UNIVERSITY FOR DEVELOPMENT STUDIES

CONSTRUCTED WETLAND FOR DOMESTIC WASTEWATER TREATMENT USING *SIDA ACUTA* AND *SYNEDRELLA NODIFLORA:* A CASE STUDY OF ZAGYURI IN THE NORTHERN REGION OF GHANA

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JULY, 2022

DECLARATION

Student

I hereby declare that this dissertation/thesis is the result of my original work and that no part of it has been presented for another degree in this University or elsewhere:

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ABSTRACT

Constructed wetlands provide sustainable alternative for wastewater treatment with the advantages of pollutants removal efficacy, economical, simple to setup, operate and maintain. Macrophytes have tremendous contribution in wastewater treatment. Their functions include gas transport, a large surface area for the attachment of microbial growth, oxygen release via roots, nutrients and heavy metals absorption and influence on soil hydraulic conductivity. The purpose of this study was to assess the effectiveness of constructed wetlands for the treatment of domestic wastewater using *Sida acuta* and *Synedrella nodiflora* as macrophytes. Eight vertical flow mesocosms with dimensions 30 cm (L) X 24cm (W) x 40cm (D) were constructed using sand and gravels amended with biochar and planted with *Sida acuta* and *Synedrella nodiflora*. Domestic wastewater was run through the mesocosms with $1.2 \times 10^{-7} m^3/sec$ average flow rate and hydraulic retention time of 3 days (72hrs). Efficiency of the treatment was compared between the unplanted (control) and planted beds. The results revealed *Sida acuta* as a better plant in enduring and tolerating wastewater. However, *Synedrella nodiflora* was highly effective in the reduction of parameters such as Phosphate, Sulphate, and Nitrate. The difference between the combined treatments and the single treatments in enhancing the wastewater quality was not significant. The study also showed that biochar amended treatments was much better as compared to the sand treatments. It is recommended that further research should be conducted to discover more endemic plants which are capable of reducing nutrients and heavy metals in our domestic wastewater.

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DEDICATION

I dedicate this work to my husband, Mr. Julius D. Nana and my children, Aseye, Delasi and Eyrame.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Water is considered as part of the fundamental natural resources for sustainable development because of its status as a basic resource and its important contribution to development, livelihoods and poverty alleviation. Water is primarily associated with industrial,, agricultural and service sector development, energy production, conservation of ecosystems and health protection (Teodosiu et al., 2007; Saravanan et al., 2009).

Water is considered as an abundant yet scarce natural resource. Out of the 70% of water covering the earth's surface, 97% is salt water, and about 2% in the form of glaciers and ice caps. Less than 3% is fresh water which is suitable for industrial and domestic use (NOAA, 2021). It is estimated that by 2030, more than 160% of the world's total water will be needed to meet global water demand. This phenomenon coupled with growth in population and change in climate has brought severe drought and water scarcity in sub-Saharan Africa and other parts of the world (Lavrnić et al., 2017; UN-WWDR, 2017).

More so, it has brought about a change in consciousness and the need for conservation and rational usage of water resource. Industrial activities within the pharmaceutical, farming, chemical production and food processing sectors often use and discharge large quantities of wastewater. The treatment of wastewater does not only provide another means to safe water supply but also helps to maintain environmental and ecosystem integrity (UN-WWDR, 2017; UN-Water, 2011). Approximately 80% of untreated discharged wastewater flows back into the ecosystem leading to the pollution of drinking water sources (UN-WWDR, 2017).

Studies also reveals there is a strong connection between the prevalence of water-related diseases like cholera, dysentery, schistosomiasis etc. and the discharged of untreated domestic and urban wastewater (UN-WWDR, 2017). It is expected that the largest increases in exposure to pollutants would occur in low- and lower-middle income countries, as a result of population growth and economic expansion, particularly in Africa (UNEP, 2016) and also to poor wastewater management (UN-WWDR, 2017).

1.2 Problem Statement and Justification

Wastewater treatment is very important in achieving the sustainable development goals (SDGs) especially goal 6.3 which seeks to "enhance the quality of water by reducing pollution, eradicating dumping and reducing the release of unsafe chemicals and materials, halving the amount of untreated wastewater and significantly increase recycling and safe reuse globally by 2030". For this reason, the development of cost-effective and sustainable wastewater treatment technologies is pivotal in achieving this goal. Technological and economic feasibility amongst other factors such as politics, socio-cultural, environment, legal etc. are prerequisites for the adoption of wastewater treatment techniques (Morris et al., 2021; El Moussaoui et al., 2019; Kirchherr et al., 2018).

Recently, constructed wetland (CW) for the treatment of wastewater has been developed as an economical, feasible and sustainable technology. Unlike the traditional wastewater treatment technologies, CWs are eco-friendly and require less personal supervision. Also, CWs has been successfully employed in municipal wastewater treatments and in various agricultural and industrial sectors sewage (Sehar and Nasser, 2019).

Constructed wetlands are basically artificial systems designed to make use of wetland vegetation, natural processes and soils to boost wastewater treatment by enhancing the processes that happens in natural wetland ecosystems (Makopondo et al., 2020; El-Khateeb et al., 2013).

The agricultural industry is currently the biggest sector that utilizes water most in the world and is more than 70% of total withdrawal (Connor et al., 2017). Increased population, climate change and urbanization have increased the need to guarantee food security in most areas of the world.

Wastewater is the main source for irrigation water particularly in the sub Saharan regions (Chopra and Pathak, 2015). The agricultural sector is the largest user of wastewater and treated wastewater globally (Zhang and Shen, 2019; Intriago et al., 2018).

In Ghana and specifically in the Northern region where agriculture is mainly rainfed, vegetable farmers rely on untreated wastewater as a source of irrigation for their crops. This poses a serious environmental and public health risk given the potential to spread waterborne diseases and pathogens such as cholera, dysentery, typhoid, *E. coli* etc.

1.3 Study Objectives

1.3.1 Main objective

To assess the potential and feasibility of wastewater treatment using adapted constructed wetland planted with locally available plants in the Northern Region of Ghana.

1.3.2 Specific Objectives

- To assess the effectiveness of *Sida acuta* and *Synedrella nodiflora*, used singularly and / or together, for treating and reusing domestic wastewater in a constructed wetland

- To assess the effect of biochar amended soil in constructed wetlands on pollutant removal efficiency
- To ascertain the aplicability of treated wastewater for irrigating Jute leaves (*Corchorus olitorius*) and Waterleaf (*Talinum triangulare*)

CHAPTER TWO

LITERATURE REVIEW

2.1 Water Resources in Ghana

About 70% of the water that covers the earth's surface naturally exist as liquid, solid and gas. Out of this 70% of water covering the earth's surface, fresh water is about 2.5% and only a small fraction of this is readily available for human consumption because most are stored within the earth (Owusu et al. 2016).

Therefore, water resource management (WRM) involves the judicious use and adequate ground planning of the resources of clean water accessible to humans. These water resources include any surface and also ground water that can be processed for the activities of man (Owusu et. al. 2016). According to the United Nations, water resource management can be classified into the following: management of resources and water service management and the management of quid pro quos required to bring demand and supply into equilibrium (United Nations, 2014).

According to USAID 2011, Ghana is not suffering from water scarcity due to the abundance of water resources. The major sectors of the economy consumes an amount of is 6.3% of freshwater of the total resource income, and this is below the standard for water scarcity. The amount of renewable water resources per person of 1,949 m^3 exceeds the Falkenmark Index threshold for water stress. Nevertheless, since almost half of fresh water comes from foreign sources, the water accessibility is affected by decisions of management and withdrawals from those nations with high abstraction rates.

Groundwater resources are more limited compared to surface water. Aquifers are generally less productive, limiting their viability for large-scale agriculture, municipal, and industrial use.

Mostly, groundwater in the rural areas is used as potable water and also for domestic use, especially in the northern regions of Ghana. The intrusion of seawater into the coastal basins reduces the quality of groundwater in the coastal cities.

There are five basins in Ghana namely, the White Volta, River Densu, Ankobra, Tano and Pra (WRC Ghana, 2015c)

Ghana's groundwater resources are generally good, except for a few instances of local contamination and areas high in iron and fluoride, and some other minerals (USAID, 2011). In some coastal aquifers, groundwater is particularly saline (WRC Ghana, 2015f).

The main uses of water resources in Ghana are drinking, irrigation and livestock watering. The city's water supply for domestic and industrial needs relies almost entirely on surface water, which is either held back behind small dams or diverted to rivers. However, water supply in rural areas is obtained almost entirely from groundwater sources. More than 10, 000 boreholes have been drilled across the country due to various groundwater development programs (UNFCC, 2011). Rainwater harvesting is becoming commonplace and has great potential for increasing water availability in some areas (WRC Ghana, 2015f).

The actual non-consumer uses of water are hydropower, inland fishing and maritime navigation. Built in 1964, 100km from the mouth of the Volta River, with an average area of 8,300 km^2 is the Akosombo Dam (Mensah, 2010). Located 20km downstream of Akosombo is another hydroelectric dam built in 1981, covering an area of 40 km^2.

Some challenges that interfere in the progress of Ghana's water resources management include illegal mining activities, popularly known as galamsey, poor agricultural practices, waste pollution, change in climate and aquatic weed growth.

2.2 Global Wastewater Production

Globally, agriculture, industry and energy are estimated to account for the largest share (70% and 20%) of water demand. The remaining 10 % is mainly used for household needs, drinking, sanitation and hygiene (WWAP, 2017). The critical factors responsible for this high demand are population growth, rapid urbanization and increasing prosperity and lifestyle. Globally, approximately 56 % of total freshwater extracted is discharged as wastewater from municipal and industrial activities or irrigation ditches (Ravina et. al., 2021). Developed countries trap and treat about 70 % of urban and industrial wastewater, while developing countries account for nearly 8 %. In Ghana and other developing countries, lack of infrastructure, technological and institutional capacity, and financing are notable limiting factors affecting the discharge of untreated wastewater (Nansubuga et al., 2016).

