STUDY OF THE USE OF SEED POWDERS OF THREE INDIGENOUS PLANTS AS BIO-COAGULANTS IN WASTEWATER AND GROUNDWATER TREATMENT

BY

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CERTIFICATION

We hereby certify that this dissertation, **"Study of the seed powders of three indigenous plants as bio-coagulants in wastewater and groundwater treatment"** was carried out by **IKECHUKWU OBIADIMMA OSINOMUMU** with Reg. No. **20194196568** in partial fulfillment of the requirements for the award of the Degree of Master of Science in Environmental Conservation and Management in the Department of Biology, Federal University of Technology, Owerri.

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DEDICATION

I dedicate this thesis to the memory of my late father and his farm, Ossy Farm Limited.

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My heartfelt appreciation is to God Almighty for his merciful guidance and protection.

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ABSTRACT

This study was designed to assess the coagulating efficacy of three indigenous plants (*Moringa oleifera, Afzelia africana,* and *Muccuna flagellipse*) seed powders as bio-coagulants in wastewater and groundwater treatment. Fresh, healthy, and mature seeds of *Moringa oleifera, Afzelia africana,* and *Muccuna flagellipse* were bought from the relief market in Owerri and processed into fine powders. Qualitative phytochemical screening of aqueous extracts of the seed powders was carried out, while physicochemical and bacterial analysis of the waste samples collected from Somachi slaughter (slaughterhouse wastewater) and Eziobodo (groundwater), Owerri Municipal was assessed before and after treatment. Three (3) different fresh stock solutions (SS) (15 g/300 ml, 30g/300ml, and 45g/300ml) of the seed powders were freshly prepared. Each 100ml of the water sample was measured into a clean 250ml beaker, and 10ml/20ml fresh stock solution were added and labeled accurately. The mixtures were stirred and allowed to stand for 24 hours for effective contact before filtering. Physicochemical parameters such as color, odor, and appearance were checked using ten (10) different observers, while pH, turbidity, electrical conductivity (EC), total dissolved solids (TDS), biological oxygen demand (BOD), dissolved oxygen (DO), and chloride (Cl^-) were assessed according to standard technique. The result revealed the presence of phytochemicals such as alkaloids, flavonoids, phenols, tannins, steroids, saponins, and anthraquinones in *M. oleifera, A. africana,* and *M. flagellipse,* except for steroids in *M. flagellipes* and *A. africana*. The physicochemical properties of the water samples before treatment show that the slaughterhouse wastewater sample was brown in color, highly turbid, poor in appearance, and almost odorless, while the groundwater sample was completely colorless, odorless, and clear with excellent appearance. After treatment with 10mL and 20mL fresh SS, there was an observed decrease in the level of turbidity, pH, temperature, and BOD and an increase in DO and Cl^- levels across different treatments and concentrations. *M. oleifera* showed the best performance with the lowest mean turbidity, followed by *A. africana* and *M. flgellipes*. Furthermore, groundwater treatment indicated changes in color, odor, appearance, and pH, which compare favorably with the control, while temperature, EC, TDS, DO, BOD, and Cl^- were statistically the same before and after treatment. The turbidity increases across the different treatments. The bacterial screening results revealed the presence of coliforms (*Escherichia coli, Streptococcus spp, Enterobacter spp*.) and pathogenic bacteria (*Pseudomonas spp., Staphylococcus aureus, and Campylobacter jejuni*). Treatment with *M. oleifera* resulted in the lowest bacterial count, followed by *A. africana* and *Muccuna flagellipes.* The bacteria count for groundwater before and after treatment was significantly the same, except for a slight increase in *E. coli* and *C. jejuni* with *M. flagellipes,* though still within the WHO allowable bacterial count for domestic water (100-500/ml) of colony-forming units. Therefore, the use of *M. oleifera* as a bio-coagulant should be adopted in wastewater treatment, as it not only improves the water quality but also reduces the pathogenic bacteria load.

Keywords: *Bio-coagulants, slaughterhouse wastewater, groundwater, water treatment.*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Water is an important natural resource for life upon which all living organisms depend for their survival and continuous existence (Hendrawati *et al*., 2016). According to Abiyu *et al.* (2018), it is considered as the most crucial natural resource on earth, whose quality and availability served as an important life determinant. It is a vital factor for global economic development because of its usage and applications in various productive sector such as industry, livestock and agricultural production, and urban/rural supply, thus leading to overuse and abuse (Déborah *et al*., 2018).

According to UNESCO, reduced water quality contributes to water scarcity. Factors such as rapid urbanization, increased farming activities, pesticide use, land degradation, high population density, and unsuitable waste disposal are affecting the quality of available fresh water sources (UNESCO, 2015). In wastewater treatment processes, one of the primary processes is coagulation followed by flocculation (Déborah *et al*., 2018). Coagulation and flocculation are physicochemical processes that are often used at the beginning or end of wastewater treatment processes. In conventional treatment processes, many different types of coagulants are commonly used depending on the chemical features of the contaminants present in the water (Vieira et al., 2010). In general, coagulants are classified as inorganic, as well as synthetic organic, or natural organic polymers (Vieira *et al*., 2010).

An ideal water has some characteristics like clear, colorless, tasteless, odorless, pathogen-free, harmful chemical-free and non-corrosive. Water is also expected not to leave sediment in all distribution organs as this will help to prevent the occurrence and the spread of waterborne diseases. To achieve this standard, there is one common technique applied in water treatment process, which is coagulation-flocculation. Coagulation is the process of coagulating colloidal particles due to the addition of synthetic materials to neutralize charged particles thus forming a precipitate due to the force of gravity and it is achieved by the use of coagulant obtain from synthetic materials such as ferrous sulfate (Fe(SO4), aluminum sulfate or alum (Al2(SO4)3), and Poly Aluminium Chloride (PAC) (Al2(OH)3Cl3) (Vieira *et al*., 2010).

Coagulation is one of the most common ways to reduce the pollutant contents in the water body that are present as turbidity, color and organic matters. Separation of these colloids can be done by the addition of synthetic coagulant or bio-coagulant followed by slow agitation (flocculation) that causes coagulation of colloidal particles so they can be separated by sedimentation. *Moringa oleifera* is a fast-growing, drought-resistant tree that can reach a height of 10-12m. It is the most

widely cultivated species of the monogeneric family, the *Moringaceae,* which is indigenous to South Asia, where it grows in the Himalayan foothills from Northeastern Pakistan to Northern West Bengal, India (Hendrawati *et al*., 2016). It has been introduced and become naturalized in other parts of India, Pakistan, Afghanistan, Bangladesh, Sri Lanka, Southeast Asia, West Asia, the Arabian Peninsula, East and West Africa, Southern Florida, throughout the West Indies, and from Mexico to Peru, Paraguay and Brazil (Vélez-Gavilán, 2017). Common names include moringa, miracle tree, drumstick tree, ben oil tree, benzolive tree, benzoil tree, horse-radish tree, horseradish tree, West Indian ben, clarifier tree, cabbage tree (Tree for Life International, 2011). The bark has a whitish-grey color and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches, and the leaves build up a feathery foliage of tripinnate leaves. *M. oleifera* is grown for human food, medicine, dye, fodder and water clarification, most especially in the West where its powdered seeds are used to flocculate contaminants and purify drinking water (Lakshmipriya *et al*. 2016). It is one of the natural coagulants that have been extensively studied in clarification of turbid water (Sajidu *et al*., 2005). *Moringa Oleifera* seed proteins have been found to possess coagulating properties similar to those of alum. Jahn, (1995) first confirmed the coagulating properties of Moringa seeds after observing women in Sudan use the seeds to clarify the turbid Nile waters (Jahn, 1981). The coagulating property of the seeds is due to water- soluble cationic polypeptides with molecular weights ranging from 6000 to 16000 daltons (Jahn, 1995).

Afzelia africana also known as counter wood tree or African Oak or Mahogany bean with trade names Àpá (Yoruba), Kawo (Hausa) and Akparata (Igbo) is leguminous plant in the family of fabaceae sub family Caesalpinaceae (Omokpariola *et al*., 2021). It is found from Senegal in West Africa to Sudan, Uganda and Tanzania in the east and south Asia (in India). It is occasionally grown in other tropical countries as an ornamental. This deciduous plant has pods containing about 6-12 elliptical, long shaped glossy black seeds with cap like waxy orange aril, which is released by explosive mechanism, if not harvested (Ikwuagwu *et al*., 2013*). Afzelia africana* contain 18-37% of oil and it is composed of palmitic and oleic acid, which required little purification and has a long shelf life. It is suitable for the production of alkyl resins and shoe polish. It is a good source of dietary protein and mineral that compare with animal protein from meat, egg and fish (Ikwuagwu *et al*., 2013). The leaves of "akparata" are sometimes eaten cooked as a vegetable, young leaves are mixed with ground cereals before cooking. The flowers are used as condiment in sauces. The flour from seeds is used as a substitute for wheat flour in biscuits and doughnuts and also as soup thickeners (Matouk *et al.,* 2014). It produces high

quality wood for constructing canoes, cooking utensils; furniture and African drum ('djembe'). *Afzelia africana* seed has been reported to contain protein, crude fibre, ash and lipid and used in providing seed flour and oil (Matouk *et al.,* 2014; Sacande, 2017). The plant is used in local medicine for general pain relief, digestive problems, such as constipation and vomiting and for internal bleedings (hemorrhagic) (Sacande, 2017). The seeds of *A. africana* exhibit ''orthodox'' storage behavior. At moisture content (MC) of about 8%, the seeds can be stored at ambient conditions for at least 33 months, without significant decrease in viability. For short term storage, the seeds can be stored with moist vermiculite or sawdust at about 25°C, ventilating frequently to ensure aerobic conditions (Sacande, 2017).

Mucuna is a genus of around 100 accepted species of climbing vines and shrubs of the family fabaceae, found worldwide in woodlands of tropical areas.

*Mucuna flagellipes (*L.) Medik (horse-eye bean) is a species of large liana from the family Fabaceae. The plant is native to tropical Central and South America and has been introduced into the Republic of the Congo. Common names include horse-eye and ox-eye bean (Yang *et al*., 2014). It is popularly known as "Ukpo" by the Igbo-speaking people of south-eastern Nigeria and used as a soup thickener in traditional soup preparation due to its high content of water dispersible polysaccharide (gum) making flour of the seed to be highly pseudoplastic and the leaves as feed for farm animals in the Northern States (Osaniyi & Eka, 2019). Its pods are covered with brownish dense whisker-like hairs called trichomes that are irritating when they come in contact with the skin or eyes. Each pod may contain 1-3 seeds with a hard coating which is white when immature and turns black when mature and dry (Enwere, 2018). The seeds are cracked, boiled, dehulled, ground to powder and added to soup. But as a choice dish, the seeds are cracked, boiled overnight and dehulled. The cotyledons are spiced to taste and served as a delicacy (Enwere, 2018).

1.2 Statement of the Problem

The provision of adequate clean potable water to communities have become a global problem, especially in developing countries like Nigeria where her rural populace depends totally on water from rivers, dams and streams which are often polluted by various anthropogenic activities like mining, application of fertilizer/pesticides, discharge of wastewater from textile industries and others without adequate treatment before discharging into this so claimed water bodies and people still depend on these water sources for their various domestic activities and also for drinking (Abiyu *et al*., 2018). The presence of these pollutants and surface run-off after heavy

rainfall affect the turbidity of the receiving water bodies, thus, increasing the level of suspended particles that do act as a shield against pathogens and some toxic materials. Conventionally, coagulation as an important means of improving water quality using majorly synthetic coagulants like ferrous sulfate (Fe(SO4)), aluminum sulfate or alum (Al2(SO4)3), and poly aluminum chloride (PAC) (Al2(OH)3Cl3) has been applied as a good method of removing these suspended particles to make this water clean for further usage, thereby averting the problems associated with water scarcity (Vieira *et al.,* 2010). These synthetic coagulants have been shown to pose some levels of health hazards, especially when used in larger quantities. Apart from the health problems caused by these synthetic coagulants, they are not easily accessed, thus making them a rare commodity. Hence, there is a need to devise a new technique for improving wastewater and groundwater quality that will not cause harm to the health and lives of people and, at the same time, will be easily accessible.

1.3 Aims of the Study

The study aims to compare the use of indigenous plants (*Moringa oleifera, Afzelia africana,* and *Muccuna flagellipes*) seed powder as a bio-coagulant to treat wastewater and groundwater.

1.4 Specific Objectives

The specific objectives are;

1. To assess the physicochemical parameters of the wastewater and groundwater before and after treatment.
2. To determine the active ingredient from the plant seed samples
3. To access the bacteria load in the wastewater and groundwater before and after treatment and on the plant, samples are used as bio-coagulants.
4. To treat wastewater and groundwater samples using the coagulation method of water treatment.

1.5 Research Hypotheses

1. Physicochemical Parameter

H_0 = There is no difference in the physicochemical parameters of wastewater and groundwater samples before and after treatment.

H_A = There is a difference in the physicochemical parameters of wastewater and groundwater. samples before and after treatment.

2. Phytochemical Analysis

H0 = There is no difference in the phytochemical analysis of the different plant seed samples.

HA = There is no difference in the phytochemical analysis of the different plant seed samples.

3. Bacteria Load

H_0 = The bacteria load in the wastewater and groundwater samples are the same before and after treatment.

H_A = The bacteria load in the wastewater and groundwater samples are not the same before and after treatment.

4. Turbidity level

H_0 = There is no difference in the turbidity of the wastewater and groundwater before and after treatment.

H_A = There is a difference in the turbidity of the wastewater and groundwater before and after treatment.

1.6 Scope of the Study

This study will cover the preparation of a stock solution of the three different leguminous plant seed powders that will be used as bio-coagulants. It will also assess their coagulating ability to improve the quality of wastewater and groundwater. The turbidity level after applying each of these bio-coagulants will be assessed using a turbidimeter.

1.7 Justification of the Study

Water, as a natural resource, has played a tremendous role in meeting man's needs and those of aquatic dwellers that cannot exist outside of water. However, the pollution of this water from different anthropogenic activities has become a major concern to man and these aquatic lives, as the chemical method of coagulation used in improving the quality of water poses some level of health challenges to man. Therefore, the need to prospect for alternative means of coagulating wastewater and groundwater that will not pose any health hazard to man and at the same time satisfy the goals of sustainability development of the present millennium justifies this study.

CHAPTER TWO

2.0 LITERATURE REVIEW

Water quality can be defined as the chemical, physical, and biological characteristics of water, usually with respect to its suitability for a designated use (Spellman, 2013). Water can be used for recreation, drinking, fisheries, agriculture, or industry. Each of these designated uses has different defined chemical, physical, and biological standards necessary to fulfill the respective purpose. For example, there are stringent standards for water to be used for drinking or swimming compared to that used in agriculture or industry. Water quality is "the physical, chemical, and biological characteristics of water" (Spellman, 2013). Water quality is a measure of the condition of water relative to the requirements of one or more biotic species and/or to any human need or purpose (Shah, 2017).

Classification of Water

Based on its source, water can be divided into ground water and surface water (Gray, 2017). Both types of water can be exposed to contamination risks from agricultural, industrial, and domestic activities, which may include many types of pollutants such as heavy metals, pesticides, fertilizers, hazardous chemicals, and oils (Davis & Masten, 2016). Water quality can be classified into four types: potable water, palatable water, contaminated (polluted) water, and infected water (Chatterjee, 2018). The most common scientific definitions of these types of water quality are as follows:

Potable water: It is safe to drink, pleasant to taste, and usable for domestic purposes. Palatable water: It is aesthetically pleasing; it considers the presence of chemicals that do not pose a threat to human health.

Contaminated (polluted) water: It is water containing unwanted physical, chemical, biological, or radiological substances, and it is unfit for drinking or domestic use.

Infected water: It is contaminated with pathogenic organisms (Chatterjee, 2018).

2.1.1 Parameters of water quality

There are three types of water quality parameters: physical, chemical, and biological (Gray, 2017).

2.1.1.1 Physical parameters of water quality

Turbidity: Turbidity is the cloudiness of water (American Public Health Association, 2015). It is a measure of the ability of light to pass through water. It is caused by suspended materials such as clay, silt, organic material, plankton, and other particulate materials in water. Turbidity in drinking water is aesthetically unacceptable, which makes the water look unappetizing. Turbidity

is measured by an instrument called a nephelometric turbidimeter, which expresses turbidity in terms of NTU or TU. A TU is equivalent to 1 mg/L of silica in suspension (APHA, 2015). Turbidity greater than 5 NTU can be visible to the average person, while turbidity in muddy water exceeds 100 NTU (APHA, 2015). Groundwater normally has very low turbidity because of the natural filtration that occurs as the water penetrates through the soil (Viessman & Hammer, 2014).

Temperature: Palatability, viscosity, solubility, odors, and chemical reactions are influenced by temperature (APHA, 2015). Thereby, the sedimentation and chlorination processes and biological oxygen demand (BOD) are temperature dependent. It also affects the biosorption process of the dissolved heavy metals in water (White et al., 1997). Most people find water at temperatures of 10–15°C most palatable (APHA, 2015).

Color: Materials decayed from organic matter, namely, vegetation, and inorganic matter such as soil, stones, and rocks impart color to water, which is objectionable for aesthetic reasons but not for health reasons (Tomar, 2016). Color is measured by comparing the water sample with standard color solutions or colored glass disks. One color unit is equivalent to the color produced by a 1 mg/L solution of platinum (potassium chloroplatinate (K2PtCl6)). The color of a water sample can be reported as follows:

1. Apparent colour is the entire water sample color and consists of both dissolved and suspended components color
2. True color is measured after filtering the water sample to remove all suspended material (APHA, 2015).

Colour is graded on scale of 0 (clear) to 70 color units. Pure water is colorless, which is equivalent to 0 color units (APHA, 2015).

Taste and odour: Taste and odor in water can be caused by foreign matter such as organic materials, inorganic compounds, or dissolved gases. These materials may come from natural, domestic, or agricultural sources (DeZuane, 2017).

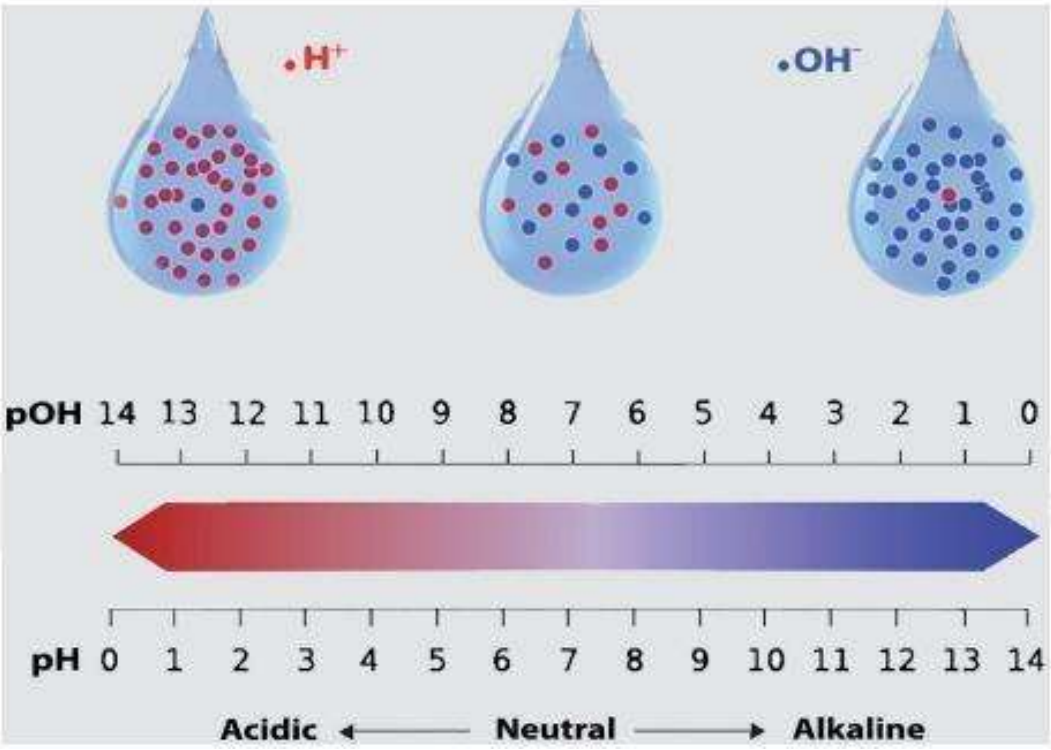
Solids: Solids occur in water either in solution or in suspension. These two types of solids can be identified by using a glass fiber filter that the water sample passes through. By definition, the suspended solids are retained on the top of the filter and the dissolved solids pass through the filter with the water (APHA, 2015). If the filtered portion of the water sample is placed in a small dish and then evaporated, the solids act as a residue. This material is usually called total dissolved solids or TDS Total solid (TS) = Total dissolved solid (TDS) + Total suspended solid (TSS)

Electrical conductivity (EC): The electrical conductivity (EC) of water is a measure of the ability of a solution to carry or conduct an electrical current.

2.1.1.2 Chemical parameters of water quality

pH: pH is one of the most important parameters of water quality. It is defined as the negative logarithm of the hydrogen ion concentration (Edzwald, 2010). It is a dimensionless number indicating the strength of an acidic or a basic solution [23]. Actually, pH of water is a measure of how acidic/basic water is (Tomar, 2016). Acidic water contains extra hydrogen ions (H^+) and basic water contains extra **hydroxyl (OH^-) ions.** pH ranges from 0 to 14, with 7 being neutral. pH of less than 7 indicates acidity, whereas a pH of greater than 7 indicates a base solution (WHO, 2011).

Pure water is neutral, with a pH close to 7.0 at 25°C. Normal rainfall has a pH of approximately 5.6 (slightly acidic) owing to atmospheric carbon dioxide gas. Safe ranges of pH for drinking water are from 6.5 to 8.5 for domestic use and living organisms need. There are two methods available for the determination of pH: electrometric and colorimetric methods.



Excessively high and low pHs can be detrimental for the use of water. A high pH makes the taste bitter and decreases the effectiveness of the chlorine disinfection, thereby causing the need for additional chlorine.

Chloride: Chloride occurs naturally in groundwater, streams, and lakes, but the presence of relatively high chloride concentration in freshwater (about 250 mg/L or more) may indicate wastewater pollution (Chatterjee, 2018). Chlorides may enter surface water from several sources including chloride-containing rock, agricultural runoff, and wastewater. Chloride ions (Cl^-) in drinking water do not cause any harmful effects on public health, but high concentrations can cause an unpleasant salty taste for most people. Chlorides are not usually harmful to people; however, the sodium part of table salt has been connected to kidney and heart diseases (WHO, 2011). Small amounts of chlorides are essential for ordinary cell functions in animal and plant life. **Chlorine residual:** Chlorine (Cl2) does not occur naturally in water but is added to water and wastewater for disinfection. While chlorine itself is a toxic gas, in dilute aqueous solution, it is not harmful to human health. In drinking water, a residual of about 0.2 mg/L is optimal. The residual concentration which is maintained in the water distribution system ensures good sanitary quality of water (Davis, 2013). Chlorine can react with organics in water forming toxic compounds called trihalomethanes or THMs, which are carcinogens such as chloroform CHCl3. Chlorine residual is normally measured by a color comparator test kit or spectrophotometer.

