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MSc thesis on:

Performance Evaluation of Bio-Coagulant for the Effective Removal of Turbidity and Microbial Pathogens from Drinking Water

By;

Zenebe Nigussie

July, 2022

Bahir Dar, Ethiopia

BAHIRDARUNIVERSITY

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FACULTY OF FOOD AND CHEMICAL ENGINEERING

Performance evaluation of Bio-coagulant for the effective removal of turbidity and microbial pathogens from drinking water

By:

Zenebe Nigussie

A thesis submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in

Chemical Engineering (Specialized in Environmental Engineering) Presented to the Faculty of Food and Chemical Engineering

Advisor: Nigus G. (Ph.D.)

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July, 2022

Bahir Dar, Ethiopia

Approval of Thesis for defense

I hereby certify that I have supervised, read, and evaluated this thesis titled "Performance evaluation of Bio-coagulant for the effective removal of turbidity and microbial pathogens from drinking water" prepared by **Zenebe Nigussie** under my guidance. I recommend the thesis to be submitted for oral defense.

Nigus Gabbiye Habtu (PhD)		06/03/2022
Advisor's name	Signature	Date

Approval of thesis for defense result

I hereby confirm that the changes required by the examiners have been carried out and incorporated in the final thesis.

Name of Student: **Zenebe Nigussie, Signature**: _____ Date: July./2022

As members of the board of examiners, we examined this thesis entitled "Performance evaluation of Bio-coagulant for the effective removal of turbidity and microbial pathogens from drinking water" by **Zenebe Nigussie**. We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Masters of Science in "**CHEMICAL ENGINEERING (ENVIRONMENTAL ENGINEERING)".**

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Declaration

This is to certify that the thesis entitled “Performance evaluation of Bio-coagulant for the effective removal of turbidity and microbial pathogens from drinking water”, submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering under the Faculty of Food and Chemical Engineering, Bahir Dar Institute of Technology, is a record of original work carried out by me and has never been submitted to this or any other institution to get any other degree or certificates. The assistance and help I received during the course of this investigation have been duly acknowledged.

_____	_____	_____
Name of the candidate	signature	Date

DEDICATION

This research work is dedicated to my father (Mr. Nigussie Woldetsadik), my grandmother (Ms. Mammit W/kidane), and my aunts (Ms. Bizuget Woldetsadik, Ms. Yehalashet Woldetsadik, and Ms. Desta Hailesilassie).

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Abstract

The bio-coagulants extracted from moringa stenopetala seed, aloe Vera leaves and cactus leaves by using 1MNaCl were examined for the removal of turbidity and microbial contaminants in drinking water. The three factors coagulant type, coagulant dose, and pH were investigated in the treated drinking water of Physico-chemical and microbial properties at the room temperature. The local plants of moringa stenopetala seed, aloe Vera leaves skin, and cactus leaves were utilized for extracting bio-coagulants of MS-SC, AV-SC & Ca-SC respectively. The characterization of the bio coagulants shows a 43.95%, 13.9% &10.94% protein content of moringa stenopetala seed, aloe Vera leaves and cactus leaves respectively. However, the Nano drop measurement of protein concentration of bio-coagulants was 2.534 mg/ml of MS-SC, 3.434 mg/ml of AV-SC, and 1.647 mg/ml of Ca-SC. Those bio coagulants have a high tendency to form large floc and settled the attached particle from the water body. This is due to the cationic positive charge of protein from bio coagulants interaction with destabilized negative charge particles of water. Six Jar test equipment was utilized to explore the coagulation activities of the Bio coagulants. The jar test was performed at three different mixing modes for 1min of rapid mixing (120rpm) followed by 19 min of slow blending (40 rpm) for flocculation followed by 15 min settling at room temperature. The Physico-chemical and microbial properties of the treated water such as pH, turbidity, alkalinity, conductivity TDS, and microbial quality (total coliform and E. coli.) were performed. It was found that Moringa stenopetala (MS-SC) and Aloe Vera plants (AV-SC) have better turbidity removal efficiency than cactus plants (Ca-SC). The turbidity removal efficiency of MS-SC, AV-SC, and Ca-SC bio coagulants were 67 - 98.83%, 59.61 - 98.74 %, and 27.4-69.83 % respectively. It is noted that the highest alkalinity percent reduction was found at a dose of 50 mg/l and pH of 7.5 for AV-SC. Complete removal of E. coli was achieved with MS-SC bio coagulant at a pH of 7.5 and dose of 150 mg/l. The main factors of Coagulant type, Coagulant dose and the interaction between (coagulant type and pH) were significantly (see in the annex table 7.5) ([F (2, 53)] =22.774; p > 0.05), ([F (2, 53) = 80.591; P > 0.05) and ([F (4, 53) = 3.081; P > 0.05)) affect the turbidity removal efficiency respectively. The result obtained in this research suggests, that using Bio coagulants as a water treatment could be a promising strategy for the reduction of turbidity and microbial community to a safe level.

Keywords: Bio-coagulant; Total coliform; E. coli, turbidity, drinking water

Table of contents

Page No.

List of Tables

List of figures

Acronyms

AOAC	Association Official Analytical Chemistry
APHA	American Public Health Association
ASTM	American Society for Testing and Materials
AV-SC	Aloe Vera- Sodium Chloride
Ca-SC	Cactus- sodium Chloride
FIB	Fecal Indicator Bacteria
PCR	Polymerase Chain reaction
POU	Point of Use
MS-SC	Moringa Stenopetall -Sodium Chloride
TDS	Total Dissolved Solids
TTC	Thermo tolerant Coliform
WHO	World Health Organization

1. Introduction

Water is one of the main important substances for humans' activities and other organisms. Pollution of water by physical, chemical, and organic causes could be a genuine issue. Water quality can be influenced by viruses, bacteria, and protozoans. The water treatment prepared by different innovations is practiced within the last decades. The treatment process has standards that are regulated by the agency or end-users (John C. Crittenden, 2012).

The water quality is continuously degrading because of fecal and physiochemical contaminants. The accessibility of those contaminants within the water at point-of-use is truly caused by waterborne diseases counting the runs, intestinal diseases, diarrhea, cholera, hepatitis, typhoid fever, vomiting, skin disease, and other related sicknesses, particularly in children and more seasoned older adults (Kifayatullah Khan1, 2017).

Cheap and easily available chemical coagulants tend to remove water impurities and microbial pathogens in a convectional water treatment system. However, those chemical coagulants have a drawback to the produced large amount of sludge and challenge in the food production processing industry. Expansion to these chemical coagulants can cause health issues like Alzheimer's disease, and neurotoxic and carcinogenic impacts. Utilizing natural coagulants is an elective solution for water treatment (L Nishia, 2012, J.E Lagasi1, 2016 & Govindan, 2018).

Scientific studies indicated that natural coagulants can remove impurities and parasitic protozoans, bacteria, and viruses (Sarahm, Miller, 2008). Plants like moringa oleifera, moringa stenopetalal, aloe Vera, cactus, and papaya plants are among the bio coagulants. Those are effectively available, secure, reasonable, and effectively multipurpose biofilter components with setting sustainability in encouraging the removal of numerous storm water pollutants (P. Galbraith, 2019). But the drawback of bio coagulants is the expensive extracting agents, low efficiency of turbidity removal, regrowth of microorganisms, and impractical to apply full-scale application (Ramavandi, 2014 & M. Megersa1, 2018). Water has been polluted within the collection, transportation, and storage. (Sungwoo Bae, 2018).

Least developing nations like Ethiopia have been utilized common sources of water supplies. Nowadays the central treatment system of water is very costly because of the chemicals coagulants imported (Ramavandi, 2014). Bio coagulants have been used as an alternative coagulant for water treatment at a point of use (Sarahm, Miller, 2008). The study was showed that the Bio coagulants extracted from moringa stenopetall seed, aloe Vera leaves skin, and cactus leaves can remove turbidity and microbial pathogens from drinking water. It was also used as a base for further investigation and contributed scientific knowledge and economic benefit.

1.1. Statement of the Problem

Water is an important component of human activities; In any case, it contains chemical, physical, and organic things which cause water pollution. Water quality is influenced by the availability of high fecal and physiochemical contaminants. Those contaminants are a public health issue that causes disease.

Water quality could be a basis to keep public health from waterborne diseases including diarrhea, intestinal infections, diarrhea, cholera, hepatitis, typhoid fever, vomiting, skin illnesses, and other related sicknesses. Water filtration may be a handle of water treatment to remove turbidity and to achieve water quality for drinking purposes. Coagulation could be a routine water treatment process that's supported by a chemical and a bio-coagulant. Chemical coagulants have been utilized overwhelmingly for centralized water treatment plants in the world, particularly for least developing nations. The centralized water treatment plants require a large investment which is one of an issue to address potable water for all citizens in provincial areas of developing nations due to the cost of water treatment. The Chemical coagulants are used for the Coagulation /flocculation of the water treatment process which is found by importing. And also, they have other constraints such as difficulty to dispose of because not biodegradable, produces a high volume of sludge, and causes neurotoxic, Alzheimer's, and other chronic diseases (L. Nishia, 2012, J.E Lagasi1, 2016, Jose Henrique et al 2016, Inas M. Al-aubadi, 2017& Govindan, 2018).

Therefore, those drawbacks of chemical coagulants lead to examining the bio coagulants which have the potential to remove turbidity and microbial from water. They are suitable for the environment i.e. eco-friendly, cheap, easy, and locally available, especially for developing countries to address potable water for rural areas. As an alternative solution, the bio coagulants are used for the Coagulation /flocculation of the water treatment process at a point of use.

1.2. Objective

1.2.1. General Objective

To evaluate the performance of bio coagulants as a coagulant for removal of turbidity and microbial pathogens from drink water.

1.2.2. Specific Objectives

- To extracted active coagulant agent from moringa stenopetala seeds, aloe Vera leaves skin, and cactus leaves through NaCl solution
- To characterize the Physico-chemical properties of the active ingredient of the natural coagulant (protein content, ash, and moisture content)
- To investigate the effect of operating conditions (solution pH and does of coagulants) for different coagulant types on the removal efficiency of microbial and turbidity of drinking water
- To determine the water quality parameters for both raw and treated water (pH, turbidity, conductivity, alkalinity, TDS, total coliform, and E. coli) and compared with WHO water quality standards

1.3. Significance of the Study

According to a World Health Organization report (1998), over 1 billion people don’t have access to and tended to satisfactory water treatment and secure water supply of which 800 million are in rural zones and about 20 million individuals around the world die each year of water-borne illnesses, especially in sub-Saharan Africa. The significance of the study was to describe the reality of developing countries' water treatment systems. Most of the developing countries have been used chemical coagulants for the process of coagulation and flocculation for the purification of the central water treatment systems. However, the chemical aid clarification of water has a long effect on the health problem, producing a huge volume of

sludge and damaging the food processing industry, so this study has contributed by solving those problems and focusing on the area of extracted bio-coagulant for replaced chemical coagulant. The main target of the research was characterized by locally available plants that have the capacity of treating water at the point of use. Therefore, the study has contributed significant knowledge for a future researchers by providing promising information about the bio-coagulants capacity of clarifying water.

1.4. Scope of the Study

The scope of the study was centered on the treatment of water by utilizing bio-coagulants. Moringa stenopetalla, aloe Vera and cactus are locally available plants that include in the study for extracted bio-coagulants. The extracted bio-coagulants were characterized protein content, ash content, and moisture content in the proximate analysis. The characterization of parameters like pH, turbidity, alkalinity, conductivity, total dissolved solids, total coliforms, and E. coli of raw water and treated water determined the capacity of those bio-coagulants in the purification of water.