In terms of use, treated wastewater in Malawi, Egypt and Ethiopia used other techniques such as anaerobic digestion, and constructed wetlands. Lagoons and drying beds have been reported in Ethiopia, Egypt and Malawi while Benin had no treatment system other than a pilot system (Ravina et. al., 2021). The treatment plant removed a total of 30% of organic waste and 50% suspended solids with bacteria respectively (Mtethiwa, et. al., 2008). Final product obtained from the treatment was used for irrigation and agriculture, but did not meet the country and WHO irrigation water permissible levels. However for a pilot in Benin, the removal efficiency was over 90 % and the treated wastewater was used for aquaculture. The optimal wastewater volume observed was approximately 47,500 m^3/day in Ethiopia (Ravina et. al., 2021). Insufficient data from other countries made comparative assessment impossible.

Evidence presented by Galletta et al., (2021) confirms significant water treatment gaps in the developing countries studied. Although the treatment plants existed and is operational, it has not provided much efficiency because the discharged effluent does not meet the regulatory value. Potential impacts and health risks have not been fully considered (Gwenzi, 2018). Similarly, all the countries studied met the requirements for environmental impact assessment regulations before and during the implementation and operation of the WWTP but did not properly apply the environmental protection standards (Galletta et. al, 2021). As important as wastewater may be, sludge is also important to the environment and public health. Sludge characterization (aromatic hydrocarbons, heavy metals) may require additional techniques to collect > 2000 m^3 methane gas from sludge digesters in general.

2.2.1 Wastewater Production in Ghana

Agodzo et. al. (2003), revealed that an estimated volume of 280 million m^3 of domestic wastewater is generated every year in Ghana. Production of industrial and commercial wastewater annually has little or no data to show except for domestic wastewater. The total estimated amount of domestic wastewater generated in Ghana in 2006 was about 280 million m^3. The amount of industrial wastewater is expected to increase as treatment plants expand in the country (Gyampo, 2012). Ghana's municipal wastewater has increased from about 530, 346 m^3/day (36%) in 2000 to about 1,452,383 m^3/day (45%) in 2020 (Agodzo, 2003). Ghana's ten regions have very poor wastewater treatment, accounting for less than 8% of Ghana's treated wastewater.

Many of the industries are sited offshore so their untreated wastewater is discharged directly into the sea while land- based industries discharges into streams and drains.

2.3 Wastewater Treatment

Approximately 10% of the country's domestic and municipal wastewater is discharged by treatment plants through sewers. As in the past, most or all of commercial and industrial wastewater is discharged into the natural environment (sea, river and wetlands) untreated.

Approximately 46% of the inhabitants of Greater Accra have access to water but it is unevenly spaced. Only 10% of the Accra metropolitan area is connected to pipe borne water sewerage lines which is connected to one of the 44 Ghana's treatment plants. More than 50% of the treatment facilities in Ghana can be located in the Greater Accra Region and out of this, only 20% are in use, whilst the rest do not meet design criteria. The main sewer covers the ministerial center in Accra, causing the UASB plant to fail shortly after commissioning.

Domestic wastewater can be treated with different techniques and they can be classified into conventional and non- conventional treatments. Conventional treatment is energy dependent and non- conventional relies solely on natural processes. Conventional treatments include activated sludge systems, aeration lagoons, trickling filters, activated sludge system and biodisc rotators. The non-conventional systems, also called eco-technologies include waste stabilization ponds (WSPs) and constructed wetlands. As technologies in use, the waste stabilization ponds and trickling filters are used in local universities, military camps, and hotels. It is a common system for large companies and institutions (Obuobie et al., 2006; Awuah, 2006). However, the most common are underground sanitary systems also known as septic tanks, which usually do not have adequate drainage areas. Since its construction in the early 1970s, there has been a little expansion of the sewerage lines. The faecal sludge treatment plants receive wastes from septic tanks, public toilets and pit latrines. As a result of the limited number and availability and/or condition of sludge treatment sites, over 60% of all collected excreta are discharged into the sea.

2.4 Wastewater and agriculture

According to FAO (2017), proper management of wastewater can safely support agriculture directly or indirectly through irrigation and aquifer recharge respectively. Treated wastewater can be used for irrigation, recharging aquifers or for entertainment purposes (Nathanson and Ambulkar, 2022)

Poor sanitation in Ghana means that, for the most part, only a fraction of domestic wastewater is treated. Most of it flows into water bodies and nearby drains used by vegetable growers for irrigation. Most urban vegetable farming is market-based and therefore depends on water availability for regular irrigation, especially during the dry season. Vegetable cultivation does not only provide a livelihood to the farmers and traders but also helps transport perishable vegetables to cities. However, high pathogen contamination has been detected in most irrigation water sources and irrigated vegetables (Keraita and Drechsel, 2004**).**

Along helping address the scarcity of water, wastewater is usually a good fertilizer due to its high nutritional value. Wastewater can be beneficial when used safely and managed properly to avoid exposure to environmental and health risks (De Souza, 2017)

2.5 Constructed Wetlands

Constructed wetlands (CWs) are an alternative technology that can be adopted for wastewater treatment. In Germany, in the early 1950s, the very first ever CW experiment using wetland vegetation to treat wastewater was carried out. Afterwards, CWs have become a definitive technique for wastewater treatment (Vymazal, 2010). They are systems designed and built to take advantage of the processes naturally associated with macrophytes, soil, and related microbes for treating wastewaters. They are constructed to make use of several processes that takes place in natural wetlands, but they do so in a much more managed environment (mesocosm). Treatment of wastewater using CWs can be categorized based on the predominant macrophyte's morphology, free-floating systems, floating leaved, emergent root and submerged wetland plants (Brix & Schierup, 1989).

Recently, constructed wetlands are widely adopted to remove different types of polluted water due to the low maintenance and operation costs, efficient pollutant removal and environmentally friendly techniques (Kadlec & Wallace, 2008). Some of the major factors that directly affect pollutant removal efficiency are hydraulic retention time (HRT), filter media and plant species growing in the system (Antover et al, 2013).

Constructed wetlands can be grouped by different parameter design. The main criteria are macrophyte growth morphology (emergent, submerged, free-floating, and floating leaved plants), flow paths (horizontal and vertical) and hydrology (water surface flow and subsurface flow) (Vymazal 2007, 2011). Sometimes CWs be hybrid (combined systems). In the 1990s and 2000s, hybrid systems were used to efficiently remove total nitrogen and ammonia (Vymazal, 2011).

There are mechanisms (physical, chemical and biological) involved in purifying treated wastewater by a constructed wetland system. Although these mechanisms are not yet fully understood, some interesting discoveries have been made recently (Selvamurugan et al., 2011).

Table 1: Pollutant removal mechanisms in Constructed wetlands (Gray and Biddlestone 1995)

Pollutant	Removal mechanism
Suspended solids	Agitation, filtration, decantation, sorption and autoflocculation.
Biochemical oxygen demand	Sedimentation, decomposition to CO_2, H_2O and NH_3 by microorganisms attached to plant and sediment surfaces.
Nitrogen	Nitrification and denitrification, NH_3 volatilization, deposition and plant uptake.
Phosphorus	Sedimentation, adsorption, complex formation, deposition reactions in lamellar substrates and plant uptake.
Heavy metals	Precipitation, sedimentation and adsorption of biomass films on plant stems, roots and bed matrices.
Pathogens	Sedimentation and filtration, competition and natural die-off, removal of antibiotics from plants roots and composting of plant waste on bed surfaces.

CWs can be classified into subsurface flow (SSF) and free water surface (FWS). The SSFs were widely employed in wastewater treatment technology because the treatment process is in contact with the environment and is effective for plant roots and rhizomes (Shutes, 2001). SSF is more efficient in reducing odour, uses less land, and has a greater capacity to adsorb pollutants (Kadlec & Wallace, 2008). Several studies have shown that SSF of wastewater through a layer of permeable root medium cultivated with aquatic plants effectively removes important inorganic contaminants from wastewater (Reddy and Smith, 1987). In the sewage treatment plant, the removal of pollutants happens via several interactions between the plants, microorganisms, media, the atmosphere and interactions in the wastewater itself (Seidel, 1966). Pollutants are removed by the simultaneous occurrence of physical, chemical and biological mechanisms.

SSFs can be grouped into vertical flow (VF) and horizontal flow (HF). Vertical flow constructed wetlands were pioneered by Seidel to provide oxygen to anaerobic septic tank effluents (Seidel, 1965). Nonetheless, VF CWs was not utilized as much as HF CWs. This is likely due to the increased operational and maintenance requirements as a result of the intermittent pumping of wastewater to the surface of the CW. Vertical subsurface flow constructed wetlands (VSSF CWs) consist of smooth gravels covered with sea sand and planted downwardly with macrophytes. They are flooded with large irregular stream of influent that crosses the bed surface, then passes within the bed and collects at an outlet at the bottom. The bed is retracted and the bed is filled with air. Thus, VSSF CW supplies more oxygen to the bed, producing a nitrifying effluent (high NO_3^-) (Cooper, 2005; Cooper et al., 1996). VF CWs can also be used to effectively remove suspended and organic matter. A newly developed algae flow system ("fill and drain") ensures good contact between the wastewater and microorganisms growth in the medium. This greatly improves the treatment process (Vymazal, 2011). The removal efficiency in VSSF CW is high due to the high fluxconcentration.

2.5.1 **Media**

Media carries out many processes that remove contaminants, particularly in controlling water infiltration, filtering particles, providing nutrient based energy for microorganisms and also growth stimulation for plants and microorganisms (De Rozari et al., 2020a, De Rozari et al., 2020b). Filter medium in wastewater treatment provides a broad surface area (Selvamurugan et al., 2011). The support media in the SSF constructed wetlands has an important function in the development and growth of microorganisms and plants as well as in pollutant removal. Materials can directly interact with contaminants via an adsorption process as well as providing physical support. Because the level of these interactions can remarkably affect the behavior and performance of the CW, the proper selection of the material used as medium is a crucial step in the optimization of CWs (Dordio et al., 2009). Several man-made media has been tested to improve the removal of nutrients in the SSF constructed wetland. This includes factory LECA (factory light-weight expanded clay aggregates), shale, granular laterite, sepiolite and ground marble (Arias & Brix, 2005). But, finding the appropriate and inexpensive filtration medium is a major problem in constructed wetland systems.

