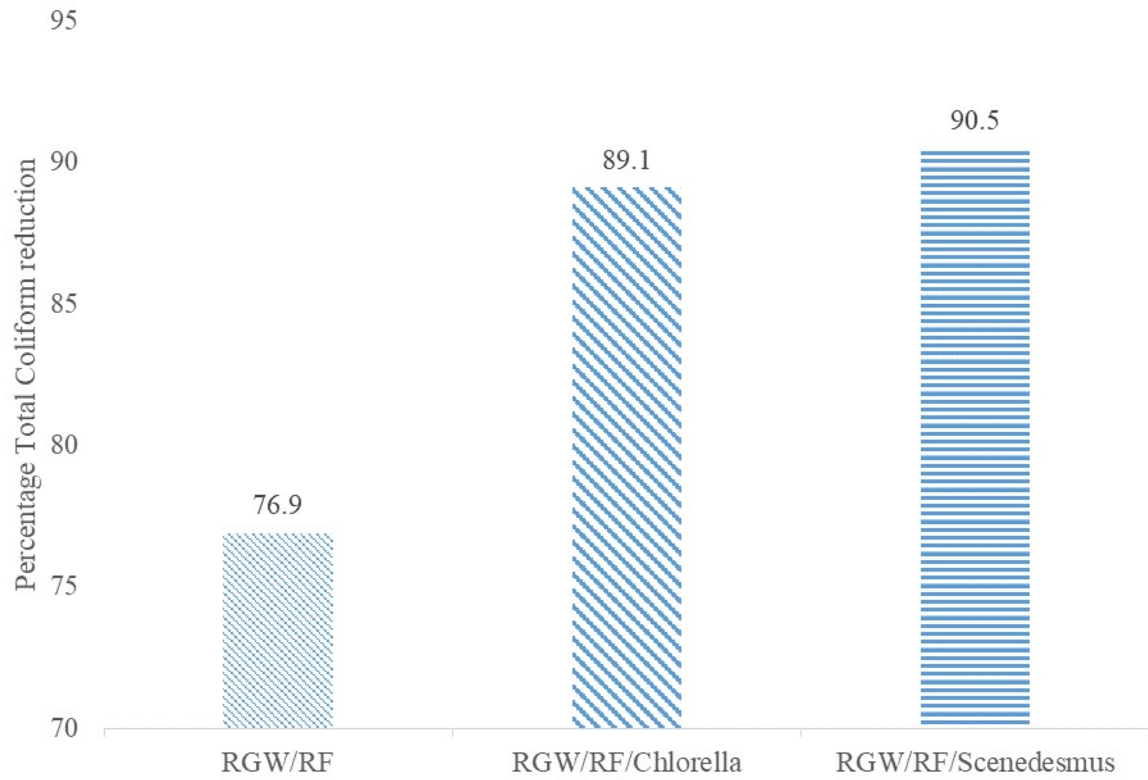
The *Eshericia coli* Log Reduction Value (LRV) was computed to assess the performance of the RF+*Chlorella* sp. and RF+*Scenedesmus* sp. treatment system in the removal of *Eshericia coli* (*E. coli*). This indicated the number of log units by which *E. coli* in the greywater was reduced during treatment with the RF+*Chlorella* and RF+*Scenedesmus* as shown in Table 4.12. In addition Figure 4.5 depicts the percentage reduction of *E. coli* from the raw greywater through the roughing filter to the *Chlorella* treatment unit and *Scenedesmus* treatment unit. This is an indication that RF+*Scenedesmus* sp. had the highest percentage *E.coli* reduction. LRV values of 0.638, 0.964 and 1.023 were recorded for the treatment of the raw greywater with roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp. respectively. The LRV values were equivalence of 76.9%, 89.1% and 90.5% reduction of *E. coli* with roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp. respectively.

Escherichia coli has often being used as an indicator of a potential disease transmission pathogens. This study found that the Log Reduction Values (LRVs) of 0.638, 0.964 and 1.023 were recorded for the treatment of the raw greywater with Roughing filter, RF+*Chlorella* and RF+*Scenedesmus* respectively. The LRV values were equivalent of 76.9%, 89.1% and 90.5% reduction of *E. coli* with Roughing filter, RF+*Chlorella* and RF+*Scenedesmus* respectively. Increase in the pH of algae media of cultivation has been attributed to an enhanced deactivation of pathogens in the bioreactor (Mun˜oz and Guieysse, 2006). Indeed, uptake of CO_2 by algae could elevate the pH in High Rate Algae ponds and closed bioreactors to about 10–11 (Posadas *et al*., 2014). The increase in pH is beneficial for the disinfection of pathogenic microorganism. Significanlty higher *E. coli* removals at pH 9.5 (100%) has been observed compared to pH 8 (50%) in a High Rate Algae Pond treating domestic wastewater (Heubeck *et al*., 2007).

Table 4.12: Log Removal Value (LRV) of *E. coli* Coliform

Treatment	**LRV**	**Final LRV**	**% reduction**
Raw Greywater → RF	0.638	0.638	76.9
Raw Greywater → RF → *Chlorella* treatment	0.638+0.326	0.964	89.1
Raw Greywater → RF → *Scenedsmus* treatment	0.638+0.385	1.023	90.5

RF = Roughing filter



Note: RGW= Raw greywater; RF=Roughing filter

Figure 4.5: Removal Efficiency of Roughing Filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp.

4.7 Predictors of *Chlorella* sp. Biomass Concentration

Predictors of chlorella sp. biomass concentrations were estimated using Mutiple Regression Models (Table 4.13). Concentrations of nitrates, phosphate, BOD_5, Crude protein and chlorophyll_a were used in the equation. Nitrate and BOD_5 concentration had a negative regression coefficient (-32.42 and -2.07). This indicated that as the Nitrate and BOD_5 level in the greywater decreased, there was an increase in *Chlorella* sp. biomass. Biochemical oxygen demand had the lowest β-value (-0.033). This shows that there are other factors apart from change in the BOD_5 which play a major role in the variation observed in the *Chlorella* sp. biomass concentration during the experiment. The highest β-value (1.054) observed for chlorophyll_a indicates that it is a major factor that explains the dependent variable (*Chlorella* sp. biomass concentration) when the variance explained by all other variables in the model is controlled for. Chlorophyll_a, Nitrate and phosphorus contributes significantly in explaining the concentration of *Chlorella* sp. biomass ($p = 0.001$). A significant fitted model is observed, hence the following equation represents the regression model:

$$Qcb = 46.46ChA + 14.98Pht - 32.42Nt - 2.07BOD - 23.86$$

Where;

Qcb = Biomass concentration of *Chlorella* sp.

ChA = Chlorophyll_a

Pht = Phosphorus

Nt = Nitrate

BOD = Biochemical Oxygen Demand

Furthermore, a significant inverse relationship existed between biomass concentration of *Chlorella* sp. and nitrate of the greywater as shown in Figure 4.6. Likewise, an inverse relationship was observed between biomass concentration of *Chlorella* sp. and phosphorus of the greywater (Figure 4.7), the relationship was significant. This shows that increase in the biomass concentration of *Chlorella* sp. brought about the reduction in phosphorus concentration of the greywater. Also, an inverse relationship existed between biomass

concentration of *Chlorella* sp. and BOD of the greywater (Figure 4.8). The relationship was not significant. A significant positive relationship was observed between biomass concentration of *Chlorella* sp. and Chlorophyll_a concentration (Figure 4.9). This indicated that an increase in the Chlorophyll_a content of the *Chlorella* sp. could bring about rise in the biomass concentration of *Chlorella* sp..

This study found that Nitrate and BOD_5 concentrations had a negative regression coefficient (-32.42 and -2.07) with the *Chlorella sp.* biomass concentration. This indicated an increase in *Chlorella sp.* biomass concentration. However, BOD showed the lowest β-value (-0.033). This indicates that there are other factors apart from change in the BOD_5 which play a major role in the variation observed in the *Chlorella* sp. biomass concentration during the experiment. In addition, Chlorophyll_a had the highest β-value (1.054), indicating that Chlorophyll_a produced the major contribution to explaining the increase in the *Chlorella* sp. biomass concentration. The regression model established in this study revealed that Chlorophyll_a of the algae, nitrate and phosphorous concentration in the greywater contributed significantly in explaining the increase in the *Chlorella* sp. biomass concentration.

This study found that high phosphorous in the greywater contibuted to an increase in the biomass concentration of *Chlorella* sp. Also, positive relationship was observed between biomass concentration of *Chlorella* sp. and Chlorophyl_a concentration. This indicated that an increase in the Chlorophyll_a content of the *Chlorella* sp. could bring about a rise in the biomass concentration of *Chlorella* sp.