2. Literature Review

Water is vital for life not only for drinking but also for, household cooking, cleaning, industrial, and irrigation, and it is also used for recreation purposes. Water is found in rivers, lakes, and groundwater (the water held in rock arrangement) and in ocean and rain.in solid state ice and snow. Found also in vapor form. Water is covered 75% of the soil but as it were 0.5% is available for drinking purposes (WHO, 2006). Usually, water that is not clean contains a different form of particulate and contamination. Globally, water-borne parasitic infections are an issue and have become an area of concern recently due to the contamination of different sources of drinking water. According to a World Health Organization report (1998), over 1 billion people in the world do not have access to and addressed adequate water treatment and safe water supply which 800 million are in rural areas and nearly 20 million people worldwide die each year of water-borne diseases, especially in sub-Saharan Africa (Gyang, 2017).

2.1. Water treatment

Water treatment may be a handle of water to attain water quality guidelines set by the end-users or its administrative organizations (John C. Crittenden, 2012). Raw water microbial quality is important to guide its suitability for use (Levi, 2015). Previously water quality standards were set based on treatment technologies quality that is not appropriate for special use (L. Nishia, 2012).

The microbial quality of water is influenced by the pollution of Microbial pathogens agents like viruses, bacteria, and parasites (Donald W. et al 2013 & L. Nishia, 2012). Water for drinking and cooking purposes must be free from pathogenic microorganisms (Davis, 2010). But the water quality is continuously corrupting within the range with the accessibility of high fecal and physiochemical contaminants at the point of utilizing, the cause of serious waterborne infections including diarrhea, intestinal infections, diarrhea, cholera, hepatitis, typhoid fever, vomiting, skin diseases, and other related sicknesses, particularly in children and older adults (Kifayatullah Khan1, 2017).

Coagulation and flocculation are basic parts of a routine water treatment framework that are designed to avoid infectious agents, remove toxic compounds that have adsorbed to the surface of particles and make the water palatable. Surface water supplies encompass organic and inorganic particles. Organic particles include algae, fungi, bacteria, and detritus from vegetables such as Inorganic clay, silt, and mineral oxides. Surface water moreover contains particulate and dissolved natural matter together referred to as common natural matter that's an item of decay and filtering of organic rubbish. Natural organic matter is essential for the arrangement of a disinfection by-product. Coagulation flocculation may be a handle of treating water by destabilizing natural water suspension by the component of: -

- compression of the electric double layers,
- Adsorption and charge neutralization
- Adsorption and interparticle bridging, and
- Enmeshment in a precipitate

Adsorption is a result of charge-charge interaction other part of the polymers extends into the solution attached to the particles forming large floc and more quickly settling. Adsorption cannot proceed beyond monolayer coverage; all surface places are identical and can put at most one adsorbed molecule (Nida M.Salem, 2011).

Routine water treatment forms are broadly utilized in numerous flocculants and coagulants. The chemical coagulant materials can be classified into inorganic coagulants (e.g., aluminum and ferric salts) and manufactured natural polymers (Ramavandi, 2014).

2.1.1. Chemical coagulants

Inorganic chemical coagulants are Aluminum sulfate (B.I. Gandiwa, 2020, Inas M. Al-aubadi, 2017, Jose Henrique et al 2016), Sodium aluminate, Aluminum chloride, Polyaluminum chloride, Polyaluminum sulfate, Polyiron chloride, Ferric chloride ad Ferric chloride the transcendent water treatment coagulant is Aluminum sulfate or Alum form as $Al_2 (SO_4)_3 .14H_2O$. it is less expensive and available used for removing impurities from water, especially in developing countries that highly consumed this chemical coagulant by

exporting, Using in most rural areas I central water treatment system in addition to its adverse health hazard. (J.E Lagasil, 2016).

Now a day's, water impurities and microbial pathogens are removed by chemical coagulants which are cheap and easily available. In any case, these chemical coagulants remaining are produced a huge sum of sludge, affect the industrial processing production of food and also cause human health problems like cause Alzheimer's disease, neurotoxic and carcinogenic effects (L. Nishia, 2012, J.E Lagasi1, 2016, Jose Henrique et al 2016 & Govindan, 2018).

2.1.2. Bio coagulants

Bio coagulants are sourced from animal and plant origin. the animal origin Bacterial Exopolysaccharides...Moringa oleifera (Ashenafi Delelegn, 2018, B.I. Gandiwa, 2020, Franciele Pereira Camacho, 2016), Moringa stenopetala (Govindan, 2018 &M. Megersa1, 2018), papaya seed (Syeda Azeem Unnisa, 2018 & Zeharaaddeen N. Garba, 2015), cactus (B.I. Gandiwa, 2020 & Inas M. Al-aubadi, 2017), aloe Vera (Azni Idris, 2011, GulmireAmruta, 2017 & Hemraj S.R, 2019), Strychnos potato rum, Jack fruits seeds (Hemraj S.R, 2019), Cassava starch (Jose Lugo-Arias, 2020) & Phaseolus vulgaris plants.

Moringa stenopetala, aloe Vera, and cactus were among the` bio coagulant included in this project. Bio coagulants extricated from Plants represent a secure, cheap, and easily adaptable biofilter component with setting up adequacy in encouraging the removal of multiple stormwater toxins (P. Galbraith, 2019).

The bio coagulants are extracted from diverse chemical-based and distilled water. The salts-based extraction is better than others and also in some research stated that increasing the fraction of molars concentration of salts also increases turbidity removal efficiency (M. Megersa1, 2018 &G.Muthuraman, 2013). And also increase the number of valent of salts in coagulant extraction increasing the efficiency of coagulation (Ramavandi, 2014). So, bio coagulants do not have a health problems and produced a small amount of sludge. These bio coagulants are easy accesses, but extracting coagulant agents are expensive low efficiency of turbidity removal, and impractical to apply full-scale application (Ramavandi, 2014). The

main disadvantage of bio coagulants extracted from plants is not found in enough amounts, turbidity removal, and some of the coagulants are also important for bacteria regrowth

i. Moringa stenopetala

One of the bio coagulant plants is the tropical plants of the family Moringaceae. it has two common species Moringa oleifera and Moringa stenopetala. Moringa oleifera lectin ability decreases the concertation of metal ions (Jose Henrique et al 2016). Moringa stenopetala also has protein and through the process of adsorption used as a flocculent agent for water purification to remove contaminants and impurities (Habauka M. kwaambwa, 2015 & Govindan, 2018).

ii. Aloe Vera

Aloe Vera is a medicinal plant aloe is a genus, it is developed in drought-prone ranges and exceptionally short-stemmed juicy plant developing to 60–100 cm. aloe Vera is a natural coagulant supported with alum it effective in high turbidity water (GulmireAmruta, 2017). The solution of Aloe Vera is as coagulation help accomplished a diminish up to 20% of the ideal dosage of aluminum sulfate in water but Aloe Vera solution demonstrated to be a poor essential coagulant in comparison with the aluminum sulfate (María Irene Kopytko 1, 2014).

iii. Cactus

Cactus is a part of the plant family Cactaceae it is one of the bio coagulants (Vasanthi Sethu1, 2019). It is utilized as a natural coagulant in water treatment through adsorption, neutralization, and formation of hydrolyzed species of positive charge within the compound (Hayelom Dargo Beyene, 2016). Cactus also contains polysaccharides and protein polymers used for coagulants. it is cost-effective and eco-friendly (Vasanthi Sethu1, 2019). The Cactus plant ecofriendly coagulant necessary for water treatment, minimizes Alzheimer's human disease, does not change pH and small volume and biodegradable sludge produced (Inas M. Al-aubadi, 2017).

2.2. Water Quality

Water Quality is determined by different parameters. This parameter can be categorized as physicochemical and microbial parameters. PH, turbidity, electro conductivity, alkalinity,

and total dissolved solids included from physicochemical parameters are determined to water quality. From microbiological parameters, total coliforms and E. coli are also the main indicators of bacteria. pH and dosage of coagulants are the main variables amid the characteristic coagulant /flocculation handle. In coagulation activities, initial turbidity of water with doses of coagulants and pH has strong relation (Sarahm, Miller, 2008).

2.2.1. Physicochemical analysis of water

2.2.1.1. Turbidity

Turbidity is one of the foremost broadly utilized parameters for measuring the quality of water. It indicates the presence of high particles which can be a shelter for harmful microorganisms like protozoa, bacteria, and viruses; it also created a barrier in filtration by forming clogging on the filter. And also, turbidity can block light for aquatic plants. Turbidity can cause materials barriers to water treatments and represent potential vehicles for bacteria (Charlotte Farrell, 2017). The surface water supplies have a high level of turbidity, so it needs treatment with flocculation /coagulation to expel turbidity (Ramavandi, 2014). Human health is affected by the contamination of consumable supplies of water. Turbidity is dependent on numerous qualities of the molecule masses such as the physical and chemical composition, size distribution, and molecule shape. Diffusing light is straightforwardly corresponding to the measured turbidity (Charlotte Farrell, 2017).

2.2.1.2. PH

pH is the foremost vital variable of water treatment in the coagulation and flocculation process (VaraSaritha, 2019). A solution that stands up to expansive changes in pH when or base is included or when the solution is diluted is called a buffer arrangement. A solution containing a powerless corrosive and its salt is a case of a buffer. Air carbon dioxide (CO_2) produces a common buffer through the following reactions:

CO_2 (g) =CO_2+ H_2 O=H2CO_3=H^++H CO_3^- =2H^++CO_3^{2-}

Typically, may be the foremost critical buffer system in water and wastewater treatment. In a water treatment plant, the reactions can be altered more quickly than the CO_2 can be replenished from the atmosphere. Reaction shifts to the right because $H_2CO_3^*$ is formed when CO2 and H2O combine CO_2 dissolves into solution pH is lowered and Reaction shifts to the left to form more $H_2CO_3^*$ to replace that removed by stripping CO_2 is removed from solution pH is raised are not common in natural settings. They are utilized in water treatment plants to amend the pH. The pH will not as it was affecting the surface charge of coagulants but too awes the suspension stability. The variation of pH in the solution for expansion of coagulant protein dissolvability in a fluid solution. In this way, the pH considered was essential to recognize the pH optimum value of the treatment system (Syeda Azeem Unnisa, 2018).

2.2.1.3. Electro conductivity

The conductivity of water is an important parameter to analyze in water treatment how much-dissolved substances, chemicals, and minerals are present in water especially indicates the presence of phosphorus and nitrogen. A higher conductivity indicates higher impurities. The capacity of a watery solution to carry out an electric current is called conductivity. This capacity depends on the nearness of particles; on their adding up to concentration, portability, and valence; and on the temperature of estimation, the Arrangement of most inorganic compounds is relatively great conductors. According to (Franciele Pereira Camacho, 2016) stated that a higher conductivity was due to the saline extraction of the bio-coagulants. And also more producing ions by dissociation of the coagulants stated (B.I. Gandiwa, 2020). the sludge formation of the coagulants during the coagulation process was increased the conductivity reported by (Hayelom Dargo Beyene, 2016).

Alternately, particles of natural compounds that don't dissociate in a watery arrangement conduct current exceptionally ineffectively. Distilled water produced in a laboratory generally has conductivity in the range of 0.5 to 3 µs.

The conductivity of potable waters in the United States is extending for the most part from 50 to 1500 (APHA, 1998). A few industrial wastewater conductivities are over 10000 µs.

Electrical conductivity comes about has expanded with alum and sago (negative concerning decrease), (VaraSaritha, 2019).