Sulfate: Sulfate ions ($SO4^{2-}$) occur in natural water and in wastewater. The high concentration of sulfate in natural water is usually caused by leaching of natural deposits of sodium sulfate (Glauber's salt) or magnesium sulfate (Epson salt) (Davis& Davis, 2008). If high concentrations are consumed in drinking water, there may be objectionable tastes or unwanted laxative effects, but there is no significant danger to public health.

Nitrogen: There are four forms of nitrogen in water and wastewater: organic nitrogen, ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen. If water is contaminated with sewage, most of the nitrogen is in the forms of organic and ammonia, which are transformed by microbes to form nitrites and nitrates. Nitrogen in the nitrate form is a basic nutrient to the growth of plants and can be a growth limiting nutrient factor (APHA, 2015). A high concentration of nitrate in surface water can stimulate the rapid growth of the algae which degrades the water quality. Nitrates can enter the groundwater from chemical fertilizers used in agricultural areas. Excessive nitrate concentration (more than 10 mg/L) in drinking water causes an immediate and severe health threat to infants. The nitrate ions react with blood hemoglobin, thereby reducing the blood's ability to hold oxygen which leads to a disease called blue baby or methemoglobinemia (APHA, 2015).

Fluoride: A moderate amount of fluoride ions (F^-) in drinking water contributes to good dental health. About 1.0 mg/L is effective in preventing tooth decay, particularly in children. Excessive

amounts of fluoride cause discolored teeth, a condition known as dental fluorosis (Davis & Davis, 2008) The maximum allowable levels of fluoride in public water supplies depend on local climate. In the warmer regions of the country, the maximum allowable concentration of fluoride for potable water is 1.4 mg/L; in colder climates, up to 2.4 mg/L is allowed.

Iron and manganese: Although iron (Fe) and manganese (Mn) do not cause health problems, they impart a noticeable bitter taste to drinking water even at very low concentration. These metals usually occur in groundwater in solution as ferrous (Fe^{2+}) and manganous (Mn^{2+}) ions. When these ions are exposed to air, they form the insoluble ferric (Fe^{3+}) and manganic (Mn^{3+}) forms making the water turbid and unacceptable to most people (APHA, 2015). These ions can also cause black or brown stains on laundry and plumbing fixtures (Chatterjee, 2018). They are measured by many instrumental methods such as atomic absorption spectrometry, flame atomic absorption spectrometry, cold vapor atomic absorption spectrometry, electrothermal atomic absorption spectrometry, and inductively coupled plasma (ICP).

Copper and zinc: Copper (Cu) and zinc (Zn) are nontoxic if found in small concentrations. Actually, they are both essential and beneficial for human health and growth of plants and animals. They can cause undesirable tastes in drinking water. At high concentrations, zinc imparts a milky appearance to the water (APHA, 2015). They are measured by the same methods used for iron and manganese measurements.

Hardness: Hardness is a term used to express the properties of highly mineralized waters. The dissolved minerals in water cause problems such as scale deposits in hot water pipes and difficulty in producing lather with soap. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions cause the greatest portion of hardness in naturally occurring waters (Spellman, 2017). They enter water mainly from contact with soil and rock, particularly limestone deposits. These ions are present as bicarbonates, sulfates, and sometimes as chlorides and nitrates (Davis & Davis, 2008). Generally, groundwaters harder than surface water. From health viewpoint, hardness up to 500 mg/L is safe, but more than that may cause a laxative effect.

Dissolved oxygen: Dissolved oxygen (DO) is considered to be one of the most important parameters of water quality in streams, rivers, and lakes. It is a key test of water pollution (APHA, 2015). The higher the concentration of dissolved oxygen, the better the water quality. Oxygen is slightly soluble in water and very sensitive to temperature. The actual amount of dissolved oxygen varies depending on pressure, temperature, and salinity of the water. Dissolved oxygen has no direct effect on public health but drinking water with very little or no oxygen tastes unpalatable to some people. There are three main methods used for measuring dissolved

oxygen concentrations: the colorimetric method—quick and inexpensive, the Winkler titration method—traditional method, and the electrometric method (APHA, 2015).

Biochemical oxygen demand (BOD): Bacteria and other microorganisms use organic substances for food. As they metabolize organic material, they consume oxygen. The organics are broken down into simpler compounds, such as CO2 and H2O, and the microbes use the energy released for growth and reproduction. When this process occurs in water, the oxygen consumed is the DO in the water. If oxygen is not continuously replaced by natural or artificial means in the water, the DO concentration will reduce as the microbes decompose the organic materials. This need for oxygen is called the biochemical oxygen demand (BOD). The more organic material there is in the water, the higher the BOD used by the microbes will be. BOD is used as a measure of the power of sewage; strong sewage has a high BOD and weak sewage has low BOD (Tchobanoglous *et al*., 2013). The quantity of oxygen used in a specified volume of water to fully decompose or stabilize all biodegradable organic substances is called the ultimate BOD or BODL. BOD is a function of time. At time = 0, no oxygen will have been consumed and the BOD = 0. As each day goes by, oxygen is used by the microbes and the BOD increases. Ultimately, the BODL is reached and the organic materials are completely decomposed.

Chemical oxygen demand (COD): The chemical oxygen demand (COD) is a parameter that measures all organics: the biodegradable and the non-biodegradable substances (Tchobanoglous *et al.,* 2013). It is a chemical test using strong oxidizing chemicals (potassium dichromate), sulfuric acid, and heat, and the result can be available in just 2 hours. COD values are always higher than BOD values for the same sample.

Toxic inorganic substances: A wide variety of inorganic toxic substances may be found in water in very small or trace amounts. Even in trace amounts, they can be a danger to public health. Some toxic substances occur from natural sources but many others occur due to industrial activities and/or improper management of hazardous waste (Tchobanoglous *et al*., 2013).

2.1.1.3 Biological parameters of water quality

One of the most helpful indicators of water quality may be the presence or lack of living organisms (Cole *et al*., 1999). Biologists can survey fish and insect life of natural waters and assess the water quality on the basis of a computed species diversity index (SDI) (Nathanson, 2004); hence, a water body with a large number of well-balanced species is regarded as a healthy system. Some organisms can be used as an indication for the existence of pollutants based on their known tolerance for a specified pollutant (Abbas *et al*., 2014). Microorganisms exist everywhere in nature. Human bodies maintain a normal population of microbes in the intestinal

tract; a big portion of which is made up of coliform bacteria. Although there are millions of microbes per milliliter in wastewater, most of them are harmless. It is only harmful when wastewater contains wastes from people infected with diseases that the presence of harmful microorganisms in wastewater is likely to occur.

2.2 WASTEWATER

Wastewater refers to any water that is not clean or is adversely affected in quality by human-induced activities (Yongabi, 2010). Wastewater originates from a combination of domestic, industrial, commercial, or agricultural activities. Wastewater is affected by domestic, industrial and commercial use, thus constantly changing its composition and making it rather difficult to define (Spellman, 2013). Wastewater is used water that has been affected by domestic, industrial and commercial use. The composition of all wastewaters is thus constantly changing and highly variable, which is why it is so difficult to pinpoint a singular definition of the word itself. The composition of wastewater is 99.9% water and the remaining 0.1% is what is removed (Shah, 2017). This 0.1% contains organic matter, microorganisms and inorganic compounds. Wastewater effluents are released to a variety of environments, such as lakes, ponds, streams, rivers, estuaries and oceans. Wastewater also includes storm runoff, as harmful substances wash off roads, parking lots and rooftops (Shah, 2017).

2.3 GROUNDWATER

Groundwater, water that occurs below the surface of Earth, where it occupies all or part of the void spaces in soils or geologic strata (Lakshmipriya *et al*. 2016). It is also called subsurface water to distinguish it from surface. Most groundwater comes from precipitation. Precipitation infiltrates below the ground surface into the soil zone (Lakshmipriya *et al.* 2016). When the soil zone becomes saturated, water percolates downward. A zone of saturation occurs where all the interstices are filled with water (Encyclopaedia Britannica, 2020). There is also a zone of aeration where the interstices are occupied partially by water and partially by air. Groundwater continues to descend until, at some depth, it merges into a zone of dense rock. Water is contained in the pores of such rocks, but the pores are not connected and water will not migrate. The process of precipitation replenishing the groundwater supply is known as recharge. In general, recharge occurs only during the rainy season in tropical climates or during winter in temperate climates. Typically, 10 to 20 percent of the precipitation that falls to the Earth enters water-bearing strata, which are known as aquifers (Lakshmipriya *et al*. 2016).

Groundwater is constantly in motion. Compared to surface water, it moves very slowly, the actual rate dependent on the transmissivity and storage capacity of the aquifer. Natural outflows of groundwater take place through springs and riverbeds when the groundwater pressure is higher than atmospheric pressure in the vicinity of the ground surface (Encyclopaedia Britannica, 2020). Internal circulation is not easily determined, but near the water table the average cycling time of water may be a year or less, while in deep aquifers it may be as long as thousands of years. Groundwater plays a vital role in the development of arid and semiarid zones, sometimes supporting vast agricultural and industrial enterprises that could not otherwise exist (Encyclopaedia Britannica, 2020). It is particularly fortunate that aquifers antedating the formation of deserts remain unaffected by increases in aridity with the passage of time. Withdrawal, however, will deplete even the largest of groundwater basins so that development based on the existence of aquifers can be only temporary at best (Lakshmipriya *et al*. 2016).

A vast amount of groundwater is distributed throughout the world, and a large number of groundwater reservoirs are still underdeveloped or uninvestigated. Scientists estimate that some 5.97 quintillion gallons (22.6 million cubic km [5.4 million cubic miles]) of groundwater reside in the upper 2 km (1.2 miles) of Earth's surface (Encyclopaedia Britannica, 2020). The most frequently investigated or exploited groundwater reservoirs are of the unconsolidated clastic (mainly sand and gravel) or carbonate hard rock type found in alluvial valleys and coastal plains under temperate or arid conditions (Encyclopaedia Britannica, 2020).

Though some groundwater dissolves substances from rocks and may contain traces of old seawater, most groundwater is free of pathogenic organisms, and purification for domestic or industrial use is not necessary. Furthermore, groundwater supplies are not seriously affected by short droughts and are available in many areas that do not have dependable surface water supplies. However, aquifers and other groundwater supplies are at risk of chemical pollution from fracking, agricultural chemicals, leaking or unfit landfills and septic tanks, and other point and nonpoint sources of pollution. Such contamination can render groundwater unfit for use and is expensive and difficult to clean up (Lakshmipriya *et al.* 2016).

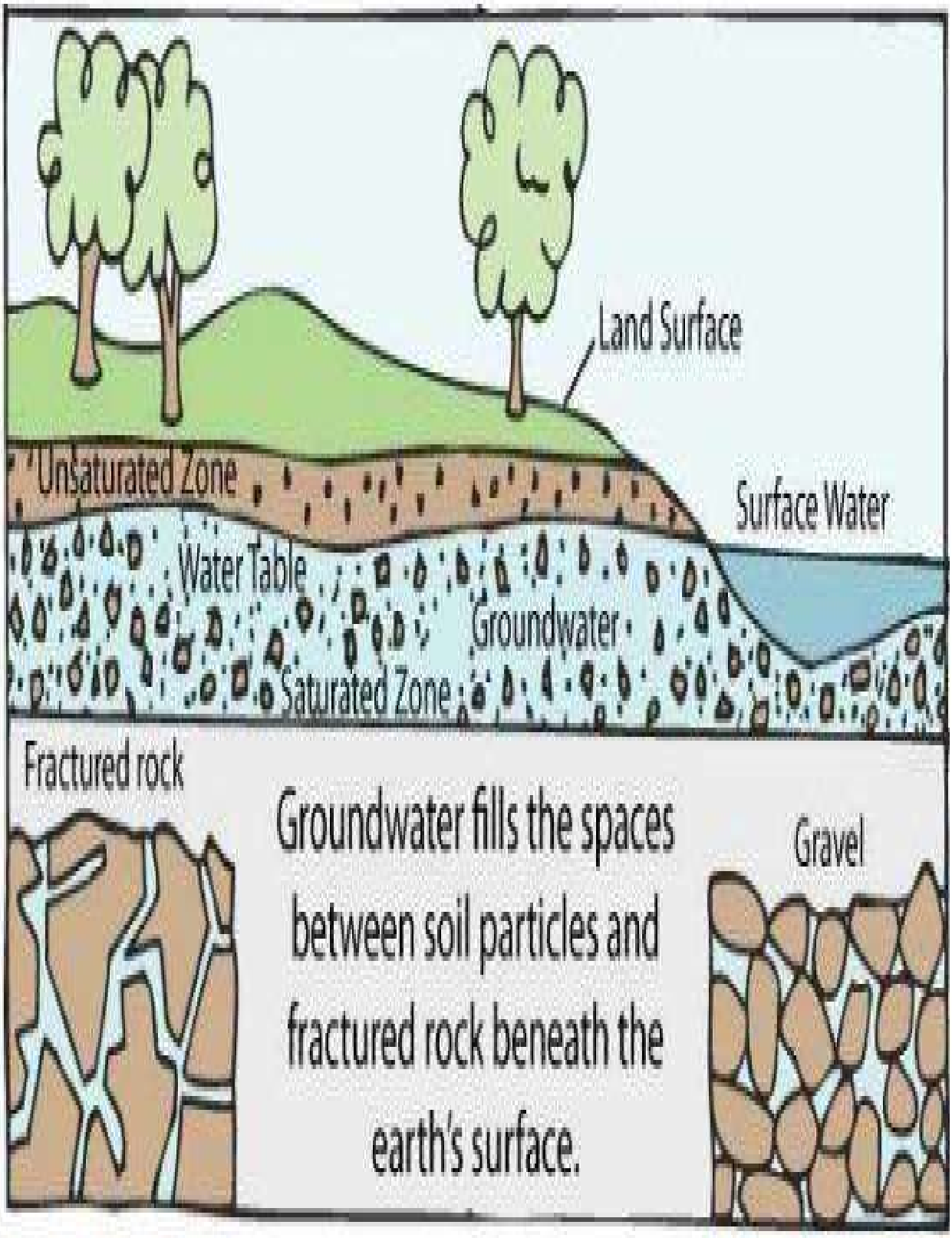


Fig 2.3: Groundwater (Lakshmipriya *et al*. 2016).

2.4 COAGULATION

Coagulation is the process by which particles become destabilized and begin to clump together due to the addition of iron or aluminum salts, such as aluminum sulphate, ferric sulphate, ferric chloride or polymers, to the water (Brain, 2021). It is an essential component in water treatment operations that removes contaminants from water by precipitation via the use of a coagulants (Sela, 2018). This is one of the common methods used by water treatment plants to provide safe, clean drinking water to public water customers. This method is often used alongside processes including filtration, disinfection and sedimentation to remove select contaminants from water (Sela, 2018).

A coagulant is a chemical that is used to remove suspended solids from drinking water (Corrosionpedia, 2018). They are made up of positively charged molecules, which help to provide effective neutralization of water (Nina, 2017). During the process of wastewater treatment, coagulation treatment is usually carried out before sedimentation and filtration. During the process, a coagulant is added to water, and its positive charge neutralizes the negative charge of suspended contaminants (Jiang,2015). Neutralization causes suspended particles to bind together (hence the term coagulation). In clumps known as “flocs”, these particles sink to the bottom of the treatment tank. They can then be more easily filtered out of water.

During this process, the coagulant is quickly added to the water and rapidly mixed, so that the coagulant is circulated throughout the water, thereby allowing it to be distributed throughout the entire sample of water (Jiang, 2015). These chemicals are called coagulants and have a positive charge. The positive charge of the coagulant neutralizes the negative charge of dissolved and suspended particles in the water. When this reaction occurs, the particles bind together, or coagulate (this process is sometimes also called flocculation) (Chekli *et al.,* 2017). The larger particles, or floc, are heavy and quickly settle to the bottom of the water supply. When the water is coagulated, it can be filtered through an ultrafiltration or microfiltration membrane, or a medium filter, to remove the settled particles. Water can also be moved into a settling tank, in which the heavy particles will sink to the bottom, where they can then be removed (Chekli *et al.,* 2017). Coagulation is most effective at removing suspended solids and natural organic matter like gravel, sand, algae, clay, iron, protozoa, and even bacteria (Brain, 2021). Many of these contaminants can give water an unpleasant taste when present in large quantities and can also give water a brown or orange colour (Brain, 2021). However, not all contaminants can be coagulated within the same timeframe, which is why other methods of water cleaning are used alongside this treatment method. Gravel, sand and fine sand can all coagulate during

neutralization within two minutes. It takes algae, clay and protozoa up to 2 hours by comparison. Hence, many pathogens essentially attach themselves to coagulated particulates, and are removed during filtration (Ramvandi, 2014). Coagulation does not guarantee safe drinking water, but it is still an essential water treatment process (Chekli *et al.,* 2017). It removes suspended substances that make water more difficult to properly treat with a disinfectant and means that less chlorine can be added to disinfect the water (Ramavandi, 2014).

The frequently used metal coagulants comprise of aluminum and iron based. Aluminum based coagulants include aluminum sulfate, aluminum chloride and sodium aluminate while that of iron based are ferric sulfate, ferrous sulfate, ferric chloride and ferric chloride sulphate (Brain, 2021). Other chemicals used as coagulants include hydrated lime and magnesium carbonate. The effectiveness of aluminum and iron coagulants arises principally from their ability to form multi-charged polynuclear complexes with enhanced adsorption characteristics (Jiang, 2015). The nature of the complexes formed may be controlled by the pH of the system. When metal coagulants are added to water the metal ions (Al and Fe) hydrolyze rapidly but in a somewhat uncontrolled manner, forming a series of metal hydrolysis species. The efficiency of rapid mixing, the pH, and the coagulant dosage determine which hydrolysis species is effective for treatment (Jiang, 2015). The importance of a coagulant is that it helps in the destabilization of the acidity of the fluid and cause flocs formation and also in purify fluid by removing unwanted active metallic or non-metallic elements (Sela, 2018).

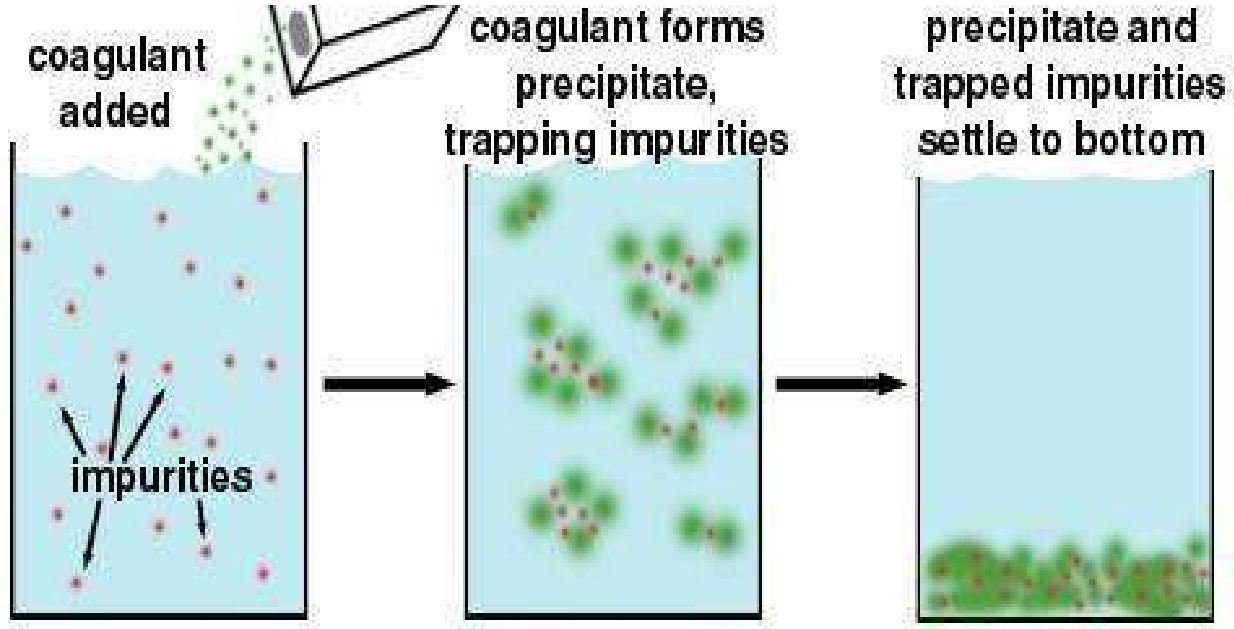


Fig. 2.4. Coagulation of Water (Sela, 2018).

2.4.1 Classification of Coagulants

Coagulants, which are the chemicals that are used to remove tiny particles in water, can be classified into two major groups, namely:

1. Inorganic coagulants
2. synthetic organic polymers and
3. Natural coagulants (Sela, 2018)

2.4.1.1 Inorganic coagulants

This class of coagulants contains inorganic compounds that have no carbon elements in their molecular structure. As such, they are considered "artificial" or unnatural (Sela, 2018). They are splinted into iron-based coagulants and aluminum-based coagulants. Iron-based coagulants are used in reducing biological decomposition products at low pH, removal of arsenic from water, sewage, and wastewater treatment, whereas aluminum-based coagulants are used in industrial waste water treatment (Sela, 2018). Some of the routinely used inorganic coagulants include the following:

Aluminum sulfate – $Al_2(SO_4)_3$: Aluminum sulfate is commonly known as alum, and it is one of the most widely used coagulants currently on the market. This chemical is a popular choice due to its high availability, cost-effectiveness, and efficacy as a coagulant. Sold in blocks and easily stored, aluminum sulfate has proved a viable option for water coagulation and treatment in third-world countries as well as in the United States. The optimal pH range of aluminum sulfate is between 5 and 7.5, which means the coagulant performs at its best in slightly acidic or neutral solutions. Ferric chloride may be a better option for coagulation below this level of pH, while ferrous sulfate may be suitable for the treatment of more alkaline water (Sela, 2018).

Sodium aluminate: Sodium aluminate is easy to produce and is widely available. It is usually deployed alongside aluminum sulfate to act as a coagulation aid, particularly in applications that require the treatment of very cold water that would be difficult to coagulate on its own without assistance. This process is sometimes referred to as double coagulation. While sodium aluminate is not usually used as a coagulating agent on its own, it is used in a number of other industrial applications, including lime soda softening (Sela, 2018).

Aluminum chloride – $AlCl_3$: Aluminum chloride works in a similar way toaluminum sulfate. However, it is less commonly used than its other aluminum-based counterpart. This is largely because AlCl3 tends to be more corrosive and difficult to store, as well as more expensive to source (Sela, 2018).

Polyaluminum chloride (PAC) – $Al_2(OH)_{24}Cl_{12}$: Polyaluminum chlorides – or PAC – make up a grouping of different compounds, many of which are suitable for coagulant applications. Different compounds within this grouping have different attributes, and they can be deployed at various pH levels for optimum impact (Sela,2018).