Table 4.13: Predictors of concentration of *Chlorella* sp. Biomass

Variables	R square	Adjusted R square	α (coefficient)	B	F/t (p Value)
Model	0.953	0.927			36.819 (<0.001)
Nitrate			-32.42	-0.251	-3.094 (0.013)
Phosphorus			14.98	0.219	2.627 (0.027)
BOD_5			-2.07	-0.033	-0.419 (0.685)
Crude protein			26.69	0.319	3.312 (0.009)
Chlorophyll_a			46.46	1.054	12.767 (<0.001)
Constant			-23.86		-0.096 (0.925)

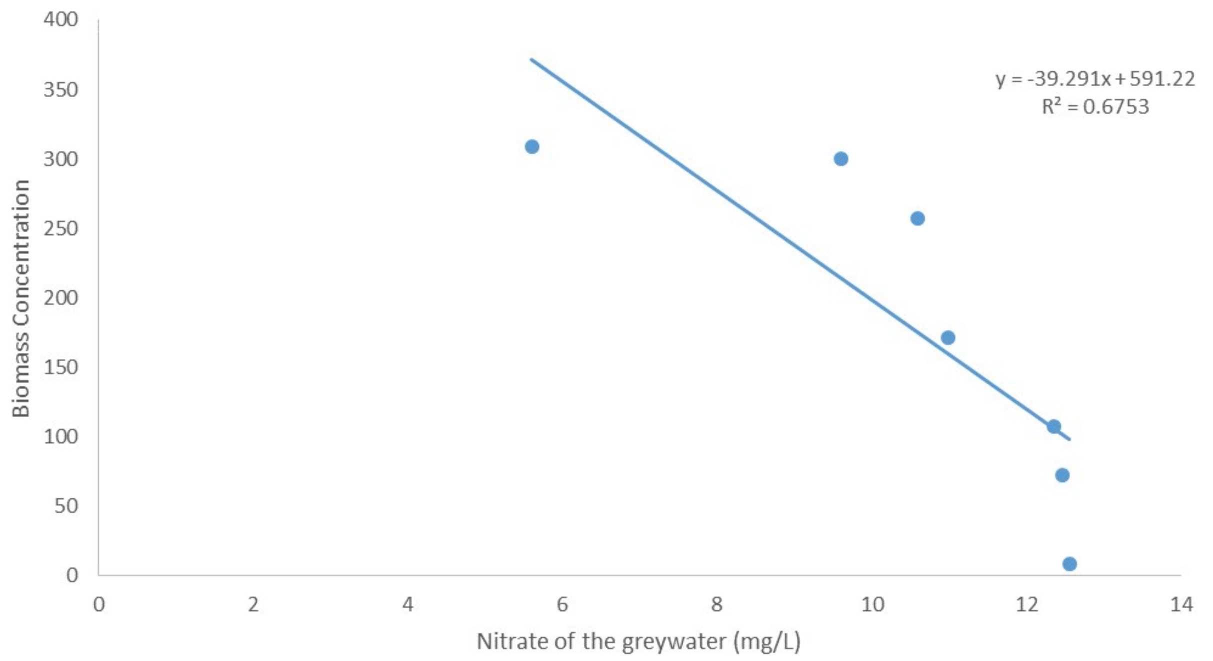


Figure 4.6: Scatter Diagram of Biomass Concentration of *Chlorella* sp. and Nitrate of the Greywater

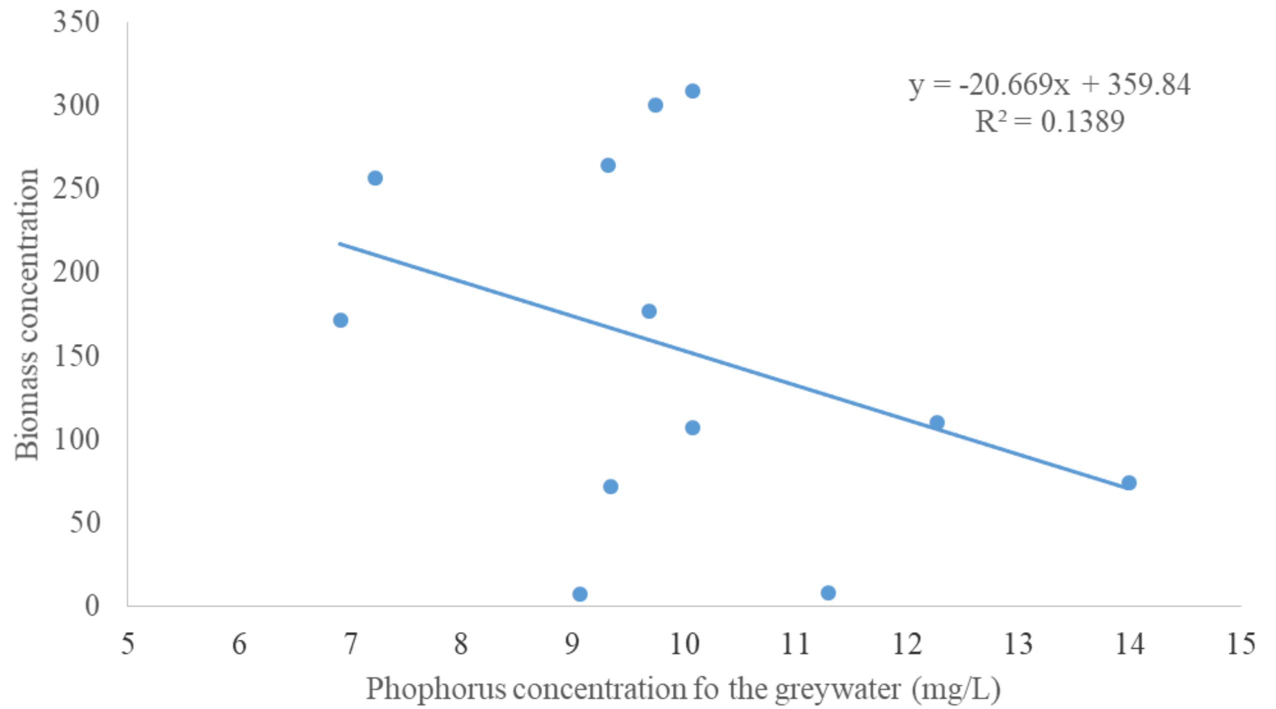


Figure 4.7: Scatter Diagram of Biomass Concentration of *Chlorella* sp. and Phosphate of Greywater

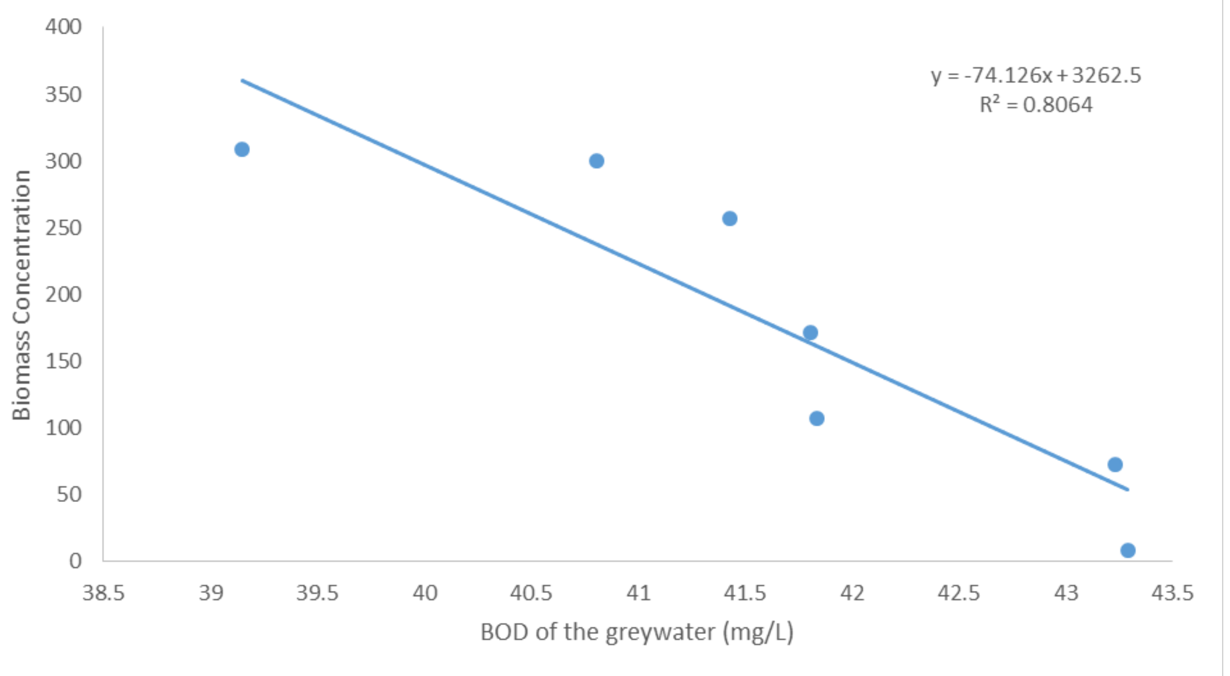


Figure 4.8: Scatter Diagram of Biomass Concentration of *Chlorella* sp. and BOD of Greywater