Biochar is a carbon rich substance that is valuable as a soil conditioner by increasing fertilizer retention (Liang et al., 2006) and stimulating beneficial microorganisms (Warnock et al., 2007). They can also amend inorganic and organic pollutants (Yu et al., 2009). Biochar has a porous structure, contains several structural groups and is known to effectively adsorbe heavy metals in water (Liu et al., 2009). Biochar, known for its strength to store carbon, can decrease or inhibit the production of N_2O, CO_2 and CH_4, in the soil and can also reduce atmospheric greenhouse gases (Yanai et al., 2007). Activated charcoal is suitable for eliminating pollutants from water, however, it is economical. Aside this, “sustainable” biochar is low-cost. Conventional biochar burns not so much compared to activated carbon. Nevertheless, this structure retains high oxygen and hydrogen with ash and absorption of hydrocarbons, organic matter and a little inorganic metal ions (Mohan et. al., 2015), showing water treatment capabilities alongside soil improvement. Biochar are acquired from several raw materials like animal and plant-based feedstock (chicken manure, pig manure, sawdust, peanut shells, straw waste; leaf litter), invasive weeds (*Eichhornia crassipes, Prosopis juliflora)* (Kloss et al., 2012). The adsorption efficiency of biochar is directly proportional to the physicochemical properties such as surface area and functional groups. Biochar has the ability to substitute conventional activated carbon such as coal, wood and coconut shell as a cheap adsorbent for pollutants and pathogens. Biochar can be used to remove pollutants from water and can be supplemented with nutrients for later use as soil conditioner, providing long-term sorption capacity and a fertilizer. Sand augmented with biochar in different amounts in VF wetland beds effectively removed coliforms, BOD_5, TVS and TSS (Rozari et al., 2015).

2.5.2 Macrophytes

Plant species are known to possess potential cellular mechanisms involved in heavy metal detoxification and thus tolerance to metal stress (Sarma, 2011). These plant species can tolerate metal toxicity by reducing the amount of metal toxicity in the polluted environment and metal absorption through the root system in the bud system without showing a toxic syndrome (Xiao-min et al., 2013)

Swamp plants, often called macrophytes or wetland plants, are adapted to grow in submerged soils. Macrophytes perform gas transport, roots oxygenation, soil permeability, nutrients uptake and other functions in wastewater treatment (Brix, 1994). Macrophytes also provide a wide surface area for the growth of microbes. In larger systems, wetland vegetation supports a variety of flora and fauna, including birds, reptiles and more. Wastewater treatment can be made aesthetically pleasing by using beautiful-looking aquatic plants such as water hyacinths or water lilies (Selvamurugan et al, 2011).

In Subsurface flow, macrophytes and media together play a vital role in pollutant removal in wastewater. Macrophytes contribute to the pollutants removal by providing favorable habitats for microorganisms, filtration processes, and rhizome production for plant absorption and uptake (Kadlec & Wallace, 2008). The success of plants in removing pollutants is bsed on the type and density of the plant (Saeed & Sun, 2012).

Sida acuta is a perennial shrub found in almost all soil type except limestone soils and clay which are seasonally flooded (APB, 1983). It is highly competitive with other plant species, but thrives in disturbed tropical or subtropical habitats with dry and wet seasons. Its taproot is buried deep into the ground and is tolerates drought, lawn mowing and shallow tillage. These weeds are found in degraded grassland, tree farms, cereals, forest farms, root crops, lawns, vegetables, roadsides, and landfills (Pitt, 1992; Flanagan et al., 2000). It is hardy and grows well in various types of soils. In a study by Agyarko et al., (2010) *S. acuta* has been used over a period of time in orthodox medicine in many countries to prevent and treat various conditions such as urinary infections, fevers, diarrhea, skin, dysentery, rheumatism, gastrointestinal and urinary infections, malaria, birth and abortion problems, heart and nervous disorders, asthma, bronchitis and other respiratory problems, weight loss supplements and other inflammations, tuberculosis and more (Biswanath et al., 2015). In Ghana, *S.acuta* is classified as a medicinal plant and its leaves are used to stop bleeding in wounds and its roots are used for foetal strength. A decoction of the entire plant is for treating heat in the body. The sap of the plant is used to treat indigestion. The plant is ground and mixed with emollient oil and sugar to form a powder used to soften and drain pus. A decoction of the leaves is used to wash wounds. Applying powdered leaves on the head relieves headache (www.csir-forig.org.gh).

Synedrella nodiflora is a weed commonly found in a variety of grassland, nurseries, wastelands, beside roads, gardens and lawns and other plots and disturbed areas, as well as in a variety of vegetable crops, farms and horticulture in the tropics. Under conditions that are suitable, *S. nodiflora* has can live more than 100 days and can thrive and reproduce rapidly multiple times annually. This plant has the characteristic of plasticity and this enables it to grow and multiply under different condition although wet and slightly shaded areas are preferred. Reproduction is solely by seed and about 6000 seeds can be produced by some species. In Papua New Guinea, the young shoots are consumed as food, and the buds are fed to swines. In Ghana, *S. nodiflora* is a common weed traditionally used to treat epilepsy, hiccups and miscarriages (Adjei et. al., 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Zagyuri, a suburb of the Tamale metropolis located 8km away from the central business town Tamale, along the Tamale-Bolgatanga highway. The climate is characterized by rains from April/May through to September/October, with an average annual rainfall of 1,100 mm, influenced by moist southwest winds (monsoon) from the Atlantic Ocean followed by a long dry season influenced by the north east (harmattan) Sahara trade winds from November to March and high temperatures from March to May.

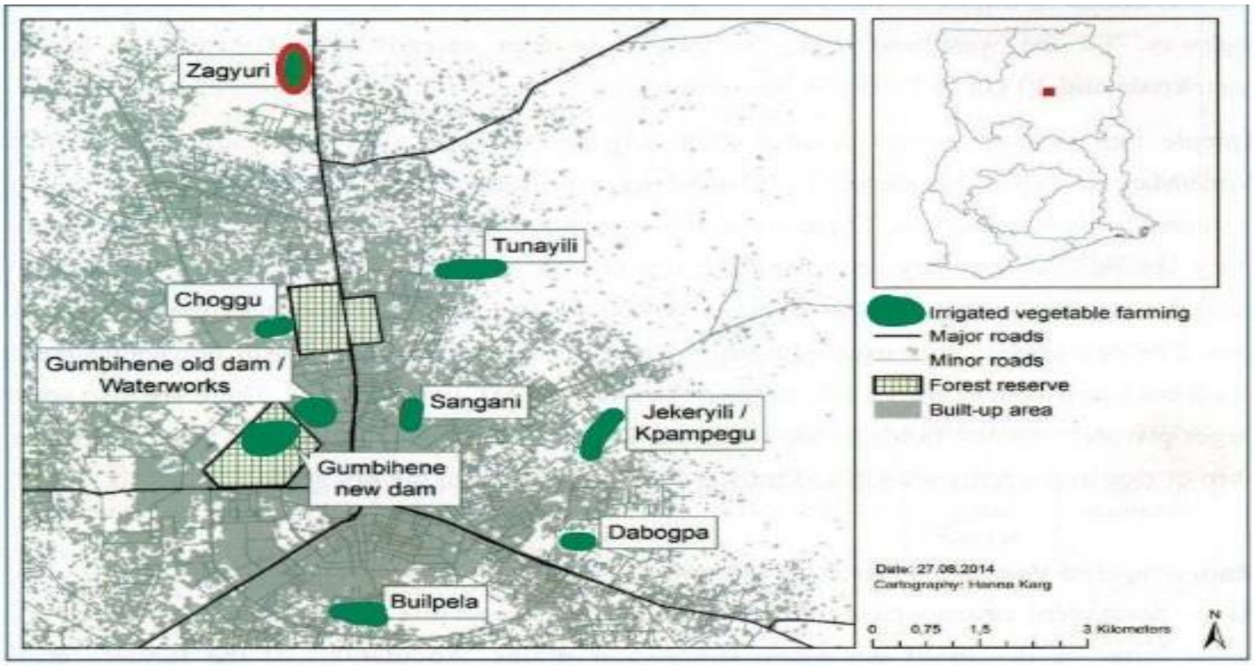

Figure 1. A map of vegetable production sites in Tamale, Ghana. Study area marked in a red circle. (Source: Karg, H, 2014, UrbanFood^{plus} project)

Zagyuri is one of the main areas for vegetable production in Tamale in the dry season. Reported total area of farming site has declined due to settlement and infrastructure – from about 12 ha in

2004 (Zibrilla & Salifu, 2004) to about 2 to 4 ha under dry- season vegetable production and 8 ha under maize farming during the rainy season (Danso et al., 2014). Untreated wastewater from broken sewage pipes at the Kamina Military Barracks and its surrounding communities is the main source of water for irrigation and on a small scale from hand-dug wells, which had been constructed on the various farms for the washing of the harvested vegetables off contaminants. Vegetables produced from these farms commonly include *Corchorus olitorius* (Ayoyo), *Amaranthus candatus* (Alefu), *Hibiscus sabdariffa* (Bra), Okro and Cowpea leaves.

The wastewater is channeled through dug-out channels into the farms where farmers fetch to irrigate their crops and a larger volume of the wastewater stored in unprotected concrete ponds as reservoirs.