2.2.1.4. Alkalinity

Alkalinity is a measure of how much acid can be added to water without causing a large change in pH used as a buffer to protect pH variation and is an important parameter to analyze in water treatment. However, the nature of chitin was expanded alkalinity of water due to the arrangement of cationic charges by amine bunches at hoisted pH reported by (Vara Saritha, 2019) Alkalinity is defined as the whole of all titratable bases down to approximately pH 4.5. It is found by experimentally determining how much acid it takes to lower the pH of the water to 4.5. In most waters, the only significant contributions to alkalinity are the carbonate species and any free H^+ or OH^-. Alkaline water has a pH greater than 7, while water with high alkalinity has a high buffering capacity.

Hence some literature stated that natural coagulants can be seen as an alternative for water purification stated by (Franciele Pereira Camacho, 2016) and it was detected that pH affects removing turbidity from water reported by (G. Muthu Raman, 2013). In alkalinity at 6 and 7 pH conditions Chitin displayed a moderate lessening, but at 8 pH alkalinities have expanded due to the application of chitin. The amine group nature of chitin is raised pH driving colloid destabilization due to the provision of cationic charges and resulting floc development advancement empowering fast settlement (VaraSaritha, 2019).

2.2.2. Microbiological analysis of water

Most pathogenic microbes exist within the environment as they were sporadical, at exceptionally low levels, and are difficult and costly to identify directly. So more commonly measured easily by indirect indicators of bacteria, but indicators of bacteria do not measure parasitic protozoans like Cryptosporidium and Giardia (Donald W. et al 2013 & Zeharaaddeen N. Garba, 2015).

2.2.2.1. Total coliform

The coliform group incorporates distinctive microscopic organisms mostly living in the digestive tract of warm-blooded animals (homeotherms). This group is Enterobacteriaceae family composed of Gram-negative rods. These microorganisms are not sporulated oxidase negative, aerobic, or facultative anaerobes and can develop within the nearness of bile salts or surfactants having the same properties and fermenting lactose with the generation of acid, aldehydes, or gas in 24–48 h at 37^{o}C. A few coliforms are displayed within the environment without any source of fecal contamination and the total coliforms are weak Living space specificity.

2.2.2.2. E. coli

Escherichia coli are the family of Enterobacteriaceae. People and warm-blooded animals' gastrointestinal tracts are the primary habitats. However, a few strains are pathogenic and mindful of gastroenteritis. In the environment it is mainly enterohemorrhagic E. coli (EHEC), enter invasive E. coli (EIEC), enterotoxigenic E. coli (ETEC), and enteropathogenic E. coli (EPEC). The responsible for food harm is EHEC, but now the waterborne transmission is recognized. The ingestion of contaminated drinking water or contact with the polluted water during recreational exercises is caused the infection. The count of both intestinal enterococci and TTC (or Escherichia coli) is in this way a great pointer to aquatic defilement by human or animal feces. The bacteriological quality of drinking and showering waters assessment can be shown by indicator bacteria of E. coli. In any case, these indicators are weak indicators of the potential nearness of viruses or pathogenic protozoa from the fecal root (Sime-Ngando, 2011). The major agent of the thermo tolerant coliform (TTC) group is Escherichia coli. This species speaks to between 70 and 95 % of TTC and is highly particular from feces. Presently the foremost often utilized FIB within the setting of microbiological investigation of water quality is Escherichia coli.

Rural families in the least developing nations depend on a central source of water. The household’s level during collection, transportation, and storage of water frequently becomes

contaminated (Sungwoo Bae, 2018). Centrally water treatment is very costly for developing communities because they imported chemical coagulants (Ramavandi, 2014). Some studies indicated the point of using simple technologies like locally available natural coagulants at the household level for removing impurities and pathogen microbes (Sarahm, Miller, 2008). Some antimicrobial plant species with suitable specificity can enhance the treatment of pathogens (P. Galbraith, 2019).

nowadays most studies in developing countries have used quantification of coliforms using cultural methods of fecal coliforms to evaluate microbial water quality but Recently, molecular techniques, especially the polymerase chain response (PCR)-based strategy, have been proposed as an elective strategy to screen and track pathogens and fecal indicator bacteria (FIB) (Sungwoo Bae, 2018).

3. Methodology (Materials and methods)

3.1. Materials and equipment

Chemicals and materials: 1M NaCl (lab graded) was used for extraction of active coagulant, 97% ethanol was purchased from NEWAY PLC and used for the preparation of 95% solvent solution for oil extraction. 1 M of NaOH solution and 1 M of HCl has been used solution for adjust pH (Vasanthi Sethu1, 2019). Denature alcohol was purchased from SIDO CHEMICAL TRADING and used for disinfecting the media preparation hood and Bunsen burner. Acetic acid was utilized for the sterilization of sample holding bottles. Glove, culture media, and reagents: EMB agar powder was purchased from MT. ET TRADING PLC was used for E. coli indicator bacteria media preparation and plate count agar were purchased from NEWAY PLC and used for the preparation of total coliform media.

The three locally available plants moringa stenopetall seed, aloe Vera and cactus leaves were used for the extraction of bio-coagulants. These plants are very important because of locally available, compatible with health, used traditionally by the community, disinfectant, and inhibited microorganisms (M. Megersa1, 2018 & Ashenafi Delelegn, 2018).

The coagulation /flocculation process was determined in the six-jar test equipment and the initial pH of the water was adjusted by 1m HCl and 1mNaOH (Ramavandi, 2014). The capacity of bio-coagulants was investigated by using 50 mg/l, 100 mg/l, and 150mg/l of doses and at pH of 6.5, 7.5 & 8.5 of water. Some literature like (Franciele Pereira Camacho, 2016) by using moringa oleifera as a bio-coagulant was obtained optimum doses of 50 mg/l and 100 mg/l, but moringa stenopetall the same family species with moringa oleifera. Moringa setnopetall and other of the two plants could be achieved by the above dose and the World health organization pH ranges from 6.5 -8.5. Turbidity was one of the imperative parameters to decide the water quality and it was happened due to the scattering of light by particles within the water. Turbidity is depending on particles' physical and chemical composition, shape and size distribution, and a strong relationship between light scattering and particles' area and volume (Charlotte Farrell, 2017). Most of the literature was determined the turbidity of water by using bio-coagulants like Moringa oleifera (Ashenafi

Delelegn, 2018, B.I. Gandiwa, 2020, Franciele Pereira Camacho, 2016), Moringa stenopetala (Govindan, 2018 & M. Megersa1, 2018), papaya seed (Syeda Azeem Unnisa, 2018 & Zeharaaddeen N. Garba, 2015), cactus (B.I. Gandiwa, 2020 & Inas M. Al-aubadi, 2017), aloe Vera (Azni Idris, 2011, Gulmire Amruta, 2017, Hemraj S.R, 2019), Strychnos potato rum, Jack fruits seeds (Hemraj S.R, 2019), Cassava starch (Jose Lugo-Arias, 2020). So, in this study, the water turbidity was determined by using the three bio-coagulants.

Equipment: the knife was used to cut Aloe Vera leaves skin separated from gel and cactus leaves into small pieces. Dried plants were reduced in size using Mortar. An electrical home machine was used for grinding well-dried moringa seeds, aloe Vera and cactus. Two different home strainers were utilized for the powder filter to induce fine. Electrical balance was used to measure powder. The furnace was used for determining ash, and Moisture Analyzer /p1028933 ML-50/ was used to characterize of moisture of the samples. Whatman filter paper 1(grade 0.7µm) was used to filter the supernatant of water and a magnetic stirrer was utilized to well blend the solution. Falcon tubes and Plates were purchased from GOOGLE TRADING PLC and used for the preparation of serial dilution and media for inoculated indicator bacteria and Centrifuge (model 800) was used to separate /eluted solution and Muslin cloth was used to filter the mixture solution.20 litter sterilized polypropylene bottle was used for water sampling. Six Jar-Test equipment (PHIPPS &BIRD) was utilized for the coagulation test. pH Meter (HI 9124) was utilized to measure pH, Turbidimeter (model: HI 98703) was utilized to measure turbidity, a small fluorescent lamp was used for colony count, Autoclave (model YX-24M) was used for sterilization, Incubator was used for bacteria growth, Microscope was used for direct observation of particles.

3.2. Collection of Bio-Coagulants

3.2.1. Bio-Coagulants Preparation

Moringa stenopetala seed was collected from south-western Ethiopia rural zone of Konso; Aloe Vera leaves were collected from Sululta is found between 9° 4'30"N to 9° 30'59"N and 38° 31'26"E to 38° 58'49"E and 40 km northwest of Addis Ababa. And Cactus plants were collected from Adam Nazareth place of Wenji exit is located at 8°32′N 39°16′E / 8.54°N 39.27°E / 8.54; 39.27 at a sea level of 1712 meters, 99 km far from southeast of Addis Ababa. Moringa seeds were removed from a cover and wings, dried in the sun for one day, and then overnight in an oven at room temperature, grinding the seed by employing a mortar and a home electrical machine to induce a fine powder. The fine Powder meshed three and more by two distinctive measure strainers. Then 60 grams of the fine powder was mixed with 350 mL of 95% ethanol and stirred by a manual for 40 min. The suspension of the solution was eluted by centrifuge at 4000 rpm for 5 minutes. And the residual solids were dried at room temperature for 24 h in the oven. The collecting New Aloe Vera leaves were washed by the tap of water to clean. And cut into small pieces and isolated the rind/skin from the gel of aloe Vera leaves. The gel was part of aloe vera known for medicinal and cosmetics research, even though the aloe vera skin was considered a waste. So as a bio coagulant aloe vera plant skin was utilized. The rind /skin of aloe vera was rinsed with hot water and dried for 3 days in the sun and the oven at 50∘C overnight. The collecting Fresh cactus pads were removed areole, glochids, and spines by knife-sharp part and it was washed or rinsed by the tap of water. Then also cut into small pieces and the outer thin skin was removed by manually using a cutting knife. The cutting tissue of the cactus was washed with refined water. The small flesh cactus was dried by sunlight for 4 days and also dried in an oven at 50∘C overnight.

Then the fine powder was weighed for analysis of protein content, moisture content, and ash content, but moringa defatted powder was analyzed in addition to raw powder. 1M NaCl solution was arranged by including 58.44gram of lab-graded NaCl to 1 liter of distilled water. Then 10 grams of the fine powders were added to 1liter of 1M NaCl solution mixed for 1 hour using a magnetic stirrer and settled for 30 min. At that point, the resulting solution was

filtered employing a muslin cloth and analyzed moisture, ash, and protein content. The 1% solution was put away at a cold temperature (4 °C) until connected to the consequent filtration forms (M. Megersa1, 2018, B.I. Gandiwa, 2020 & Azni Idris, 2011).

3.3. Experiments

3.3.1. Experimental setup and description

The mixing solution of Coagulation tests was carried out by using 1L beakers with standard six Jar-Test equipment (PHIPPS &BIRD).

Figure 3:1 Jar test equipment with samples (sourced from photo)

The treatment tests were conducted at coagulant dose of 50 mg/l, 100 mg/l & 150 mg/l and pH of 6 .5, 7.5 & 8.5 at room temperature.

The solution to the Jar test was arranged by the taking after equation:

$C1V1 = C2V$ …… (3.1)

C_1 (Stock solution concentration) V_1 Stock solution volume C_2 the dosage of bio coagulant, V_2 the volume of water. The procedure involved in the jar test was three different mixing

modes for 1min of rapid mixing (120rpm) followed by 19 min of slow mixing (40 rpm) for flocculation and followed by 15 min settling at a room temperature (Jose Lugo-Arias, 2020). And the sample was taken from the middle of the supernatant withdrawn by a syringe and filtered by grade 0.7 µm of Whatman paper1 for assist examination of Physico chemical and microbiological parameters. The general experimental framework of the experiments is displayed in figure 3.2.