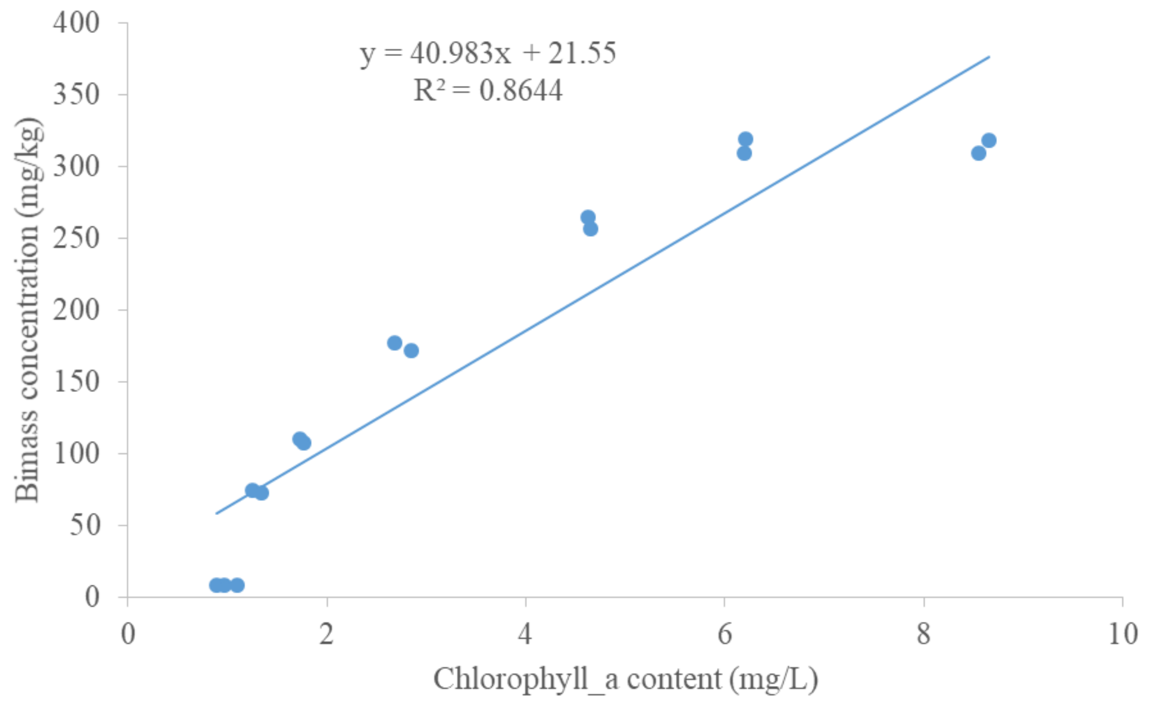


Figure 4.9: Scatter Diagram of Biomass Concentration and Chlorophyll_a Content of *Chlorella* sp.

4.8: Predictors of *Scenedesmus* sp. Biomass Concentration

Table 4.14 presents the Regression Model to relationship between biomass concentration of *Scenedesmus* sp. and concentration of nitrates, phosphate, BOD_5, crude protein and chlorophyll_a. The table revelaed that Nitrate and BOD_5 concentration produced a negative regression coefficient (-20.6 and -0.109). This indicated an increase in *scenedesmus* sp. biomass in the greywater. Biochemical oxygen demand had the lowest β-value (-0.003). This indicates that there are other factors apart from change in the BOD_5 which play a major role in the variation observed in the *Scenedesmus* sp. biomass concentration during algae cultivation. The highest β-value was recorded for Chlorophyll_a (1.071) which revealed that chlorophyll_a had the main contribution to explaining the increase in *Scenedesmus* sp. biomass concentration when the variance explained by all other variables in the model is controlled for. Significantly Chlorophyll_a, Nitrate and Phosphorous contributed to the increase in biomass concentration of *Scendesmus* sp. A significant fitted model is observed, hence the following equation represents the regression model:

$$QScb = 25.57ChA + 4.46Pht - 20.6Nt - 0.109BOD + 27.87$$

Where;

QScb = Biomass concentration of Scenedesmus sp.

ChA = Chlorophyll_a

Pht= Phosphorous

Nt = Nitrate

BOD = Biochemical Oxygen Demand

Furthermore, a significant inverse relationship existed between biomass concentration of *Scenedemus* sp. and nitrate of the greywater as depicted in Figure 4.10. Also, an inverse, non-significant relationship was observed between biomass concentration of *Scenedesmus* sp. and phosphorus concentration of the greywater (Figure 4.11). Figure 4.12 illustrates that an inverse relationship existed between biomass concentration of *Scenedesmus* sp. and BOD of the greywater. The relationship was not significant. Furthermore, there was a

significant positive relationship between biomass concentration of *Scenedesmus* sp. and Chlorophyll_a concentration (Figure 4.13).

This study found that Nitrate and BOD_5 concentration of the greywater produced a negative regression coefficient (-20.6 and -0.109) with *Scenedesmus* sp. biomass concentration. This shows an increase in *Scenedesmus* sp. biomass in the greywater. Also, this shows that there are other factors apart from change in the BOD_5 which play a major role in the variation observed in the *Scenedesmus* sp. biomass concentration during algae cultivation. The highest β-value was recorded for Chlorophyll_a (1.071) which revealed that chlorophyll_a had the main contribution to explaining the increase in *Scenedesmus* sp. biomass concentration. Significantly, Chlorophyll_a, Nitrate and Phosphorous contribute in explaining the increase in biomass concentration of *Scendesmus* sp.

Furthermore, a significant, positive relationship existed between biomass concentration of *Scenedemus* sp. and nitrate concentration of the greywater. Also, positive relationship was observed between biomass concentration of scenedesmus sp. and phosphorous concentration of the greywater.

Table 4.14: Predictors of Concentration of *Scenedesmus* sp. Biomass

Variables	R square	Adjusted R square	α (coefficient)	B	F/t (p Value)
Model	0.942	0.910			29.229 (<0.001)
Nitrate			-20.60	-0.250	-2.546 (0.031)
Phosphorus			4.46	0.102	1.164 (0.274)
BOD_5			-0.109	-0.003	-0.033 (0.974)
Crude protein			10.47	0.277	2.522 (0.033)
Chlorophyll_a			25.57	1.071	11.173 (<0.001)
Constant			27.87		0.182 (0.860)

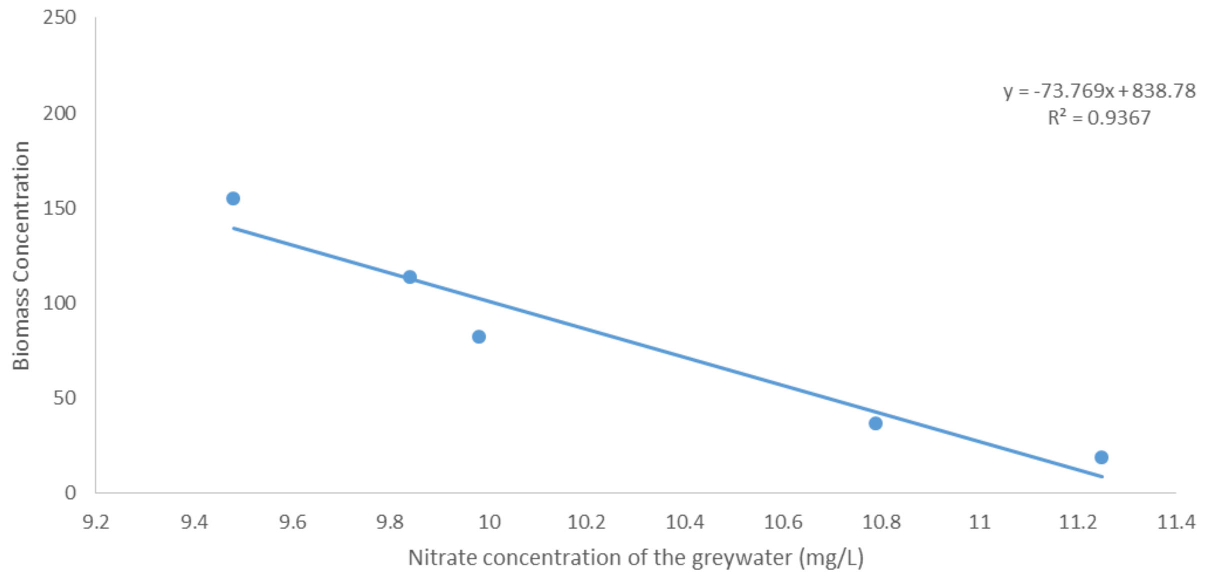


Figure 4.10: Scatter Diagram of Biomass Concentration of *Scenedesmus* sp. and Nitrate of the Greywater

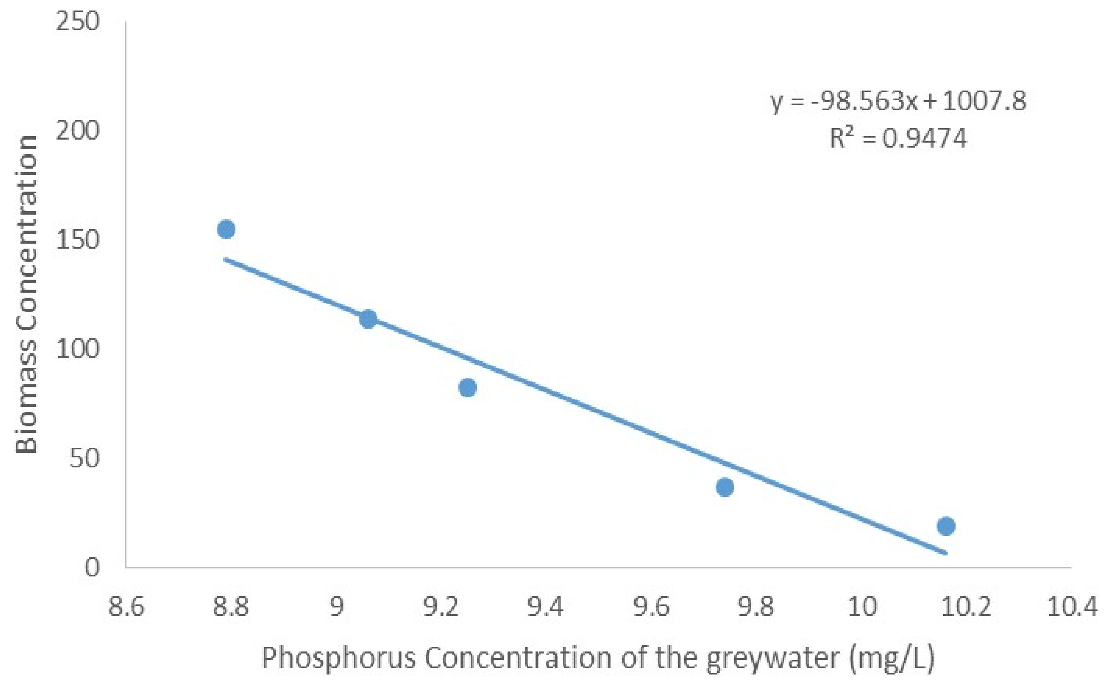


Figure 4.11: Scatter Diagram of Biomass Concentration of *Scenedesmus* sp. and Phosphate of Greywater