Figure 2: Domestic Wastewater at Zagyuri flowing from Kamina Barracks

3.2 Selection of Macrophytes

Two traditional plants, *Sida acuta* and *Synedrella nodiflora* were purposively selected for this study to evaluate their tolerance and accumulation ability to heavy metals and nutrients. The choice of these plants was based on their widespread availability at the study area and tolerance to wastewater. These plants species also have the ability to co-exist on the same soil. This quality brought about their concurrent study under the same experimental conditions.

Figure 3: A picture of *Sida acuta* and *Synedrella nodiflora*

3.3 Biochar

Biochar was produced in a pyrolysis furnace at 350℃ using Peanut Shells as feedstocks. The biochar produced from pyrolysis was grinded and passed through a 2mm sieve.

3.4 Experimental Setup and operation

The experiment was conducted at CSIR-Water Research Institute, Tamale, from November 2021 – March 2022. Eight rectangular plastic tanks with vertical subsurface flow CWs were constructed and labelled CW1- CW8. The Vertical flow (VF) mesocosms were of dimensions 30 cm (L) X 24cm (W) x 40cm (D). The media used consisted of a layer of gravel 5cm thick (diameter 10 – 15 mm) placed at the bottom of each tank, followed by a layer of sand 15 cm in the middle and 5 cm of biochar above. The base of the tanks was fitted with a PVC pipe to act as the outlet. Domestic wastewater flowed continuously from a 100L plastic basin which served as an inlet. The beds were filled weekly with domestic wastewater with hydraulic loading rate of $0.11 m^3/m^2.d$ ($4.6 L/m^2.h$). The media used in this study were sea sand, biochar and a combination of the two planted with and without *Sida acuta* and *Synedrella nodiflora* (Table 2)

Table 2. Treatment Unit arrangement with media Composition

Media Treatment	% Media			Plant
	Sand	Gravel	Biochar	
CW1	58%	42%	0%	S.A
CW2	58%	42%	0%	S.N
CW3	58%	42%	0%	S.A + S.N
CW4	42%	25%	33%	S.A
CW5	42%	25%	33%	S.N
CW6	42%	25%	33%	S.A + S.N
CW7(control)	58%	42%	0%	-
CW8(control)	42%	25%	33%	-

S.A= *Sida acuta*, S.N = *Syndrella nodiflora*

CW 1 planted with only *Sida acuta*

CW 2 planted with only *Synedrella nodiflora*

CW 3 with *Sida acuta* and *Synedrella nodiflora*

CW 4 amended with biochar and planted with *Sida acuta* only

CW 5 amended with biochar and planted with *Synedrella nodiflora* only

CW 6 amended with biochar and planted with *Sida acuta* and *Synedrella nodiflora*

CW7 unplanted (control)

CW 8 Unplanted amended with biochar (control)

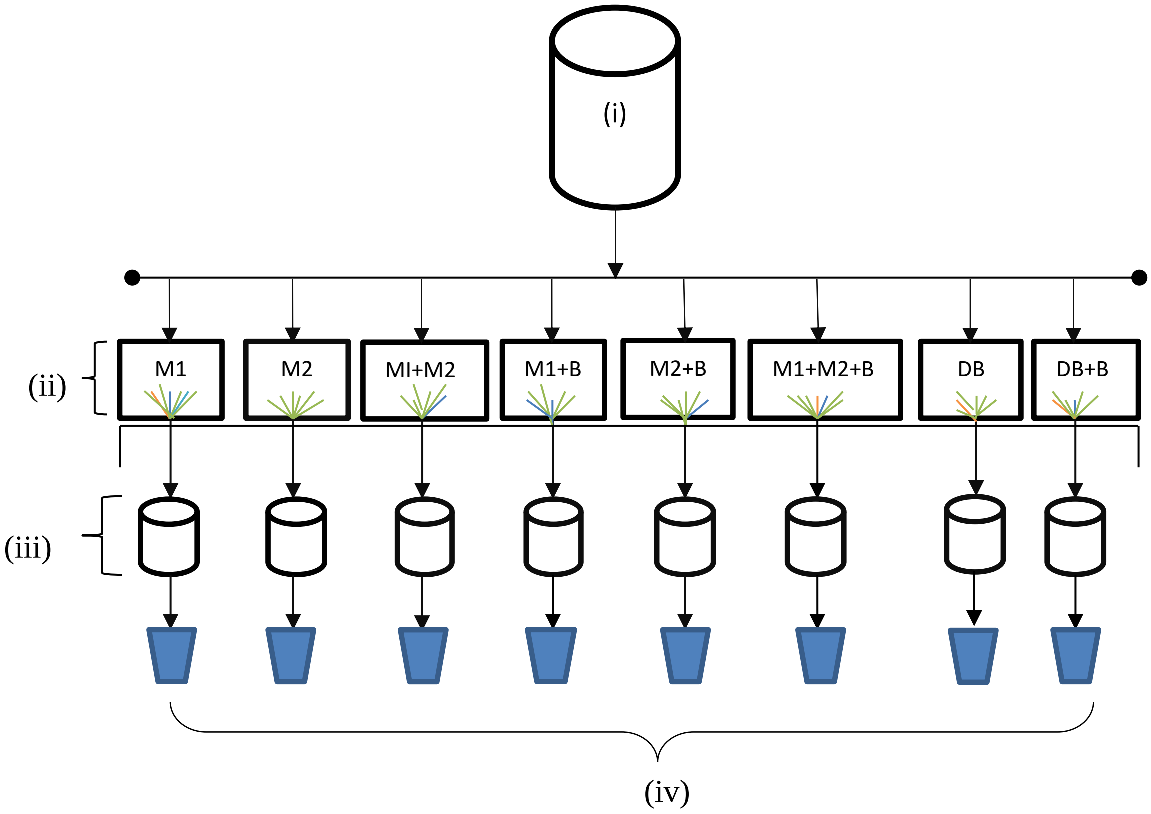

Figure 4: Schematic diagram of experimental set up (i) storage tank, (ii) VF CW unit, (iii) Effluent, (iv) vegetable pots. (M1*) Sida acuta*, (M2) *Synedrella nodiflora*, (B) Biochar, (DB) Dry bed

Figure 5: Experimental set up of the vertical flow CW with planted macrophytes

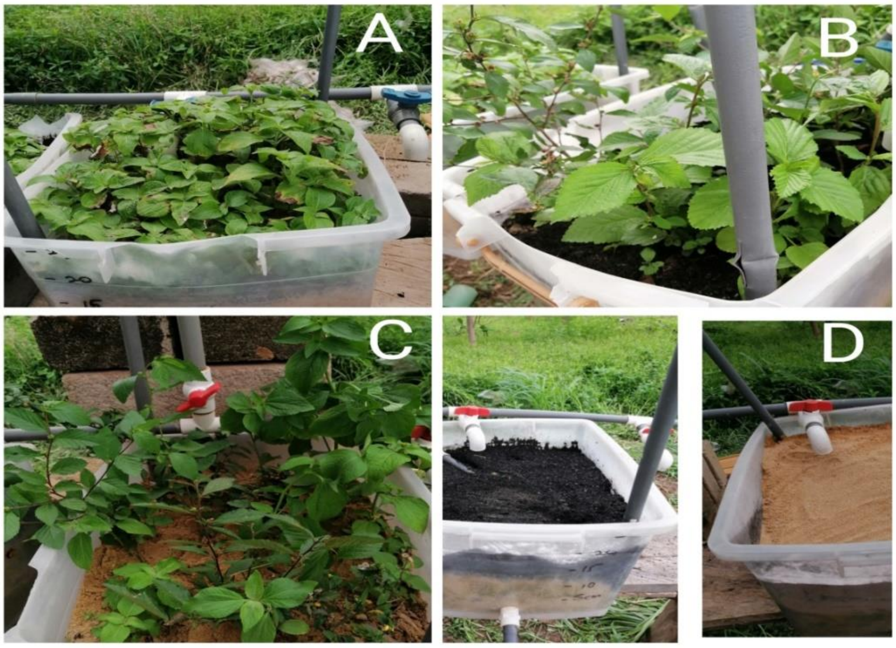

Figure 6: View of the planted and unplanted wetland beds

A= a bed planted with *Synedrella nodiflora* only

B= a bed planted with *Sida acuta* only

C= a bed planted with a combination of *Sida acuta* and *Synedrella nodiflora*

D= unplanted beds (controls)

3.5 Wastewater Quality Monitoring

Laboratory analysis was carried out on the raw wastewater before it was discharged into the wetland beds. The hydraulic retention time (HRT) of domestic wastewater through the beds was 3 days (72hrs) and the average flow rate of 1.2×10^{-7} m^3/sec. Flow rate was monitored daily to maintain flow stability.

Effluents were collected at the outlet at the end of day 3 from outlet over four weeks. The treated wastewater samples were immediately analyzes in the laboratory for physico chemical parameters and microbial content before used to irrigate selected vegetables.

Wastewater quality analysis was performed using the appropriate APHA standard methods (1998).

Figure 7: A section of the treated wastewater (effluent) from outlet

3.6 Irrigated Vegetables

The vegetables were irrigated with potable water for a week before introduced to the treated wastewater. Then potable water was mixed with treated wastewater and used for watering in low quantities for the plant to acclimatize and establish .Irrigation with only the treated wastewater was used afterwards. Laboratory analysis was performed to check for pathogens before treated wastewater was introduced and after harvesting.