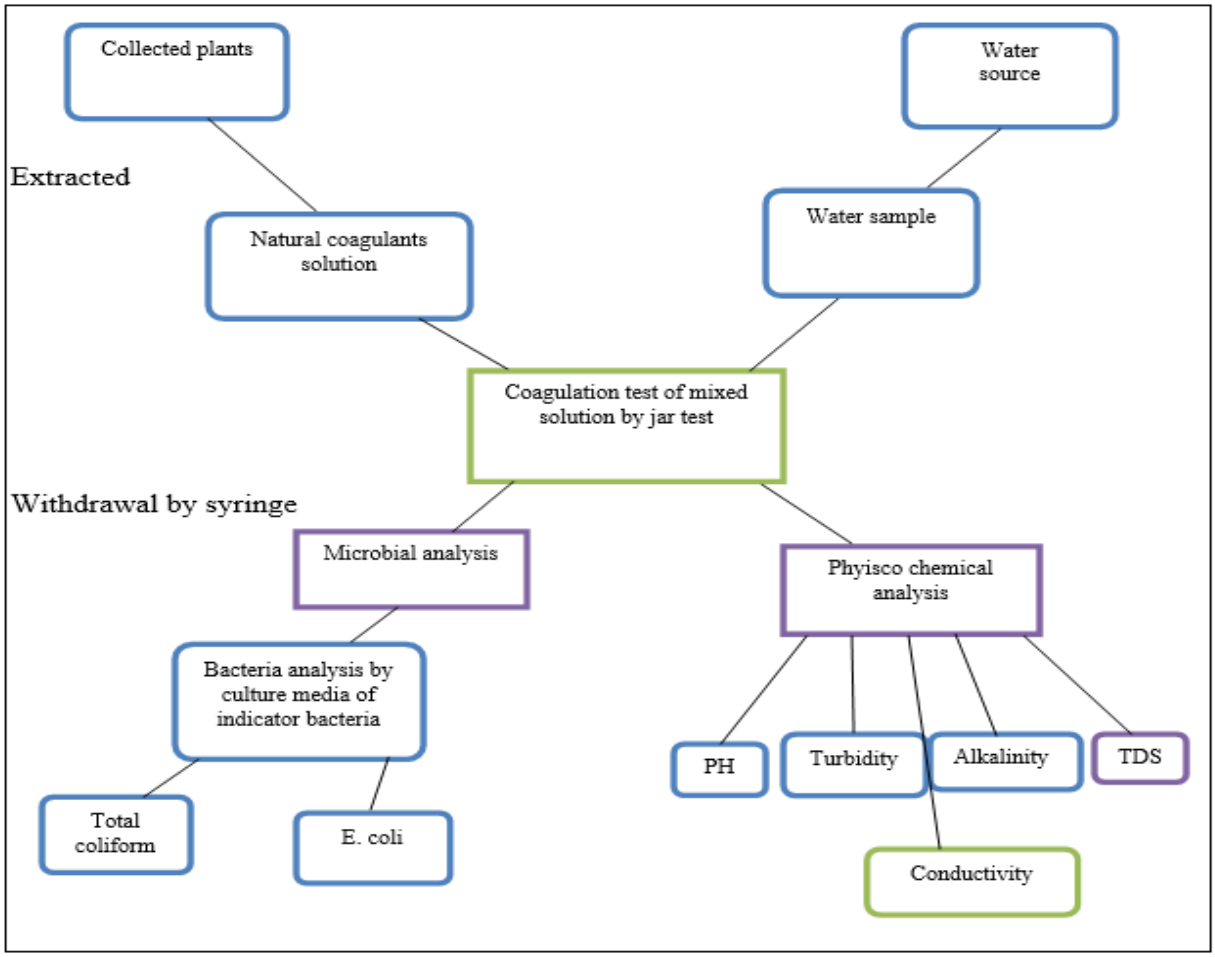


Figure 3: 2 schematics of the general framework of the experiment

3.4. Physicochemical characterization of plants powder and extracting bio coagulants

Moringa stenopetalla seed, Aloe Vera leaves skin, Cactus leaves to powder and their extracted bio coagulants were characterized for protein content, moisture content, and Ash content. Protein in the sample was determined by the Kjeldahl distillation method (AOAC, 2005). For protein content determination first nitrogen was found by 0.5 gram of the fine powder sample measured and added to the tube. Then 6 ml concentrated Sulphuric acid 98 % (H2SO4) was added into a tube with samples for digesting overnight. In the morning 3 grams of copper sulfate ($CUSO_4$) and Potassium sulfate (K_2SO_4) catalyst,3.5ml H_2O_2added to the tube for digesting for 3 hours. Then 30ml of distilled water and 40 ml of 35% NaOH were mixed into the digested samples for the distillation process. The mixture was put on the left side and then 25ml boric acid was added into the conical flask on the distillation machine for 8 minutes. The mixture was made alkaline with 35% NaOH and also the mixture was formed with Ammonium sulfate. The Ammonium sulfate was changed into ammonia in the mixture. Then ammonia was collected in a 25 ml boric acid solution of the conical flask on the right side of the distillation process and the boric acid solution was titrated by standard HCl. The percent nitrogen content of the sample was calculated by using the following formula.

$$\%\,\mathrm{N} = \frac{14\;X\;(mL\;HCl - \text{mL blank})\;\text{X Conc.of HCl}}{Weight\;of\;sample\;(g)} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.2)$$

$$\%\,Protein = \%\,N * Factor\;(6.25) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.3)$$

Where %N = percentage of Nitrogen ml HCl = volume of HCl in L consumed to the endpoint of titration blank = volume of HCl consumed to the endpoint of titration without samples Conc. of HCl= 0.1 m HCl (often used 0.1) 14 = molecular weight of nitrogen

Moisture content: The samples of moisture content were determined by weighing 5 grams of a sample with a crucible. The crucible was cleaned and heated to 105^{o}C for 30 minutes. And also cooled in the desiccators and weighed. At that point, the dry and cool crucible with

the sample was warmed in an electrical oven for approximately 3 hours at 105^0C. the warmed sample was cooled in the desiccators and weighed. The rate of the moisture content was calculated by utilizing the taking after equation:

$$\text{Moisture content (\%)} = \frac{W2-W3}{W2-W1} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(3.4)}$$

Where W_2= weight of the cooled crucible, W_1 = weight of sample plus crucible
W_3 = weigh of dried sample plus crucible

Figure 3: 3 Moisture Analyzer (sourced from photo)

Ash content: The samples of the ash content were decided by weighing around 2.5 grams of the sample with a porcelain crucible. The porcelain crucible was cleaned and warmed to 550oC for 30 minutes additionally cooled within the desiccators and weighed. The samples were burned on the hot plate under a fume hood up until the smoking stopped. At that point, the crucible with its content was placed in a muffle furnace; the time began from the muffle furnace after coming to 550 ^{0}C for almost five hours. The samples of the ash were cooled within the desiccators and weighed.

$$\text{Total Ash (\%)} = \frac{M3-M1}{M2-M1} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(3.5)}$$

Where M_1= weight of the cooled crucible, M_2 = weight of samples with cooled crucible
M_3 = weigh of Ash with cooled crucible

Figure 3: 4 Muffle Furnace (sourced from photo)

3.5. Preparation of water samples and characterization

The grab sampling strategy was utilized to gather water samples from the surface water of the Legedadi dam. The Legedadi dam is found between $9^{o}20'$ N and $38^{o}45'$ E at a sea level of 2450.

Figure 3: 5 Google Map of Legedadi Dam

It is built for the drinking purpose of Addis Ababa city in 1971. The water samples were collected at depth of 40 cm of the dam by using polypropylene bottles for analysis of physicochemical and microbiological and the samples were taken from the Dam by taking after the methods expressed in (ASTM (American Society of Testing and Materials), 1996). The water samples were collected in triplicate at 8:30 AM. The experiment was done in Addis Ababa water and sewerage Authority (AAWASA) and Addis Ababa University

College of Natural and Computational Sciences: Food Science and Nutrition Department and Department of Microbial, Cellular and Molecular Biology. According to the method reported elsewhere (United State Environmental protection Agency, 2017) and (United State Environmental protection Agency, 2005) method, 1623 and 1623.1 requirements for the amount of sample for microbial with sterile glass bottles for laboratory examination. The collecting water Samples were analyzed for their pH, alkalinity, conductivity, turbidity, TDS, total coliform, and E. coli.

3.6. Physico-chemical water quality measurement:

pH, alkalinity, conductivity, and TDS, of the raw and treated water with bio coagulant at different pH and coagulant doses were determined as shown in figure 3.6. Alkalinity was determined by Titration with 0.02 N sulfuric acid standard solution,50 ml water samples and indicator solution if pH < 8.3, Bromocresol Green Methyl Red Solution (changed to pink), and pH >8.3 Phenolphthalein indicator solution. It is calculated using the formula (HACH, 1997).

$$ml\ titrant * 20\ (multiplier\ used) = mg/l\ total\ alkalinity \quad \ldots\ldots\ldots\ldots (3.6)$$

Figure 3:6 pH meter &TDS meter (sourced from photo)

3.6.1. Turbidity analysis

The sample of raw and treated water of turbidity was decided by Turbid meter (model: HI 98703) (American Public Health Association, 1999 & United State Environmental protection Agency, 2017). The finding of turbidity was expressed as NTU (Nephelometric Turbidity Units) (L. Nishia, 2012, Hayelom DargoBeyene, 2016 & Sarahm, Miller, 2008).

Figure 3:7 Turbidity meter (sourced from photo)

$$\text{Turbidity removal (\%)} = \frac{\text{TurbidityRaw water} - \text{TurbidityTreated water}}{\text{TurbidityRaw water}} * 100 \ldots\ldots\ldots \text{(3.7)}$$

3.7. Biological contaminants analysis

3.7.1. Enumeration of total coliform and E. coli bacteria

The bacteriological quality of raw and treated water was decided in triplicate from each sample by total coliform and E. coli. Total coliform was inoculated on 20 ml Petri dish media prepared by count plate agars and E. coli was inoculated by EMB agar. The agar powders were prepared based on the grams of powder with litters of distilled water from the manual then assume total ml Petri dish from 20 ml each of Petri dish and calculated how many grams of agars were needed for the prepared media. The calculated grams of agar heated with balanced distilled water and this hot media, Falcon tube, and distilled water for serial dilution were sterilized in an autoclave for 15 minutes beginning from a gauge reading of 121^{o}C. The cooling media was filled on a plate in the hood overnight and then inoculated for the detection of total coliform and E. coli bacteria. A sample was taken from the collected

raw water and treated water using and cultivation of microbes was done immediately after collection and treatments.

The analysis of total coliforms and E. coli was conducted utilizing spread plate strategies based on Standard Strategies (9215C of American Public Health Association 1998). 1 ml of sample was serially weakened with 9 ml distilled water for 3 serials to diminish the bacterial thickness. Subsequently, 0.1 ml of the appropriate dilutions were spread directly onto the count plate agar and EMB agar plates for total coliforms and E. coli respectively. The Petri-dishes were put in an incubator and kept up at 37 0C. At long last, the number of colonies was tallied by the colony counter after 24 hours of incubation periods (APHA, 1998).

$$\text{Percent Reduction } (\%) = \frac{Initial\ No.\ Micro\ organism - Final\ No.\ Micro\ organism}{Initial\ Micro\ organism} * 100 \ldots\ldots (3.8)$$

3.8. Zeta potential

Zeta potential investigation was used to determine the Zeta potential of Bio-coagulants extracted from moringa stenopetall, Aloe Vera, and cactus. This investigation was carried out using the electrophoresis strategy by electrical Microscope coordinate perception of particles. Zeta potential provides the estimation of the electrostatic charges of the particles that influence the attraction and repulsion powers between their particles. In this manner, conducting a ZP investigation approves the effectiveness of moringa stenopetall, Aloe Vera, and cactus as coagulants.