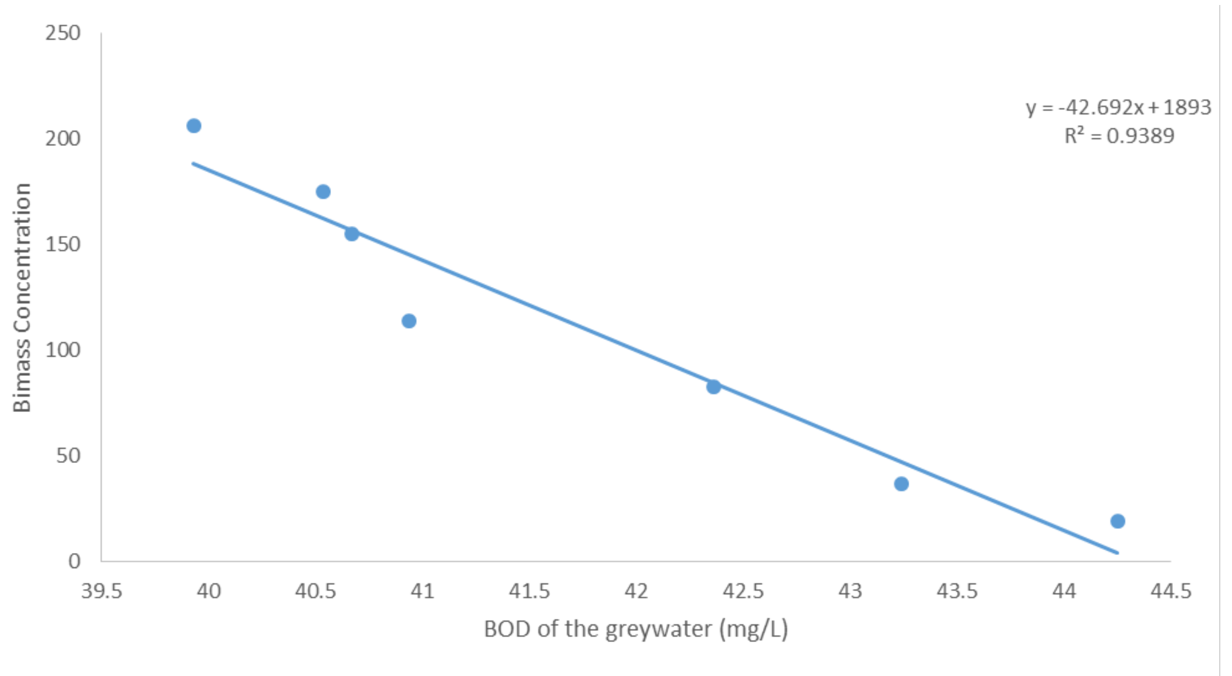


Figure 4.12: Scatter Diagram of Biomass Concentration of *Scenedesmus* sp. and BOD of Greywater

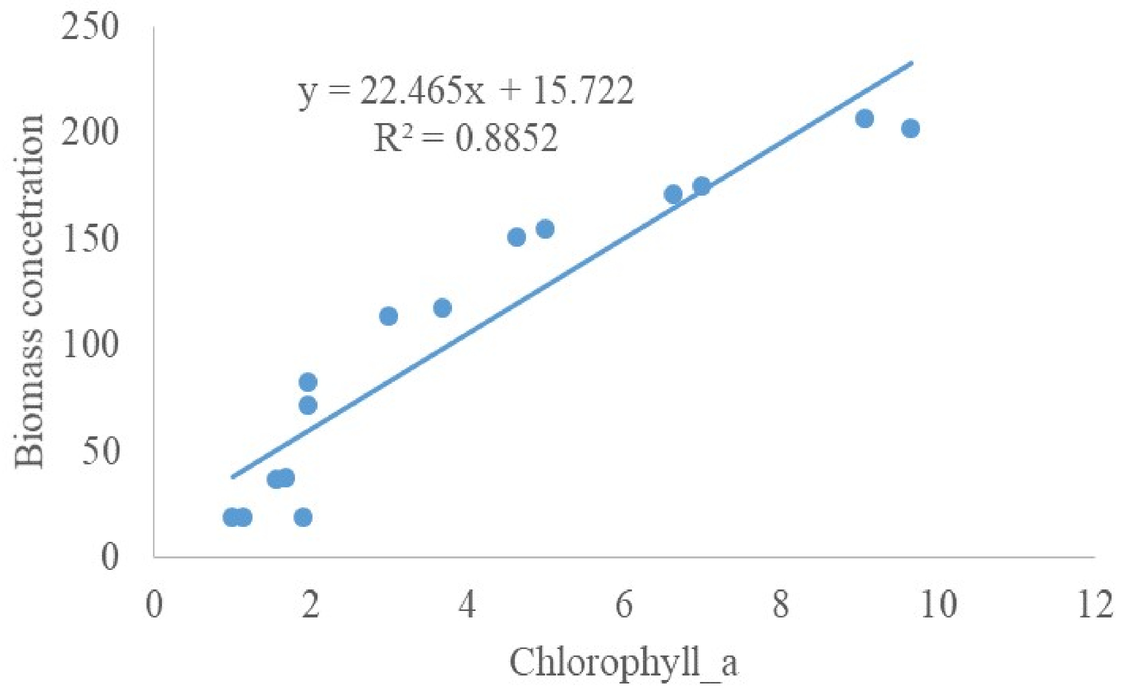


Figure 4.13: Scatter Diagram of Biomass Concentration and Chlorophyll_a Content of *Scenedesmus* sp.

4.9 Distribution of Rainfall, Relative Humidity, Sun Cycles and Temperature in Ibadan (April. 2017 to June 2019)

Table 4.15 presents the distribution of meteorological information: rainfall, temperature (minimum and maximum), relative humidity and Sun cycle. Highest average monthly rainfalls in the year 2017, 2018 and 2019 was recorded in the month of July (22.7±21.8 mm), May (23.4±20.9 mm) and June (26.7±36.1 mm) respectively. The maximum mean temperature (^{0}C) was recorded in the month of January of year 2019 (35.2±1.4) and 2018 (34.5±1.2). Furthermore, the normality of the rainfall and relative humidity data was assessed as shown in Appendices VIII and IX. The tables reveal that the rainfall and relative humidity differ across the twelve months in a year. This is an indication that the meteorological conditions were not similar across the months. Furthermore, highest sun cycles (hours) were observed in the month of December in the year 2017 (7.3±1.2) and year 2018 (7.1±1.5). The monthly variations in sun cycle over the 12 months (Jan-December) in the three years were presentend in Apendices X, XI and XII. The figures revealed that the least sun cycle (hours period of sun/day) was observed in the month of August of the three years while higher sun cycle was recorded in months of January and December.

Temperature is one of the essential weather conditions that affect the growth of algae. For example, Munoz, *et al.* (2006) reported an increased treatment efficiency of symbiotic culture containing *Chlorella sorokiniana* and *Ralstonia basilensis* at an elevated temperature of between 25 °C to 30 °C. Temperature higher than the room temperature could increase the activities of some green algae species during cultivation. The meteorological data however revealed that the ambient temperature of the study area was within the temperature range suitable for the growth of the algae (*Chlorella and Scenedesmus*). The rainfall and relative humidity varied across the twelve months. This variation indicates that the meteorological conditions in an area are not similar across the months. Furthermore, the highest sun cycles (hours) observed were in the month of January and December. Variations in sun cycle were recorded across other months within the three years of data collection. These variations could affect the algal cultivation and production.

Table 4.15: Distribution of Rainfall, RH, Sun Cycles and Temperature in Ibadan from April. 2017 to June 2019