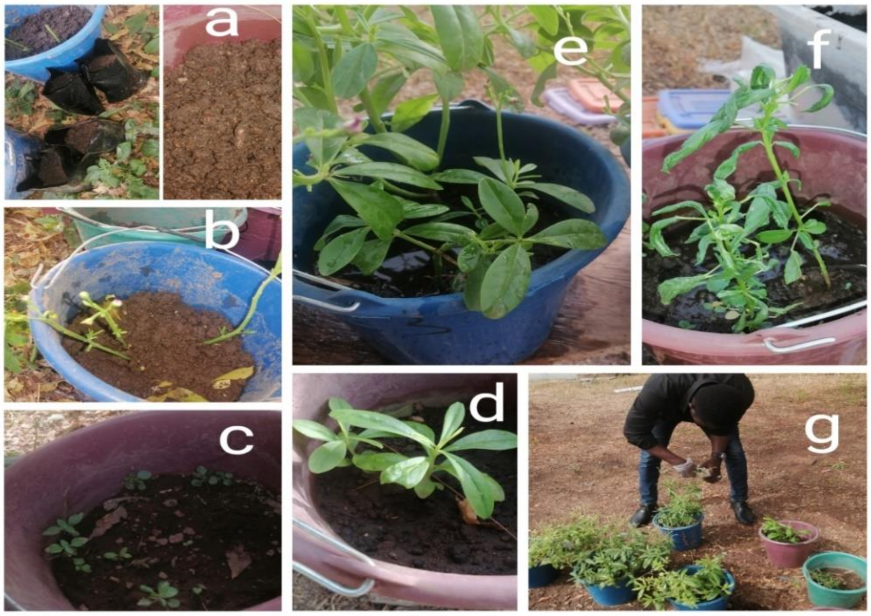

Figure 8: A view of irrigated vegetables: a - b= vegetables at the time of nursing, c - d= time of growth, e - f =time of maturity, g = harvesting of vegetables for laboratory analysis

3.7 Statistical Analysis

The mean percentage removal was calculated from the data collected over a four-week period using the equation for percentage removal efficiency, where C_{in} and C_{ef} are concentrations for influent and effluent respectively.

$$(\%R) = \frac{C_{in} - C_{ef}}{C_{in}} x\ 100\%$$

ANOVA (one-way) using Microsoft Office Excel 2010 was used to statistically analyze the data. Significant differences were considered at α = 0.05 level. This was followed by a pair-wise comparison using Bonferroni corrected Tukey HSD post-hoc tests to further address the significant difference of each treatment.

CHAPTER FOUR

RESULTS

4.1 Physico – Chemical results

Results of physico-chemical analysis done on domestic wastewater treated with *S. acuta* and *S. nodiflora* in the study area are presented in Tables 3-10.

Table 3. Results of physico-chemical parameters of CW planted with *S. acuta*

Parameter	**Unit**	**Influent** (Min-Max Mean±STD)	**Effluent** (Min-Max Mean±STD)	**Removal efficiency %**
TDS	Mg/l	1012 – 1802 1398 ± 400.7	962-1271 1070 ± 138.1	23.5
BOD	Mg/l	13.1 – 20.6 17.53 ± 3.160	10.3 – 11.1 10.65 ± 0.342	39.2
COD	Mg/l	153 – 410 306.3 ± 111.3	49 – 96 74.25 ± 20.95	75.8
NO_3	Mg/l	11.9 – 21.17 16.67 ± 4.985	5.146 – 11.92 9.246 ± 3.080	44.5
PO_4	Mg/l	0.796 – 3.677 2.402± 1.300	0.005 – 1.816 0.809 ± 0.749	66.3
SO_4	Mg/l	10.6 – 154 87.45 ± 58.96	0.001 – 118 43.30 ± 55.62	50.4
Fe	Mg/l	0.302 – 0.416 0.377 ± 0.052	0.07 – 0.205 0.130 ± 0.066	65.5
Zn	Mg/l	1.143 – 2.132 1.863 ± 0.480	0.008 – 0.028 0.017 ± 0.010	99.1
Mn	Mg/l	0.105 – 0.13 0.112 ± 0.012	0.034 – 0.27 0.103 ± 0.112	8.0
Overall %R				**52.5**

The results suggest that nutrients were better removed (44.5- 66.3%) although COD had a high removal of 75.8%. Manganese removal was very poor (8%) as compared to Fe (65.5%) and Zn (99.1%). The overall performance for this treatment planted with *S. acuta* was 52.5%.

The study (Table 4) revealed that removal of heavy metals was highly satisfactory (1.8-97.6%) Manganese (Mn) was the least. The overall removal efficiency of *Synedrella nodiflora* planted in sand was 58.4%. This treatment can be said to be satisfactory as compared to the treatment with *Sida acuta* planted in sand (52.5%), (Table 3). Removal of Sulphate (SO_4) was excellent as compared to the treatment with *Sida acuta* .

Table 4. Results of physico-chemical parameters of CW planted with *S. nodiflora*

Parameter	**Unit**	**Influent** (Min-Max Mean±STD)	**Effluent** (Min-Max Mean±STD)	**Removal efficiency %**
TDS	Mg/l	1012 – 1802 1398 ± 400.7	961 – 1350 1129±188.6	19.2
BOD	Mg/l	13.1 – 20.6 17.53 ± 3.160	10.1 – 11.6 10.8± 0.627	38.4
COD	Mg/l	153 – 410 306.3 ± 111.3	62 – 105 85.25±20.32	72.2
NO_3	Mg/l	11.9 – 21.17 16.67 ± 4.985	8.032 – 10.95 9.106± 1.289	45.4
PO_4	Mg/l	0.796 – 3.677 2.402± 1.300	0.01 – 1.922 1.007± 0.838	58.1
SO_4	Mg/l	10.6 – 154 87.45 ± 58.96	0.094 – 0.175 0.136 ± 0.033	99.8
Fe	Mg/l	0.302 – 0.416 0.377 ± 0.052	0.02 – 0.019 0.009 ± 0.006	97.6
Zn	Mg/l	1.143 – 2.132 1.863 ± 0.480	0.034 – 0.270 0.122 ± 0.091	93.5
Mn	Mg/l	0.105 – 0.13 0.112 ± 0.012	0.041 – 0.177 0.110 ± 0.107	1.8
Overall %R				**58.4**

In the treatment with both plants in sand media, phytoremediation of heavy metals was very high, ranging from 53.7 - 87.7 %. Removal of organic pollutants (COD, BOD) followed best than the inorganic parameters (NO_3, PO_4, SO_4). Phosphate (PO_4) was removed better in this treatment. This may be due to the combination effect of the plants. Contrary to CW1 (Table 3) and CW2 (Table 4), Mn removal was rather higher than Fe and Zn.

Table 5. Results of physico-chemical parameters of CW planted with *S. acuta and S. nodiflora*

Parameter	**Unit**	**Influent** (Min-Max Mean±STD)	**Effluent** (Min-Max Mean±STD)	**Removal efficiency %**
TDS	Mg/l	1012 – 1802 1398 ± 400.7	919 – 1108 1011 ± 77.3	27.7
BOD	Mg/l	13.1 – 20.6 17.53 ± 3.160	9.8 – 11.0 10.3 ± 0.510	41.2
COD	Mg/l	153 – 410 306.3 ± 111.3	63 – 89 82 ± 12.67	73.2
NO_3	Mg/l	11.9 – 21.17 16.67 ± 4.985	5.357 – 12.05 9.443 ± 3.391	43.4
PO_4	Mg/l	0.796 – 3.677 2.402± 1.300	0.034 – 1.258 0.593 ± 0.513	75.3
SO_4	Mg/l	10.6 – 154 87.45 ± 58.96	0.001 – 154 69.60 ± 63.88	10.3
Fe	Mg/l	0.302 – 0.416 0.377 ± 0.052	0.025 – 0.216 0.086 ± 0.090	77.2
Zn	Mg/l	1.143 – 2.132 1.863 ± 0.480	0.004 – 0.027 0.012 ± 0.011	53.7
Mn	Mg/l	0.105 – 0.13 0.112 ± 0.012	0.02 – 0.213 0.101 ± 0.085	87.7
Overall %R				**54.4**

Table 6 presents the treatment with *Sida acuta* in biochar amended soil. Removal efficiencies of all parameters are satisfactory except Sulphate (SO_4). Metal removal was the highest, followed by organic pollutants then inorganic pollutants. Overall performance was 45.7%. This is slightly better than CW1 (Table 3) which was also planted with *Sida acuta* in sand but without biochar. It can therefore be stated that the presence of biochar alongside *Sida acuta*, clearly had an impact on the removal efficiency of the treatment bed CW4 (Table 6).

Table 6. Results of physico-chemical parameters of CW planted with *S.acuta* and amended with biochar

Parameter	**Unit**	**Influent** (Min-Max Mean±STD)	**Effluent** (Min-Max Mean±STD)	**Removal efficiency %**
TDS	Mg/l	1012 – 1802 1398 ± 400.7	919 – 1791 1216 ± 395	13.0
BOD	Mg/l	13.1 – 20.6 17.53 ± 3.160	8.9 – 10.1 9.525 ± 0.506	45.7
COD	Mg/l	153 – 410 306.3 ± 111.3	66 – 92 79.75 ± 10.84	74.0
NO_3	Mg/l	11.9 – 21.17 16.67 ± 4.985	6.22 – 10.7 9.513 ± 2.198	42.9
PO_4	Mg/l	0.796 – 3.677 2.402± 1.300	0.465 – 2.697 1.228 ± 0.999	48.9
SO_4	Mg/l	10.6 – 154 87.45 ± 58.96	0.001 – 133 69.45 ± 54.80	20.5
Fe	Mg/l	0.302 – 0.416 0.377 ± 0.052	0.091 – 0.191 0.150 ± 0.043	60.2
Zn	Mg/l	1.143 – 2.132 1.863 ± 0.480	0.002 – 0.014 0.033 ± 0.054	98.2
Mn	Mg/l	0.105 – 0.13 0.112 ± 0.012	0.091 – 0.114 0.103 ± 0.011	8.0
Overall %R				**45.7**

The study revealed that biochar amendment in CW4 (Table 7) caused a raise in some parameters like BOD, Zn and Mn but no significant difference in the nutrients, COD, Fe. Removal efficiency of CW5 was 50.0%, which is lower in performance than the non-biochar treatment CW2 (Table 4).This implies most of the removal was performed by *Synedrella nodiflora* and to some extent the sand media. The addition of biochar did not significantly enhance the treatment.