3.9. Experimental Design and data analysis

The Experimental design was contained three components coagulants type, pH, and does of natural coagulants and it has seven responses with two replication and three levels which means a total of 54 experiments were conducted in the study. SPSS software version 20 was utilized for generating the Analysis of variance (ANOVA) tables to decide the significance of the components and their interaction. Additionally, it was utilized to analyze the relationship between factors. The experimental design was built up utilizing Design Expert v7.0.0 software with General factorial designs (see table 7.1 in the annex section) and it was used to develop the factorial model. And also, the Response surface method was utilized to decide the optimum working condition of the parameters.

4. Results and Discussion

4.1. Extraction of Bio-coagulants

Currently, the Extraction of bio coagulants from plants are the subject matter and much interesting in the research area, but their extraction as part of phytochemical and/or biological investigations presents specific challenges. Scientists have been developed many protocols of extraction of the bio coagulants ingredients to ensure their effectiveness. The Extraction methods are solvent extraction, distillation method, pressing, and sublimation according to the extraction principle. However, in this study Moringa stenopetall, Aloe Vera, and Cactus plants were used as raw materials for the extraction of bio-coagulants by using Solvent extraction techniques. Those three locally available plants of moringa stenopetall seed, aloe Vera and cactus leaves were used for the extraction of bio-coagulants. These plants are very important because of locally available, compatible with health, used traditionally by the community, disinfectant, and inhibited microorganisms (M. Megersa1, 2018 & Ashenafi Delelegn, 2018). The above plants have cationic characteristics of protein content to remove water impurities studies like (Jose Henrique et al 2016) was investigated Moringa oleifera lectin ability decreases the concertation of metal ions; (Habauka M. kwaambwa, 2015 & Govindan, 2018) also investigated on the Moringa stenopetala has protein cations to remove water contaminants and impurities; aloe Vera was investigated as a bio coagulant to remove turbidity of water (GulmireAmruta, 2017) and Cactus also contains polysaccharides and protein polymers used for coagulants (Vasanthi Sethu1, 2019). So, the 1% liquid solution (the ratio of 10 mg :1ml) of bio coagulants of MS-SC, AV-SC & Ca-SC were prepared from Plants of Moringa stenopetala seeds, Aloe Vera leaves skins and Cactus leaves respectively (Azni Idris, 2011). Those bio coagulants were used with 1M NaCl solution as a solvent for extraction. The fraction of NaCl solution also affected the efficiency and the 1M NaCl solution was done best performance (M. Megersa1, 2018 & G. Muthuraman, 2013). Solvent extractions are the most widely used method especially saline extraction and ethanol extraction. It can be seen in table 4.1 those bio coagulants extracted with Solvents were improved the extraction efficiency of the bio coagulants than raw powder similar to results reported by (M. Megersa1, 2018, Franciele Pereira Camacho, 2016 & G. Muthuraman, 2013).

4.2. Characterization of Bio-coagulants

Moringa stenopetall, Aloe Vera, and Cactus plants were used as raw materials for the extraction of bio-coagulants. The extraction of bio-coagulants is important for improving the amount of protein content of raw powder by using the solvent. So, in this study, the bio-coagulants were extracted by utilizing a 1M NaCl solution. The extricated bio coagulants were characterized by their protein, moisture, and Ash content. The characterization result of the Protein, moisture, and ash content of the three bio-coagulants are presented in table 4:1.

Table 4:1 Physico-chemical properties of the powder and extracted bio-coagulants

types of plant species	Parameters	Raw powder	Ethanol extraction	1M NaCl extraction
Moringa stenopetall	Protein (%)	39.73 ± 0.25	43.01 ± 0.09	43.95 ± 0.49
	Moisture (%)	5 ± 1.13	6.5 ± 0.71	9.85 ± 0.07
	Ash (%)	4.4 ± 0	4.6 ± 0.28	5.05 ± 0.21
Aloe Vera	Protein (%)	13.56 ± 0.12	13.9 ± 0.42	
	Moisture (%)	3.7 ± 0.71	11.05 ± 0.78	
	Ash (%)	13.8 ± 0.28	31.6 ± 0.28	
Cactus	Protein (%)	10.5 ± 0.74	10.94 ± 0.37	
	Moisture (%)	2.4 ± 0	9.35 ± 0.21	
	Ash (%)	26.2 ± 0.28	35.43 ± 0.81	

It is seen in table 4:1 that moringa stenopetala raw powder and ethanol and NaCl extraction of Bio-coagulants have a protein content of 39.73 ± 0.25, 43.01 ± 0.09and 43.95 ± 0.49 respectively. While Aloe Vera leaves of skin protein content were 13.56 ± 0.12 for raw powder, and 13.9 ± 0.42 for NaCl extraction of Bio-coagulants respectively. And Cactus leave to have a protein content of 10.5 ± 0.74 for raw powder and 10.94 ± 0.37 for NaCl extractions of Bio-coagulants respectively. As it can be seen in figure 4.2. the Nano drop

measurement of protein concentration of bio-coagulants was 2.534 mg/ml of MS-SC, 3.434 mg/ml of AV-SC, and 1.647 mg/ml of Ca-SC. It can be inferred from the characterization result that moringa stenopetala has a higher protein content compared to aloe Vera and cactus plants for raw and extracted bio-coagulants. Besides, the protein content of extracted Bio-coagulants has better protein than raw powder in all three plants due to the salt-based extraction process. Similarly, other studies by (M. Megersa1, 2018, G.Muthuraman, 2013 & Ramavandi, 2014) stated that the Bio-coagulants amount of protein content was increased because of the salt-based extraction process.

With respect to the solvent effect, it is noted that salt-based extracted bio coagulant has a higher protein content than ethanol extracted bio coagulant (see table 4.1). Because of the salt-based extraction protein-protein dissociations are increasing and protein solubility increases with increasing the salt ionic strength. A similar result has been reported elsewhere (Franciele Pereira Camacho, 2016). However, in this study, a relatively, higher protein content, ash, and moisture content of Aloe Vera skin and Cactus leave were recorded compared with other studies (Karina Di Scala1, 2012, Muñoz Om, (2015, M. Z. Haque , 2014 & Sarahi Rodríguez-González1, 2014).

4.3. Zeta potential (ZP)

Zeta potential (ZP) is a dynamic analysis conducted for controlling the parameters through a mechanism of coagulation and flocculation by using the Zetasizer. The Zetasizer analyzes the stability of dispersed systems and measures the magnitude of electrostatic charge attraction or repulsion forces among the particles (Lanan Fabm, 2020). Be that as it may, in this study, the Zeta potential (ZP) was analyzed through the component of Electrophoresis. The movement of charged colloidal particles or polyelectrolytes immersed in a fluid, beneath the influence of the connection outside the electric field is called electrophoresis. The Electrophoresis experiment was processed with materials of 83% ethanol for solvent, 17% water, and 0.5g/l concentration of each sample of bio-coagulant, Borosilicate beaker, 18 voltage batteries, electric wire, glass slides, and optical Microscope for direct observation. As it can be seen in figure 4.1, the direct electric field is applied to the solution and the positive charges move to the negative anode of the magnifying instrument glass slides. A few writings argue that the experimentally decided zeta potential leads to strong underestimation

(Henriëtte E. Bakker, 2017). The electrophoretic speed is the speed during electrophoresis and the electrophoretic versatility is the size of the velocity divided by the magnitude of the electric field quality. According to (A.V. Delgado, 2007) if the particles move toward lower potential (negative anode), the mobility of the charged colloidal particles is tallied as positive and negative within the inverse case. So, within the experiment of this study, the charged colloidal particles were moved toward the negative electrode. The glass slides were used to direct microscopic observation of the particles and by employing a direct electric field (A.R. Boccaccini1, 2010). Electrophoretic deposition may be a special method of charging particles to the attached strong portion of the cathode.

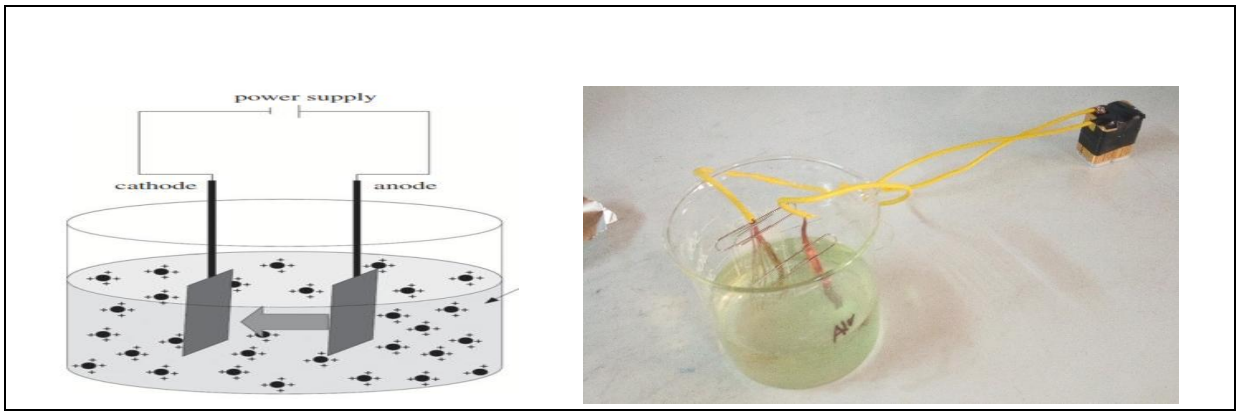
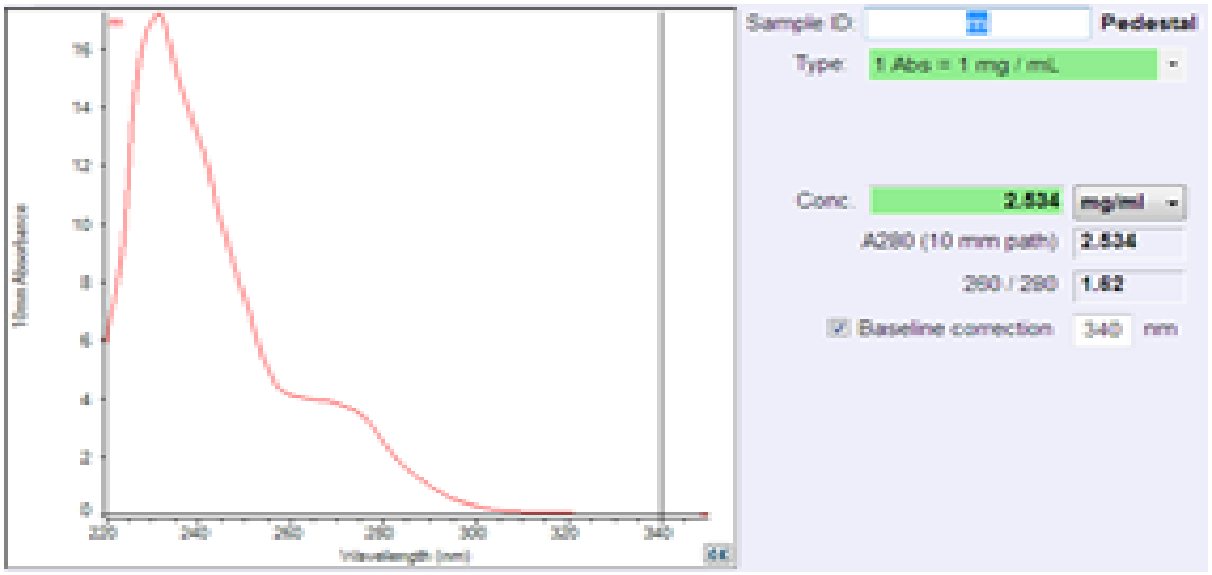