Year	Month	TempMax. (^{0}C)	TempMin. (^{0}C)	Rainfall (mm)	RH (%)	Sun cycle (Hrs)
2017	April	33.5±1.2	23.6±1.7	15.5±14.6	77.6±13.7	6.6±1.6
	May	32.1±1.9	23.5±1.6	19.1±14.5	79.5±9.6	6.3±2.4
	June	30.9±1.6	22.9±1.2	18.5±20.3	82.0±11.1	4.4±1.8
	July	29.1±1.7	22.6±0.8	22.7±21.8	78.4±14.6	2.9±1.6
	Aug.	27.7±1.6	21.9±0.8	12.9±8.9	79.3±15.1	2.4±1.3
	Sept.	29.2±1.5	22.1±0.9	20.9±17.4	79.2±11.5	3.7±1.8
	Oct.	31.9±1.5	23.1±1.4	10.2±9.1	80.8±8.1	5.8±1.8
	Nov.	34.4±1.1	24.3±0.7	NR	78.2±6.9	6.5±1.7
	Dec.	34.9±1.5	23.5±1.4	27.6±27.4	80.4±9.5	7.3±1.2
2018	Jan.	34.5±1.2	22.1±1.4	NR	81.5±4.7	5.5±3.2
	Feb.	35.2±2.3	24.4±1.6	4.3±2.8	83.3±4.1	5.7±2.1
	Mar.	34.5±1.2	23.9±1.7	12.1±13.0	86.7±3.8	6.9±1.5
	Apr.	32.7±1.7	23.9±1.5	22.5±20.9	86.7±3.7	5.4±3.2
	May	31.9±1.4	23.1±1.7	23.4±20.9	86.0±4.6	5.9±2.9
	June.	30.4±1.9	22.7±1.3	22.8±18.7	82.5±2.7	4.5±2.7
	Jul.	28.7±1.8	22.4±0.8	14.2±14.0	81.6±3.1	2.8±2.5
	Aug.	28.2±1.6	21.9±0.7	14.8±16.1	59.9±10.5	1.6±1.9
	Sep.	29.9±2.2	22.5±1.0	16.8±24.6	79.9±10.9	4.2±2.6
	Oct.	31.9±1.4	22.8±1.3	22.7±29.1	80.6±9.0	6.3±3.0
	Nov.	33.1±1.4	23.7±1.4	13.7±10.2	81.7±8.6	7.0±2.7
	Dec.	34.6±1.1	25.1±0.0	NR	82.2±10.2	7.1±1.5
2019	Jan.	35.2±1.4	23.9±1.6	NR	63.3±13.2	6.0±2.3
	Feb.	35.5±2.0	24.7±1.4	4.8±4.8	68.8±14.8	3.6±2.8
	Mar.	34.9±1.4	24.1±1.8	22.6±24.8	74.9±4.8	6.7±2.0
	Apr.	34.1±1.9	24.5±1.4	17.0±27.1	78.0±3.1	6.8±2.8
	May	32.5±1.8	23.8±1.2	23.2±27.2	80.3±3.0	6.0±3.3
	June.	30.4±1.3	23.1±1.1	26.7±36.1	85.2±3.4	4.3±2.8

Note: NR=No rain

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1: Conclusion

This study found that high quantity of water is consumed by households within the community and laundry activity consumed more water compared to bathing and kitchen. Greywater is generated in high quantity from all the three sources (laundry, bathing and kitchen) in Kube-atenda community, more greywater is generated from laundry activity.

The values of turbidity, Biochemical Oxygen Demand the greywater before the treatment were higher compared to the NESREA and WHO's recommended limits for wastewater. Similarly, Nitrate, phosphate concentration in the greywater exceeded the recommended limit of wastewater by NESREA. Also, Iron, manganese and lead were found in varying concentration in the greywater while cadmium was not detected. The study reveals that bacteria and coliform counts were very high in the greywater before the treatment. Also, untreated greywater contained disease-causing bacteria such as Coliform organisms such as *Enterobacter, Aeromonas, and faecal indicator organisms-Salmonella* and *Eschericia coli*.

It was found that variations occur among the optimal concentration of algae (*Chlorella* sp. and *Scenedesmus* sp.) inoculum during the laboratory experiment. The highest growth of the cultured algae was observed on the 10th day of culture in both (BBM and greywater) media. However, among the different concentrations of the inoculum, 20% *Chlorella sp.* and *Scenedesmus sp.* showed better growth performance than other concentrations in the 250 ml greywater. This optimal (20%) algae inoculum (*Chlorella* sp. and *Scenedesmus* sp.) produced the highest Chlorophyll-a and biomass concentration and was therefore selected for the out-door greywater treatment.

Furthermore, concentration of nitrate and phosphorus were reduced at 20% algae (*Chlorella sp. and Scenedesmus sp.*) inoculum. The study found a higher biomass growth

rate for both the *Chlorella* sp. and *Scenedesmus* sp. algae during cultivation. Chlorophyll_a, nitrate and phosphorous contribute to the increase in biomass concentration of *Chlorella sp. and Scendesmus* sp.. The treatment process produced *Chlorella sp. and Scenedesmus sp.* biomass with higher protein content. However, Crude protein (%) of the *Scenedesmus* sp. was significantly higher compared to the value obtained for *Chlorella* sp. after the experiment.

Furthermore, data from the study revealed a high reduction in the turbidity level of the greywater water after RF+*Chlorella* sp. and RF+*Scenedesmus* sp. treatments. The study found that the heavy metal values for the raw greywater, roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* sp. treated greywater were similar. Also, this study shows high reduction in the BOD_5 (mg/L), nitrates and phosphorous values of greywater after the outdoor treatment with roughing filter, RF+*Chlorella* sp. and RF+*Scenedesmus* Sp.. However, the finding has revealed that the combined method of treatment- RF+*Chlorella* sp. and RF+*Scenedesmus* sp. was more effective in terms of BOD_5, nitrates and phosphorous for the treatment of the greywater source as the starting point of the experiment.

5.2: Recommendations

Based on the findings of this study, therefore, the following recommendations are proposed:

i. The findings of this study have shown that greywater from households contain nutrients in quantity that is enough for plant growth, particularly small plants like algae. The greywater could be used to grow algae for economic gain instead of indiscriminate greywater disposal at the community level.

ii. There should be further study on the effect of the algae produced from greywater management on the growth of a specified animals like fish, chicken etc. this would improve animal production, food security, job creation and better environmental sanitation

iii. People at the community level should be trained and involved in greywater management for economic gain in Nigeria

iv. There should be effective greywater management technology at the household or community level across the country to reduce the menace of indiscriminate disposal of greywater into the environment.

v. There is need for more research on the design and implementation of simple and easy to maintain algae-based greywater management technology at the community level.

vi. There should be a further research on the effect of varying pH and temperature on the algae growth rate.

vii. Households should be encourage to develop an easy to maintan greywater collection and treatment unit that would promote resource recovery for sustainable development.

5.3: Limitation of the Study

The results presented in this study relied strictly on the data obtained from the pilot experiments. The study was conducted on a batch scale for 12 days per batch over a three months period. This is a limited period for the study and perhaps not adequate to determine the variation in the outdoor environmental condition on the cultivation of the algae on the greywater and to explain the trends in the biomass yield of the biomass. The study did not analyse the algae biomass for the possible accumulation of potential health risk constituents. The current research scope and means could not cover this complex theme.

5.4: Contributions to Knowledge

This study recorded high percentage reduction in BOD_5, nitrate and phosphorous values of greywater treated with RF+*Chlorella* sp. and RF+*Scenedesmus* sp.. The finding has revealed that the combined method of treatment- RF+*Chlorella* sp. and RF+*Scenedesmus* sp. was more effective in terms of BOD_5, nitrates and phosphorous for the treatment of the greywater source as the starting point of the experiment. Also, the treatment process produced *Chlorella* sp. and *Scenedesmus* sp. biomass with higher protein content.