Aluminum chlorhydrate (ACH) – Al2(OH)5Cl•2H2O: Aluminum chlorhydrate is in the PAC family of chemicals, but it is the most concentrated form, with the highest level of alumina and basicity provided by a stable solution. It is a highly effective coagulant, and it is not too difficult or expensive to source (Brain, 2021).

Ferric sulfate – Fe2(SO4)3: Ferric sulfate works in a similar way to aluminum sulfate, and it is considered to be a highly effective coagulant for industrial usage. Depending on availability, ferric sulfate may be cheaper to source than aluminum sulfate – or other aluminum-based coagulants – although this may not be true in every location. Ferrous sulfate tends to be more effective in situations where reducing agents are required as the compound increases the availability of iron ions. But it may not be as effective as aluminum sulfate when treating water with an acidic or neutral pH level (Brain, 2021).

Ferric chloride – FeCl2: Ferric chloride is perhaps the easiest and cheapest coagulant to source, which makes it a popular choice in some industries. It is sourced from waste material from steelmaking facilities, so it may be considered a relatively green option as it is largely recycled. Ferric chloride is the most corrosive of all the commonly used inorganic coagulants. This may reduce the lifespan of equipment, and it can cause serious damage if released into the water table. Ferric chloride is effective even at pH levels as low as 4.5 (Brain, 2021).

2.4.1.2 Synthetic Organic Polymer Coagulants

These are coagulants synthetized chemically consisting of majorly organic polymers, amines, and acrylamides (Nina, 2017).

2.4.1.3 Natural Coagulants

This type of coagulant is also known as a bio-coagulant because it is derived from biological materials like plants and animals, but especially plants with coagulating abilities as revealed by some previous studies (Yongabi, 2010). Native plants like *Moringa oleifera*, locust bean seeds (*Parkia biglobossa*) etc. have traditionally been used to improve the quality of water in many developing countries around the world (Kebreab, 2004; Miller *et al*, 2008). Similarly, water hyacinth (Eichhornia crassipes) has been widely used for the treatment of wastewater, amongst other plants like duck weed, seaweed, and alligator weed (Shuaibu & Yongabi, 2005). These plants are able to remove nutrients, suspended solids, heavy metals, and other contaminants.

Coagulant Aid: A coagulant aid is a chemical or material, which is not a coagulant, used to assist or modify coagulation (Ayekoe *et al*., 2017). Coagulant aids add density to slow- settling flocs and add toughness to the flocs so that they do not break up during the mixing and settling processes. A coagulant aid improves the effectiveness of a coagulant by:

- Forming larger or heavier particles
- Speeding reactions
- Permitting a reduced coagulant dosage

The primary reason to use coagulant aids is to reduce the amount of alum used, which, in turn, decreases the amount of alum sludge produced (Ayekoe *et al*., 2017). Coagulant aids may be of Nonionic, cationic or anionic polymers, Sodium aluminate, activated silica, Clay, Acids, and Alkalis etc. the use of coagulant aid helps in reducing or eliminating the problem of weak flocs that do not stay together long enough to settle completely or flocs that settle poorly (Ayekoe *et al*., 2017). The addition of a coagulant aid may also reduce the amount of coagulant that is required.

2.5 Factors Affecting Coagulation Processes

The process of coagulation of water depends on various factors like pH of the medium, temperature of water, coagulant feed concentration, coagulant dosage, type of coagulant, mass and initial turbidity (Chekli *et al.,* 2017). Moreover, it is also depending on pre-treatment and type of pollutants present. Effect of pH on coagulation: pH effects on the activities of coagulants. The optimum pH for alum coagulation is 6 to 7.5 whereas 5.0 to 8.0 are for iron. If the alkalinity is lower or higher, then the floc does not form properly. As a result, more coagulant is consumed. In this case, it is beneficial to correct the pH by adding acid or base (Chekli *et al.,* 2017).

Temperature: Temperature is another factor for coagulation water treatment process. It is more significant at lower turbidity. In case of alum, at low temperatures aluminum hydroxide form a strongly hydrated and very stable sol. So, in winter season high coagulant are consumed. When the temperature becomes below the 5ºC, then alum or ferric salts do not work properly. So, it should be considering another coagulant like polyaluminum chloride (PACl) (Chekli *et al.,* 2017).

Type of pollutants: The salt composition of soft water and hard water are not same. Hard water contains Ca and Mg ions. They can alter the charge on the colloidal particles (Chekli *et al.,* 2017). **Optimum dosage:** It is very significant to determine the optimum dosage of a coagulant which will give the maximum clarifying effect. Insufficient amount of coagulant will not be able to destabilize properly the colloidal particles. On the other hand, higher dosage can cause

excessive sludge production, corrosion and loss of money (Chekli *et al.,* 2017).

Type of coagulant: All the coagulants are not suitable for all cases. Different temperatures, pH, type of medium may vary the effectiveness of the coagulant. At lower temperature the polyaluminum chloride (PACl) may be more effective than the traditional coagulants like alum or iron salt. Same way, some pH range can be beneficial to use iron salt instead of alum (Chekli *et al.,* 2017).

2.6 Afzelia africana

Plate 2.6: Seed of *Afzelia africana* Plant (Orwa *et al*., 2009).

Afzelia africana, a timber tree species of a family Fabaceae is a tropical African tree, medium to large, deciduous, up to 40 m high (Bationo *et al.,* 2011). It is mostly used for its high-grade timber but has good potential to provide fodder for livestock and food (Bationo *et al*., 2011). *Afzelia africana* is a multipurpose tree suitable for use in agroforestry systems. It has been considered to be vulnerable because of pressure put by wood exploitation but also because of poor regeneration of stands due to browsing animals or intensive lopping. It is popularly called African oak or African mahogany, with trade names Àpá (Yoruba), Kawo (Hausa) and Akpalata

(Igbo) is one of the most widely distributed species in Africa.

Afzelia africana can grow up to 30–40 m high in forests and up to 10-18 m in savannah (Orwa *et al*., 2009). It is tap-rooted but also develops secondary roots that explore the first centimeters of the soil (Bationo *et al*., 2011). The trunk has small, unequal buttresses at its base (Gérard & Louppe, 2011; Orwa *et al.*, 2009). The trunk is straight, cylindrical, branchless, up to 20 m high, and can reach 1-1.8 m in diameter above buttresses (Donkpegan *et al*., 2014). The bark is 2 cm thick, scaly, very aromatic, and grey to dark brown in color. The crown is large, spreading. Its shape (flat or rounded) depends on age and growing conditions (Gérard & Louppe,2011; Orwa *et al*., 2009). The branches are tortuous, more or less upright, and the and the branchlets are glabrous with lenticels. The leaves are alternate, petiolated, and paripinnate, up to 30 cm long, with 7–17 pairs of leaflets. The leaflets are opposite, elliptic to ovate-elliptic in shape, 5–15 cm long x 3-8.5 cm broad. The inflorescence is a terminal or axillary panicle, 3–13 cm long. The flowers are sweet scented, white to yellowish, zygomorphous, bearing 5 petals, of which one is 1.5 cm by 1 cm, red-striped, and the other 4 are very minute. *Afzelia africana* flowers in the rainy season. The fruit requires six months to ripen. It is an oblong, straight, flattened, dehiscent pod, 10-20 cm long by 5-8 cm broad, brown to black in color. Pods can remain on the tree for six months after ripening. Each pod contains several potentially toxic seeds, 2-3 cm long, inserted in a conspicuous edible bright orange aril covering one-third of their length. The other 2/3 of the seeds are black. Seeds are spread by birds, which feed on the arils (Gérard & Louppe, 2011).

2.6.1 Taxonomical Classification of *Afzelia Africana*

Kingdom: Plantae

Subkingdom: Viridiplantae, green plants

Infrakingdom: Streptophyto—Land plants

Superdivision: Embryophyta

Division: Tracheophyta—Vascular Plants

Subdivision: Spermatophyta (seed plants, phanerogames)

Class: Magnoliopsida

Superorder: Rosanae

Order: Fabales

Family: Fabaceae: Peas, Legumes

Genus: Afzelia Sm.-Mahogany

Species: *Afzelia africana* Sm. ex Pers., African mahogany

2.6.2 Geographical Distribution and Ecology

Afzelia africana is the one of the most widely distributed in Africa (Sacande, 2017). It thrives in the several countries: Benin, Burkina Faso, Cameroon, Central African Republic, Côte d'Ivoire, Ghana Guinea, Guinea-Bissau, Mali, Niger, Nigeria, Senegal, Sierra Leone, Sudan, Togo, Uganda, Senegal, and Uganda (Sacande, 2017). It shows great adaptation to the shift in environmental conditions. In dry areas, it is found on deep and well-drained soil (Gerard & Louppe, 2011). The main habitats of *Afzelia africana* are: dense humid semi-deciduous forests, woodlands, gallery forests and mountainous vegetation. The species spreads more in Sudanian savannas. Afzelia africana is a legume species and known with its symbiotic association with ectomycorrhizal fungis (Yorou *et al*., 2008). It is a light demanding species (Sinsin *et al*., 2014; Orwa *et al*., 2009; Djodjouwin *et al*., 2011), even though at sapling andseedling stage, the species can tolerate shade and develop under close canopy condition (Biaou *et al*., 2011). Seedlings establishment and recruitment are challenging by many environmental pressures as well as climate stress particularly in arid and semi-arid sudanian zones.

2.6.3 Uses of Afzelia Africana

Afzelia africana is considered to be one of the most important woody fodder plants in many parts of Africa. Its foliage is reported to be good for cattle particularly during the dry season and the beginning of the rainy season when grass has not grown yet and other forages are rare (Gérard & Louppe, 2011; Ikhimioya *et al*., 2007).

Afzelia africana leaves, fruits and seeds are browsed by wildlife animals, and many parts of the tree are edible. The leaves can be cooked and used as vegetables while young leaves are mixed with ground cereals before cooking. The flowers are used as condiment in sauces and the seed aril is reported to be sweet. The seed is rich in protein and oil. It is possible to make flour out the seed and to use it in mixture with wheat flour in order to increase protein value (Gérard & Louppe, 2011; Ejikeme *et al*., 2010). Due to the presence of a water-soluble gum (xyloglucan), the seed is used as a thickening agent for soup in South-Eastern parts of Nigeria though itis reported to have some toxicity (Igwenyi *et al*., 2010). The oil has long shelf-life, contains valuable PUFA (polyunsaturated fatty acids) and can be used for cooking (Gérard & Louppe, 2011; Ejikeme *et al*., 2010). The oil of *Afzelia africana* is reported to be a semi-drying oil that can have industrial applications in surface coatings of alkyd resins (Gérard & Louppe, 2011; Ejikeme *et al*., 2010). The product of oil extraction is a seed cake that can be fed to livestock. *Afzelia africana* is mainly used for its heavy wood, which is light brown to red brown in colour, durable, termite-proof, and of high quality (dimensional stability and durability). It does not

require treatment prior to usage in permanent humid conditions or in places where insects are abundant. It can compare to high grade timbers like teak or merbau and is used for carpentry, canoes, house building, paneling, parquet floors, doors, frames stairs and many types of furniture and kitchen utensils. *Afzelia africana* wood makes good firewood and charcoal. It is used as an ornamental and for rituals and considered a fetish tree in many regions (Gérard & Louppe, 2011). This species has been considered vulnerable due to the pressure put by wood exploitation (IUCN, 1998).

2.7 Mucuna Plant

Plate 2.7: Seeds of *Muccuna flagellipes* Plant (Ndubisi et al. 2020).

Mucuna flagellipes (L.) Medik is a large, vigorous, much-branched, twining liana that climbs into the tree canopy (Ndubisi *et al*. 2020). The plant is harvested from the wild for local use as a medicine, source of beads and fibre, and possibly also as a food. It is popularly known as “ukpo” by the Igbo speaking people of south-eastern Nigeria and used as a soup thickener in traditional soups preparation due to its high content of water dispersible polysaccharide (gum) making flour of the seed to be highly pseudoplastic and the leaves as feed for farm animals in the Northern States (Osaniyi & Eka, 2019). The stems are thick and soft, and bear alternate, trifoliate leaves with petioles up to 15 cm (6 in) long. The leaflets are ovate or elliptical, and up to 15 cm (6 in)

long; the lateral leaflets are somewhat oblique, and all leaflets have rounded bases and apiculate tips. The inflorescences grow laterally or in the axils of the leaves and are pendulous racemes with peduncles up to a metre long, with the flowering part near the tip. The calyx has a 1 cm (0.4 in) long tube and the petals are thick, waxy and yellowish. The standard is slightly longer than the wings and keel. The flowers are followed by transversely ridged, oblong pods about 15 cm × 5 cm (6 in × 2 in) bearing orange-brown bristly stinging hairs; the pods have a suture underneath and two longitudinal, undulating wings. The one to four seeds are rounded, almost surrounded by hilum and 2.5 cm (1 in) or more in diameter (Quattrocchi, 2016).

2.7.1 Taxonomical Classification of *Mucuna flagellipes*

Kingdom: Plantae

Clade: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Rosids

Order: Fabales

Family: Fabaceae

Genus: *Mucuna*

Specie: *Mucuna urens*

2.7.1.1 Uses of *Mucuna flagellipes*

Mucuna flagellipes is used in traditional medicine. A tincture made from the powdered bean macerated in alcohol is a soothing remedy used against hemorrhoids, especially those inclined to bleed. The stinging hairs that grow on the pods can be taken internally against intestinal worms, which are expelled alive (Quattrocchi, 2016). These hairs are irritating to the skin and cause intense itching, with reddening and the formation of tiny pustules, soon after contact; the active chemical is the proteolytic enzyme, mucunain. Potable water can be obtained from the fleshy stems. Fibres from the stem are used to make strong rope, and the seeds are used to make beads and ornaments, as well as being used as famine food (Ndubisi *et al.* 2020). An extract of the seeds given to male guinea pigs at low dosages was found to cause the degeneration of sperm, raising the possibility that the plant could be used as a male anti-fertility agent (Quattrocchi, 2016; Udoh & Ekpenyong, 2018*)*

2.8 Moringa oleifera

Fig. 2.7. Tree of *Moringa oleifera* Plant (Mataka *et al*., 2010)

Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae mostly found in the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly- growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree), is now widely cultivated in many locations in the tropics (Mataka *et a*l., 2010; Madrona *et al*., 2010; Madrona *et al*., 2012; Mangale *et al.,* 2012). It is commonly referred to as the miracle tree because of the multipurpose uses of the plant parts. Moringa oleifera seed kernels contain a significant amount of oil that is commercially known as Ben oil or Behen oil which is high in tocopherols (Anwar & Rashid, 2007).

2.8.1 Taxonomical Classification of *Moringa oleifera*

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Capparidales

Family: Moringaceae

Genus: Moringa

Species: *Moringa oleifera*

All parts of the Moringa tree (leaves, seeds, roots and flowers) are suitable for human and animal consumption. The leaves, which are rich in protein, minerals, β-carotene and antioxidant compounds, are used not only for human and animal nutrition but also in traditional medicine (Leone *et al.,* 2015b). The seeds of M. oleifera seed kernels contain a significant amount of oil (up to 40%) with a high-quality fatty acid composition (oleic acid>70%) and after refining, a notable resistance to oxidative degradation (Ahaotu *et al*., 2013a; Anwar *et al*., 2005). The oil is known commercially as Ben oil. Its properties make it suitable for both human consumption and commercial purposes. Indeed, moringa oil could be a good substitute for olive oil in the diet as well as for non-food applications, like biodiesel, cosmetics, and a lubricant for fine machinery. Moreover, after oil extraction, the seed cake can be used in waste water treatment as a natural coagulant (Ndabigengesere and Subba, 1998) or as an organic fertilizer to improve agricultural productivity (Ahaotu, 1997; Boskou, 2011; Mangale *et al*., 2012; Ahaotu *et al*., 2013b).

The powder from Moringa oleifera seeds is used as a coagulant to precipitate solids, including bacteria, from dirty water. After mixing one powdered seed in 1 liter of turbid water, almost all the solids coagulated and fell to the bottom of the container after two hours. When Moringa oleifera is compared with conventional chemical coagulants, it has the following advantages: cost effectiveness, availability, biodegradable sludge, eco-friendliness, low sludge volume, it does not produce harmful by-products, ease of handling as it is not corrosive, and it does not affect the pH of water.

2.8.2 *Moringa oleifera* seeds

Plate 2.8.2: Seeds of *Moringa oleifera* (Ogunsina *et al*., 2014)

Moringa oleifera seeds are globular, about 1 cm in diameter. They are three-angled, with an average weight of about 0.3 g, 3-winged, with wings produced from the base of the seed to the apex, 2-2.5 cm long and 0.4–0.7 cm wide; the kernel is responsible for 70%–75% of the weight (Ogunsina *et al*., 2014; Rockwood *et al*., 2013). Among all the plant materials that have been tested over the years, powder processed from the seeds of Moringa oleifera has been shown to be one of the most effective as a primary coagulant for water treatment and can be compared to that of alum (a conventional chemical coagulant).

2.8.2.1 Water treatment with Moringa seeds

According to Jennifer (2015), solutions for Moringa seeds for water treatment may be prepared from seed kernels or from the solid residue left over after oil extraction (press cake). Moringa seeds, seed kernels, or dried press cake can be stored for long periods, but moringa solutions for treating water should be prepared fresh each time. In general, 1 seed kernel will treat 1L (1.056 qt) of water.

2.8.2.1.1 Procedures used in treating wastewater with Moringa oleifera

- Jennifer (2015) explained that mature Moringa seed pods should be harvested and the seeds removed from the pods.
- The seeds should then be shelled to obtain clean seed kernels, discarding any discolored seeds.
- The quantity of kernels needed should be determined based on the amount and turbidity of the water, noting that generally, one seed kernel will treat one liter of water.
- The appropriate number of seed kernels should be crushed using a grinder, mortar and pestle, or similar tools to obtain a fine powder, which should then be sifted through a screen or small mesh.
- The seed powder should be mixed with a small amount of clean water to form a paste.
- This paste should then be mixed with 250 ml of clean water in a bottle and shaken for one minute to activate the coagulant properties and form a solution.
- Finally, the solution should be filtered through a muslin cloth or mesh screen to remove insoluble materials before being added to the water to be treated.

2.9 Related Literature

Ojiako (2014), working on phytochemical analysis and antimicrobial screening of *Moringa oleifera* leaf extract, reveals the following phytochemicals: alkaloids, tannins, saponins, and phenols from ethanolic extracts.

Gupta & Gupta (2014), working on preliminary photochemical screening of leaves of *Moringa oleifera* Lam, indicate that leaf extract has the presence of alkaloids, flavonoids, tannins, saponins, steroids, glycosides, and terpenoids as bioactive compounds.

The work of Nweze & Nwafor (2014) on Phytochemical, Proximate, and Mineral Composition of Leaf Extracts of Moringa oleifera Lam. from Nsukka, South-Eastern Nigeria, reveals the presence of flavonoids, anthraquinones, alkaloids, saponins, steroids, terpenoids, cardiac glycosides, and tannins in the aqueous leaf extract of *M. oleifera*.

The work of Olorunmaiye *et al*. (2019) on the proximate and phytochemical composition of African mahogany (*Afzelia africana*) seed and African mesquite (*Prosopis africana*) pod, revealed the presence of alkaloids, tannins, saponins, cardiac glycosides, flavonoids, terpenoids, and phenols. Okwu & Okoro (2007), working on the phytochemical composition of *Brachstegia eurycoma* and *Mucuna flagellipes* seeds, revealed the presence of alkaloids, flavonoids, tannins, and saponins.

Elzein *et al*. (2018), working on qualitative and quantitative phytochemical analysis of *Moringa oleifera* (Lam) pods, indicated the presence of phytochemicals like alkaloids, saponins, tannins, flavonoids, and sterols in the different extracts.

Desta & Bote's (2021) work on wastewater treatment using a natural coagulant (*Moringa oleifera* seeds): optimization through response surface methodology shows that *Moringa oleifera* seeds were found to be particularly successful at removing color, turbidity, and COD from water samples with diverse characteristics. However, as compared to acidic water, it is more effective in terms of its basic qualities.

Shan *et al*. (2017) reveal that the seeds of *Moringa oleifera* contain water-soluble, positively charged proteins that act as an effective coagulant for water and wastewater treatment. Based on this, the water quality of the ‘‘Sungai Baluk’’ river was examined before and after the treatment using MO seed. MO seed exhibited high efficiency in the reduction and prevention of bacterial growth in both wastewater and ‘‘Sungai baluk’’ river samples. The turbidity was removed up to 85–94%, and dissolved oxygen (DO) was improved from 2.58 ± 0.01 to 4.00 ± 0.00 mg/L. The chemical oxygen demand (COD) and biological oxygen demand (BOD) increased after the treatment from 99.5 ± 0.71 to 164.0 ± 2.83 mg/L for COD and from 48.00 ± 0.42 to 76.65 ± 2.33

mg/L for BOD, respectively.

The work of Hendrawati *et al*. (2016) on the use of *Moringa oleifera* seed powder as a coagulant to improve the quality of wastewater and groundwater revealed that *M. oleifera* was able to reduce 98.6% of the turbidity of wastewater, 10.8 % of its conductivity, 11.7% of its BOD, and remove its metal contents (Cd, Cr, and Mn). When applied to ground water, M. oleifera removed the turbidity of ground water by as much as 97.5% while reducing the conductivity and BOD of ground water by 53.4 % and 18%, respectively. The use of M. oleifera also reduced the total number of coliforms.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The Study Are

The study was carried out in Imo State, Nigeria, lying within latitudes $4^{0}45^{11}$N and $7^{0}15^{11}$ N and longitudes $6^{0}50^{11}$ E and $7^{0}25^{11}$ E (Ndubisi et al. 2020). It has a total area of 5,100 square kilometers and a population of 4.8 million people. The capital city is Owerri. Imo has three geopolitical zones, namely Owerri, Orlu, and Okigwe, and twenty-nine local government areas (Ndubisi et al. 2020). There are two distinct seasons within this area, namely the rainy season, which begins in April and lasts until October, with annual rainfall varying from 1,500-2,200mm (60–80 inches), while the dry season is ushered in by the harmattan period and is characterized by hot weather and low humidity (Lakshmipriya et al. 2016). The rainy season is associated with very high humidity (about 80–85%) and very heavy rainfall. Temperature varies according to season, between 25°C and 32°C on sunny days. The forest/vegetation in Owerri is a rain forest with lots of plant diversity, growing under the described climatic conditions. The population is predominantly Igbos and Christians (Ndubisi et al. 2020).

Figure 3.1: Map of the study area (Lakshmipriya *et al*. 2016).

3.2 Samples collection and authentication

3.2.1 Plant Seed Samples

The three plant seeds were collected from Owerri metropolis. Matured and naturally dry seedpods of *Moringa oleifera* (brown in color) were harvested from a healthy *Moringa* tree in the botanical garden of the Federal University of Technology, Owerri. While dried, mature, and healthy seeds of *Afzelia africana* and *Muccuna flagellipes* were bought from Relief Market Owerri, the samples were identified and authenticated by Dr. C. M. Duru, plant biosystematist, Biology Department, Federal University of Technology Owerri.