Table 7. Results of physico-chemical parameters of CW planted with *S.nodiflora* and amended with biochar

Parameter	**Unit**	**Influent** (Min-Max Mean±STD)	**Effluent** (Min-Max Mean±STD)	**Removal efficiency %**
TDS	Mg/l	1012 – 1802 1398 ± 400.7	937 – 1791 1295 ± 372.6	7.4
BOD	Mg/l	13.1 – 20.6 17.53 ± 3.160	8.7 – 9.9 9.325 ± 0.568	46.8
COD	Mg/l	153 – 410 306.3 ± 111.3	72 – 101 85.75± 12.84	71.9
NO_3	Mg/l	11.9 – 21.17 16.67 ± 4.985	6.218 – 10.7 9.512 ± 2.199	42.9
PO_4	Mg/l	0.796 – 3.677 2.402± 1.300	0.743 – 2.438 1.213 ± 0.821	49.5
SO_4	Mg/l	10.6 – 154 87.45 ± 58.96	0.001 – 128 66.55 ± 52.34	23.9
Fe	Mg/l	0.302 – 0.416 0.377 ± 0.052	0.001 – 0.127 0.057 ± 0.052	84.9
Zn	Mg/l	1.143 – 2.132 1.863 ± 0.480	0.002 – 0.106 0.034 ± 0.049	98.2
Mn	Mg/l	0.105 – 0.13 0.112 ± 0.012	0.053 – 0.124 0.085 ±0.037	24.1
Overall %R				**50.0**

Comparing the removal efficiency of CW6 (Table 8) to the performance of CW3 (Table 5), it can be concluded that addition of biochar to the treatment performed slightly higher than or almost like the treatment without biochar. But it is important to note that removal of some pollutants such as Zn, SO4 and BOD was high. Metal removal was generally satisfactory. In general, treatment by CW8 was fair. The combined effect of the plants clearly had a general influence on the removal efficacy. The overall efficiency of CW6 was 57.2% (Table 8) which is slightly higher than 54.4% in CW3 (Table 5).

Table 8. Results of physico-chemical parameters of CW planted with *S. acuta* and *S. nodiflora* and amended with biochar

Parameter	**Unit**	**Influent** (Min-Max Mean±STD)	**Effluent** (Min-Max Mean±STD)	**Removal Efficiency %**
TDS	Mg/l	1012 – 1802 1398 ± 400.7	890 – 1708 1234 ± 346.1	11.7
BOD	Mg/l	13.1 – 20.6 17.53 ± 3.160	6.2 – 9.6 8.375 ± 1.493	52.2
COD	Mg/l	153 – 410 306.3 ± 111.3	63 – 163 102.3 ± 42.91	66.6
NO_3	Mg/l	11.9 – 21.17 16.67 ± 4.985	5.75 – 10.14 8.423 ± 1.991	49.5
PO_4	Mg/l	0.796 – 3.677 2.402± 1.300	0.299 – 1.796 0.869 ± 0.655	63.8
SO_4	Mg/l	10.6 – 154 87.45 ± 58.96	0.001 – 87.4 64.28 ± 42.87	26.5
Fe	Mg/l	0.302 – 0.416 0.377 ± 0.052	0.018 – 0.239 0.124 ± 0.117	67.1
Zn	Mg/l	1.143 – 2.132 1.863 ± 0.480	0.003 – 0.016 0.009 ± 0.006	99.5
Mn	Mg/l	0.105 – 0.13 0.112 ± 0.012	0.019 – 0.039 0.025 ± 0.009	77.7
Overall %R				**57.2**

Removal efficiency in unplanted wetland (Table 9) was 39.0%. This is lower than the efficiencies of the treated wetlands with sand only; Table 3 (52.5%), Table 4 (58.4%), Table 5 (54.4%). This is obvious that the macrophytes played an important role in the wastewater treatment. Performance of treatment (Table 9) was below average except for Zn removal which was highly satisfactory.

Table 9. Results of physico-chemical parameters of CW unplanted (sand control)

Parameter	**Unit**	**Influent** (Min-Max Mean±STD)	**Effluent** (Min-Max Mean±STD)	**Removal Efficiency %**
TDS	Mg/l	1012 – 1802 1398 ± 400.7	611 – 1562 1170 ± 446.6	16.3
BOD	Mg/l	13.1 – 20.6 17.53 ± 3.160	10.6 – 12.4 11.6 ± 0.748	33.8
COD	Mg/l	153 – 410 306.3 ± 111.3	111 – 320 200.5 ± 87.76	34.5
NO_3	Mg/l	11.9 – 21.17 16.67 ± 4.985	8.168 – 11.12 10.11 ± 1.351	39.4
PO_4	Mg/l	0.796 – 3.677 2.402± 1.300	0.577 – 2.922 1.798 ± 1.176	25.1
SO_4	Mg/l	10.6 – 154 87.45 ± 58.96	0.001 – 135 49.65 ± 63.77	43.2
Fe	Mg/l	0.302 – 0.416 0.377 ± 0.052	0.255 – 0.306 0.276 ± 0.022	15.6
Zn	Mg/l	1.143 – 2.132 1.863 ± 0.480	0.005 – 0.194 0.057 ± 0.092	96.9
Mn	Mg/l	0.105 – 0.13 0.112 ± 0.012	0.049 – 0.091 0.07 ± 0.018	46.4
Overall %R				**39.0**

Table 10 (CW8) showed that there were differences in the removal efficiencies of planted and unplanted beds (control) even though the difference for the planted beds treated with biochar (Table 6-54.4%, Table 7- 50.0%, Table 8-57.1%) was slightly better than the unplanted bed (46.7%).

Table 10. Results of physico-chemical parameters of CW unplanted (biochar control)

Parameter	**Unit**	**Influent** (Min-Max Mean±STD)	**Effluent** (Min-Max Mean±STD)	**Removal Efficiency %**
TDS	Mg/l	1012 – 1802 1398 ± 400.7	961 – 1867 1283 ± 419.1	8.2
BOD	Mg/l	313.1 – 20.6 17.5 ± 3.160	10.2 – 12.2 11.15 ± 0.835	36.4
COD	Mg/l	153 – 410 306.3 ± 111.3	108 – 232 170.5 ± 51.28	44.3
NO_3	Mg/l	11.9 – 21.17 16.67 ± 4.985	8.022-13.29 10.93 ± 2.315	34.4
PO_4	Mg/l	0.796 – 3.677 2.402± 1.300	0.395 – 2.587 1.355 ± 0.997	43.6
SO_4	Mg/l	10.6 – 154 87.45 ± 58.96	0.001 – 123 62.20 ± 55.84	28.9
Fe	Mg/l	0.302 – 0.416 0.377 ± 0.052	0.049 – 0.205 0.150 ± 0.073	60.2
Zn	Mg/l	1.143 – 2.132 1.863 ± 0.480	0.005 – 0.012 0. 009 ± 0.005	99.5
Mn	Mg/l	0.105 – 0.13 0.112 ± 0.012	0.028 – 0.049 0.039 ± 0.009	65.2
Overall %R				**46.7**

4.1.1 Metal accumulation levels in macrophytes

Table 11. Metal levels in plants before treatment

	S. Acuta		*S. nodiflora*	
Mg/l				
	Root	Shoot	Root	Shoot
Fe	0.797	0.736	0.569	0.553
Zn	2.167	2.232	2.181	2.154
Mn	0.150	0.195	0.199	0.164

The metal levels in the *Sida acuta* and *Synedrella nodiflora* was determined before it was planted in the beds (Table 11).This was only to ascertain if the plants were able to accumulate metals from the wastewater after the treatment. From the results, Zn was high in the roots and shoots in both plants (Table 11). Metal levels in *S. nodiflora* were high in the root and high in the shoot for *S. acuta.* Manganese was generally low in in both plants

Table 12. Metal levels in plants after treatment

Metal		***S.acuta***		***S. nodiflora***	
		Root	**Shoot**	**Root**	**Shoot**
Fe	Range	1.83 - 2.00	3.50 - 3.80	2.40 -3.13	3.30-3.70
	mean±std	1.92 ± 0.12	3.65 ± 0.21	2.77±0.52	3.50±0.28
Zn	Range	2.00 - 2.01	2.02 - 2.04	2.01 - 2.02	2.10 - 2.12
	mean±std	2.01 ± 0.01	2.03 ± 0.01	2.02 ± 0.01	2.11 ± 0.01
Mn	Range	0.233 -0.251	0.286 - 0.361	0.270-0.297	0.005-0.372
	mean±std	0.242 ±0.013	0.324 ±0.053	0.284±0.019	0.186±0.263

After the treatment, there were significant rise in the metal levels measured before planting. On the contrary, the level of Zn in both plants declined (Table 12) comparing it to the levels measured before planting (Table 11). This means both plants are not good extractors of Zn. Zinc (Zn) was best removed in the effluents (Figure 9). This implies treatment was performed by the media and to a very small extent the macrophytes. Extraction of Manganese (Mn) by both plants was satisfactory in both the roots and the shoots. Mn removal in the effluents was high in the planted beds than in the controls (Figure 9).