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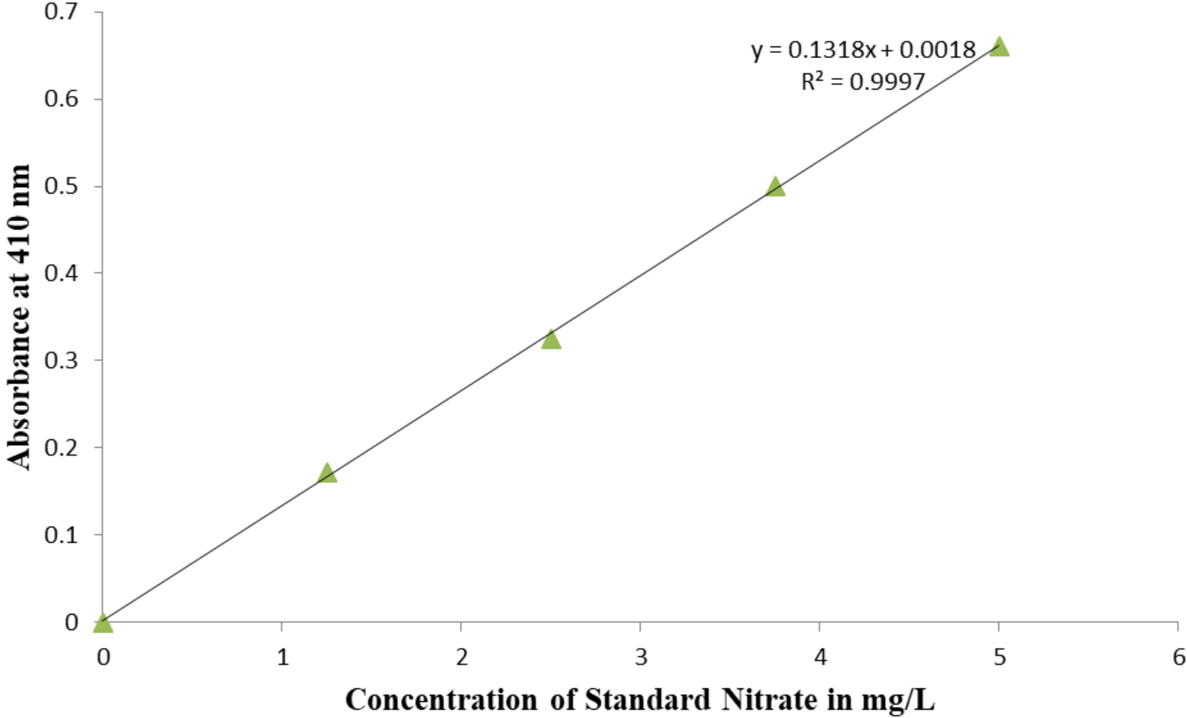
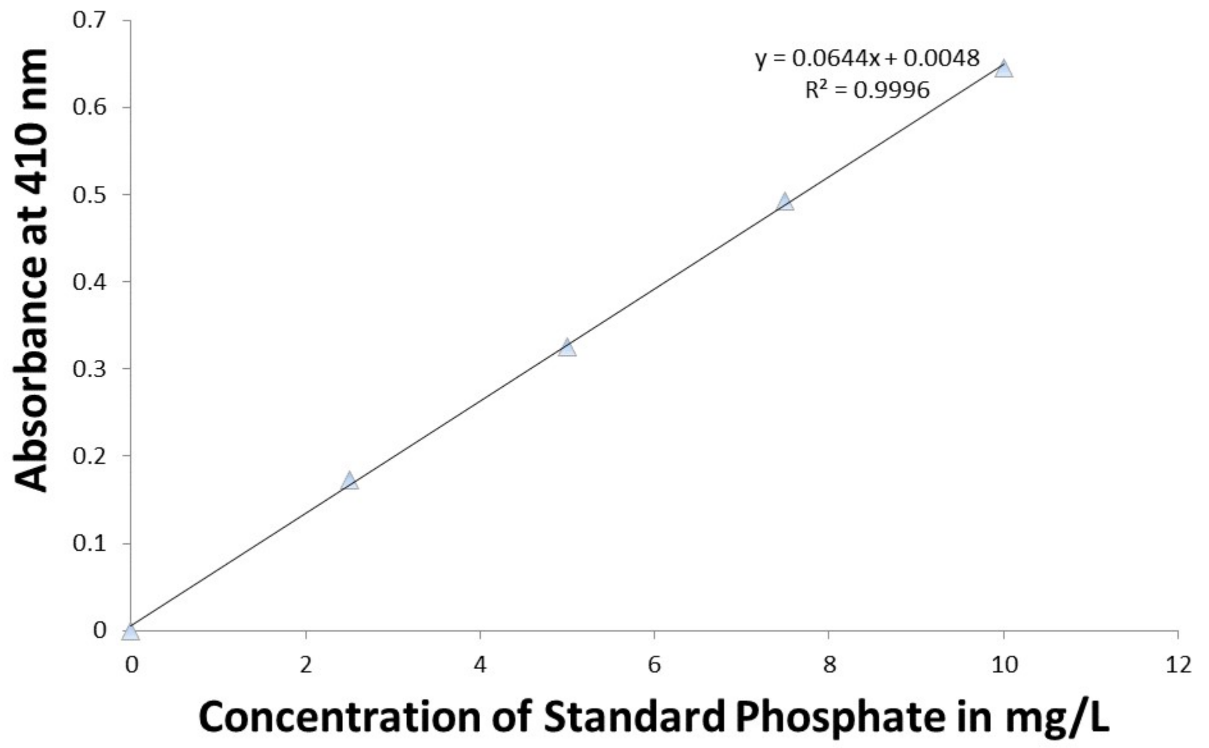
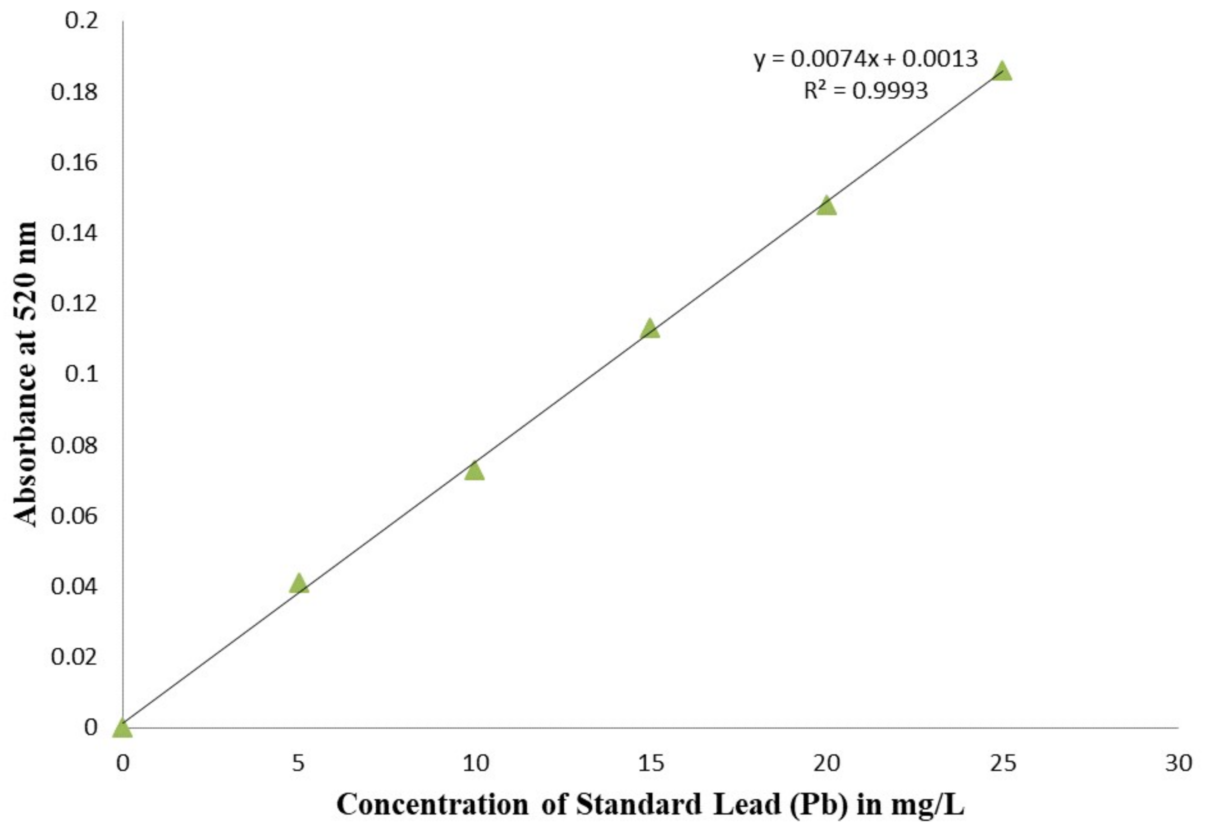
APPENDIX I: Standard curves of Nitrate



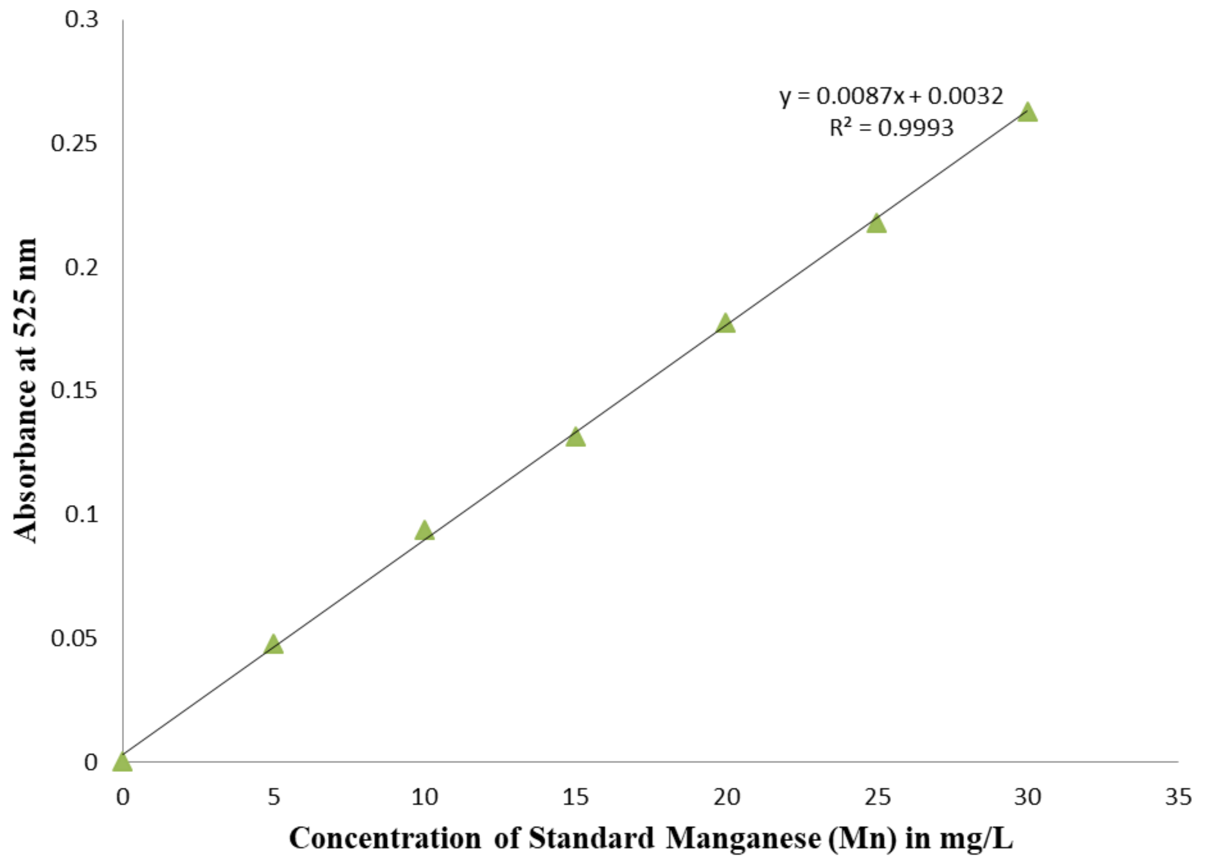
APPENDIX II: Standard curves of phosphate-Phosphorous