3.2.2 Wastewater and Groundwater sample Collection

Wastewater used in this study was collected randomly at different points (upper stream, midstream, and downstream) from the Somachi slaughterhouse in Owerri Municipality into 10 liters, while groundwater was collected from different boreholes in Eziobodo village into 10litres gallon. The water samples were transported immediately to the laboratory for analysis.

3.3 Preparation of Samples

3.3.1 Preparation of Seeds of *Moringa oleifera*

The methodology of Dehghani & Alizadeh (2016), with slight modifications, was used. The seeds of *Moringa oleifera* were dehusked manually using a kitchen knife and then dried in an oven at 35 °C for 5 hours to make sure they were properly dried before being triturated into a fine powder with a home blender and then stored in an airtight plastic container for further

3.3.2 Preparation of Seeds of *Afzelia africana* (Akparata)

The seeds of Afzelia Africana were soaked in water overnight to soften them before cracking with a hammer. The seed kernels were dried in an oven at 35 °C for 5 hours using the method described by Dehghani and Alizadeh (2016). The dried seeds were triturated into a fine powder using a home blender and then stored in an airtight plastic container for further use.

3.3.3 Preparation of Seeds of *Muccuna flagellipes* (Ukpo)

The methodology of Dehghani & Alizadeh (2016), with a slight modification, was used. The seeds of *Muccuna flagellipes* were cracked manually with a hammer to remove the seed kernels and then dried in an oven at 35°C for 5 hours to make sure they were properly dried before being triturated into a fine powder with a home blender and then stored in an airtight plastic container for further use.

3.4 Stock solution preparation

The methodology of Hoa & Hue (2018) was adopted with a slight modification, different grams of the seed powder (15g, 30g and 45g) for each sample were dissolved in 300 mL of distilled water to prepare a fresh stock solution that was used in the treatment process. The mixture was blended with a magnetic stirrer for 30 minutes at high speed to extract the active proteins from the powdered seed samples. The suspension was filtered through a Whatman No. 1. filter paper into a beaker to obtain a homogenous stock solution that is free from suspended materials. This solution was to be prepared fresh each time it was to be used in water treatment and kept in a cool place with a minimum temperature of 20^0C to prevent changes in pH and viscosity (Katayon *et al*., 2006).

3.5 Physicochemical Parameters

Physicochemical parameters such as color, odor, taste, appearance, turbidity, pH, electrical conductivity, total dissolved solids, biological oxygen demand, dissolved oxygen, and chloride ion were determined following the standard protocols and methods of the American Public Health Organization (APHA) (1995) and the American Society for Testing and Materials (ASTM) using different calibrated standard instruments. The temperature, pH, and conductivity were analyzed on-site immediately after sample collection.

3.5.1 Determination of Temperature

The temperature of the samples was determined on-site and in the laboratory. The temperature was measured using a mercury-in-glass thermometer. The value of each sample was taken after submerging the temperature probe in the water sample and holding it for a couple of minutes to achieve a stabilized reading. After the measurement of each sample, the probe was rinsed with deionized water to avoid cross-contamination among different samples.

3.5.2 Determination of pH

The pH of the water samples was measured using a pH meter (model HI 98130 HANNA). The pH meter was calibrated with three standard solutions (pH 4.0, 7.0, and 10.0), before taking the measurements. The value of each sample was taken after submerging the pH probe in the water sample and holding it for a couple of minutes to achieve a stabilized reading. After the measurement of each sample, the probe was rinsed with deionized water to avoid cross contamination among different samples.

3.5.3 Determination of Electrical Conduction

The conductivity of the samples was measured using a conductivity meter (model HI 98130 HANNA). The probe was calibrated using a standard solution with a known conductivity. The

probe was submerged in the water sample and the reading was recorded after the disappearance of stability indicator. After the measurement of each sample, the probe was rinsed with deionized water to avoid cross contamination among different samples.

3.5.4 Determination of Turbidity

The turbidity of the water samples was measured using a spectrophotometer (Unico S-2150 model). Each sample was poured in a cuvette and then placed inside the cuvette holder inside the spectrophotometer and the absorbance read against visible light of 450nm, the value of the absorbance was recorded. The cuvette was rinsed with deionized water to avoid cross-contamination among different samples.

3.5.5 Determination of Total Dissolved Solid (TDS)

The TDS of the water samples were determined by the gravimetric method as described by Sawyer *et al*. (1994). The weight of empty filter paper was weighted and recorded as W1 for each sample, and then a known volume of the sample was measured and filtered. After filtration, the filter paper was air-dried, and the weight was read and recorded as W_2. The value of TDS was calculated by using the formula below;

$$\text{TDS} = \frac{W2-W1}{Amount\ of\ sample} \times 1000$$

3.5.6 Determination of Biological Oxygen Demand

Biochemical oxygen demand was determined using azide modification of Winkler's method. BOD bottle was prepared and incubated at 20°C for 5 days in the dark for each sample. After five days, the incubated BOD bottle was poured by mixing 2 mL of orthophosphoric acid, which was shaken gently and titrated with sodium thiosulphate to the end point where there was a color change. The titre value represents dissolved oxygen on day five. BOD was then calculated as the difference between dissolved oxygen on day one and that on day five.

3.5.7 Determination of Dissolved Oxygen

Dissolved Oxygen (DO) was determined using azide modification of Winkler's method as decribed by Biwas, (2015). 200 mL of the water sample was carefully transferred into a 300 ml BOD bottle. 1 mL of manganese sulphate solution was added followed by 1 mL of the alkaline alkali-iodide-azide reagent. The resulting mixture was titrated against 0.025 N sodium thiosulphate to the end point where there was colour change, the titre value was recorded as DO.

3.6 Phytochemical Analysis

The preliminary qualitative phytochemical screening was carried out according to the method described by Harbone (1998), Parekh & Chanda (2007). The extracts from the different samples were assessed for the presence/absence of the following phytochemicals parameter: saponins, flavonoids, alkaloids, tannins, phenols, anthraquinones and steroids.

3.6.1 Test for Alkaloids

Methodology is as reported by Ejikeme *et al.,* (2014). Two (2) ml of extract was added to 2 ml of concentrated hydrochloric acid. Then few drops of Mayer's reagent will be added. Presence of green color or white precipitate indicates the presence of alkaloids.

3.6.2 Test for Tannins

About 1 g of the plant extract was dissolved in 5 ml of distilled water, filtered and ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate indicate the presence of tannins

3.6.3 Test for saponins

About 1 g of the powdered sample was dissolved in a 20 ml distilled water and then boiled in a water bath, before filtering. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth, which shows the presence of saponins.

3.6.4 Test for flavonoids

Five (5) ml of dilute ammonia solution was added to a portion of aqueous filtrate of the extract followed by addition of concentrated H_2SO_4. A yellow colouration observed indicates the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminum solution was added to a portion of each filtrate. A yellow colouration was observed, thus, indicating the presence of flavonoids.

3.6.5 Test for Phenols

Two (2) ml of distilled water followed by few drops of 10% ferric chloride was added to 1ml of the extract. Formation of blue or green color indicates presence of phenols.

3.6.6 Test for Anthraquinones

To 1 ml of the extract add few drops of 10% ammonia solution and the appearance of pink color precipitate indicates the presence of anthraquinones.

3.6.7 Test for Steroids

To 1 ml of the extract, equal volume of chloroform was added and a few drops of concentrated sulphuric acid. The appearance of brown ring indicates the presence of steroids and appearance

of bluish brown ring indicates the presence of phytosteroids.

3.7 Bacteria Screening

3.7.1 Bacteria Culture

The bacteria load for each sample was checked before and after treatment to ensure that the water is safe for domestic use. This was achieved by culturing the samples using nutrient media according to the method described by Prescott, (1999). Nutrient agar was prepared and pour in an autoclaved petri-dishes after autoclaving the media. Upon solidification of the media, a sterilized wire loop was used in inoculating the sample via streak plate method and then incubated at 37^0C for 24 hours. The growth was subjected to biochemical test for proper identification of the isolates.

3.7.2 Isolate Identification

The bacteria isolates were identified by macroscopic and biochemical method as described by Prescott, (1999). the following biochemical tests were carried out;

Coagulase: This test was carried out by combining blood plasma with each of the cultured plate with growth. Bacteria generate the coagulase enzyme, which causes the blood plasma to coagulate, indicating a positive reaction.

Catalase: The test was done on a slide or in a test tube by mixing a colony of bacteria with a few drops of 3 percent H2O2 and looking for bubble formation within 10 seconds, indicates a positive reaction.

Oxidase: This test was carried out by impregnating a filter paper with 1 percent tetramethyl-p-phenylenediamine dihydrochloride (Kovac's) reagent, which acts as an artificial electron donor, and drying it. The bacterial colonies are smeared on a paper strip, and the color change is checked after 10 seconds. Bacteria isolates are oxidase positive when the color changes to dark purple, delayed oxidase positive when the color changes to purple within 60 to 90 seconds and oxidase negative if the color does not change or it takes longer than 2 minutes.

Indole: The test was carried out by the use of Kovac's reagents. 1ml of bacteria isolate from the nutrient broth was pipetted into a clean test-tube, then Kovac's reagent was added and the presence of red rosindole dye indicates the presence of indole positive.

Methyl Red: The test was carried out by the use of methyl red. 1ml of bacteria isolate from the nutrient broth was pipetted into a clean test-tube, then methyl red was added and the presence of red colour indicates the presence of methyl red positive.

Voges-Proskauer: This test was carried out by pipetting 2ml of broth to clean test tube. 6 drops

of % alpha-naphthol and mix well to aerate. Then, 2 drops of 40% potassium hydroxide were added before mixing. The presence of pink-red color at the surface within 30 min, indicates a positive VP test.

3.8 Statistical Data Analysis

The data generated from the study was analyzed using tables, charts, Analysis of Variance (ANOVA). All analysis was determined at a significant level of $P>0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Analysis of the Plant Seed Samples Used as Bio-coagulants

The phytochemical parameter of the plant seed samples used as bio-coagulant is shown in Table 4.1. The result reveals the presence of the following phytochemicals: alkaloids, flavonoids, phenols, tannins, steroids, and anthraquinones in all the plant seed samples. While steroids were only present in *M. oleifera*.

Table 4.1: Phytochemical Analysis of the Plant Seed Samples Used as Bio-coagulants

S/N	PARAMETERS	*Moringa oleifera*	*Afzelia africana*	*Muccuna flagellipse*
1	**Alkaloids**	+	+	+
2	**Flavonoids**	+	+	+
3	**Phenols**	+	+	+
4	**Tannins**	+	+	+
5	**Steroids**	+	**-**	**-**
6	**Saponins**	+	+	+
7	**Anthraquinones**	+	+	+

Legend:

+ = Present

- = Absent

4.2 Physicochemical parameters of water samples collected from Somachi slaughterhouse and Eziobodo groundwater before and treatment with alum

The result of physicochemical analysis before treatment and after treatment with alum shows a significant difference in the color, odor, appearance, turbidity, temperature, dissolved oxygen, biological oxygen demand and chloride ion after treatment with alum for slaughterhouse wastewater, as there was an increase in the mean value of color from (33-100), odor (92-100), appearance (31- 100), dissolved oxygen (4.20-5.78) and chloride ion (1.42-2.14) and decreased in turbidity (0.86-0.13), Temperature (31-29), and biological oxygen demand (7.59-2.05). Groundwater before treatment and slaughterhouse wastewater after treatment were significantly higher than slaughterhouse wastewater before treatment in color, odor and appearance (Table 4.2).

Table 4.2: Physicochemical properties of slaughterhouse wastewater and groundwater before and after treatment with alum

Physicochemical properties	WHO Standard	Before treatment with alum		After treatment with alum	
		Slaughter house wastewater	Groundwater	Slaughter house wastewater	Groundwater
Colour	-	33^{b}±4.3	100^{a}±1.0	100^{a}±1.0	100^{a}±1.0
Odour	-	92^{b}±7.2	100^{a}±1.0	100^{a}±1.0	100^{a}±1.0
Appearance	-	31^{b}±4.1	100^{a}±1.0	100^{a}±1.0	100^{a}±1.0
Turbidity (Opt. Index)	-	0.86^{a}±0.3	0.02^{c}±0.01	0.13^{b}±0.2	0.02^{c}±0.01
pH	6.5-8.5	6.62^{a}±1.3	7.19^{a}±1.5	7.12^{a}±1.2	7.14^{a}±1.4
Temperature (^{O}C)	-	31^{b}±4.1	21^{b}±3.3	29^{a}±5.4	21^{b}±3.3
Electrical conductivity (µs/Cm)	1000	195^{b}±21.4	214^{a}±26.6	187^{b}±20.2	215^{a}±26.7
Total dissolved solids (Mg/L)	1000	327^{a}±31.7	253^{b}±27.8	305^{a}±29.6	252^{b}±27.7
Dissolved oxygen (Mg/L)	6.5- 8.0	4.20^{c}±1.2	7.13^{a}±1.8	5.78^{b}±1.5	7.14^{a}±1.9
Biological oxygen demand (Mg/L)	6	7.59^{a}±1.7	1.56^{b}±0.09	2.05^{b}±0.1	1.54^{b}±1.7
Chloride ion	250	1.42^{c}±0.7	3.80^{a}±1.1	2.14^{b}±0.9	3.83^{a}±0.7

Mean along the row having different superscripts of letters differ significantly at P = 0.05 using the Duncan Multiple Range Test.

4.3 Physicochemical properties before and after treatment with 15g/300mL Stock Solution from the plant material used

The Physicochemical properties before and after treatment with 10mL and 20mL of 15g/300mL Stock Solution (SS) indicates that there was a significant difference in the colour, appearance, odour, turbidity, pH, Temperature, dissolved oxygen, biological oxygen demand and chloride after treatment of the slaughterhouse wastewater with 15g/300mL SS at 10mL and 20mL accordingly. The increase in mean colour from (33-41) at 10mL; (33-42) at 20mL and (33-43) at 20mL, appearance (31-37) at 10mL; (31-39) at 20mL and (31-37) at 20mL was seen in the treatment with *Moringa oleifewra* and *Afzelia africana* respectively. There was a decrease in odour of which treatment with *M. oleifera* at 10mL and *Muccuna flagellipes* at 10mL and 20mL are significantly the same while, *A. africana* at 10mL and 20mL and *M. oleifera* at 20mL were significantly the same. Also, there was an decrease in turbidity of which the treatment with *M. oleifera* had the least mean turbidity (0.31) at 10mL and (0.40) at 20mL. in addition, the decrease in pH and temperature and increase in dissolved oxygen were significantly the same across the different treatment Furthermore, the decrease in biological oxygen demand and increase in chloride ion were the same for treatment with *M. oleifera* and *A. africana* at 10mL and 20mL than that of *M. flagellipes* accordingly.

Conversely, there was a decrease in the colour, odour, appearance and pH, of which the change in colour odour and pH were statistically the same across the different treatment while in appearance, treatment with 10mL *M. oleifera* and *A. africana* were significant the same and better than 20mL of *M. oleifera* and *A. africana* and *M. flagellipes* at 10mL and 20mL. temperature, electrical conductivity, total dissolved solids, dissolved oxygen, biological oxygen demand and chloride were statistically the same before and after treatment (Table 4.3).

Table 4.3: Physicochemical properties before and after treatment with 15g/300mL Stock Solution from the plant material used

Slaughter house wastewater before and after treatment at 15g/300ml stock solution								
Physicochemical properties	**WHO standard**	**Before treatment**	**10ml**			**20 ml**		
			Moringa olifera	***Afzelia africana***	***Muccuna flagellipes***	***Moringa olifera***	***Afzelia africana***	***Muccuna flagellipes***
Colour	-	$33^{b}\pm4.3$	$41^{a}\pm5.8$	$34^{b}\pm2.6$	$34^{b}\pm2.6$	$42^{a}\pm5.9$	$43^{a}\pm3.0$	$35^{b}\pm2.7$
Odour	-	$92^{a}\pm7.2$	$37^{c}\pm4.6$	$41^{b}\pm3.4$	$38^{c}\pm2.8$	$41^{b}\pm5.8$	$45^{b}\pm3.7$	$36^{c}\pm2.6$
Appearance	-	$31^{b}\pm4.1$	$37^{a}\pm4.6$	$28^{b}\pm1.7$	$27^{b}\pm1.6$	$39^{a}\pm4.9$	$37^{a}\pm2.2$	$28^{b}\pm1.7$
Turbidity (Opt. Index)	-	$0.86^{b}\pm0.3$	$0.31^{e}\pm0.2$	$0.69^{c}\pm0.5$	$0.46^{d}\pm0.3$	$0.40^{d}\pm0.4$	$0.92^{b}\pm0.8$	$1.01^{a}\pm0.5$
pH	6.5-8.5	$6.62^{a}\pm1.3$	$4.79^{a}\pm0.6$	$5.12^{a}\pm1.2$	$5.55^{a}\pm1.6$	$4.87^{a}\pm0.8$	$5.02^{a}\pm1.0$	$5.55^{a}\pm1.6$
Temperature (^{O}C)	-	$31^{a}\pm4.1$	$23^{b}\pm2.4$	$23^{b}\pm2.4$	$22^{b}\pm2.3$	$23^{b}\pm2.4$	$23^{b}\pm2.4$	$22^{b}\pm2.2$
Electrical conductivity (µs/Cm)	1000	$195^{a}\pm21.4$	$196^{a}\pm21.6$	$191^{a}\pm28.2$	$182^{a}\pm26.9$	$194^{a}\pm23.3$	$190^{a}\pm27.4$	$182^{a}\pm26.9$
Total dissolved solids (Mg/L)	1000	$327^{a}\pm31.7$	$287^{a}\pm23.9$	$291^{a}\pm33.6$	$298^{a}\pm34.5$	$293^{a}\pm24.2$	$296^{a}\pm35.1$	$299^{a}\pm35.2$
Dissolved oxygen (Mg/L)	6.5- 8.0	$4.20^{b}\pm1.2$	$6.27^{a}\pm1.6$	$5.58^{a}\pm1.5$	$5.05^{a}\pm1.2$	$6.27^{a}\pm1.6$	$5.58^{a}\pm1.5$	$5.08^{a}\pm1.4$
Biological oxygen demand (Mg/L)	6.00	$7.59^{a}\pm1.7$	$3.84^{c}\pm0.9$	$4.02^{c}\pm1.1$	$5.12^{b}\pm1.8$	$3.98^{c}\pm1.5$	$4.09^{c}\pm1.4$	$5.16^{b}\pm2.0$
Chloride ion	250	$1.42^{c}\pm0.7$	$3.08^{a}\pm1.1$	$2.87^{a}\pm0.2$	$2.11^{b}\pm0.1$	$3.06^{a}\pm1.0$	$2.63^{a}\pm0.1$	$2.14^{b}\pm0.2$
		Groundwater before and after treatment at 15g/300ml stock solution						
Colour	-	$100^{a}\pm1.0$	$46^{b}\pm6.2$	$47^{b}\pm4.0$	$43^{b}\pm3.4$	$49^{b}\pm6.2$	$46^{b}\pm3.7$	$44^{b}\pm3.4$
Odour	-	$100^{a}\pm1.0$	$40^{b}\pm4.9$	$46^{b}\pm3.8$	$39^{b}\pm2.9$	$46^{b}\pm6.6$	$41^{b}\pm3.2$	$45^{b}\pm2.9$
Appearance	-	$100^{a}\pm1.0$	$40^{b}\pm4.9$	$43^{b}\pm22$	$37^{c}\pm2.5$	$39^{c}\pm4.9$	$38^{c}\pm2.3$	$39^{bc}\pm4.9$
Turbidity	-	$0.02^{d}\pm0.01$	$0.12^{c}\pm0.1$	$0.22^{b}\pm0.3$	$0.42^{a}\pm0.1$	$0.40^{a}\pm0.4$	$0.30^{a}\pm0.3$	$0.32^{a}\pm0.4$
pH	6.5-8.5	$7.19^{a}\pm1.5$	$5.00^{b}\pm0.8$	$5.56^{b}\pm1.6$	$6.46^{ab}\pm1.9$	$5.02^{b}\pm0.9$	$5.22^{b}\pm1.3$	$5.43^{b}\pm2.0$
Temperature (^{O}C)	-	$21^{a}\pm3.3$	$20^{a}\pm2.1$	$20^{a}\pm2.2$	$23^{a}\pm2.4$	$20^{a}\pm2.1$	$23^{a}\pm2.4$	$22^{a}\pm3.2$
Electrical conductivity (µs/Cm)	1000	$214^{a}\pm26.6$	$201^{a}\pm23.2$	$200^{a}\pm29.3$	$199^{a}\pm28.1$	$201^{a}\pm26.8$	$198^{a}\pm29.2$	$197^{a}\pm29.0$
Total dissolved solids (Mg/L)	1000	$253^{a}\pm27.8$	$265^{a}\pm28.8$	$270^{a}\pm34.7$	$270^{a}\pm31.6$	$262^{a}\pm21.2$	$216^{a}\pm28.4$	$258^{a}\pm32.4$
Dissolved oxygen (Mg/L)	6.5- 8.0	$7.13^{a}\pm1.4$	$6.53^{a}\pm1.7$	$6.10^{b}\pm1.8$	$6.03^{b}\pm1.7$	$5.51^{b}\pm1.1$	$6.11^{b}\pm1.8$	$6.01^{b}\pm1.4$
Biological oxygen demand (Mg/L)	6.00	$1.56^{a}\pm0.9$	$1.65^{a}\pm0.2$	$1.67^{a}\pm0.4$	$1.90^{a}\pm0.7$	$1.66^{a}\pm1.0$	$1.73^{a}\pm0.6$	$1.89^{a}\pm0.6$
Chloride ion	250	$3.80^{a}\pm1.1$	$3.45^{a}\pm1.3$	$3.52^{a}\pm0.5$	$3.48^{a}\pm0.6$	$4.36^{a}\pm1.8$	$3.48^{a}\pm0.3$	$3.51^{a}\pm0.4$

Mean along the row having different superscript of letters differ significantly at P = 0.05 using Duncan Multiple Range Test.

4.4 Physicochemical properties before and after treatment with 30g/300mL Stock Solution from the plant material used.

Table 4.4 shows that there was a significant change in the colour, odour, appearance, turbidity, pH, temperature, dissolved oxygen, biological oxygen demand and chloride ion for slaughterhouse wastewater treatment. An increase in colour (33-41) at 10mL; (33-40) at 20mL and (33-39) at 20mL was recorded against treatment with *Moringa oleifera* and *Afzelia africana* respectively, appearance (31-40) at 10mL and (31-44) at 20mL for *M. oleifera*. The decrease in turbidity was least in treatment with *M. oleifera* (0.86-0.102) at 10mL and (0.86-0.21) at 20mL, pH, treatment with 10mL of *M. oleifear* was the least while others were significantly the same. The decrease in temperature and increase in dissolved oxygen were significantly the same. While, in biological oxygen demand and chloride ion, *M. oleifera* and *A. africana* were significantly the same. In addition, electrical conductivity and total dissolved solids were statistically the same before and after treatment.