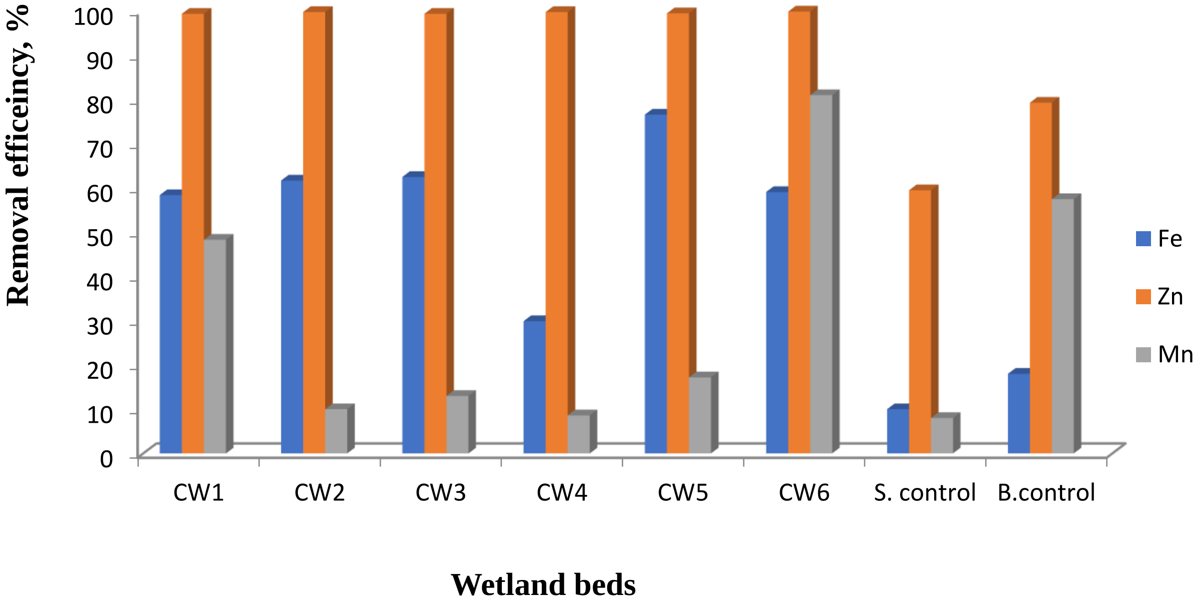

Figure 9. Average Removal efficiencies of metals in effluent in wetland beds

Table 13. Analysis of Variance for BOD, COD and Mn

Sand treatment	**Control (CW7)**	**CW1**	**CW2**	**CW3**
BOD_{mean}	11.6^{x}	10.7^{x}	10.8^{x}	10.3^{x}
COD_{mean}	201^{x}	74.3^{y}	85.3^{y}	82^{y}
Mn	0.265^{x}	0.103^{x}	0.126^{x}	0.154^{x}
Biochar treatment	**Control (CW8)**	**CW4**	**CW5**	**CW6**
BOD_{mean}	11.2^{x}	9.5^{y}	9.3^{z}	8.4^{x}
COD_{mean}	171^{x}	79.8^{x}	85.8^{x}	102^{x}
Mn	0.047^{x}	0.103^{y}	0.085^{y}	0.023^{z}

Note: Averages followed by same letter indicates non-significant difference, and those following with different letters indicate significant difference by Tukey test at 5% of probability.

4.2 Bacteriological results

A significant difference was observed in the microbial load between the raw wastewater and the treated wastewater (Table 14). Biochar amended soil performed better in removing microbes. *E. coli* and Total coliform removal was of average. Vibrio cholerae was removed better in the biochar amended beds. Unplanted beds reduced microbes to some extent. This means media has some ability to treat wastewater.

Irrigated vegetables showed a reduced microbial level (Table 14) compared to the level of microbes in the treated water (Table 14). *Vibro cholera* and *Samonella* was best removed in the treatments with biochar. Microbial levels in treated wastewater and irrigated vegetables were compared to FAO limits and WHO guidelines for wastewater quality for irrigation or reuse (Appendix 1).

Table 14. Microbial load (mean values) in Raw and Treated water

Table 11 (100ml/cfu)	**Raw Waste water**	**CWI**	**CW2**	**CW3**	**CW4**	**CW5**	**CW6**	**CW7 Control**	**CW8 Control (biochar)**
Total Coliform	67,000	5,100	7,300	6,200	3,005	4,010	2,900	3,200	2,710
E.coli	9,000	3,200	3,520	4,200	1,710	1,100	1,050	2,004	1,300
Vibrio cholerae	2,310	51	78	33	0	0	0	15	0
Salmonella	1,970	101	87	83	32	47	51	84	22

Table 15. Microbial load (Average) in irrigated vegetables after harvesting

	Mean Values			
100ml/cfu	**Water leaf watered with Biochar amended treated water**	**Water leaf watered with non Biochar amended treated water**	**Jute leaves watered with Biochar amended treated water**	**Jute leaves watered with non Biochar amended treated water**
Total Coliform	1300	1700	1,860	2,100
E.coli	900	1,600	78	119
Vibro cholerae	1005	1850	0	0
Salmonella	1,070	1,100	0	0

CHAPTER FIVE

DISCUSSION

In this study, eight VF mesocosms containing combinations of sand and biochar were used to treat domestic wastewater. The inflow and otflow concentrations in the different wetland beds were characterized and the removal efficiencies based on pH, TDS, COD, BOD, NO_3, PO_4, SO_4, Fe, Zn and Mn values were evaluated.

TDS

The results presented in Table 1 to 8 shows that the efficiency of the CW in removing TDS was only 15.88 ± 7.23% which was below satisfactory. The lowest removal of 7.4 % was found in Table 7 (planted with *S. nodiflora* and biochar) and the highest of 27.7% found in Table 5 (planted with combination of plants). Analysis of variance proved no significant differences between influent and effluent concentrations indicating that the eight treatments were not effective in TDS reduction. This suggests that neither the biochar amendment in sand nor the macrophytes nor both influenced the efficiency of wetland beds in reducing TDS. Total Dissolved Solids (TDS) can be defined as solids dissolvable in water, consisting of a small amount of organic matter and inorganic salts mostly calcium, magnesium, potassium, sodium, bicarbonates, chlorides and sulfates (Muigai et. al., 2010). Almeida et al., 201, noted an increase in the TDS levels in the treated wastewater compared to the raw wastewater of each treatment but there were significantly different. The study indicated that TDS levels were high in the effluents because there was no salt retention in the wetland beds. Sudarsan et al. hypothesized that, some of the bio-films fell out and got dissolved after the bio-films were growing in the media especially gravels in large quantities and therefore enhanced TDS value through the outlet.

Nitrate

In comparison, there was no significant difference in all the treatments in reducing nitrate, there was no significant difference in reducing nitrate. The controls had no beneficial results from the other beds. The values for nitrate removal were lower in all the wetland beds in comparison to the organic matter values. This could be attributed to the fact that removal of total nitrogen and phosphorus require s a prolonged Hydraulic Retention Time, HRTs (Gupta et al., 2015). According to Table 7-8, the removal efficiencies of the controls were the lowest and this denotes controls showed increment in nitrate concentrations. This increment may be as a result of the formation of NO_3 due to nitrification. Nitrification is the conversion of NO_2 to NO_3 by some heterotroph bacteria such as Nitrospina, Nitrospira and Nitrobacter (Kurniadie, 2011). Because of the small differences between unplanted and planted treatments, it can be concluded that vegetation played an important role in NO_3 uptake (Kadlec and Wallace, 2008). The highest removal efficiency in nitrate (49.5%) was found in the combination treatment amended with biochar (Table 8) although there was no important difference with other treatment beds. Aside the plants, biochar played a role in removing nitrate. Many studies have demonstrated the ability of biochar to retain nitrogen (Ding et al, 2010). Wetland denitrification occurs in the anoxic zones of the sediments beneath an aerobic water surface layer or in anoxic microsites of a biofilm attached to plant tissue or substrata. Very porous biochar has a large surface area, which offers an anaerobic state of the microbial bioactive film, and this could be the possible reasons for the high removal efficiency (49.5%) in nitrate in the wetland planted with both plants (Table 8), as compared to the other beds. Saeed & Sun, 2012, reported that denitrification by facultative bacteria produces nitrogen gas (N_2), nitrogen oxides (N_2O) or nitric oxide (NO). Some of the facultative bacteria like Aerobacter, Proteus and Flavobacterium, were capable of reducing NO_3 to NO_2. Overall, single planted *S. acuta* and *S. nodiflora* also performed well in NO_3 reduction

(43% and 44% respectively). The processes might be similar; however, due to the different root sizes and shapes of the roots, different microenvironment can exist in the soil, depending on the diversity of microorganisms. Almeida et al., 2017 reported that plants support NO_3 removal but other effects are species dependent.

Phosphate

Phosphate was generally removed by all treatments. However, the results showed that there was no significant difference between the control treatments and the other treatments in reducing phosphate. The highest removal was in the combination treatment Table 5 (75.3%) and the lowest found in Table 9 (25.1%). However, combination treatment with biochar gave better results at 63.8%.Therefore, in this study, using a combination of both plants in phosphate reduction was better than using a single plant. However, single planted *S. acuta* proved to be the most effective in reducing phosphate by 54.6% phosphate reduction. This indicates that this species has a great potential for absorbing phosphate. It was observed that treatment in sand media yielded better results than in the biochar amended sand with removal efficiencies between 58% - 75.3%. In addition to the absorption of plants, phosphate removal can also occur through ion exchange and adsorption from membranes (Mojiri et al., 2017). Brix et al., 2001noted P-sorption in subsurface constructed wetlands can be enhanced by a mixture of materials like sand or gravel. Haritash et al., 2017 found that phosphate removal relies on the uptake of plant and is therefore imperative to control the build-up in plant tissues. This is consistent with the hypothesis that planted system play a greater role than unplanted systems. Studies (Sudarsan et al., 2016) have shown that nutrients uptake is mostly by root due to symbiotic microorganism existing in the roots.

Sulphate

High effluent concentration of sulphate was observed in the sandy medium planted with single *S. nodiflora* (Table 4) at 99.8%, followed by single *S.acuta* at 50% while the lowest performance was observed in the treatment with biochar, Table 6 and Table 8 at 20.5%. There was no significant difference in sulphate concentrations among the eight VF mesocosms. This indicates that the addition of biochar to the sand media or the presence of plants in mesocosms had a significant effect on the sulphate removal. These results were consistent with those of Chen et al., 2016, who showed that the presence of vegetation only had little effect on sulphate removal.