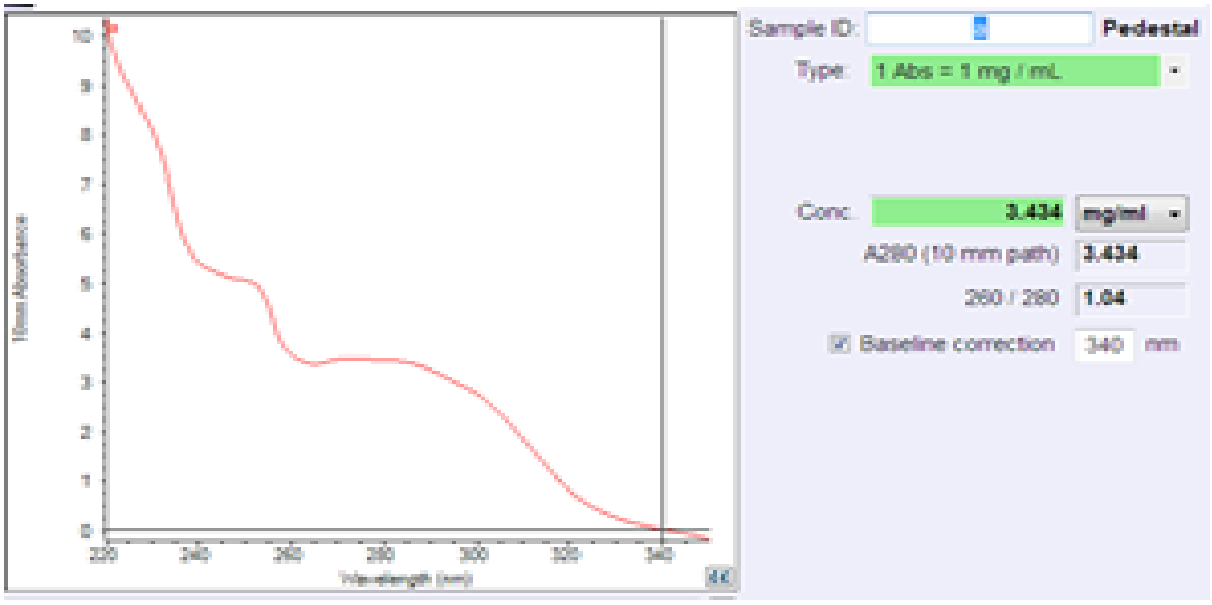
Figure 4.1. Showing the Electrophoresis experiment

Zeta potential is a vital parameter that tells to suspension firmness and movement. However, in this study, the Zeta potential was analyzed by using the equation of *Helmholtz–Solutions* (HS) through electrophoresis. Electrophoresis is the counterpart of electro-osmosis, the liquid moves concerning a strong body when an electric field is connected, though during electrophoresis the fluid as an entire is at rest, whereas under the impact of the electric field the particle moves concerning the liquid (A.V.Delgado, 2007) and the equation of Hamaker. So, the Zeta potential of moringa, aloe Vera and cactus were 18 MV, 22 MV, and 33 MV respectively. Therefore, as it can be seen in figure 4.3, the image showed that the aloe Vera and moringa bio-coagulants were displayed the layers of the image were denser and moringa in some content was aggregated in the particles. In general, the two bio-coagulants have higher aggregated particles than the cactus which showed a scatter of particles attached to the negative electrode of glass slides. Thus, the prepared solution by moringa and aloe Vera has been showing a weak repulsion between macromolecules and they have a high tendency for

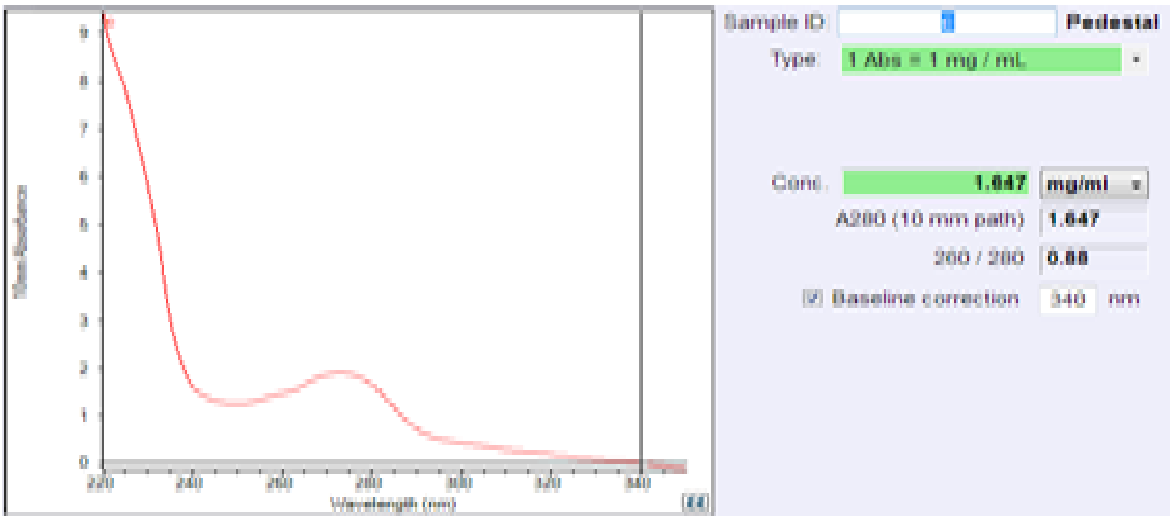
the particle to aggregate. But the cactus solution has a strong repulsion between macromolecules which resulted in good stability. However, low absolute values which are normally lower than 30, indicate a weak repellent force thus introducing a high tendency for the particle to aggregate (A. R. Boccaccini1, 2010).



a)

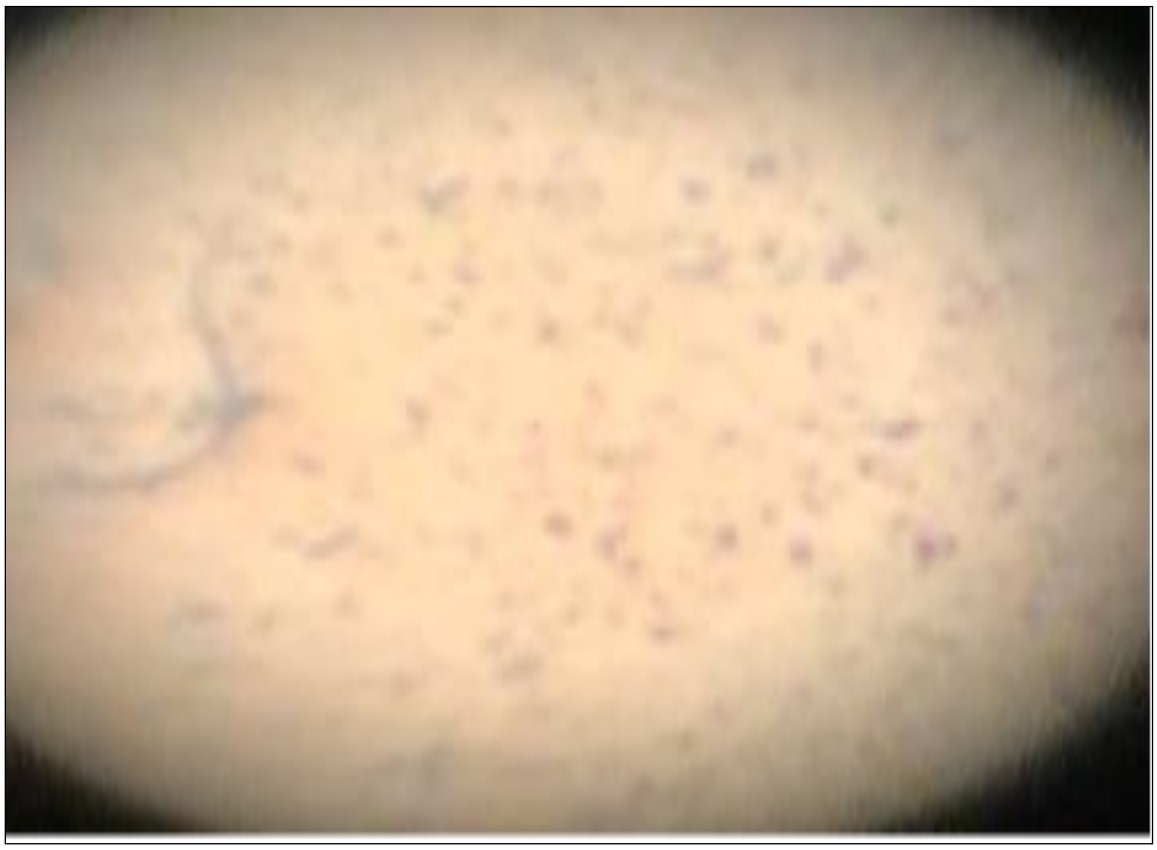


b)



c)

Figure 4.2. Nanodrop absorbance graph: a) MS-SC, b) AV-SC & c) Ca-SC



a)

b)

c)

Figure 4.3 Microscopic 100xs. Images electrophoretic deposited on glass slides electrode from its suspension in ethanol: a) MS-SC, b) AV-SC & c) Ca-SC

4.4. Characterization of raw water

The characteristic of the raw water gotten from Legedadie Dam is displayed in table 4.2. As it can be seen from Table 4.2, most water quality parameters surpass the WHO drinking water limit. In specific, the Legedadie dam has high turbidity and E. coli. This shows that the water was contaminated by photogenic organisms.

Table 4.2: Selected Physico-chemical characteristics of raw water

Parameters	Raw water	WHO Guidelines for Drinking water
pH	7.53	6.5-8.5
Turbidity	239.67 NTU	less than 5 NTU
Alkalinity	68.8 mg/l	less than 50 mg/l
Conductivity	113.44 µS/cm	
TDS	60.33 mg/l	less than 1000 mg/l
T. Coliform	593CFU/0.1 ml	Absent
E. coli	305CFU/0.1 ml	Absent

The diverse parameters analyzed in water experiments treated by bio-coagulants extricated from moringa; aloe Vera and cactus are displayed in table 4.3. The parameters were investigated in the coagulation and flocculation process of drinking water with the doses of 50 mg/l, 100 mg/l, and 150 mg/l of the three Bio-coagulants and at the pH of 6.5, 7.5, and 8.5. All three plants as coagulants were performed well to diminish the turbidity of treated water to the standard WHO limit as the doses expanded from 50 mg/l to 150 mg/l. In this study, the treated water was achieved a pH range from 6.64 to 8.03.