Similarly, the physicochemical properties for groundwater treatment with 30g/300mL SS of the different plant materials used. Physicochemical properties such as colour, odour, appearance and pH decreased after treatment but the decrease in colour (100-47) and odour (100-40) were statistically the same while in appearance, *M. oleifera* and *A. africana* at 10mL have the highest appearance than *M. oleifera* and *A. africana* at 20mL and *M. flagellipes* at 10mL and 20mL respectively. The turbidity increased across the different treatment, with the highest turbidity recorded at 20mL SS of *M. oleifera, A. africana* and *M. flagellipes* from 0.02-0.38 and the least at 10mL SS of *M. oleifera* (0.02). Furthermore, the temperature, electrical conductivity, total dissolved solids, biological oxygen demands and chloride ions were statistically the same before and after treatment.

Table 4.4 Physicochemical properties before and after treatment with 30g/300mL Stock Solution from the plant material used.

Physicochemical properties	WHO standard	Before treatment	10ml *Moringa olifera*	10ml *Afzelia africana*	10ml *Muccuna flagellipes*	20 ml *Moringa olifera*	20 ml *Afzelia africana*	20 ml *Muccuna flagellipes*
Slaughter house wastewater before and after treatment at 30g/300ml stock solution								
Colour	-	$33^{b}\pm4.3$	$41^{a}\pm5.8$	$32^{b}\pm2.6$	$24^{c}\pm2.6$	$40^{a}\pm5.9$	$39^{a}\pm0.8$	$25^{b}\pm2.2$
Odour	-	$92^{a}\pm7.2$	$33^{c}\pm4.6$	$33^{b}\pm3.4$	$23^{c}\pm2.8$	$39^{b}\pm5.8$	$38^{b}\pm0.7$	$24^{c}\pm2.6$
Appearance	-	$31^{b}\pm4.1$	$40^{a}\pm4.6$	$34^{b}\pm3.7$	$33^{b}\pm3.6$	$44^{a}\pm4.9$	$31^{b}\pm3.4$	$34^{b}\pm3.7$
Turbidity (Opt. Index)	-	$0.86^{c}\pm0.7$	$0.10^{e}\pm0.2$	$0.83^{c}\pm0.5$	$0.92^{b}\pm0.9$	$0.21^{d}\pm0.4$	$1.0^{b}\pm0.2$	$1.12^{a}\pm0.13$
pH	6.5-8.5	$6.62^{a}\pm1.3$	$4.82^{b}\pm0.6$	$5.01^{a}\pm1.2$	$5.44^{b}\pm1.6$	$4.76^{a}\pm0.8$	$5.26^{a}\pm1.0$	$5.59^{a}\pm1.7$
Temperature (^{O}C)	-	$31^{a}\pm4.1$	$25^{b}\pm2.5$	$23^{b}\pm2.4$	$21^{b}\pm2.3$	$24^{b}\pm2.4$	$22^{b}\pm2.3$	$22^{b}\pm2.3$
Electrical conductivity (µs/Cm)	1000	$195^{a}\pm21.4$	$187^{a}\pm21.6$	$192^{a}\pm28.2$	$180^{a}\pm26.9$	$198^{a}\pm28.4$	$190^{a}\pm2784$	$178^{a}\pm26.9$
Total dissolved solids (Mg/L)	1000	$327^{a}\pm31.7$	$294^{a}\pm23.9$	$289^{a}\pm33.6$	$300^{a}\pm34.5$	$301^{a}\pm24.2$	$301^{a}\pm35.1$	$300^{a}\pm35.2$
Dissolved oxygen (Mg/L)	6.5- 8.0	$4.20^{b}\pm1.2$	$6.34^{a}\pm1.6$	$5.54^{a}\pm1.5$	$5.03^{b}\pm1.2$	$5.61^{a}\pm1.6$	$5.61^{a}\pm1.5$	$5.03^{b}\pm1.4$
Biological oxygen demand (Mg/L)	6.00	$7.59^{a}\pm1.7$	$4.0^{c}\pm0.9$	$4.04^{c}\pm1.1$	$5.28^{b}\pm1.8$	$4.00^{c}\pm1.5$	$5.12^{c}\pm1.4$	$5.02^{b}\pm2.0$
Chloride ion	250	$1.42^{c}\pm0.7$	$3.20^{a}\pm1.1$	$2.62^{a}\pm0.2$	$2.14^{b}\pm0.1$	$3.70^{a}\pm1.0$	$2.45^{a}\pm0.1$	$2.11^{b}\pm0.2$
Groundwater before and after treatment at 30g/300ml stock solution								
Colour	-	$100^{a}\pm1.0$	$47^{b}\pm6.2$	$42^{b}\pm4.0$	$42^{b}\pm3.4$	$47^{b}\pm6.2$	$46^{b}\pm3.7$	$38^{b}\pm3.4$
Odour	-	$100^{a}\pm1.0$	$40^{b}\pm4.9$	$39^{b}\pm3.8$	$38^{b}\pm3.9$	$40^{b}\pm6.3$	$40^{b}\pm3.2$	$39^{b}\pm2.9$
Appearance	-	$100^{a}\pm1.0$	$48^{b}\pm4.9$	$48^{b}\pm22$	$44^{c}\pm2.5$	$49^{c}\pm4.9$	$46^{c}\pm2.3$	$39^{c}\pm4.9$
Turbidity	-	$0.02^{d}\pm0.01$	$0.12^{c}\pm0.1$	$0.23^{b}\pm0.3$	$0.43^{a}\pm0.1$	$0.11^{a}\pm0.4$	$0.29^{a}\pm0.3$	$0.38^{a}\pm0.4$
pH	6.5-8.5	$7.19^{a}\pm1.5$	$5.05^{b}\pm0.8$	$5.56^{b}\pm1.6$	$6.43^{ab}\pm1.9$	$5.14^{b}\pm0.9$	$5.55^{b}\pm1.3$	$6.44^{ab}\pm2.0$
Temperature (^{O}C)	-	$21^{a}\pm3.3$	$22^{a}\pm2.1$	$22^{a}\pm2.2$	$22^{a}\pm2.4$	$21^{a}\pm2.1$	$21^{a}\pm2.4$	$21^{a}\pm3.2$
Electrical conductivity (µs/Cm)	1000	$214^{a}\pm26.6$	$202^{a}\pm23.2$	$200^{a}\pm29.3$	$189^{a}\pm28.1$	$202^{a}\pm26.8$	$200^{a}\pm29.2$	$197^{a}\pm29.0$
Total dissolved solids (Mg/L)	1000	$253^{a}\pm27.8$	$266^{a}\pm19.8$	$268^{a}\pm34.7$	$267^{a}\pm31.6$	$267^{a}\pm21.2$	$268^{a}\pm19.7$	$262^{a}\pm32.4$
Dissolved oxygen (Mg/L)	6.5- 8.0	$7.13^{a}\pm1.4$	$6.12^{b}\pm1.7$	$6.10^{b}\pm1.8$	$6.03^{b}\pm1.7$	$6.26^{b}\pm1.1$	$6.10^{b}\pm1.8$	$6.02^{b}\pm1.4$
Biological oxygen demand (Mg/L)	6.00	$1.56^{a}\pm0.9$	$1.7^{a}\pm0.2$	$1.71^{a}\pm0.4$	$1.94^{a}\pm0.7$	$1.74^{a}\pm1.0$	$1.89^{a}\pm0.6$	$1.90^{a}\pm0.6$
Chloride ion	250	$3.80^{a}\pm1.1$	$3.46^{a}\pm1.3$	$3.54^{a}\pm0.5$	$3.51^{a}\pm0.6$	$3.61^{a}\pm1.8$	$3.47^{a}\pm0.3$	$3.49^{a}\pm0.4$

Mean along the row having different superscripts of letters differ significantly at P = 0.05 using the Duncan Multiple Range Test.

4.5 Physicochemical properties before and after treatment with 45g/300mL Stock Solution from the plant material used.

Table 4.5 shows that physicochemical properties such as colour, odour, appearance, turbidity, pH, temperature, dissolved oxygen, biological oxygen demand and chloride ion changes significantly after treatment of slaughterhouse wastewater with 45g/300mL SS of the three different plant materials used. There was an increase in colour across the treatment of which *Moringa oleifera* and *A. africana* (33- 40); (33-42) and (33-40); (33-40) at 10mL and 20mL respectively were the highest, for appearance, *M. oleifera* and *A. africana* at 20mL have the best appearance at (45) and (40), while for odour, *M. oleifera* and *A. africana* at 20mL have the best odour at 37 and 38 respectively. Also, turbidity decreased across the different treatment but with a lesser turbidity recorded against treatment with *M. oleifera* (0.31) at 20mL, the decreased in temperature was significantly the same across the different treatments. In addition, electrical onductivity and total dissolved solids statistically the same before and after treatment. Th increase in dissolved oxygen and chloride were the same statistically while the increase in biological oxygen demand was more in treatment with *M. flagellipes* (5.17) and lesser at *M. oleifera* (3.98) both at 20mL.

Similarly, the treatment of groundwater shows that physicochemical properties such as colour, odour, appearance, and turbidity changes significantly as the colour decreased from 100-41, 38 and 48, odour, 100-39, 49, appearance, 100-44, 47 and turbidity, 0.02-0.15, 0.22, 0.42 accordingly. The decrease in pH across the different treatments were significantly the same after treatment. While the temperature, electrical conductivity, total dissolved solids, biological oxygen demand and chloride ion were statistically the same before and after treatment.

Table 4.5: Physicochemical properties before and after treatment with 45g/300mL Stock Solution from the plant material used.

Physicochemical properties	WHO standard	Before treatment	10ml *Moringa olifera*	10ml *Afzelia africana*	10ml *Muccuna flagellipes*	20 ml *Moringa olifera*	20 ml *Afzelia africana*	20 ml *Muccuna flagellipes*
Slaughter house wastewater before and after treatment at 45g/300ml stock solution								
Colour	-	$33^{b}\pm4.3$	$40^{a}\pm5.8$	$40^{a}\pm2.6$	$36^{b}\pm2.6$	$42^{a}\pm5.9$	$40^{a}\pm3.0$	$27^{c}\pm2.7$
Odour	-	$92^{a}\pm7.2$	$32^{c}\pm4.6$	$37^{b}\pm3.4$	$28^{d}\pm2.8$	$37^{b}\pm5.8$	$38^{b}\pm3.7$	$26^{d}\pm2.6$
Appearance	-	$31^{c}\pm4.1$	$39^{b}\pm4.6$	$39^{b}\pm1.7$	$31^{c}\pm1.6$	$45^{a}\pm4.9$	$40^{b}\pm2.2$	$34^{c}\pm1.7$
Turbidity	-	$0.86^{d}\pm0.3$	$0.33^{e}\pm0.2$	$0.76^{c}\pm0.5$	$0.72^{d}\pm0.3$	$0.31^{e}\pm0.4$	$1.6^{b}\pm0.8$	$1.71^{a}\pm0.5$
pH	6.5-8.5	$6.62^{a}\pm1.3$	$4.78^{b}\pm0.6$	$5.19^{b}\pm1.2$	$5.44^{b}\pm1.6$	$4.79^{b}\pm0.8$	$5.50^{b}\pm1.0$	$5.46^{b}\pm1.6$
Temperature (^{O}C)	-	$31^{a}\pm4.1$	$24^{b}\pm2.4$	$22^{b}\pm2.4$	$22^{b}\pm2.3$	$24^{b}\pm2.4$	$22^{b}\pm2.4$	$23^{b}\pm2.2$
Electrical conductivity (µs/Cm)	1000	$195^{a}\pm21.4$	$189^{a}\pm21.6$	$188^{a}\pm28.2$	$178^{a}\pm26.9$	$187^{a}\pm23.3$	$189^{a}\pm27.4$	$178^{a}\pm26.9$
Total dissolved solids (Mg/L)	1000	$327^{a}\pm31.7$	$293^{a}\pm23.9$	$289^{b}\pm33.6$	$300^{a}\pm34.5$	$296^{b}\pm24.2$	$291^{b}\pm35.1$	$303^{a}\pm35.2$
Dissolved oxygen (Mg/L)	6.5- 8.0	$4.20^{b}\pm1.2$	$6.20^{a}\pm1.6$	$5.30^{a}\pm1.5$	$5.13^{a}\pm1.2$	$6.32^{a}\pm1.6$	$5.40^{a}\pm1.5$	$5.21^{a}\pm1.4$
Biological oxygen demand (Mg/L)	6.00	$7.59^{a}\pm1.7$	$4.00^{c}\pm0.9$	$4.02^{c}\pm1.1$	$5.04^{b}\pm1.8$	$3.98^{c}\pm1.5$	$4.09^{c}\pm1.4$	$5.17^{b}\pm2.0$
Chloride ion	250	$1.42^{c}\pm0.7$	$3.02^{a}\pm1.1$	$2.52^{a}\pm0.2$	$2.02^{b}\pm0.1$	$3.04^{a}\pm1.0$	$2.64^{a}\pm0.1$	$2.13^{b}\pm0.2$
Groundwater before and after treatment at 45g/300ml stock solution								
Colour	-	$100^{a}\pm1.0$	$48^{b}\pm6.2$	$41^{c}\pm4.0$	$41^{c}\pm3.4$	$49^{b}\pm6.2$	$41^{c}\pm3.7$	$38^{d}\pm3.4$
Odour	-	$100^{a}\pm1.0$	$41^{b}\pm4.9$	$39^{c}\pm3.8$	$37^{b}\pm2.9$	$49^{b}\pm6.6$	$38^{b}\pm3.2$	$38^{b}\pm2.9$
Appearance	-	$100^{a}\pm1.0$	$47^{b}\pm4.9$	$44^{c}\pm22$	$44^{c}\pm2.5$	$41^{c}\pm4.9$	$44^{c}\pm2.3$	$44^{c}\pm4.9$
Turbidity	-	$0.02^{d}\pm0.01$	$0.15^{c}\pm0.1$	$0.22^{b}\pm0.3$	$0.42^{a}\pm0.1$	$0.11^{c}\pm0.4$	$0.21^{b}\pm0.3$	$0.39^{a}\pm0.4$
pH	6.5-8.5	$7.19^{a}\pm1.5$	$5.01^{b}\pm0.8$	$5.55^{b}\pm1.6$	$6.42^{ab}\pm1.9$	$5.03^{b}\pm0.9$	$5.53^{b}\pm1.3$	$6.43^{b}\pm2.0$
Temperature (^{O}C)	-	$21^{a}\pm3.3$	$22^{a}\pm2.1$	$21^{a}\pm2.2$	$22^{a}\pm2.4$	$22^{a}\pm2.1$	$22^{a}\pm2.4$	$22^{a}\pm3.2$
Electrical conductivity (µs/Cm)	1000	$214^{a}\pm26.6$	$198^{a}\pm23.2$	$201^{a}\pm29.3$	$189^{a}\pm28.1$	$268^{a}\pm26.8$	$198^{a}\pm29.2$	$183^{a}\pm29.0$
Total dissolved solids (Mg/L)	1000	$253^{a}\pm27.8$	$276^{a}\pm19.8$	$268^{a}\pm34.7$	$269^{a}\pm31.6$	$264^{a}\pm21.2$	$264^{a}\pm19.7$	$270^{a}\pm32.4$
Dissolved oxygen (Mg/L)	6.5- 8.0	$7.13^{a}\pm1.4$	$6.47^{a}\pm1.7$	$6.08^{b}\pm1.8$	$6.03^{b}\pm1.7$	$6.03^{b}\pm1.1$	$6.12^{b}\pm1.8$	$6.03^{b}\pm1.4$
Biological oxygen demand (Mg/L)	6.00	$1.56^{a}\pm0.9$	$1.79^{a}\pm0.2$	$1.92^{a}\pm0.4$	$1.93^{a}\pm0.7$	$1.91^{a}\pm1.0$	$1.91^{a}\pm0.6$	$1.90^{a}\pm0.6$
Chloride ion	250	$3.80^{a}\pm1.1$	$3.48^{a}\pm1.3$	$3.53^{a}\pm0.5$	$4.52^{a}\pm0.6$	$3.53^{a}\pm1.8$	$3.53^{a}\pm0.3$	$3.49^{a}\pm0.4$

Mean along the row having different superscript of letters differ significantly at P = 0.05 using Duncan Multiple Range Test.

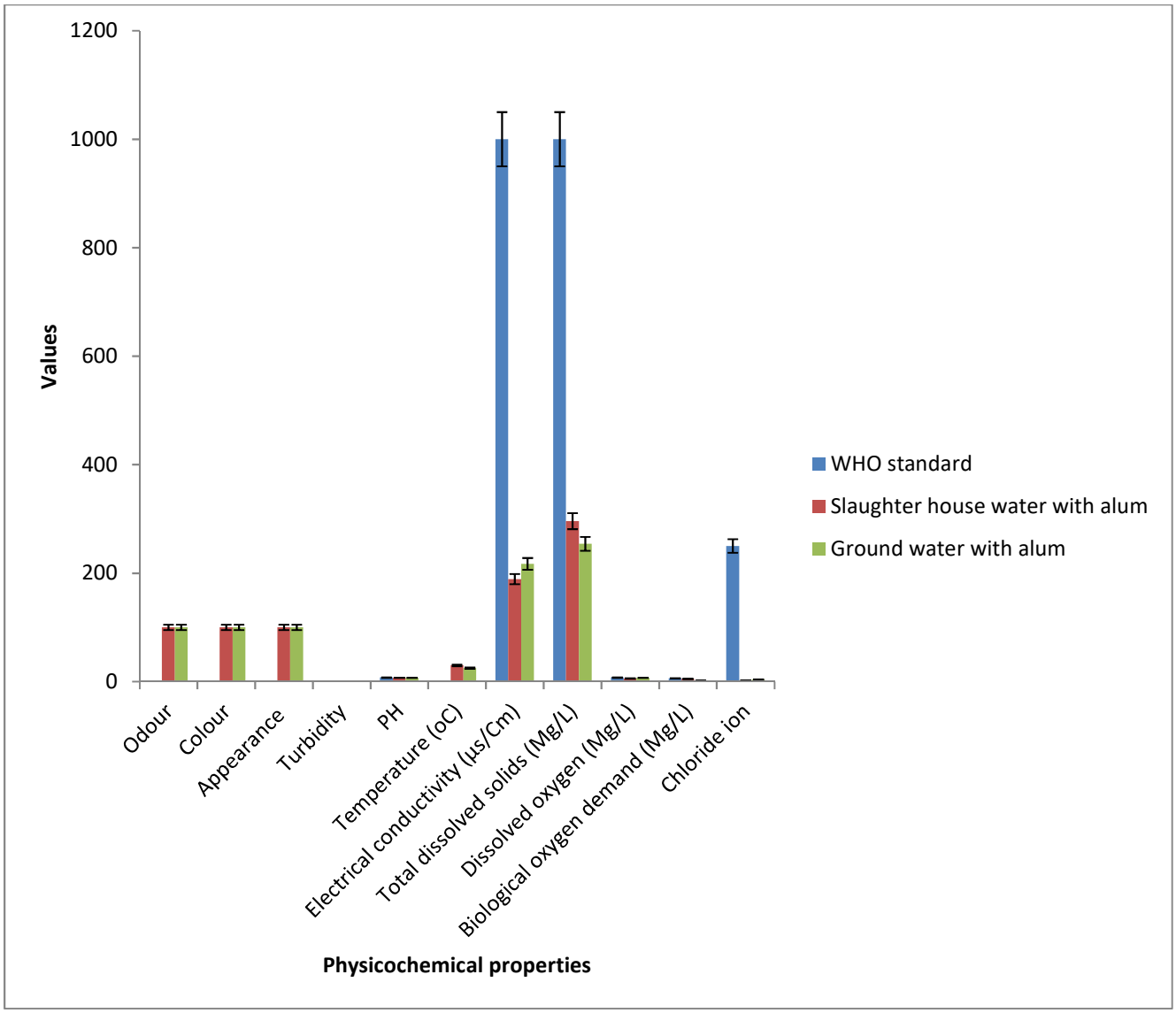


Figure 4.2: Overall physicochemical properties of water samples after treatment with alum

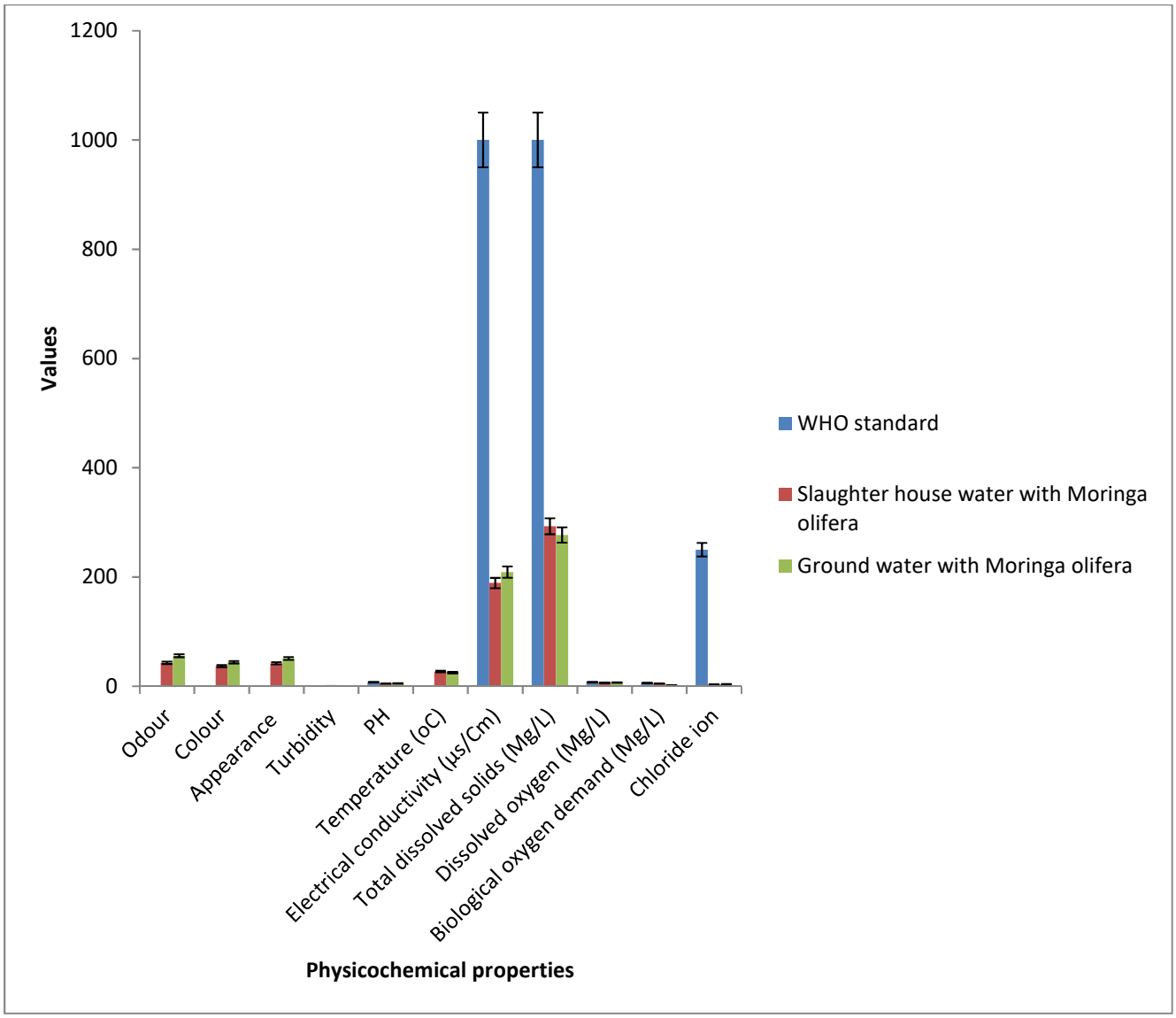


Figure 4.3: Overall physicochemical properties of water samples after treatment with *Moringa olifera.*