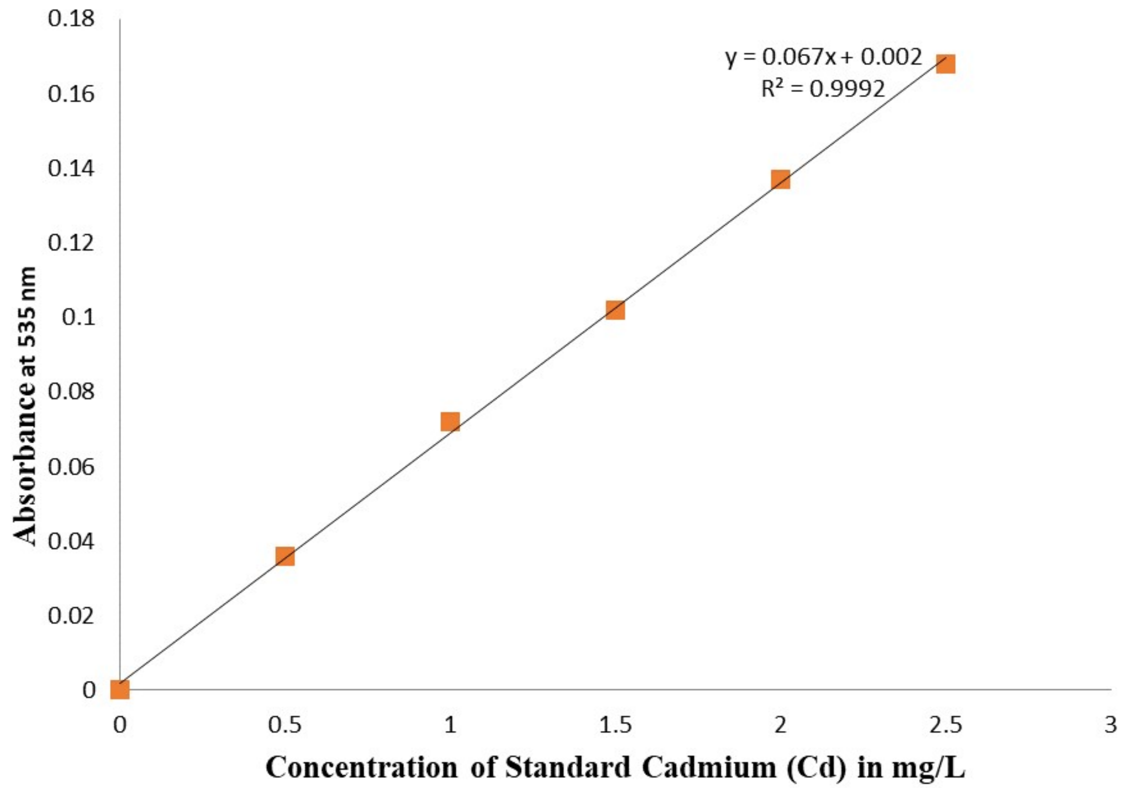
APPENDIX III: Standard curves of Lead (Pb)



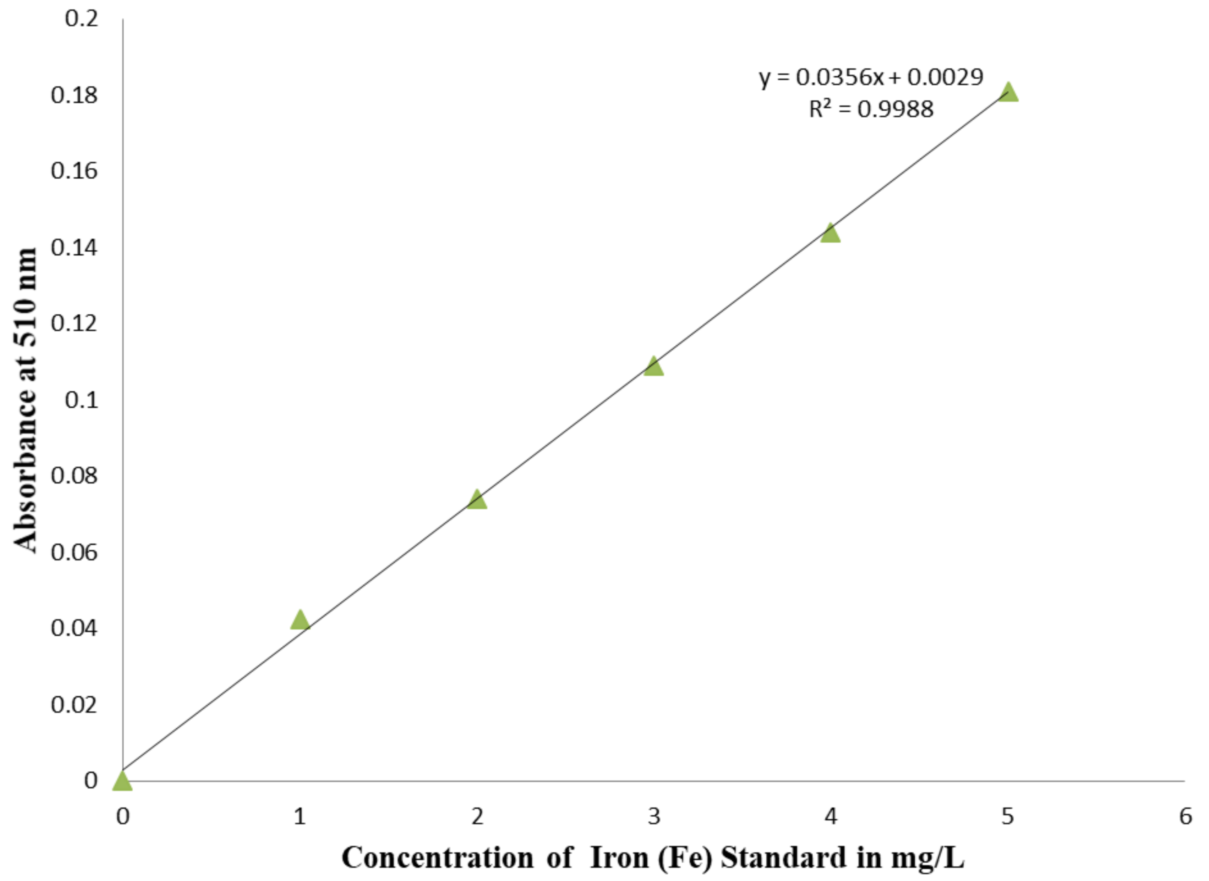
APPENDIX IV: Standard curves of Manganese (Mn)



APPENDIX V: Standard curves of cadmium (Cd)



APPENDIX VI: Standard curves of Iron (Fe)



Appendix VII: Ethical approval

INSTITUTE FOR ADVANCED MEDICAL RESEARCH AND TRAINING (IAMRAT)

College of Medicine, University of Ibadan, Ibadan, Nigeria.

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UI/UCH EC Registration Number: **NHREC/05/01/2008a**

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Improving Greywater quality and Harnessing water Resources through Algae based Technology in a Low to Middle-Income Community in South-West, Nigeria

UI/UCH Ethics Committee assigned number: UI/EC/17/0144

Name of Principal Investigator: **Adejumo Mumuni**

Address of Principal Investigator: Department of Environmental Health Sciences, College of Medicine, University of Ibadan, Ibadan

Date of receipt of valid application: 02/05/2017

Date of meeting when final determination on ethical approval was made: **N/A**

This is to inform you that the research described in the submitted protocol, the consent forms, and other participant information materials have been reviewed and *given full approval by the UI/UCH Ethics Committee.*

This approval dates from **07/11/2017 to 06/11/2018.** If there is delay in starting the research, please inform the UI/UCH Ethics Committee so that the dates of approval can be adjusted accordingly. Note that no participant accrual or activity related to this research may be conducted outside of these dates. *All informed consent forms used in this study must carry the* UI/UCH EC *assigned number and duration of* UI/UCH EC *approval of the study.* It is expected that you submit your annual report as well as an annual request for the project renewal to the UI/UCH EC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the Code including ensuring that all adverse events are reported promptly to the UI/UCH EC. *No changes are permitted in the research without prior approval by the* UI/UCH EC *except in circumstances outlined in the Code. The* UI/UCH EC *reserves the right to conduct compliance visit to your research site without previous notification.*

Professor Catherine O. Falade

Director, IAMRAT

Chairperson, UI/UCH Ethics Committee

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Research Units • Genetics & Bioethics • Malaria • Environmental Sciences • Epidemiology Research & Service • Behavioural & Social Sciences • Pharmaceutical Sciences • Cancer Research & Services • HIV/AIDS

Appendix VIII: Distribution patern of rainfall across the year 2017, 2018 and 2019