According to (Charlotte Farrell, 2017) stated that turbidity was one of the important parameters to determine the water quality and it happened due to the scattering of light by particles within the water. Moringa is a bio-coagulant that has got the best performance of turbidity reduction for treated water as low as 2.83±0.03 NTU at a pH of 6.5 and a coagulant dose of 150 mg/l. On the other hand, Aloe Vera and cactus were recorded at 2.93± 0.10 NTU and 73.35±1.48 NTU at pH of 8.5 and 150 mg/l dose respectively. Moringa and cactus plants as coagulants were performed lower alkalinity of treated water as the dose decreased from 150 mg/l to 50 mg/l and pH from 8.5 to 6.5. However, aloe Vera was achieved 18.2± 5.66 mg/l of total alkalinity with the dose of 50 mg/l and at a pH of 7.5. The minimum conductivity and TDS were recorded at 532.5± 67.18 µs/cm and 234± 1.41 mg/l by using a cactus plant with a dose of 50 mg/l at pH 6.5 respectively. Moringa and aloe Vera plants as coagulants were performed lower T. coliform decrease from treated water as the dose expanded from 50 mg/l to 150 mg/l. Aloe Vera bio-coagulant has got the best performance of T. coliform removal from treated water of 128.5± 2.12 (CFU)/0.1ml of the experiment at pH of 7.5 and the dos of 150 mg/l. Aloe Vera and moringa plants as coagulants completely removed E. coli from treated water as the dose of coagulant increments from 100 mg/l and 150 mg/l at all three pH respectively.

1 *Table 4:3 Different parameters in the analysis of water sample using Bio-coagulants extracted from moringa, aloe Vera and cactus powder*

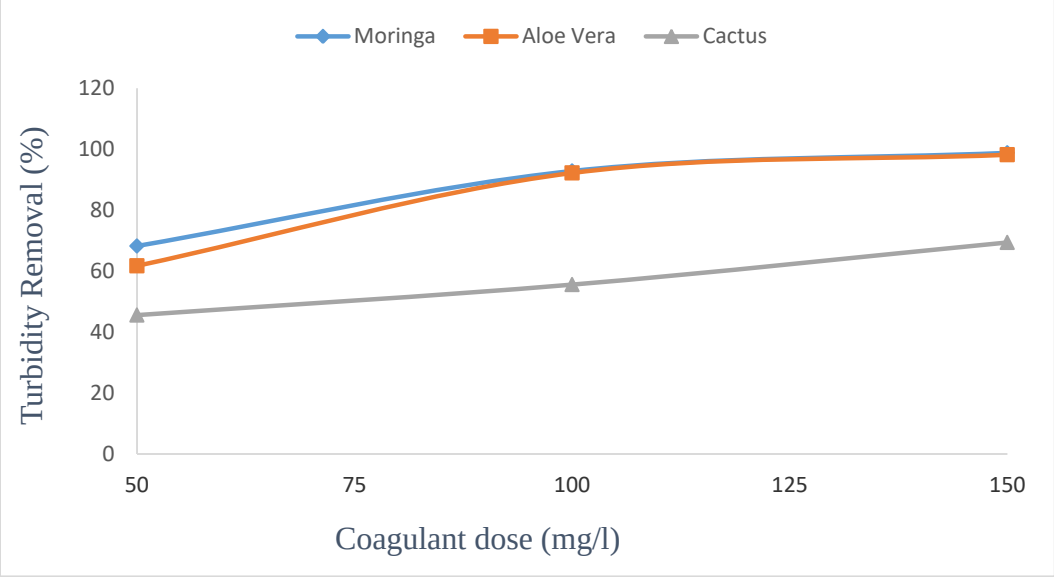
Parameters	pH	Dose of moringa Coagulant			Dose of aloe Vera Coagulant			Dose of cactus Coagulant		
		50 mg/l	100 mg/l	150 mg/l	50 mg/l	100 mg/l	150 mg/l	50 mg/l	100 mg/l	150 mg/l
	6.5	6.75±0.06	6.64 ± 0.04	6.82± 0.04	6.82±0.03	6.69± 0.03	6.74±0.04	6.64 ± 0.04	6.64± 0.02	6.8±0.01
	7.5	7.41±0.01	7.65± 0.02	7.64± 0.04	7.41 ± 0.03	7.49± 0.04	7.43 ± 0.04	7.3 ± 0.02	7.43± 0.04	7.405 ± 0.08
Response pH	8.5	7.68±0.02	8± 0.01	8.03± 0.03	7.63 ±0.02	7.7± 0.01	7.64 ± 0.02	7.47± 0.08	7.82± 0.01	7.81 ± 0.06
	6.5	76.17±1.23	17.1±0.42	2.83±0.03	91.75±7.14	18.65±0.08	4.3±0.03	130.5±2.12	106.5±1.12	73.35±1.48
	7.5	73.76±0.36	12.35±0.07	3.29±0.04	34.25±5.16	4.6±0.01	8.23±0.04	120.5±4.95	172.5±0.71	90.05±1.48
Turbidity (NTU)	8.5	62.9±0.71	13.5±0.45	4.52±0.06	29.05±1.91	13.97±0.06	2.93±0.10	161.5±3.54	173.6±0.57	105±1.41
	6.5	28.2± 0.85	38.4± 0.28	121.1± 0.42	26.3± 1.27	33.6± 0.28	26.8± 0.28	23.4± 0.28	24.5± 0.42	37.3± 0.14
	7.5	29.4± 0.28	58± 0.28	137.7± 0.42	18.2± 5.66	53.3± 0.14	48.8± 0.57	50.5± 0.42	52± 0.45	59.4± 0.57
Alkalinity (mg/l)	8.5	42± 0.28	56.6± 0.57	55.3± 0.14	50.4± 0.85	71.4± 0.28	70.2± 0.57	50.5± 0.42	52.8± 0.28	62.1± 2.69
	6.5	706± 53.74	838± 4.24	1369.5± 2.12	552± 69.30	887± 4.24	1782.5± 2.25	532.5± 67.18	589.5± 21.92	1365.5± 7.78
Conductivity	7.5	757.5± 9.19	987.5± 3.54	1534± 2.83	1467± 8.49	994.5± 4.95	1404.5± 2.12	824.5± 6.36	925± 7.07	1536± 1.41
(µs/cm)	8.5	1306± 21.21	1054.5± 64.35	1537.5± 0.71	1664.5± 7.78	1659.5± 2.12	1653.5± 3.54	830± 14.14	949.5± 6.36	1542± 1.41
	6.5	299± 4.24	337± 2.83	606± 1.41	271.5± 31.82	368± 3.46	874± 2.83	234± 1.41	297± 5.66	664.5± 3.54
	7.5	308.5± 0.87	481± 1.41	675± 2.82	712± 7.07	478± 9.90	724± 2.83	437± 2.83	456± 1.41	752± 1.41
TDS (mg/L)	8.5	717± 5.66	533.5± 3.54	757± 1.41	727.5± 3.54	827± 2.83	809± 1.41	440± 7.07	467± 2.83	763± 2.83
	6.5	546.5± 4.95	344.5± 3.54	137.5± 2.12	557± 4.24	336.5± 4.95	132.5± 3.54	539± 1.41	576± 2.83	458± 2.83
T. coliform	7.5	362± 2.83	300.5± 0.71	141..5± 2.12	351± 4.24	215± 4.24	128.5± 2.12	336± 5.66	582.5± 0.71	467.5± 3.54
((CFU)/0.1ml)	8.5	320± 2.83	312± 1.41	132.5± 3.54	333± 4.24	215± 2.83	144.5± 3.54	560± 5.66	568.5± 2.12	456.5± 3.54
	6.5	236± 1.41	76± 2.83	60± 2.83	209± 1.41	65± 4.24	N. D	196± 8.49	210± 1.41	212± 2.83
E. coli	7.5	181± 2.83	77± 2.83	N. D	187± 4.24	N. D	82.5± 3.54	168± 5.66	217± 2.83	218.5± 0.71
((CFU)/0.1ml)	8.5	176.5± 2.12	81± 4.47	N. D	187± 1.41	67± 2.83	81.5± 2.12	198± 4.24	223.5± 0.71	221± 1.41

4.5. Factors affecting Physico-chemical properties of treated water

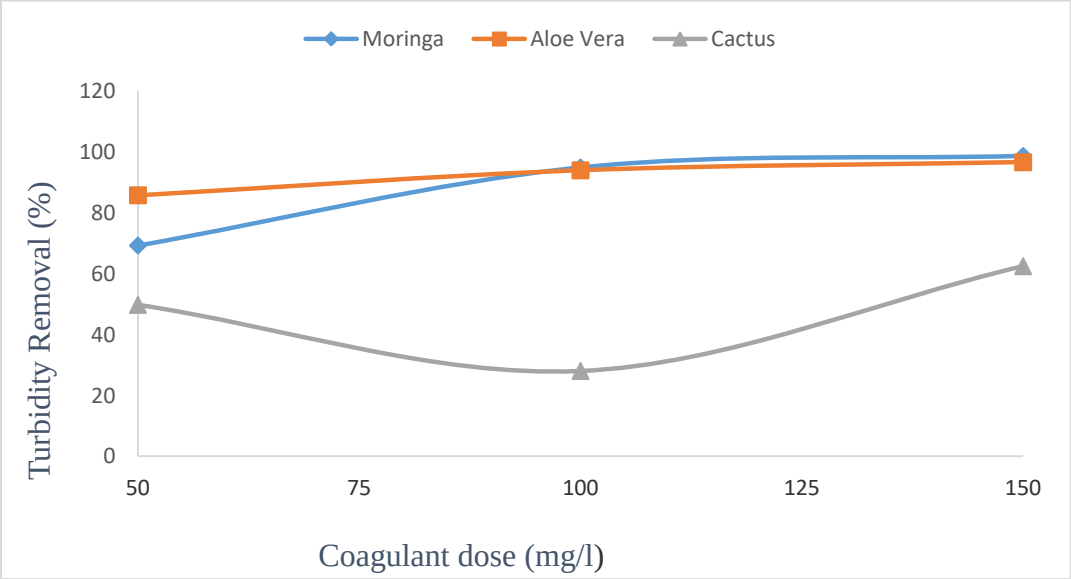
4.5.1. Effect of Bio-coagulants, dose, and pH on Turbidity

The dosage of coagulant and pH are the foremost vital variables of water treatment in the coagulation and flocculation process (VaraSaritha, 2019). So, in this study, the three factors of coagulant type, coagulant dose, and pH were investigated for treating drinking water at room temperature. The results obtained at different coagulant types, and pH with a variation of coagulant doses are presented in figure 4.4.a-c. As it can be seen in figure 4.4.a, the turbidity removal was slightly expanded for the three bio-coagulants by expanding their dose. When the amount of moringa, aloe Vera and cactus bio- coagulants increased from 50mg/l to 150 mg/l, the turbidity removal increased from 68.22%: to 98.82%, 61.72%: to 98.205% & 45.55%: to 69.395% respectively (see table 4.3). Such turbidity reduction is due to the increment of protein content (active ingredient) of those bio-coagulants at a higher dosage. Thus, increasing of protein caused the effective collision, neutralization/bridging between the active ingredient and colloids of water (Lek LeeChoong [Lek, et al., 2018). The turbidity removal was slightly increased for the moringa and aloe Vera bio-coagulants with increasing their dose. When the amount of moringa and aloe Vera bio-coagulants increased from 50mg/l to 150 mg/l, the turbidity removal increased from 69.225 to 98.63 and 85.71 to 96.565 respectively. As it can be seen in figure 4.4b. at a pH of 7.5, the turbidity removal was slightly decreased for the cactus bio-coagulant with increasing its dose. When the amount of cactus bio-coagulant increased from 50mg/l to 100 mg/l, the turbidity removal decreased from 49.72 to 28.025. The turbidity removal was slightly increased for the moringa and aloe Vera bio-coagulants are presented in figure 4.4.c. at pH of 8.5. As it can be seen in figure 4.4 the turbidity removal increased from 73.755 to 98.115 & 87.88 to 98.475 respectively. When the amount of moringa and aloe Vera bio-coagulants were increased from 50mg/l to 150 mg/l, the turbidity removal was somewhat increased for the two bio-coagulants by expanding their dosage. So, the bio-coagulants are leading to colloid destabilization, the rapid growth of floc, and rapid settlement due to the provision of cationic charges by amine groups at those doses and the nature of water pH. However, the two bio coagulants do not have a similar