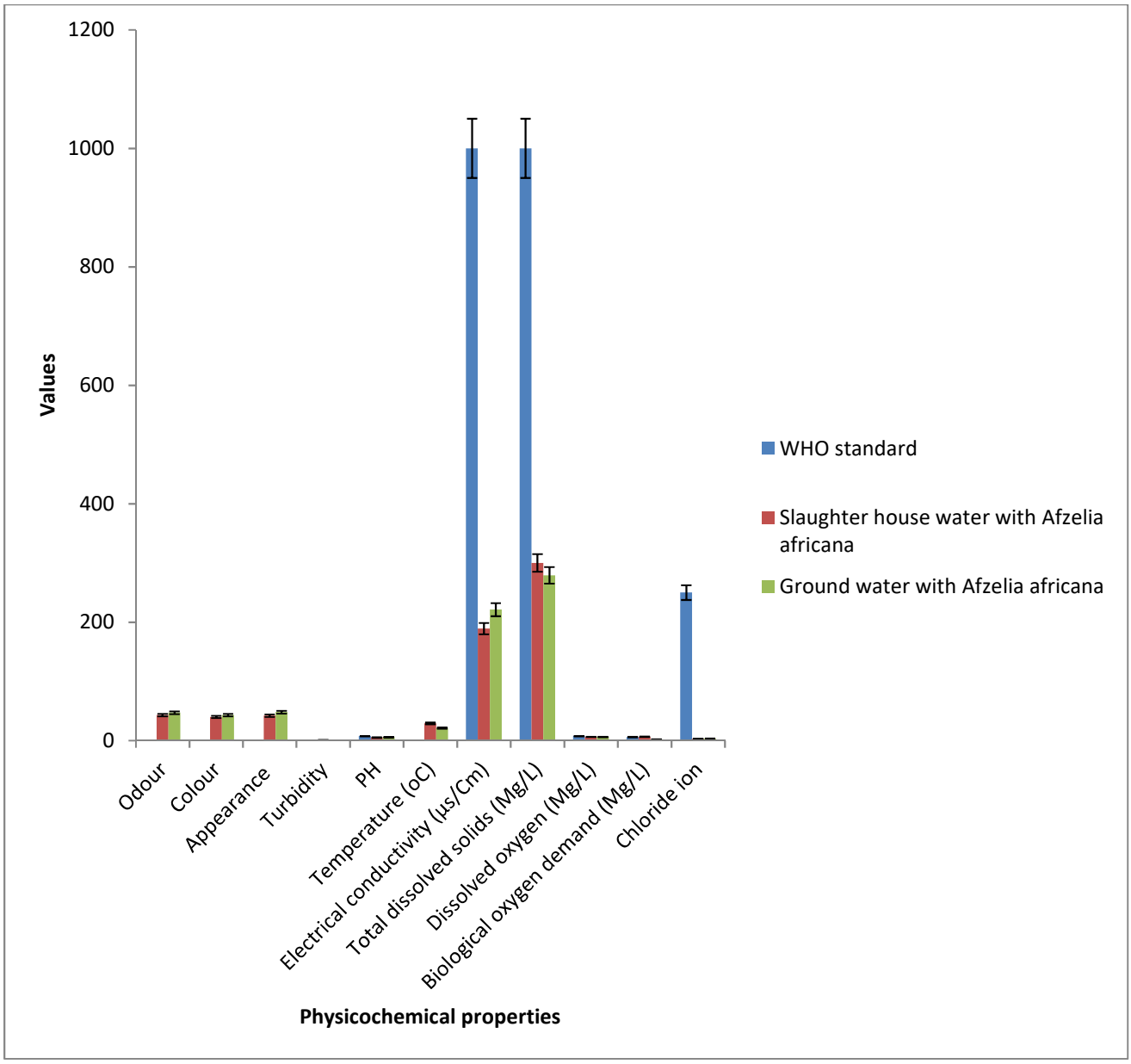


Figure 4.4: Overall physicochemical properties of water samples after treatment with *Afzelia africana*

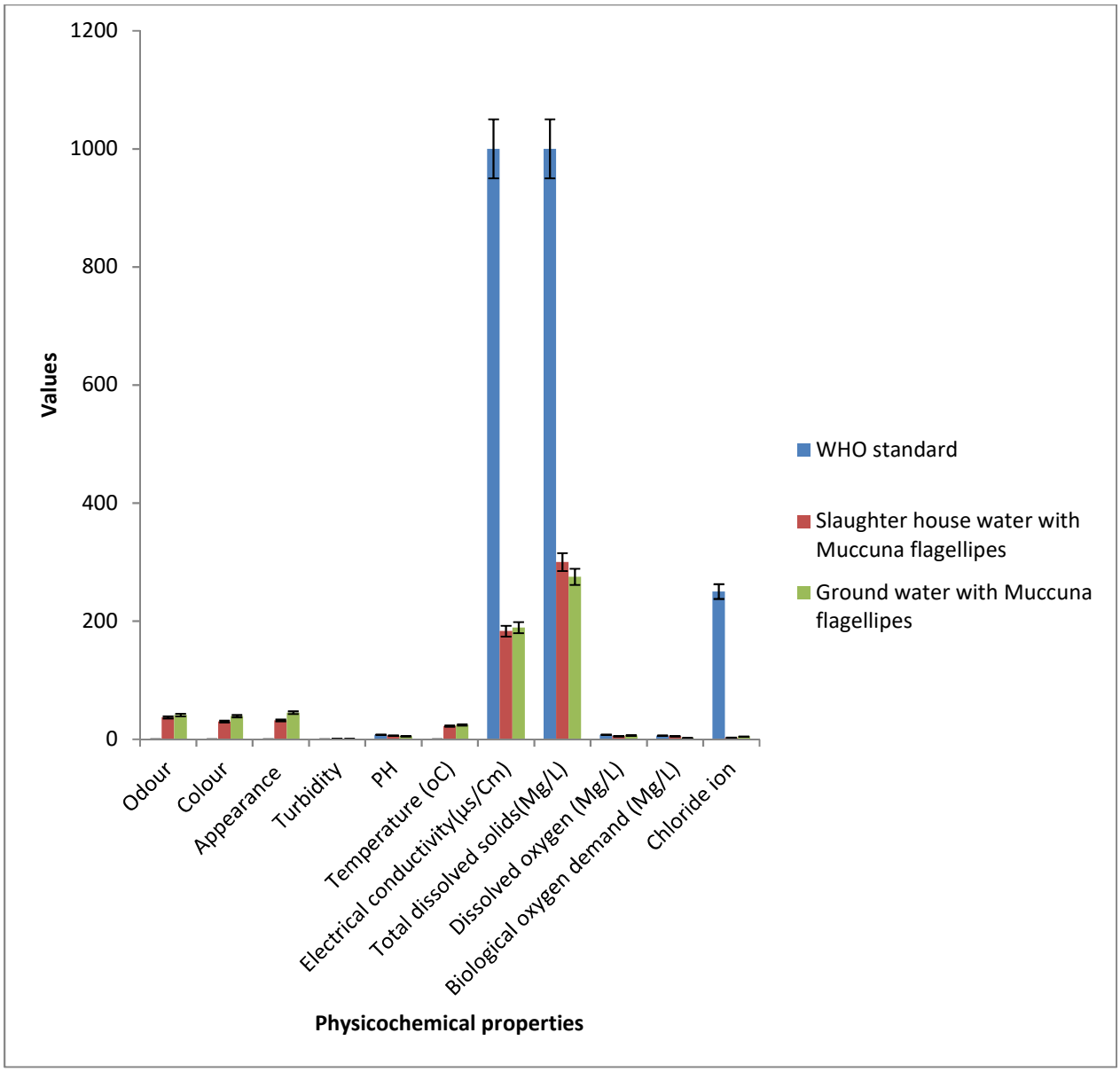


Figure 4.5: Overall physicochemical properties of water samples after treatment with *Muccuna flagellipse.*

4.6 Bacterial Analysis of the Bio-coagulant, Slaughterhouse wastewater and Groundwater samples Before and After Treatment

The result of the bacterial screening before and after treatment, reveals the presence of the following bacteria; *Escherichia coli, Enterobacter spp., Salmonella spp., Pseudomonas spp., Streptococcus spp., Staphylococcus aureus,* and *Campylobacter jejuni*. The bacteria load for slaughterhouse wastewater before treatment was high for all the bacteria present with a higher colonial count against *E. coli* for the different sampling point [upper ($4.5x10^3$cfu), mid ($4.0x10^3$cfu) and down ($3.8x10^3$cfu) slaughterhouse wastewater] with an exception of *Campylobacter jejuni* which was below detection limit (<10). While the colonial count of the bio-coagulant shows that on *Salmonella spp* and *Staphylococcus aureus* were detected at a count of (<50) but others were below detectable limit (<10). After treatment of slaughterhouse wastewater with the three different bio-coagulants (*M. oleifera*, *A. africana* and *M. flagellipes*) shows that the bacteria load decreased of which *M. oleifera* has the least bacteria count across the different sampling point which compared favorably to the control followed by *A. africana* and *M. flagellipes* having the highest count. Again, *C. jejuni* was below detection limit (<10). Conversely, the bacteria count for groundwater sample before and after treatment was significantly the same with exception the treatment with *M. flagellipes* of which *E. coli* and *C. jejuni* in was higher after treatment (Table 4.6).

Table 4.6: Bacterial load of the bio-coagulant, slaughterhouse wastewater and groundwater samples before and after treatment with bio-coagulant used and control (alum)

SAMPLES			BACTERIA ISOLATES						
			Escherichia Coli	***Enterobacter spp.***	***Salmonella spp.***	***Pseudomonas spp.***	***Streptococcus spp.***	***Staphylococcus aureus***	***Campylobacter jejuni***
Bio-coagulant	Before Treatment	USH	4.5×10^3	3.7×10^3	3.8×10^3	4.1×10^3	3.3×10^3	3.2×10^3	<10
		MSH	4.0×10^3	3.6×10^3	3.5×10^3	3.8×10^3	3.0×10^3	3.0×10^3	<10
		DSH	3.8×10^3	3.3×10^3	3.4×10^3	3.6×10^3	3.1×10^3	$3..1 \times 10^3$	<10
		GW1	1.0×10^2	< 10	1.0×10^2	< 10	< 10	< 10	<50
		GW2	1.0×10^2	< 10	1.0×10^2	< 10	< 10	< 10	<50
		GW3	1.0×10^2	< 10	1.0×10^2	< 10	< 10	< 10	<50
		Mo	< 10	< 10	<50	< 10	< 10	<50	<10
		Aa	< 10	< 10	<50	< 10	< 10	<50	<10
		Mf	< 10	< 10	<50	< 10	< 10	<50	<10
	Mo	USH	2.0×10^2	2.5×10^2	2.7×10^2	2.1×10^2	1.5×10^2	1.8×10^2	<10
		MSH	3.0×10^2	3.1×10^2	2.9×10^2	2.7×10^2	3×10^2	1.5×10^2	<10
		DSH	<10	<10	3.0×10^2	<10	<10	<10	<10
	Aa	USH	3.5×10^2	3.0×10^3	2.9×10^2	3.1×10^3	2.6×10^3	2.0×10^2	<10
		MSH	3.2×10^2	2.7×10^3	2.8×10^2	2.9×10^3	3.1×10^3	1.7×10^2	<10
		DSH	2.5×10^2	2.1×10^2	3.2×10^2	1.8×10^2	1.65×10^2	1.53×10^2	<10
	Mf	USH	2.4×10^3	2.1×10^3	2.13×10^3	2.3×10^3	2.6×10^3	2.11×10^3	< 10
		MSH	2.9×10^3	3.1×10^3	3.0×10^3	2.5×10^3	2.8×10^3	2.35×10^2	<10
		DSH	2.7×10^2	2.4×10^2	3.4×10^2	2.0×10^3	1.7×10^3	2.5×10^3	<10
After treatment		COU	2.1×10^2	2.5×10^2	2.3×10^2	2.1×10^2	1.5×10^2	1.8×10^2	<10
	Mo	GW1	1.2×10^2	<10	1.1×10^2	<10	<10	<10	x 10^2
		GW2	1.3×10^2	<10	1.05×10^2	<10	<10	<10	x 10^2
		GW3	1.2×10^2	<10	1.2×10^2	<10	<10	<10	x 10^2
	Aa	GW1	1.4×10^2	<10	1.5×10^2	<10	<10	<10	1.2×10^2
		GW2	1.5×10^2	<10	1.4×10^2	<10	<10	<10	1.5×10^2
		GW3	1.6×10^2	<10	1.7×10^2	<10	<10	<10	1.4×10^2
	Mf	GW1	1.8×10^3	<10	1.8×10^2	<10	<10	<10	1.6×10^2
		GW2	1.9×10^3	<10	1.79×10^2	<10	<10	<10	1.8×10^3
		GW3	2.0×10^2	<10	1.6×10^2	<10	<10	<10	1.9×10^3
		COG	1.0×10^2	1.0×10^2	1.0×10^2	1.0×10^2	1.0×10^2	1.0×10^2	1.0×10^2

Legend: <10 = Below detection limit, USH = Upper slaughterhouse wastewater, MSH= Mid- slaughterhouse wastewater, DSH = Down- slaughterhouse wastewater, COU= Control for slaughterhouse wastewater, COG = Control for groundwater GW1-GW3 = Groundwater station 1-3, Mo = *Moringa oleifera*, Aa = *Afzelia africana* Mf = *Muccuna flagellipes*

CHAPTER FIVE

5.0 DISCUSSION, COCLUSION, AND RECOMMENDATION

5.1 DISCUSSION

5.1.1 Phytochemical Analysis

The result of the phytochemical analysis shows that the aqueous extract of the three Indigenous plant seed powders (*Moringa. oleifera*, *Afzelia. africana,* and *Muccuna. flagellipes*) has the presence of alkaloids, flavonoids, phenols, tannins, steroids, and anthraquinones. While steroids were only present in *M. oleifera,* this implies that *M. oleifera* has all the phytochemical parameters assessed, while *A. africana* and *M. flagllipes* lack steroids but have the remaining phytoconstituents. This result is in line with the works of Okwu & Okoro, (2007), Nweze & Nwaform (2014), Ojiako (2014), Elzein *et al*. (2018), and *Olorunmaiye et al*. (2019).

5.1.2 Physicochemical properties of the water samples before and after treatment

5.1.2.1 Slaughterhouse Wastewater (SHW)

The physicochemical properties of the slaughterhouse wastewater (SHW) before treatment show that the water was brown in colour and poor in appearance but almost odourless. The turbidity as measured with a spectrophotometer was 0.86 with a pH (6.62) which was within WHO standard, while electrical conductivity (195), total dissolved solids (327), dissolved oxygen (4.20) and chloride ion (1.42) were all below WHO standard. Biological oxygen demand (7.59) was above WHO standard. After treatment with alum, which serves as the control, the wastewater becomes colourless, odourless with a better appearance and a significant decreased in turbidity (0.86-0.13), temperature (31-29) and biological oxygen demand (7.59-2.05). The decrease in electrical conductivity (195-187) and total dissolved oxygen (327-305) were not significant while the increase in pH (6.62-7.12) and chloride ion (1.42- 2.14) were statistically significant different at p=0.05.

The result of the physicochemical properties of the slaughterhouse wastewater treated with 15g/300mL stock solution (SS) from the different three plant samples used in this present study at a concentration of 10mL and 20mL SS revealed a significant difference in the colour, odour, appearance, turbidity, pH, Temperature, dissolved oxygen, biological oxygen demand and chloride after treatment. The treatment with the best significant increase in colour was *Afzelia africana* (33-43) at 20mL SS, followed by *Moringa oleifera* (33-42) at 20mL SS but in appearance, *M. oleifera* (31-39) was the best followed by *A. africana* (31-37) at 20mL SS, although, the observed difference was not statistically significant. The decrease in turbidity was least in treatment with *M. oleifera* at (0.86-0.31) at 10mL SS, which agrees with the work

(Muyibi *et a*l. 2004; Ghebremichael *et al.* 2005; Arnoldsson *et al.* 2008; Lea 2010; Subramanium *et al*. 2011; Vikashni *et al.* 2012) as their finding indicated that the turbidity was reduced from water samples after treatment with *M. oleifera* seed cake. In addition, the decrease in pH and temperature and increase dissolved oxygen were significantly the same across the different treatment. The decrease in pH after treatment below the pH before treatment, shows that the plant materials used were acidic as their pH values were *M. oleifera* (3.82), *Afzelia africana* (4.87), and *Muccuna flagellipes* (5.01). thus, these decrease in pH after treatment does not corroborate with the work of (Ghebremichael *et al*. 2005; Arnoldsson *et al*. 2008; Alo *et al*. 2012), whose work showed a significant increase in pH after treatment. The reason for the observed difference may be due to the differences in ecological and edaphic factors from where these plants were cultivated, which affect the pH of the stock solutions prepared from the study plant samples. Furthermore, the decrease in biological oxygen demand and increase in chloride ion was the same statistically for treatment with *M. oleifera* and *A. africana* at 10mL and 20mL than that of *M. flagellipes* accordingly.

The physicochemical properties of the slaughterhouse wastewater before and after treatment with 30g/300mL stock solution prepared from the three different study samples shows that there was a significant difference in the colour, odour, appearance, turbidity, pH, temperature, dissolved oxygen, biological oxygen demand and chloride ion after treatment, of which the treatment with *M. oleifera* and *A. africana* have the highest increase in colour (33-41) at 10mL; (33-40) at 20mL and (33-39) at 20mL respectively and appearance, *M. oleifera* (31-44) at 20mL was the best while others were significantly the same. The decrease in turbidity was least in treatment with *M. oleifera* (0.86-0.10) at 10mL and (0.86-0.21) at 20mL, pH was least in *M. oleifera* (6.62-4.76) at 20mL. Also, the decrease in temperature and increase in dissolved oxygen were significantly the same but increase biological oxygen demand and chloride ion were statistically significant in treatment with *M. oleifera* and *A. africana* while, electrical conductivity and total dissolved solids were statistically the same before and after treatment.

Furthermore, physicochemical properties of the slaughterhouse wastewater treated with 10mL and 20mL stock solution prepared from the three different plant samples used reveals an increase in colour and appearance across the treatment of which *Moringa oleifera* and *A. africana* were the highest at (33-40); (33-42) and (33-40); (33-40) at 10mL and 20mL for colour and in appearance at 20mL (45) and (40) respectively. A decrease in turbidity across the different treatment was observed but the least was seen in *M. oleifera* (0.31) at 20mL. In addition, electrical conductivity and total dissolved solids were statistically the same before and after

treatment. The increase in dissolved oxygen and chloride were the same statistically while the increase in biological oxygen demand was more in treatment with *M. flagellipes* (5.17) and lesser at *M. oleifera* (3.98) both at 20mL.

5.1.2.2 Groundwater (GW)

The physicochemical properties of groundwater before treatment showed that the water was colourless, odourless and excellent appearance. A turbidity of (0.02), pH (7.19), temperature (21), electrical conductivity of (214), total dissolved solid (253), biological oxygen demand (1.56), dissolved oxygen (7.13) and chloride (3.80). after treatment with alum (control), there was no significant difference in the physicochemical properties. While upon treatment with the different stock solutions (15g/300mL, 30g/300mL and 45g/300mL) prepared from the three different plant materials used for this study at 10mL and 20mL concentrations showed that there was a significant decreased in the different physicochemical properties assessed, which did not compare favorably with the control. There was a change in the colour, odour, appearance and pH, of which the change in colour odour and pH were statistically the same across the different treatment while in appearance, treatment with 10mL *M. oleifera* and *A. africana* were significant the same and better than 20mL of *M. oleifera* and *A. africana* and *M. flagellipes* at 10mL and 20mL. Temperature, electrical conductivity, total dissolved solids, dissolved oxygen, biological oxygen demand and chloride were statistically the same before and after treatment but the turbidity increases after treatment with highest increase observed in treatment with *M. flagellipes* (0.42, 0.43 and 0.42) at 10mL and (0.32, 0.38 and 0.39) at 20mL respectively as opposed to the turbidity before treatment (0.02).

Thus, treatment with *M. oleifera* was be best bio-coagulants among the other bio-coagulant used in this present study and the reason for this may be as result of the high saponin content as compared to others while the poor coagulating ability seen in *M. flagellipes* may be due to the oily nature of the endocarp which limit its chelating ability.

5.1.3 Bacterial Screening of the Bio-coagulant, Slaughterhouse wastewater and Groundwater samples Before and After Treatment

The result of the bacterial screening before and after treatment, reveals the presence of the following bacteria; *Escherichia coli, Enterobacter spp., Salmonella spp., Pseudomonas spp., Streptococcus spp., Staphylococcus aureus,* and *Campylobacter jejuni*. The bacteria load for slaughterhouse wastewater before treatment was high for all the bacteria present with a higher colonial count against *E. coli* for the different sampling point [upper ($4.5x10^3$cfu), mid

($4.0x10^3$cfu) and down ($3.8x10^3$cfu) slaughterhouse wastewater] with an exception of *Campylobacter jejuni* which was below detection limit (<10). While the colonial count of the bio-coagulant shows that *Salmonella spp* and *Staphylococcus aureus* were detected with a colonial count of (<50) while others were below detectable limit (<10). After treating slaughterhouse wastewater with the three different bio-coagulants (*M. oleifera*, *A. africana* and *M. flagellipes*), the bacteria load decreased of which the treatment with *M. oleifera* has the least bacteria count across the different sampling point with the down-slaughterhouse wastewater having the least colonial count, which was below detection limit (<10) for all the identified isolates with exception of *Salmonella spp* (3.0×10^2) while that of upper-stream and mid-stream compared favorably to the control. Treatment with *A. africana* shows that the least colonial count as seen across down-slaughterhouse wastewater, which compared well to the control with an exception of *Enterobacter spp., Pseudomonas spp.* and *Streptococcus spp.* which was still the same before treatment. In addition, treatment with *Muccuna flagellipes* shows that the colonial count for *Escherichia coli, Enterobacter spp.* and *Salmonella spp.* compared well with the control for down-slaughterhouse wastewater but their colonial count for upper and mid-slaughterhouse wastewater were all higher than the control. Conversely, the bacteria count for groundwater sample before and after treatment was significantly the same with exception the treatment with *M. flagellipes* of which *E. coli* and *C. jejuni* were slightly high after treatment, but all compared with control and were still within range for WHO allowable bacterial count of domestic water (100-500/ml).