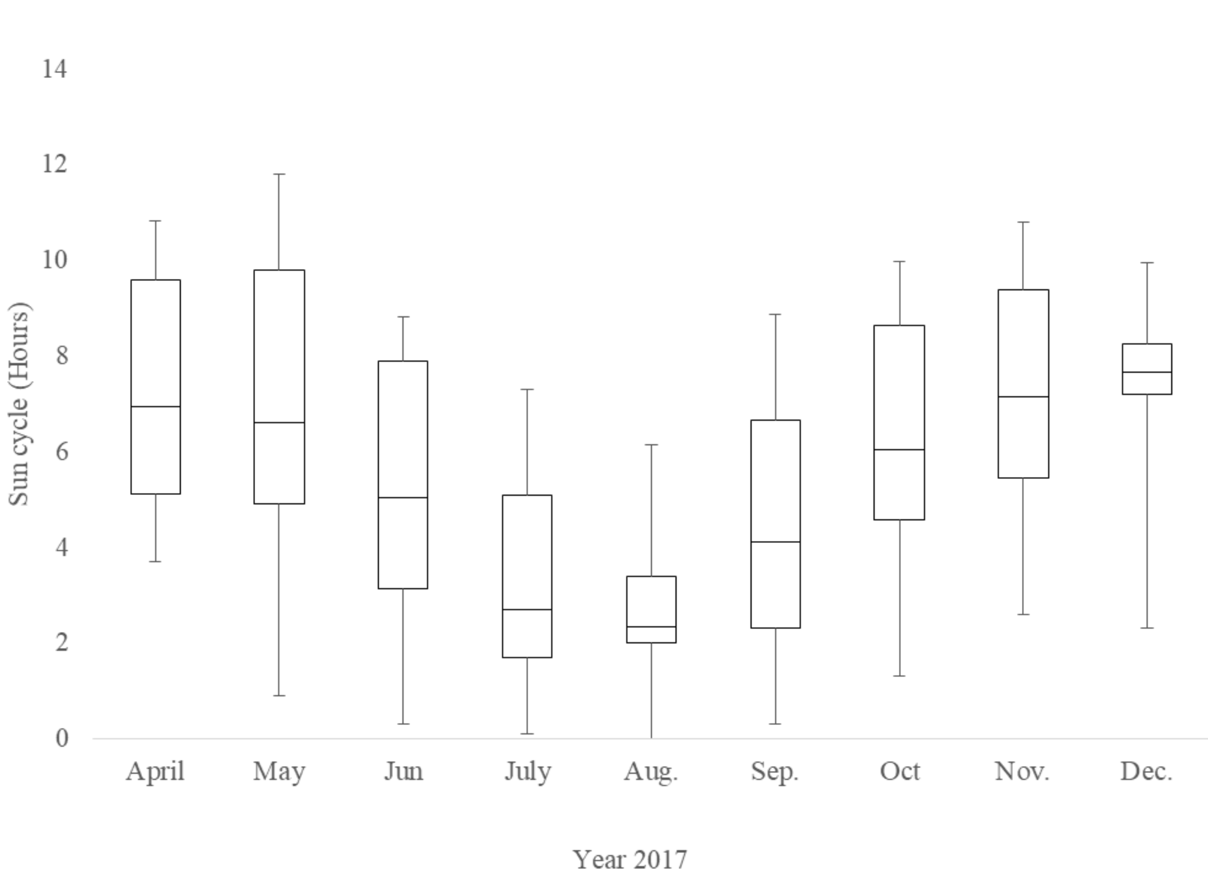
Year	Month	Rainfall (mm)	Min. – Max. values	Kurtosis
2017	April	15.5±14.6	0.5-42.4	0.881
	May	19.1±14.5	1.2-42.0	1.361
	June	18.5±20.3	1.1-73.4	2.696
	July	22.7±21.8	0.8-71.5	1.004
	Aug.	12.9±8.9	1.0-25.7	1.198
	Sept.	20.9±17.4	2.0-50.0	0.938
	Oct.	10.2±9.1	1.5-25.3	0.926
	Nov.	NR	NR	NR
	Dec.	27.6±27.4	8.2-47.0	-
2018	Jan.	NR	NR	NR
	Feb.	4.3±2.8	1.6-7.2	-
	Mar.	12.1±13.0	1.2-37.2	1.613
	Apr.	22.5±20.9	6.0-57.4	2.491
	May	23.4±20.9	2.5-76.0	2.857
	Jun.	22.8±18.7	4.5-64.1	1.407
	Jul.	14.2±14.0	0.6-46.0	1.580
	Aug.	14.8±16.1	1.3-57.0	3.094
	Sep.	16.8±24.6	0.4-78.0	1.802
	Oct.	22.7±29.1	0.4-105.2	6.235
	Nov.	13.7±10.2	1.2-26.0	1.047
	Dec.	NR	NR	NR
2019	Jan.	NR	NR	NR
	Feb.	4.8±4.8	2.1-7.5	-
	Mar.	22.6±24.8	1.0-61.3	1.457
	Apr.	17.0±27.1	2.0-77.8	6.579
	May	23.2±27.2	0.4-80.2	2.161
	Jun.	26.7±36.1	1.8-135.0	6.339

Note: NR=No Rain

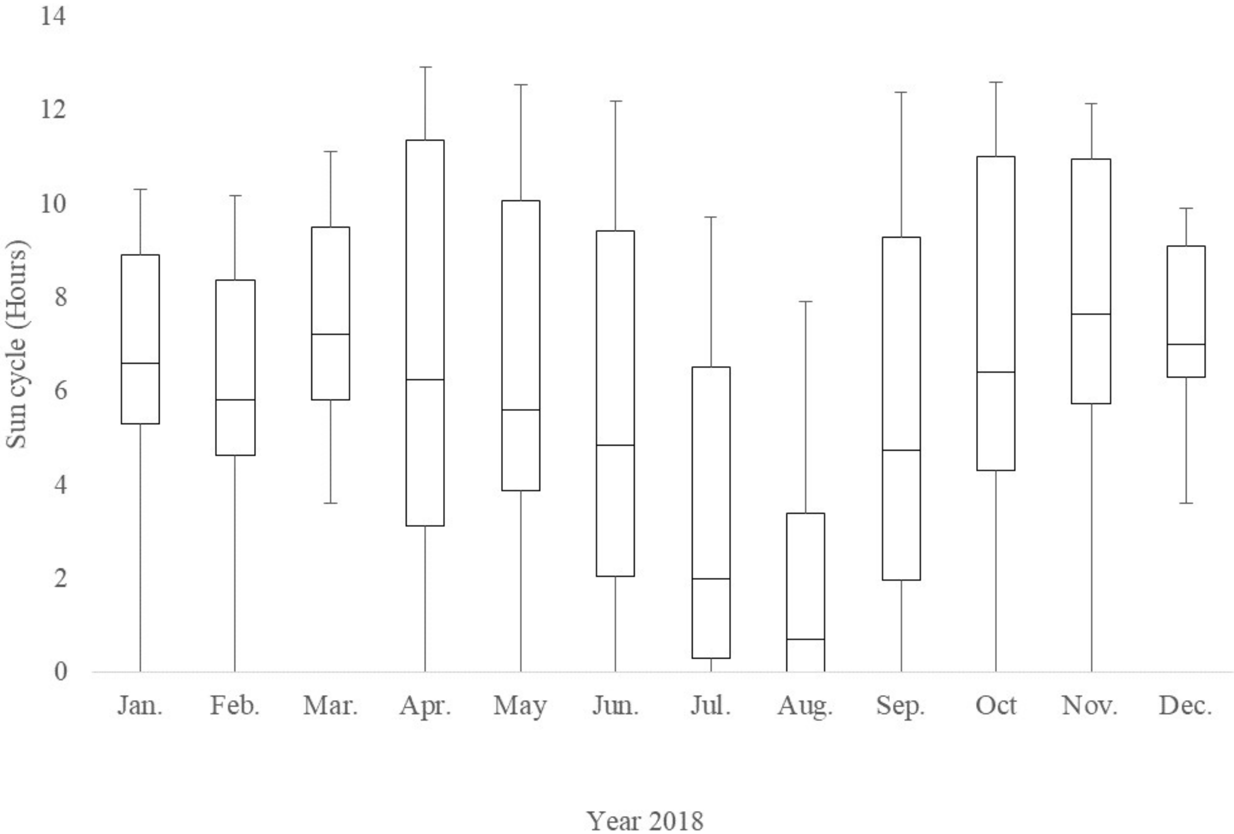
Appendix IX: Distribution patern of relative humidity across year 2017, 2018 and 2019

Year	Month	RH (%)	Min. – Max. values	Kurtosis
2017	April	77.6±13.7	30.0-91.0	5.289
	May	79.5±9.6	50-0-94.0	1.862
	June	82.0±11.1	34.0-95.0	12.010
	July	78.4±14.6	30.0-95.0	6.326
	Aug.	79.3±15.1	39.0-95.0	1.538
	Sept.	79.2±11.5	34.0-93.0	8.098
	Oct.	80.8±8.1	55.0-92.0	1.745
	Nov.	78.2±6.9	65.0-89.0	0.723
	Dec.	80.4±9.5	48.0-93.0	3.781
2018	Jan.	81.5±4.7	72.0-90.0	0.314
	Feb.	83.3±4.1	77.0-95.0	1.032
	Mar.	86.7±3.8	79.0-94.0	0.794
	Apr.	86.7±3.7	80.0-94.0	0.559
	May	86.0±4.6	76.0-97.0	0.004
	Jun.	82.5±2.7	77.0-89.0	0.691
	Jul.	81.6±3.1	75.0-89.0	0.063
	Aug.	59.9±10.5	41.0-74.0	1.175
	Sep.	79.9±10.9	44.0-92.0	5.238
	Oct.	80.6±9.0	48.0-92.0	5.509
	Nov.	81.7±8.6	60.0-93.0	2.131
	Dec.	82.2±10.2	41.0-94.0	8.804
2019	Jan.	63.3±13.2	35.0-85.0	0.727
	Feb.	68.8±14.8	36.0-98.0	0.528
	Mar.	74.9±4.8	63.0-86.0	0.637
	Apr.	78.0±3.1	73.0-87.0	2.162
	May	80.3±3.0	75.0-87.0	0.404
	Jun.	85.2±3.4	80.0-93.0	0.518

Appendix X: Distribution patern of sun cycle across the months of the year 2017



Appendix XI: Distribution patern of sun cycle across the months of the year 2018



Appendix XII: Distribution patern of sun cycle across the months of Year 2019