Some mechanisms that aid sulphate removal in CW systems include uptake by plants, release of hydrogen sulphide by the bacteriological reduction of sulphate, precipitation and adsorption of sulphur compounds in the media due to calcium and iron solubility (Chen et al., 2014; Baldwin and Mitchell, 2012). Since the VF mesocosms were aerobic, hydrogen sulphide release may not have contributed in removing sulphate significantly. Since all the treatment beds had a high pH (about 8), the main potential mechanisms involved in sulphate removal may be the simultaneous occurrence of adsorption and precipitation. Also, competition with other compounds can affect the efficiency of sulphate removal. Since the raw wastewater was obtained from domestic wastewater and all the media contains silicon compound, competition between sulphates and other compounds and elements is inevitable. This situation could reduce the removal efficiency of all media in sulphate removal (De Rozari et al., 2020). This may be relevant for this study because sulphate removal was low in almost all the treatments (Table 3-10).

BOD and COD

According to the results obtained, the average BOD and COD of the eight treatment beds were 61.7 and 64.1 percent, respectively. Higher removal efficiency among the various planted treatments was observed for COD, 66.6 – 75.3% with CW1 (single *S. acuta* in sand) showing the highest, 75.3%.

One way ANOVA analysis showed a significantly different treatment among some treatments for BOD and COD. This implies that each treatment had some potential in reducing BOD and COD. A further t-test, Table 13, comparing means of the unplanted treatments (control) to the planted treatments at a more detailed level at 5% significance ($p< 0.05$) and Bonferroni corrected revealed that single *S. acuta* planted in biochar amended soil (CW4) differed significantly from the unplanted biochar treatment (CW8) and also from single S. nodiflora planted in biochar amended soil (CW5) for BOD. In the case of COD, single S.acuta planted in sand media (CW1) was significantly different from the unplanted sand treatment (CW7), Table 13. Several studies have shown differences in the removal activity between the planted and unplanted wetlands (He and Mankin, 2002). This study also shows similar pattern. The results show that the plants played a role in the BOD and COD removal (Kadlec and Wallace, 2008). However, combination treatment amended with biochar had the best performance for reducing BOD value (52.2 %). It is assumed that due to the presence of both plants, the diversity of microorganism in rhizosphere area was high.

Sehar et al., (2015) suggested that interactions between microbes and physical mechanisms support BOD and COD removal by attracting dissolved oxygen. BOD removal involves precipitation and microbial decomposition by aerobic bacteria attached to plant roots, while COD removal involves precipitation and filtration rather than biological processes. Both plants performed well for these parameters. The controls were not as beneficial as the planted treatments since the rhizosphere had no additional oxygen supply as it was unplanted.

Heavy Metal Concentration

Removal efficiency of heavy metals in descending order in the different wetland beds was Zn > Fe > Mn (Table 3-Table 10). Zn showed the highest removal in the combination treatment with biochar (CW 6) and in the biochar control (CW8), both at 99.5%. The lowest removal was in the combination treatment (CW3) at 53.7%. Single *S. nodiflora* (CW2) performed well in removing Fe at 99.8% and sand control performed least at 15.6%. Mn removal was highest in the combination treatment CW3, 87.7%, followed by combination treatment with biochar CW6 at 77.7%. Single *S. nodiflora* performed least at 1.8 %. Despite the variations, the concentrations of the metals in the treated wastewater were below the FAO guidelines for irrigation water (Table 5). The metal concentrations in the treated water between the planted treatments and the unplanted treatments (controls) for Fe and Zn were not significantly different. However, analysis of variance for Manganese (Mn) was significant at $p< 0.05$ and the Bonferroni corrected post hoc t-test revealed a significant difference between single *S. acuta* biochar treatment(CW4) and unplanted biochar treatment (CW8) and a high significant difference between CW4 and combination treatment with biochar CW6 (Table 11).

Sida acuta and *Synedrella nodiflora* showed differing bioaccumulation abilities after being exposed to different levels of Fe, Zn and Mn pollution. Metal accumulation in plants was in the order *Combination* >*Sida acuta* >*Synedrella nodiflora.* Combination plants accumulated very high Fe and Mn in the roots. *S.acuta* extracted more Fe, Zn and Mn in the shoot than in the root. *S. nodiflora* accumulated Fe and Zn in the shoot and Mn in the root. Metal results (Table 9-10), shows that *S. acuta* and *S. nodiflora* were able to undergo absorption and bioaccumulation of Fe, Zn and Mn concentrations significantly. It was also observed that biochar augmented the digestibility of metals in the soil (media).

A successful phytoremediation process through the techniques of phytoextraction of the contaminant is solely dependent on the plant's ability to transport pollutants from below-ground biomass to the top layer. On the other hand, the success of the plant remediation processes through plant pollutant stabilization methods often depends on the limited ability of the plant to stabilize the pollutants below ground (Baker, 2000; Dada and Awotoye, 2013). Metal contamination level determines the metal in all the studied plants. This observation is similar to that reported by Wang et al. (2007). This may be because plant roots first come into direct contact with pollutants through passive transport.

Pathogen removal

High pathogen removal was achieved in the treatments amended with biochar with the highest being observed in the combination wetland amended with biochar CW6. In this study, pathogens of interest were Total coliform, *E. coli, Vibro cholera* and *Salmonella.* The values obtained for these pathogens for the treated wastewater and irrigated vegetables were compared to the FAO wastewater quality guidelines for irrigation and WHO guidelines for agriculture reuse. It was observed that treatments with biochar gave a lower microbial load compared to the treatments from sand beds and also vegetables irrigated with biochar amended treatments showed lower values (Table 13).

Removal of pathogens in treatments was performed by both plants and biochar. There is a limited number of studies on the effect of biochar amendment on pathogen removal from domestic wastewater (Gwenzi et al., 2017) and these few studies focused on E. coli removal

(Boehm et al., 2020). Anaerobic biofiltration of untreated wastewater using biochar showed significantly higher removal rates of E. coli (99.5%), enterococcus (99.6%) and bacteriophages (98.6%) compared to sand filters (Kaetzl et al., 2019). Nevertheless, the effectiveness of the actual removal of the pathogen may be different due to the hydrophobicity of the pathogen and the atmosphere at the electrical point (i.e., the pH at which a molecule shows electrical neutrality) (Perez-Mercado et al., 2019).

Jensen et al., 1991, revealed that wastewater purification occurs through microbial interactions in plant stems and the reaction of water with upper sediments. Microbial growth on plant roots, chemical processes and filtrations of the substrate itself together provide the system with water purifying properties (Jensen, 1993). Although the microbial population in the rhizosphere region of the plant root system is dominated by bacteria, bacteria are expected to degrade most of the common organic matter, i.e. those contributing most to the biochemical oxygen demand, which is the most important material due to relatively low enzymatic activity on the tested organic substrates (Jensen, 1993).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

Conclusion

Constructed wetlands are feasible alternative for treating wastewater with high efficiency in the removal of pollutants, economical, ease of operation and maintenance, and possible use of the treated water for agriculture purposes. The Vertical Subsurface Flow (VSSF) constructed wetland employed proved to be effective and efficient in reducing pollutants from domestic wastewater. Physical, chemical, and biological mechanisms occurred in the constructed wetland to effectively reduce nutrients. The role of media (sand, gravel, and biochar), plants and microorganisms in the wetland improved the conditions of the environment, thereby improving the quality of the wastewater. In terms of vegetation, this study found out that *Sida acuta* was best in maintaining endurance and tolerance towards wastewater. However, *Synedrella nodiflora* was better in reducing some parameters such as Sulphate, Phosphate, and Nitrate. A difference in improving the quality of wastewater between single treatment and combination treatment was not significant. Nonetheless, there were significant differences in the reduction of Nitrate and Phosphate between control (unplanted) and planted treatments. In this study, both macrophytes were better in removing heavy metal and organic matter, rather than inorganic matter. It was observed that both the macrophytes and biochar amendment contributed to the treatment of the wastewater. The two traditional plants, *Sida acuta* and *Synedrella nodiflora* used in this experiment showed a significant potential to bioaccumulate heavy metals in treating wastewater. The absorption of metals in these plants was in the order *S. acuta* > *S. nodiflora* in both the shoots and the roots. However, combination treatment significantly accumulated Manganese (Mn) much better. Comparing the results of this study to the WHO and FAO guidelines for agricultural reuse and wastewater quality for irrigation, all parameters were within the given range. This makes the treatment a success and satisfies the third objective of this study.

Recommendations

Regarding the application of Constructed wetland in the treatment of domestic wastewater;-

- It is important to further explore the use of local media that remove pollutants effectively from domestic wastewater. The application of local media in constructed wetlands may decrease the cost of investment remarkably and encourage farmers to practice.
- It is recommended that further research should be conducted to discover endemic plants which are capable of reducing nutrients and heavy metals in our domestic wastewater.
- The use of biochar as media in CWs has to be extensively researched to a significant extent even though biochar has been investigated widely for its remediation capability in the environment. The large availability of biomass resources in Ghana would make biochar production and its use in CWs convenient for farmers.
- It is recommended that the performance of the system be improved by providing a proper condition in terms of system design and operational parameters.
- The rate of flow of the pipes discharging wastewater from tanks to the beds should be regulated so that the minimum required retention time in beds is ensured and the beds would be capable of tolerating hydraulic shocks.
- Economic aspects of constructed wetlands should be considered. Such systems can be used to produce plant and vegetable fibers which are utilized in the paper and cloth industries. Also, constructed wetlands create beautiful landscapes that can be considered in terms of tourism.

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APPENDIX

Appendix 1. GUIDELINES FOR INTERPRETATION OF WASTEWATER QUALITY FOR IRRIGATION

	Unit	FAO limit	WHO guideline
E. Conductivity	µS/m	2000	
TDS	Mg/l		
p H	pH units	6.5-8.5	
DO	Mg/l		
BOD	Mg/l	30	
COD	Mg/l	90	
NO_3	Mg/l	15	
PO_4	Mg/l		
SO_4	Mg/l	500	
Fe	Mg/l	5.0	
Zn	Mg/l	2.0	
Mn	Mg/l	0.2	
Feacal coliform	Per 100ml	1000	
Total coliform			<3000
Vibro Cholerae			<1000