5.2 CONCLUSION

This present study revealed that leguminous plant seed powders from *Moringa oleifera, Afzelia africana,* and *Muccuna. flagllipes* used as bio-coagulants for treating wastewater showed possible potential in treating wastewater but not in groundwater. *Moringa oleifera* showed the highest potential, with evidence of a sharp reduction in turbidity, color, odor, TDS, BOD, EC, and increased DO and Cl^- after treatment as compared to the untreated wastewater samples. Again, *M. oleifera* was also seen as the best in the reduction of total coliforms and some pathogenic bacteria resulting from the wastewater samples.

5.3 RECOMMENDATION

- ✓ The use of *M. oleifera* should be adopted in wastewater treatment both locally and commercially.
- ✓ Further research should be carried out on other plant materials.
- ✓ The use of synthetic coagulants should be discouraged.

5.4 CONTRIBUTION TO KNOWLEDGE

- This is the first time *Afzelia africana* and *Muccuna flagellipse* have been used as bio-coagulants in wastewater treatment.
- The research has shown that wastewater from slaughterhouses can be treated with *M. oleifera* seed powder.
- The study has shown that the use of synthetic coagulants should be discouraged as they pose some level of toxin to the human body.
- The study has also proven that *M. oleifera* seed powder exhibits some level of antibacterial activity and should be used as a substitute in water chlorination.

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APPENDIX

Appendix 1: Rating sample form for physicochemical parameters before and after treatment

OBSERVER NO:

Before Treatment

WATER SAMPLE	PARAMETERS		
	Colour	Odour	Appearance
Upstream from slaughterhouse effluent (USHW)			
Midstream from slaughterhouse effluent (MSHW)			
Downstream from slaughterhouse effluent (DSHW)			
Groundwater from station 1			
Groundwater from station 2			
Groundwater from station 3			

KEY:

C = COLOUR
Rating:
Colourless = 5 points Fairly colourless = 4 points
Rather colourless = 3 points
Coloured = 2 points
Very colourful = 1 point

O = ODOUR
Rating:
Odourless = 5 points
Fairly Odourless = 4 points
Rather Odourless = 3 points
Has Odour = 2 points
Very Odourful = 1 point

A = APPEARNCE
Rating:
Excellent = 5 points
Very good = 4 points
Good = 3 points
Poor = 2 points
Very poor = 1 point

USHW = Upper stream slaughterhouse wastewater
MSHW = Mid-stream slaughterhouse wastewater
DSHW = Downstream slaughterhouse wastewater
GWST1 = Groundwater station 1
GWST2 = Groundwater station 2
GWST3 = Groundwater station 3

M. o = *Moringa oleifera*
A. a = *Afzelia africana*
M. f = *Muccuna flagellipse*

After Treatment

WATER SAMPLES		STOCK SOLUTIONS FROM PLANT SAMPLE USED																							
		15g/300mL Stock Solution								30g/300mL Stock Solution								45g/300mL Stock Solution							
		10mL				20mL				10mL				20mL				10mL				20mL			
		C	T	O	A	C	T	O	A	C	T	O	A	C	T	O	A	C	T	O	A	C	T	O	A
M	USHW																								
. o	MSHW																								
	DSHW																								
A.	USHW																								
a	MSHW																								
	DSHW																								
M	USHW																								
. f	MSHW																								
	DSHW																								
M	GWST1																								
. o	GWST2																								
	GWST3																								
A.	GWST1																								
a	GWST2																								
	GWST3																								
M	GWST1																								
. f	GWST2																								
	GWST3																								

Table 1: Physicochemical Parameters Before Treatment

WATER SAMPLES			PHYSICOCHEMICAL PARAMETERS										
			C	O	A	TB	pH	T (^{0}C)	EC (µS/Cm)	TDS (Mg/L)	DO (Mg/L)	BOD (Mg/L)	Cl^-
WHO STANDARD			-	-	-	-	6.5-8.5	-	1000	1000	6.5-8.0	6	250
R1	SHW	U	33	92	31	0.75	6.62	31	159	327	4.20	7.59	1.42
		M	33	92	31	0.83	6.62	31	159	327	4.20	7.59	1.42
		D	33	92	31	0.89	6.62	31	159	327	4.20	7.59	1.42
		CO	**100**	**100**	**100**	**0.13**	**7.12**	**29**	**187**	**295**	**5.78**	**5.05**	**2.14**
	GW	S1	100	100	100	0.02	7.21	21	214	253	7.21	1.56	3.80
		S2	100	100	100	0.01	7.24	21	214	252	7.13	1.56	3.80
		S3	100	100	100	0.03	7.23	22	214	255	7.13	1.56	3.80
		CU	**100**	**100**	**100**	**0.02**	**7.14**	**21**	**215**	**252**	**7.14**	**1.54**	**3.83**
R2	SHW	U	35	91	30	0.75	7.32	32	157	325	4.21	7.61	1.42
		M	34	90	31	0.83	7.32	32	157	325	4.21	7.61	1.42
		D	34	91	30	0.89	7.32	32	157	325	4.21	7.61	1.42
		CO	**100**	**100**	**100**	**0.15**	**7.21**	**29**	**185**	**293**	**5.80**	**5.04**	**2.12**
	GW	S1	100	100	100	0.02	7.21	21	214	253	7.21	1.56	3.80
		S2	100	100	100	0.01	7.24	21	214	252	7.13	1.56	3.80
		S3	100	100	100	0.03	7.23	22	214	255	7.13	1.56	3.80
		CU	**100**	**100**	**100**	**0.01**	**7.12**	**21**	**215**	**250**	**7.15**	**1.53**	**3.82**
R3	SHW	U	34	92	31	0.75	6.97	32	158	326	4.21	7.6	1.42
		M	34	91	31	0.83	6.97	32	158	326	4.21	7.6	1.42
		D	34	92	31	0.89	6.97	32	158	326	4.21	7.6	1.42
		CO	**100**	**100**	**100**	**0.14**	**7.23**	**28**	**184**	**294**	**5.81**	**5.02**	**2.13**
	GW	S1	100	100	100	0.02	7.21	21	214	253	7.21	1.56	3.80
		S2	100	100	100	0.01	7.24	21	214	252	7.13	1.56	3.80
		S3	100	100	100	0.03	7.23	22	214	255	7.13	1.56	3.80
		CU	**100**	**100**	**100**	**0.01**	**7.12**	**21**	**215**	**250**	**7.15**	**1.54**	**3.79**

Where CO = CU = Control for slaughterhouse wastewater and groundwater respectively
R1=R2=R3= replicates, SHW= slaughterhouse wastewater

Physicochemical properties after treatment at 15g/300mL Stock Solution

Water Samples		Replicate 1 15g/300mL Stock Solution																					
		10mL											20mL										
		A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-	A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-
M. o	USHW	42	43	39	0.24	4.83	25	187	290	6.35	4.01	3.1	42	43	38	0.43	4.73	25	187	292	6.32	4.02	3.12
	MSHW	40	39	36	0.28	4.77	23	196	295	6.23	3.98	3.04	41	40	40	0.37	4.78	23	196	297	6.24	3.96	3.05
	DSHW	43	42	38	0.38	4.80	24	198	287	6.33	3.78	3.05	43	43	39	0.42	4.83	24	198	289	6.31	3.79	3.07
A. a	USHW	38	41	31	0.63	5.02	24	184	305	5.65	4.32	2.87	39	40	37	1.00	5.05	24	184	306	5.62	4.31	2.88
	MSHW	33	40	30	0.74	5.01	23	192	295	5.54	4.03	2.76	47	48	38	0.90	5.00	23	192	294	5.53	4.02	2.77
	DSHW	35	43	24	0.65	5.04	22	190	287	5.6	4.04	2.54	46	46	37	0.71	5.07	22	190	289	5.61	4.05	2.53
M. f	USHW	30	37	22	0.79	5.44	21	178	300	5.03	5.35	2.15	31	32	22	1.05	5.42	21	178	303	5.04	5.34	2.18
	MSHW	38	38	35	0.26	5.57	22	180	302	5.13	5.04	2.13	37	32	31	1.00	5.59	22	180	302	5.11	5.02	2.14
	DSHW	37	39	22	0.60	5.55	24	189	290	5.06	5.05	2.05	37	39	28	0.44	5.53	24	189	294	5.02	5.04	2.07
M. o	GWST1	49	46	40	0.12	5.00	20	201	265	6.53	1.65	3.45	49	50	40	0.12	5.02	20	201	262	6.51	1.66	3.46
	GWST2	48	47	40	0.17	5.03	23	200	260	6.43	1.87	3.5	48	47	37	0.12	5.03	23	200	260	6.45	1.89	3.53
	GWST3	49	46	40	0.12	5.05	21	202	268	6.5	1.76	3.47	49	50	40	0.11	5.04	21	202	267	6.52	1.75	3.46
A. a	GWST1	46	45	39	0.23	5.47	22	198	265	6.12	1.7	3.46	46	41	38	0.30	5.52	22	198	266	6.11	1.73	3.48
	GWST2	47	46	43	0.22	5.56	20	200	270	6.10	1.67	3.52	48	40	38	0.31	5.55	20	200	272	6.10	1.68	3.51
	GWST3	47	45	38	0.22	5.55	21	201	268	6.08	1.89	3.48	48	40	40	0.29	5.57	21	201	268	6.07	1.87	3.47
M. f	GWST1	40	39	37	0.42	6.46	23	189	270	6.03	1.9	3.54	40	40	38	0.40	6.48	23	189	273	6.04	1.91	3.53
	GWST2	43	45	37	0.43	6.42	20	196	264	6.04	1.94	4.51	45	38	37	0.39	6.43	20	196	267	6.02	1.93	4.52
	GWST3	45	38	38	0.42	6.46	23	197	257	6.02	1.85	3.47	43	39	39	0.38	6.49	23	197	258	6.01	1.85	3.47
		Replicate 2																					
		A	**C**	**O**	**T**	**pH**	**Tp**	**EC**	**TDS**	**DO**	**BOD**	**Cl^-**	**A**	**C**	**O**	**T**	**pH**	**Tp**	**EC**	**TDS**	**DO**	**BOD**	**Cl^-**

M. o	USHW	43	42	40	0.24	4.83	25	187	290	6.35	4.01	3.1	41	42	37	0.43	4.73	25	187	292	6.32	4.02	3.12
	MSHW	41	40	37	0.28	4.77	23	196	295	6.23	3.98	3.04	40	39	41	0.37	4.78	23	196	297	6.24	3.96	3.05
	DSHW	42	41	39	0.38	4.80	24	198	287	6.33	3.78	3.05	42	42	38	0.42	4.83	24	198	289	6.31	3.79	3.07
A. a	USHW	39	40	32	0.63	5.02	24	184	305	5.65	4.32	2.87	38	41	36	1.00	5.05	24	184	306	5.62	4.31	2.88
	MSHW	34	39	31	0.74	5.01	23	192	295	5.54	4.03	2.76	46	47	37	0.90	5.00	23	192	294	5.53	4.02	2.77
	DSHW	35	42	23	0.65	5.04	22	190	287	5.6	4.04	2.54	45	45	36	0.71	5.07	22	190	289	5.61	4.05	2.53
M. f	USHW	32	36	21	0.79	5.44	21	178	300	5.03	5.35	2.15	32	31	21	1.05	5.42	21	178	303	5.04	5.34	2.18
	MSHW	37	37	34	0.26	5.57	22	180	302	5.13	5.04	2.13	36	36	30	1.00	5.59	22	180	302	5.11	5.02	2.14
	DSHW	36	38	21	0.60	5.55	24	189	290	5.06	5.05	2.05	36	38	27	0.44	5.53	24	189	294	5.02	5.04	2.07
M. o	GWST1	48	47	41	0.12	5.00	20	201	265	6.53	1.65	3.45	48	50	41	0.12	5.02	20	201	262	6.51	1.66	3.46
	GWST2	47	46	40	0.17	5.03	23	200	260	6.43	1.87	3.5	47	48	38	0.12	5.03	23	200	260	6.45	1.89	3.53
	GWST3	48	45	41	0.12	5.05	21	202	268	6.5	1.76	3.47	48	49	41	0.11	5.04	21	202	267	6.52	1.75	3.46
A. a	GWST1	47	44	38	0.23	5.47	22	198	265	6.12	1.7	3.46	45	40	37	0.30	5.52	22	198	266	6.11	1.73	3.48
	GWST2	48	45	44	0.22	5.56	20	200	270	6.10	1.67	3.52	47	39	36	0.31	5.55	20	200	272	6.10	1.68	3.51
	GWST3	47	44	37	0.22	5.55	21	201	268	6.08	1.89	3.48	47	40	41	0.29	5.57	21	201	268	6.07	1.87	3.47
M. f	GWST1	40	38	36	0.42	6.46	23	189	270	6.03	1.9	3.54	41	41	37	0.40	6.48	23	189	273	6.04	1.91	3.53
	GWST2	43	44	35	0.43	6.42	20	196	264	6.04	1.94	4.51	44	37	36	0.39	6.43	20	196	267	6.02	1.93	4.52
	GWST3	44	37	37	0.42	6.46	23	197	257	6.02	1.85	3.47	42	38	38	0.38	6.49	23	197	258	6.01	1.85	3.47
		Replicate 3																					
		A	**C**	**O**	**T**	**pH**	**Tp**	**EC**	**TDS**	**DO**	**BOD**	**Cl^-**	**A**	**C**	**O**	**T**	**pH**	**Tp**	**EC**	**TDS**	**DO**	**BOD**	**Cl^-**
M. o	USHW	43	43	40	0.24	4.83	25	187	290	6.35	4.01	3.1	42	43	38	0.43	4.73	25	187	292	6.32	4.02	3.12
	MSHW	41	40	37	0.28	4.77	23	196	295	6.23	3.98	3.04	41	40	41	0.37	4.78	23	196	297	6.24	3.96	3.05
	DSHW	43	42	39	0.38	4.80	24	198	287	6.33	3.78	3.05	43	43	39	0.42	4.83	24	198	289	6.31	3.79	3.07
A. a	USHW	39	41	32	0.63	5.02	24	184	305	5.65	4.32	2.87	39	41	37	1.00	5.05	24	184	306	5.62	4.31	2.88
	MSHW	34	40	31	0.74	5.01	23	192	295	5.54	4.03	2.76	47	48	38	0.90	5.00	23	192	294	5.53	4.02	2.77
	DSHW	35	43	24	0.65	5.04	22	190	287	5.6	4.04	2.54	46	46	37	0.71	5.07	22	190	289	5.61	4.05	2.53
M.	USHW	31	37	22	0.79	5.44	21	178	300	5.03	5.35	2.15	32	32	22	1.05	5.42	21	178	303	5.04	5.34	2.18

f	MSHW	38	38	35	0.26	5.57	22	180	302	5.13	5.04	2.13	37	34	31	1.00	5.59	22	180	302	5.11	5.02	2.14
	DSHW	37	39	22	0.60	5.55	24	189	290	5.06	5.05	2.05	37	39	28	0.44	5.53	24	189	294	5.02	5.04	2.07
M. o	GWST1	49	47	41	0.12	5.00	20	201	265	6.53	1.65	3.45	49	50	41	0.12	5.02	20	201	262	6.51	1.66	3.46
	GWST2	48	47	40	0.17	5.03	23	200	260	6.43	1.87	3.5	48	48	38	0.12	5.03	23	200	260	6.45	1.89	3.53
	GWST3	49	46	41	0.12	5.05	21	202	268	6.5	1.76	3.47	49	50	41	0.11	5.04	21	202	267	6.52	1.75	3.46
A. a	GWST1	47	45	39	0.23	5.47	22	198	265	6.12	1.7	3.46	46	41	38	0.30	5.52	22	198	266	6.11	1.73	3.48
	GWST2	48	46	44	0.22	5.56	20	200	270	6.10	1.67	3.52	48	40	37	0.31	5.55	20	200	272	6.10	1.68	3.51
	GWST3	47	45	38	0.22	5.55	21	201	268	6.08	1.89	3.48	48	40	41	0.29	5.57	21	201	268	6.07	1.87	3.47
M. f	GWST1	40	39	37	0.42	6.46	23	189	270	6.03	1.9	3.54	41	41	38	0.40	6.48	23	189	273	6.04	1.91	3.53
	GWST2	43	45	36	0.43	6.42	20	196	264	6.04	1.94	4.51	45	38	37	0.39	6.43	20	196	267	6.02	1.93	4.52
	GWST3	45	38	38	0.42	6.46	23	197	257	6.02	1.85	3.47	43	39	39	0.38	6.49	23	197	258	6.01	1.85	3.47

Physicochemical properties after treatment at 30g/300mL Stock Solution

Water Samples		Replicate 1 30g/300mL Stock Solution																					
		10mL											20mL										
		A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-	A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-
M. o	USHW	41	41	32	0.19	4.83	25	187	290	6.35	4.01	3.1	40	40	38	0.70	4.73	25	187	292	6.32	4.02	3.12
	MSHW	38	40	40	0.24	4.77	23	196	295	6.23	3.98	3.04	38	41	39	0.24	4.78	23	196	297	6.24	3.96	3.05
	DSHW	43	42	31	0.14	4.80	24	198	287	6.33	3.78	3.05	41	40	38	0.21	4.83	24	198	289	6.31	3.79	3.07
A. a	USHW	33	32	30	1.14	5.02	24	184	305	5.65	4.32	2.87	39	40	37	0.98	5.05	24	184	306	5.62	4.31	2.88
	MSHW	40	42	37	0.84	5.01	23	192	295	5.54	4.03	2.76	48	41	38	0.88	5.00	23	192	294	5.53	4.02	2.77
	DSHW	40	40	39	0.83	5.04	22	190	287	5.6	4.04	2.54	40	39	37	0.91	5.07	22	190	289	5.61	4.05	2.53
M. f	USHW	32	24	24	0.77	5.44	21	178	300	5.03	5.35	2.15	32	26	24	1.12	5.42	21	178	303	5.04	5.34	2.18
	MSHW	34	25	23	0.92	5.57	22	180	302	5.13	5.04	2.13	34	25	24	0.99	5.59	22	180	302	5.11	5.02	2.14
	DSHW	36	24	22	0.68	5.55	24	189	290	5.06	5.05	2.05	34	24	22	1.16	5.53	24	189	294	5.02	5.04	2.07
M. o	GWST1	46	47	40	0.12	5.00	20	201	265	6.53	1.65	3.45	49	50	40	0.12	5.02	20	201	262	6.51	1.66	3.46
	GWST2	47	48	40	0.17	5.03	23	200	260	6.43	1.87	3.5	50	47	41	0.12	5.03	23	200	260	6.45	1.89	3.53
	GWST3	48	47	40	0.12	5.05	21	202	268	6.5	1.76	3.47	49	47	40	0.11	5.04	21	202	267	6.52	1.75	3.46
A. a	GWST1	46	42	38	0.23	5.47	22	198	265	6.12	1.7	3.46	48	48	38	0.30	5.52	22	198	266	6.11	1.73	3.48
	GWST2	41	40	38	0.22	5.56	20	200	270	6.10	1.67	3.52	45	46	38	0.31	5.55	20	200	272	6.10	1.68	3.51
	GWST3	46	41	40	0.22	5.55	21	201	268	6.08	1.89	3.48	48	45	40	0.29	5.57	21	201	268	6.07	1.87	3.47
M. f	GWST1	43	39	37	0.42	6.46	23	189	270	6.03	1.9	3.54	45	45	37	0.40	6.48	23	189	273	6.04	1.91	3.53
	GWST2	44	41	36	0.43	6.42	20	196	264	6.04	1.94	4.51	43	37	37	0.39	6.43	20	196	267	6.02	1.93	4.52
	GWST3	43	39	37	0.42	6.46	23	197	257	6.02	1.85	3.47	44	38	38	0.38	6.49	23	197	258	6.01	1.85	3.47
		Replicate 2																					
		A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-	A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-

M. o	USHW	42	42	33	0.19	4.83	25	187	290	6.35	4.01	3.1	41	41	39	0.70	4.73	25	187	292	6.32	4.02	3.12
	MSHW	39	41	41	0.24	4.77	23	196	295	6.23	3.98	3.04	39	42	40	0.24	4.78	23	196	297	6.24	3.96	3.05
	DSHW	44	43	32	0.14	4.80	24	198	287	6.33	3.78	3.05	42	41	39	0.21	4.83	24	198	289	6.31	3.79	3.07
A. a	USHW	35	33	31	1.14	5.02	24	184	305	5.65	4.32	2.87	40	42	38	0.98	5.05	24	184	306	5.62	4.31	2.88
	MSHW	41	43	38	0.84	5.01	23	192	295	5.54	4.03	2.76	49	42	39	0.88	5.00	23	192	294	5.53	4.02	2.77
	DSHW	42	41	40	0.83	5.04	22	190	287	5.6	4.04	2.54	41	40	38	0.91	5.07	22	190	289	5.61	4.05	2.53
M. f	USHW	33	25	25	0.77	5.44	21	178	300	5.03	5.35	2.15	33	27	25	1.12	5.42	21	178	303	5.04	5.34	2.18
	MSHW	35	26	24	0.92	5.57	22	180	302	5.13	5.04	2.13	35	26	25	0.99	5.59	22	180	302	5.11	5.02	2.14
	DSHW	37	25	23	0.68	5.55	24	189	290	5.06	5.05	2.05	35	25	23	1.16	5.53	24	189	294	5.02	5.04	2.07
M. o	GWST1	47	48	41	0.12	5.00	20	201	265	6.53	1.65	3.45	50	50	41	0.12	5.02	20	201	262	6.51	1.66	3.46
	GWST2	48	49	42	0.17	5.03	23	200	260	6.43	1.87	3.5	50	48	42	0.12	5.03	23	200	260	6.45	1.89	3.53
	GWST3	49	47	41	0.12	5.05	21	202	268	6.5	1.76	3.47	48	48	41	0.11	5.04	21	202	267	6.52	1.75	3.46
A. a	GWST1	48	43	39	0.23	5.47	22	198	265	6.12	1.7	3.46	49	49	39	0.30	5.52	22	198	266	6.11	1.73	3.48
	GWST2	42	41	39	0.22	5.56	20	200	270	6.10	1.67	3.52	47	47	39	0.31	5.55	20	200	272	6.10	1.68	3.51
	GWST3	47	42	41	0.22	5.55	21	201	268	6.08	1.89	3.48	48	46	41	0.29	5.57	21	201	268	6.07	1.87	3.47
M. f	GWST1	44	40	38	0.42	6.46	23	189	270	6.03	1.9	3.54	46	46	38	0.40	6.48	23	189	273	6.04	1.91	3.53
	GWST2	45	42	37	0.43	6.42	20	196	264	6.04	1.94	4.51	44	38	38	0.39	6.43	20	196	267	6.02	1.93	4.52
	GWST3	44	40	38	0.42	6.46	23	197	257	6.02	1.85	3.47	45	39	39	0.38	6.49	23	197	258	6.01	1.85	3.47
		Replicate 3																					
		A	**C**	**O**	**T**	**pH**	**Tp**	**EC**	**TDS**	**DO**	**BOD**	**Cl^-**	**A**	**C**	**O**	**T**	**pH**	**Tp**	**EC**	**TDS**	**DO**	**BOD**	**Cl^-**
M. o	USHW	42	42	33	0.19	4.83	25	187	290	6.35	4.01	3.1	41	41	39	0.70	4.73	25	187	292	6.32	4.02	3.12
	MSHW	39	41	41	0.24	4.77	23	196	295	6.23	3.98	3.04	39	42	40	0.24	4.78	23	196	297	6.24	3.96	3.05
	DSHW	44	43	32	0.14	4.80	24	198	287	6.33	3.78	3.05	42	41	39	0.21	4.83	24	198	289	6.31	3.79	3.07
A. a	USHW	34	33	31	1.14	5.02	24	184	305	5.65	4.32	2.87	40	41	38	0.98	5.05	24	184	306	5.62	4.31	2.88
	MSHW	41	43	38	0.84	5.01	23	192	295	5.54	4.03	2.76	49	42	39	0.88	5.00	23	192	294	5.53	4.02	2.77
	DSHW	41	41	40	0.83	5.04	22	190	287	5.6	4.04	2.54	41	40	38	0.91	5.07	22	190	289	5.61	4.05	2.53
M.	USHW	33	25	25	0.77	5.44	21	178	300	5.03	5.35	2.15	33	27	25	1.12	5.42	21	178	303	5.04	5.34	2.18

f	MSHW	35	26	24	0.92	5.57	22	180	302	5.13	5.04	2.13	35	26	25	0.99	5.59	22	180	302	5.11	5.02	2.14
	DSHW	37	25	23	0.68	5.55	24	189	290	5.06	5.05	2.05	35	25	23	1.16	5.53	24	189	294	5.02	5.04	2.07
M. o	GWST1	47	48	41	0.12	5.00	20	201	265	6.53	1.65	3.45	50	50	41	0.12	5.02	20	201	262	6.51	1.66	3.46
	GWST2	48	49	41	0.17	5.03	23	200	260	6.43	1.87	3.5	50	48	42	0.12	5.03	23	200	260	6.45	1.89	3.53
	GWST3	49	47	41	0.12	5.05	21	202	268	6.5	1.76	3.47	49	48	41	0.11	5.04	21	202	267	6.52	1.75	3.46
A. a	GWST1	47	43	39	0.23	5.47	22	198	265	6.12	1.7	3.46	49	49	39	0.30	5.52	22	198	266	6.11	1.73	3.48
	GWST2	42	41	39	0.22	5.56	20	200	270	6.10	1.67	3.52	46	47	39	0.31	5.55	20	200	272	6.10	1.68	3.51
	GWST3	47	42	41	0.22	5.55	21	201	268	6.08	1.89	3.48	48	46	41	0.29	5.57	21	201	268	6.07	1.87	3.47
M. f	GWST1	44	40	38	0.42	6.46	23	189	270	6.03	1.9	3.54	46	46	38	0.40	6.48	23	189	273	6.04	1.91	3.53
	GWST2	45	42	37	0.43	6.42	20	196	264	6.04	1.94	4.51	44	38	38	0.39	6.43	20	196	267	6.02	1.93	4.52
	GWST3	44	40	38	0.42	6.46	23	197	257	6.02	1.85	3.47	45	39	39	0.38	6.49	23	197	258	6.01	1.85	3.47

Physicochemical properties after treatment at 45g/300mL Stock Solution

Water Samples		Replicate 1 45g/300mL Stock Solution																					
		10mL											20mL										
		A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-	A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-
M. o	USHW	40	39	32	0.34	4.83	25	187	290	6.35	4.01	3.1	40	33	37	0.32	4.73	25	187	292	6.32	4.02	3.12
	MSHW	38	40	31	0.29	4.77	23	196	295	6.23	3.98	3.04	40	39	37	0.26	4.78	23	196	297	6.24	3.96	3.05
	DSHW	44	41	39	0.16	4.80	24	198	287	6.33	3.78	3.05	50	50	46	0.18	4.83	24	198	289	6.31	3.79	3.07
A. a	USHW	31	40	31	1.00	5.02	24	184	305	5.65	4.32	2.87	43	41	38	1.21	5.05	24	184	306	5.62	4.31	2.88
	MSHW	41	39	39	0.62	5.01	23	192	295	5.54	4.03	2.76	41	41	37	0.60	5.00	23	192	294	5.53	4.02	2.77
	DSHW	40	41	36	0.85	5.04	22	190	287	5.6	4.04	2.54	41	40	39	0.75	5.07	22	190	289	5.61	4.05	2.53
M. f	USHW	35	38	28	0.72	5.44	21	178	300	5.03	5.35	2.15	39	39	33	0.71	5.42	21	178	303	5.04	5.34	2.18
	MSHW	29	25	20	0.94	5.57	22	180	302	5.13	5.04	2.13	29	24	20	1.11	5.59	22	180	302	5.11	5.02	2.14
	DSHW	31	24	22	0.66	5.55	24	189	290	5.06	5.05	2.05	30	24	22	1.02	5.53	24	189	294	5.02	5.04	2.07
M. o	GWST1	49	50	40	0.12	5.00	20	201	265	6.53	1.65	3.45	49	49	40	0.12	5.02	20	201	262	6.51	1.66	3.46
	GWST2	49	46	40	0.17	5.03	23	200	260	6.43	1.87	3.5	49	50	41	0.12	5.03	23	200	260	6.45	1.89	3.53
	GWST3	48	47	40	0.12	5.05	21	202	268	6.5	1.76	3.47	48	50	40	0.11	5.04	21	202	267	6.52	1.75	3.46
A. a	GWST1	47	47	39	0.23	5.47	22	198	265	6.12	1.7	3.46	44	46	38	0.30	5.52	22	198	266	6.11	1.73	3.48
	GWST2	46	39	39	0.22	5.56	20	200	270	6.10	1.67	3.52	47	47	39	0.31	5.55	20	200	272	6.10	1.68	3.51
	GWST3	46	46	38	0.22	5.55	21	201	268	6.08	1.89	3.48	45	46	38	0.29	5.57	21	201	268	6.07	1.87	3.47
M. f	GWST1	44	39	37	0.42	6.46	23	189	270	6.03	1.9	3.54	38	39	37	0.40	6.48	23	189	273	6.04	1.91	3.53
	GWST2	44	38	37	0.43	6.42	20	196	264	6.04	1.94	4.51	43	40	37	0.39	6.43	20	196	267	6.02	1.93	4.52
	GWST3	44	39	38	0.42	6.46	23	197	257	6.02	1.85	3.47	42	39	37	0.38	6.49	23	197	258	6.01	1.85	3.47
		Replicate 2																					
		A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-	A	C	O	T	pH	Tp	EC	TDS	DO	BOD	Cl^-

M. o	USHW	42	42	33	0.34	4.83	25	187	290	6.35	4.01	3.1	41	41	39	0.32	4.73	25	187	292	6.32	4.02	3.12
	MSHW	39	41	41	0.29	4.77	23	196	295	6.23	3.98	3.04	39	42	40	0.26	4.78	23	196	297	6.24	3.96	3.05
	DSHW	44	43	32	0.16	4.80	24	198	287	6.33	3.78	3.05	42	41	39	0.18	4.83	24	198	289	6.31	3.79	3.07
A. a	USHW	35	33	31	1.00	5.02	24	184	305	5.65	4.32	2.87	40	42	38	1.21	5.05	24	184	306	5.62	4.31	2.88
	MSHW	41	43	38	0.62	5.01	23	192	295	5.54	4.03	2.76	49	42	39	0.60	5.00	23	192	294	5.53	4.02	2.77
	DSHW	42	41	40	0.85	5.04	22	190	287	5.6	4.04	2.54	41	40	38	0.75	5.07	22	190	289	5.61	4.05	2.53
M. f	USHW	33	25	25	0.72	5.44	21	178	300	5.03	5.35	2.15	33	27	25	0.71	5.42	21	178	303	5.04	5.34	2.18
	MSHW	35	26	24	0.94	5.57	22	180	302	5.13	5.04	2.13	35	26	25	1.11	5.59	22	180	302	5.11	5.02	2.14
	DSHW	37	25	23	0.66	5.55	24	189	290	5.06	5.05	2.05	35	25	23	1.02	5.53	24	189	294	5.02	5.04	2.07
M. o	GWST1	47	48	41	0.12	5.00	20	201	265	6.53	1.65	3.45	50	50	41	0.12	5.02	20	201	262	6.51	1.66	3.46
	GWST2	48	49	42	0.17	5.03	23	200	260	6.43	1.87	3.5	50	48	42	0.12	5.03	23	200	260	6.45	1.89	3.53
	GWST3	49	47	41	0.12	5.05	21	202	268	6.5	1.76	3.47	48	48	41	0.11	5.04	21	202	267	6.52	1.75	3.46
A. a	GWST1	48	43	39	0.23	5.47	22	198	265	6.12	1.7	3.46	49	49	39	0.30	5.52	22	198	266	6.11	1.73	3.48
	GWST2	42	41	39	0.22	5.56	20	200	270	6.10	1.67	3.52	47	47	39	0.31	5.55	20	200	272	6.10	1.68	3.51
	GWST3	47	42	41	0.22	5.55	21	201	268	6.08	1.89	3.48	48	46	41	0.29	5.57	21	201	268	6.07	1.87	3.47
M. f	GWST1	44	40	38	0.42	6.46	23	189	270	6.03	1.9	3.54	46	46	38	0.40	6.48	23	189	273	6.04	1.91	3.53
	GWST2	45	42	37	0.43	6.42	20	196	264	6.04	1.94	4.51	44	38	38	0.39	6.43	20	196	267	6.02	1.93	4.52
	GWST3	44	40	38	0.42	6.46	23	197	257	6.02	1.85	3.47	45	39	39	0.38	6.49	23	197	258	6.01	1.85	3.47
		Replicate 3																					
		A	**C**	**O**	**T**	**pH**	**Tp**	**EC**	**TDS**	**DO**	**BOD**	**Cl^-**	**A**	**C**	**O**	**T**	**pH**	**Tp**	**EC**	**TDS**	**DO**	**BOD**	**Cl^-**
M. o	USHW	42	42	33	0.34	4.83	25	187	290	6.35	4.01	3.1	41	41	39	0.32	4.73	25	187	292	6.32	4.02	3.12
	MSHW	39	41	41	0.29	4.77	23	196	295	6.23	3.98	3.04	39	42	40	0.26	4.78	23	196	297	6.24	3.96	3.05
	DSHW	44	43	32	0.16	4.80	24	198	287	6.33	3.78	3.05	42	41	39	0.18	4.83	24	198	289	6.31	3.79	3.07
A. a	USHW	34	33	31	1.00	5.02	24	184	305	5.65	4.32	2.87	40	41	38	1.21	5.05	24	184	306	5.62	4.31	2.88
	MSHW	41	43	38	0.62	5.01	23	192	295	5.54	4.03	2.76	49	42	39	0.60	5.00	23	192	294	5.53	4.02	2.77
	DSHW	41	41	40	0.85	5.04	22	190	287	5.6	4.04	2.54	41	40	38	0.75	5.07	22	190	289	5.61	4.05	2.53
M.	USHW	33	25	25	0.72	5.44	21	178	300	5.03	5.35	2.15	33	27	25	0.71	5.42	21	178	303	5.04	5.34	2.18

f	MSHW	35	26	24	0.94	5.57	22	180	302	5.13	5.04	2.13	35	26	25	1.11	5.59	22	180	302	5.11	5.02	2.14
	DSHW	37	25	23	0.66	5.55	24	189	290	5.06	5.05	2.05	35	25	23	1.02	5.53	24	189	294	5.02	5.04	2.07
M. o	GWST1	47	48	41	0.12	5.00	20	201	265	6.53	1.65	3.45	50	50	41	0.12	5.02	20	201	262	6.51	1.66	3.46
	GWST2	48	49	41	0.17	5.03	23	200	260	6.43	1.87	3.5	50	48	42	0.12	5.03	23	200	260	6.45	1.89	3.53
	GWST3	49	47	41	0.12	5.05	21	202	268	6.5	1.76	3.47	49	48	41	0.11	5.04	21	202	267	6.52	1.75	3.46
A. a	GWST1	47	43	39	0.23	5.47	22	198	265	6.12	1.7	3.46	49	49	39	0.30	5.52	22	198	266	6.11	1.73	3.48
	GWST2	42	41	39	0.22	5.56	20	200	270	6.10	1.67	3.52	46	47	39	0.31	5.55	20	200	272	6.10	1.68	3.51
	GWST3	47	42	41	0.22	5.55	21	201	268	6.08	1.89	3.48	48	46	41	0.29	5.57	21	201	268	6.07	1.87	3.47
M. f	GWST1	44	40	38	0.42	6.46	23	189	270	6.03	1.9	3.54	46	46	38	0.40	6.48	23	189	273	6.04	1.91	3.53
	GWST2	45	42	37	0.43	6.42	20	196	264	6.04	1.94	4.51	44	38	38	0.39	6.43	20	196	267	6.02	1.93	4.52
	GWST3	44	40	38	0.42	6.46	23	197	257	6.02	1.85	3.47	45	39	39	0.38	6.49	23	197	258	6.01	1.85	3.47

Where; A = Appearance, C = Colour, O= Odour, Tp = Temperature, EC = Electrical Conductivity, TDS = Total dissolved solids, DO = Dissolved oxygen, BOD = Biological oxygen demand, Cl^- = Chloride ion.
USHW = Upper slaughterhouse wastewater, MSHW = Mid-slaughterhouse wastewater, DSHW = Down slaughterhouse wastewater, GWST1-3 = Groundwater station 1-3.

Physicochemical Parameter for Control after treatment

Samples		**A**	**C**	**O**	**TB**	**pH**	**T (0C)**	**EC (µS/Cm)**	**TDS (mg/L)**	**DO (mg/L)**	**BOD (mg/L)**	**Cl-**
WHO Standard	**Control (Alum)**	-	-	-	-	6.5-8.5	-	1000	1000	6.5-8.0	6.00	250.00
	CO (SHW)	100	100	100	0.13	7.12	29	187	295	5.78	5.05	2.14
	CU (GWST)	100	100	100	0.02	7.14	21	215	252	7.14	1.54	3.83

Appendix 2: Morphological characteristics of bacteria isolates from water samples before treatment

Bacteria Isolates	Shape	Color	Reaction to Grams	Formation of Spore
Escherichia coli	Rod	Bright pink	Negative (-ve)	Nil
Streptococcus spp.	Cocci	Gray to whitish	Positive (+ve)	Nil
Enterobacter spp.	Rod	Yellow	Negative (-ve)	Nil
Salmonella spp.	Rod	Pale green	Positive (+ve)	Nil
Staphylococcus aureus	Rod	Yellow or White	Positive (+ve)	Nil
Pseudomonas spp.	Rod	Greenish blue	Negative (-ve)	Nil
Campylobacter jejuni	Spiral	Grayish	Negative (-ve)	Nil

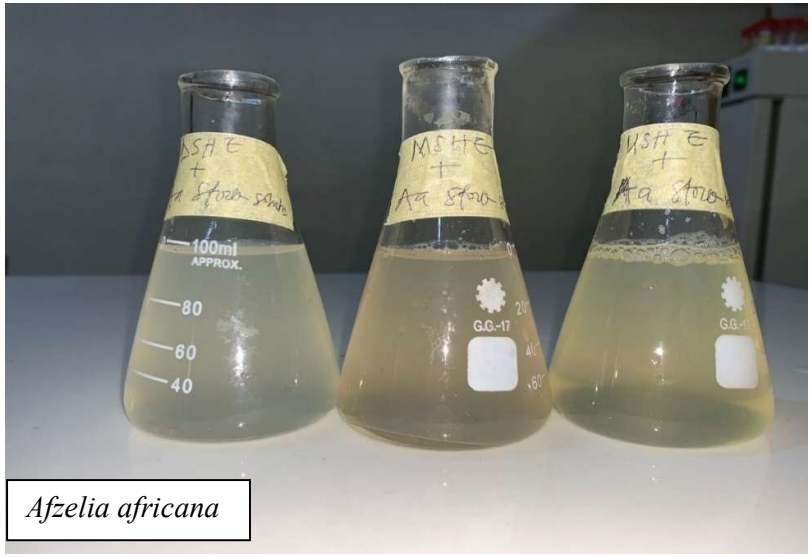
Appendix 3: Biochemical test for isolates identification from water samples before treatment

Bacteria Isolates	Motility	Catalase	Coagulase	Indole	Oxidase	Methyl Red	Voges-Proskauer
Escherichia coli	$+Ve$	$+Ve$	$-Ve$	$+Ve$	$-Ve$	$+Ve$	$-Ve$
Streptococcus spp.	$-Ve$	$-Ve$	$-Ve$	$-Ve$	$-Ve$	$-Ve$	$-Ve$
Enterobacter spp.	$+Ve$	$+Ve$	$-Ve$	$-Ve$	$-Ve$	$-Ve$	$+Ve$
Salmonella spp.	$+Ve$	$+Ve$	$-Ve$	$-Ve$	$-Ve$	$+Ve$	$-Ve$
Staphylococcus aureus	$-Ve$	$+Ve$	$+Ve$	$-Ve$	$-Ve$	$+Ve$	$+Ve$
Pseudomonas spp.	$+Ve$	$+Ve$	$-Ve$	$-Ve$	$+Ve$	$-Ve$	$-Ve$
Campylobacter jejuni	$+Ve$	$+Ve$	$-Ve$	$-Ve$	$+Ve$	$-Ve$	$-Ve$

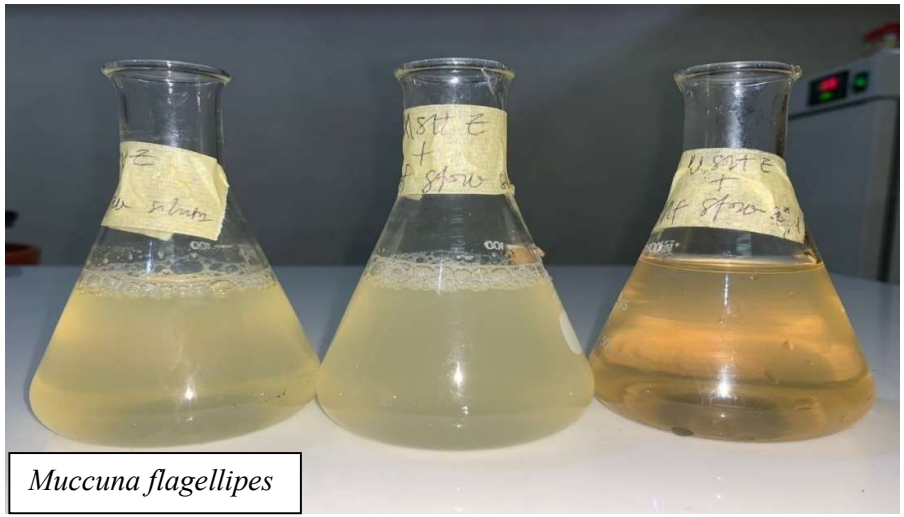
Key
$+Ve$ = Positive;
$+Ve$ = Negative

Appendix 4: Slaughterhouse sample before treatment

Appendix 5: Slaughterhouse samples after treatment



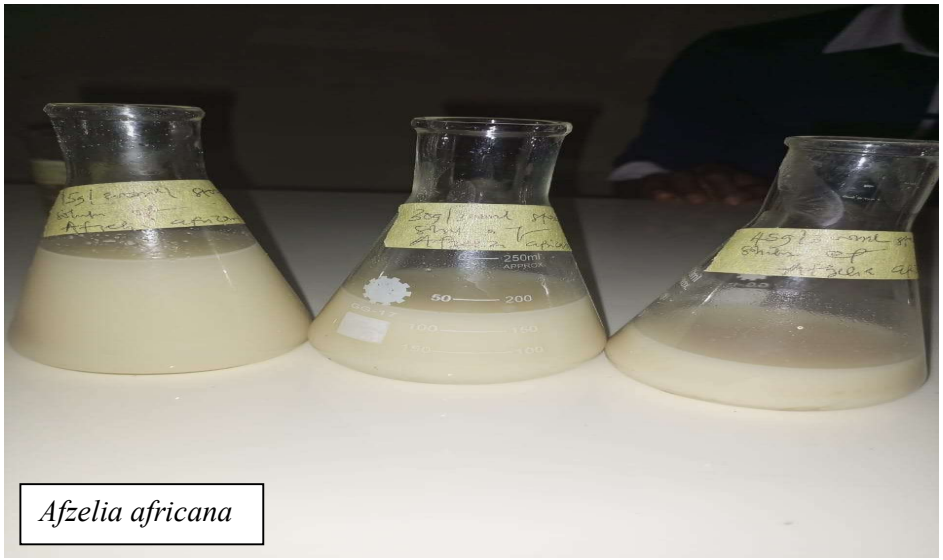
Afzelia africana



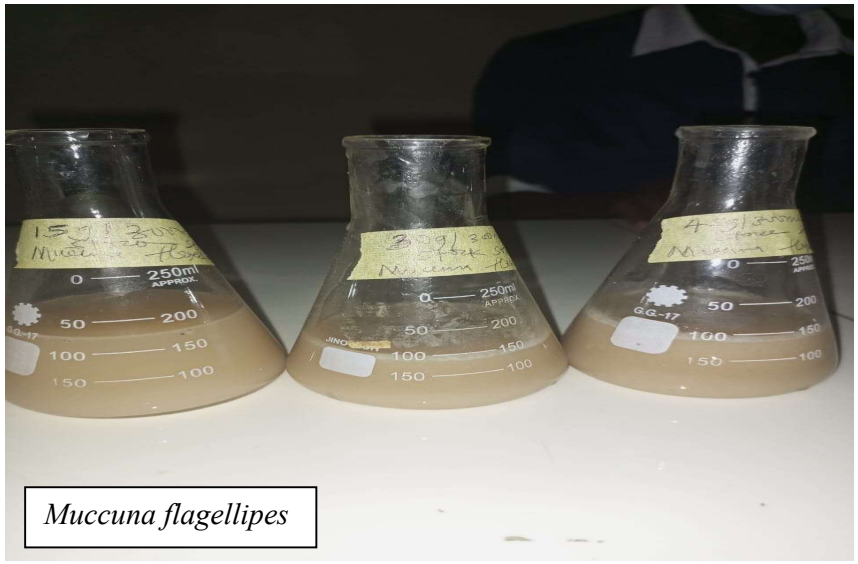
Muccuna flagellipes

Moringa

Appendix 6: Stock solutions prepared from the three different plant samples.



Afzelia africana



Muccuna flagellipes

Moringa