amount of protein. When we come to the case of proximate analysis the moringa protein amount is the highest. In the proximate analysis, the protein content was estimated from nitrogen. so, the one scenario the nitrogen element was found not only in protein form but also in other forms another scenario the aloe Vera bio coagulant of the protein concentration was actively soluble, and the treatment condition (Karina DI SCALA1, 2012, Milene C. et al, 2014, M. Z. Haque *, 2014 & M. Megersa1, 2018) comfortable for it. As it can be seen in the figure 4.2 shows the Nanodrop measurement of aloe Vera bio coagulant is the highest protein concentration. When the amount of cactus bio-coagulant increased from 50mg/l to 100 mg/l, the turbidity removal decreased from 32.615 to 27.565. Some literature (Lanan Fabm, 2020) stated that turbidity removal decreased due to redispersion of aggregated particles with added bio-coagulant above optimum dose and pH. This situation indicated that the bridge mechanism occurred when a 100 mg/l dose of cactus bio-coagulant was used. The adsorption of the bio coagulant particle surface is destabilized during the interparticle bridging mechanism. A single adsorbed cactus polymer chained on a water colloidal particle as the tails and coils of it can overhang into a medium and clatter with another colloidal particle stated by (Shak, 2014). In agreement with the work of (G. Muthu Raman, 2013), pH has an impact on expelling turbidity from drinking water. Generally, those bio coagulants positively charged particle colloid destabilization of the negatively charged colloid particle of water through the process of adsorption and charge neutralization, and inter-particle bridging.



(a)



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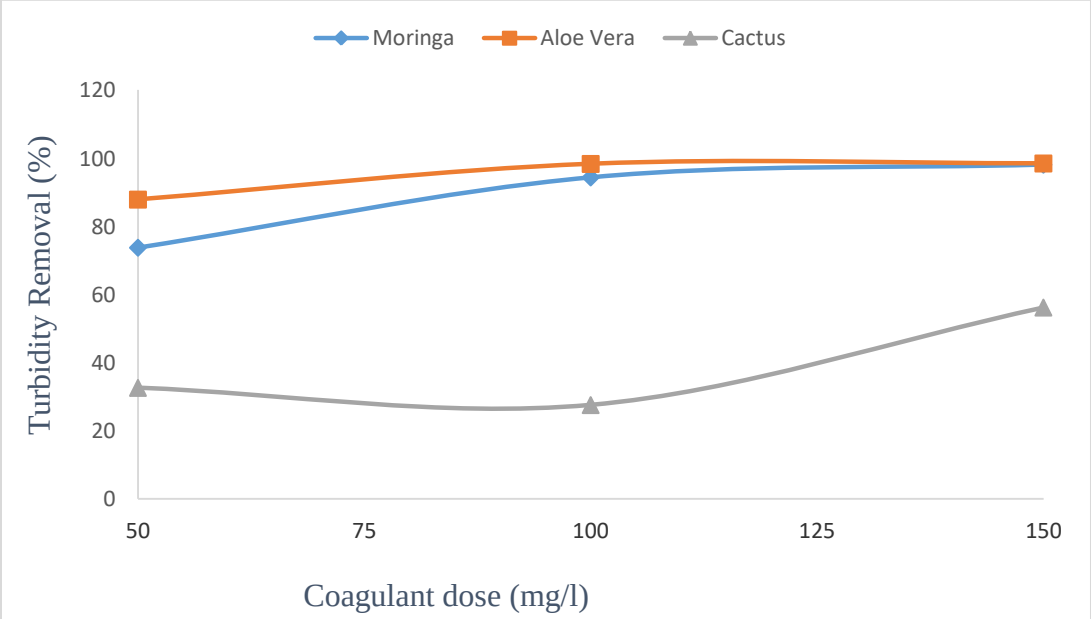


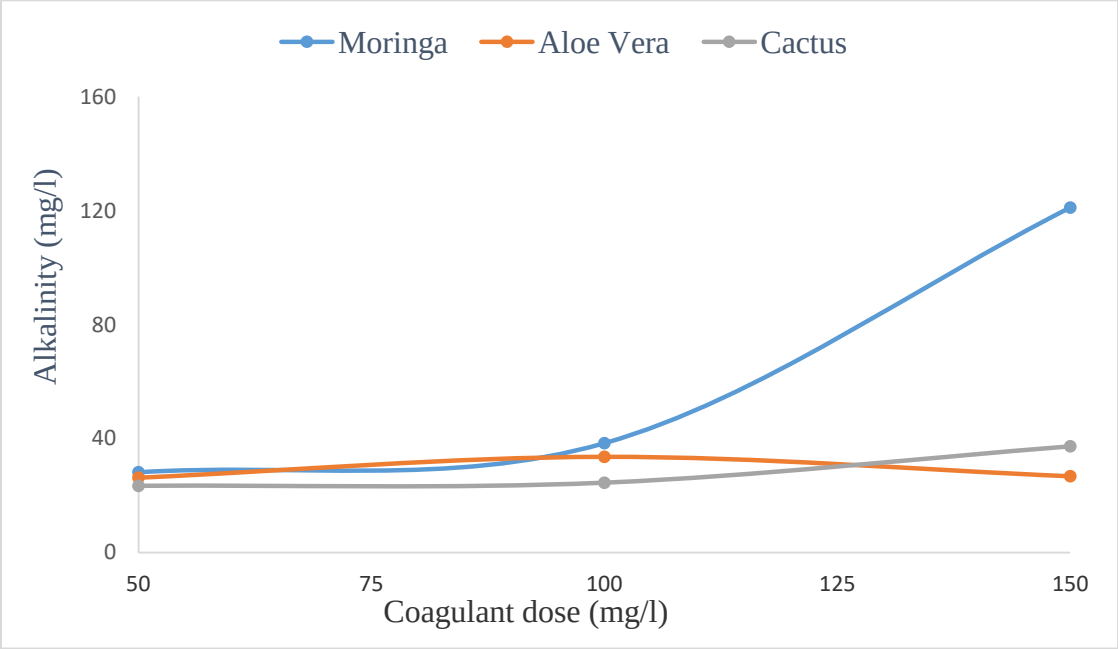
(c)

Figure 4:4 Dose of Coagulant versus Turbidity removal (a) at pH of 6.5(b) at pH of 7.5 & (c) at pH of 8.5

4.5.2. Effect of Bio-coagulants, dose, and pH on Alkalinity

Alkalinity is the capability of water to accept H+ particles (protons) and deciding the Alkalinity of water is critical to choosing the number of coagulants to be added within the treatment of water. The nature of chitin was expanded the alkalinity of water due to the arrangement of cationic charges by amine bunches at hoisted pH (Vara Saritha, 2019). As it can be presented in figure 4.5.a, higher alkalinity is observed for moringa bio-coagulant and increase with an increasing adsorbent dose for a solution pH of 6.5, while alkalinity is marginally increased for cactus and Aloe Vera bio-coagulants when the bio coagulants dose increased. When the amount of moringa and cactus was increased from 50mg/ to 150 mg/l, the alkalinity increased from 28.2 to 121.1 and 23.4 to 37.3 respectively. Similarly, when the amount of aloe Vera was increased from 50mg/l up to 100mg/l the alkalinity increased from 26.3 to 33.6 but when the amount increased from 100mg/l up to 150 mg/l the alkalinity decreased from 33.6 to 26.8 as presented in table 4.3. The alkalinity was clearly increased for the moringa bio-coagulant with increasing its dose is presented in figure 4.5 at a pH of 6.5 and 7.5. The situation indicated that during utilization of 150 mg/l doses of moringa bio-coagulant was increased alkalinity. So, the nature of moringa bio-coagulant is leading to colloid destabilization, the rapid growth of floc, and rapid settlement due to the provision of cationic charges by amine groups at this dose (VaraSaritha, 2019). But the reverse condition was indicated at 50 mg/l dosages by aloe Vera bio-coagulant which means less colloid destabilization, slow growth of floc, and slow settlement at this dose. Generally, those bio coagulants positively charged particle colloid destabilization of the negatively charged colloid particle of water through the process of adsorption and charge neutralization, and inter-particle bridging. When the amount of Moringa and Cactus bio-coagulants were increased from 50mg/l to150 mg/l (figure 4.5), the alkalinity increased from 29.4 to 137.7 and 50.5 to 59.4 respectively. A pH of 7.5 showed an increase of alkalinity from 18.2 to 53.3 with the dose increased from 50 mg/l to 100 mg/L in aloe Vera bio-coagulant but the dose increased from 100 mg/l to 150 mg/l the alkalinity increased from 53.3 to 48.8. The alkalinity was clearly decreased for the moringa and aloe Vera bio-coagulants with an increase from 50 mg/l to 100 mg/l of the dose presented in figure 4.5.c.at pH of 8.5. And the alkalinity was increased when the amount of moringa and aloe Vera increased from 100 mg/l to 150 mg/l.

As shown table 4.3 indicated that all doses of moringa, aloe Vera and cactus bio-coagulants were achieved as a result of pH between ranges of safe drinking water (figure 4.6). Hence some literature stated that natural coagulants can be seen as an alternative for water purification (Franciele Pereira Camacho, 2016) and it was detected that pH affects removing turbidity from water (G. Muthu Raman, 2013).



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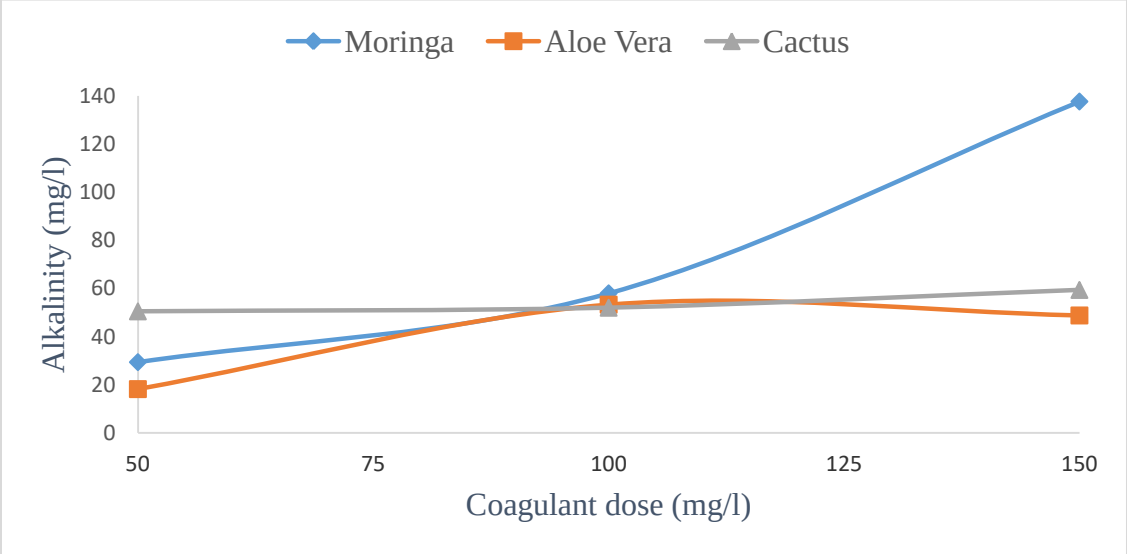

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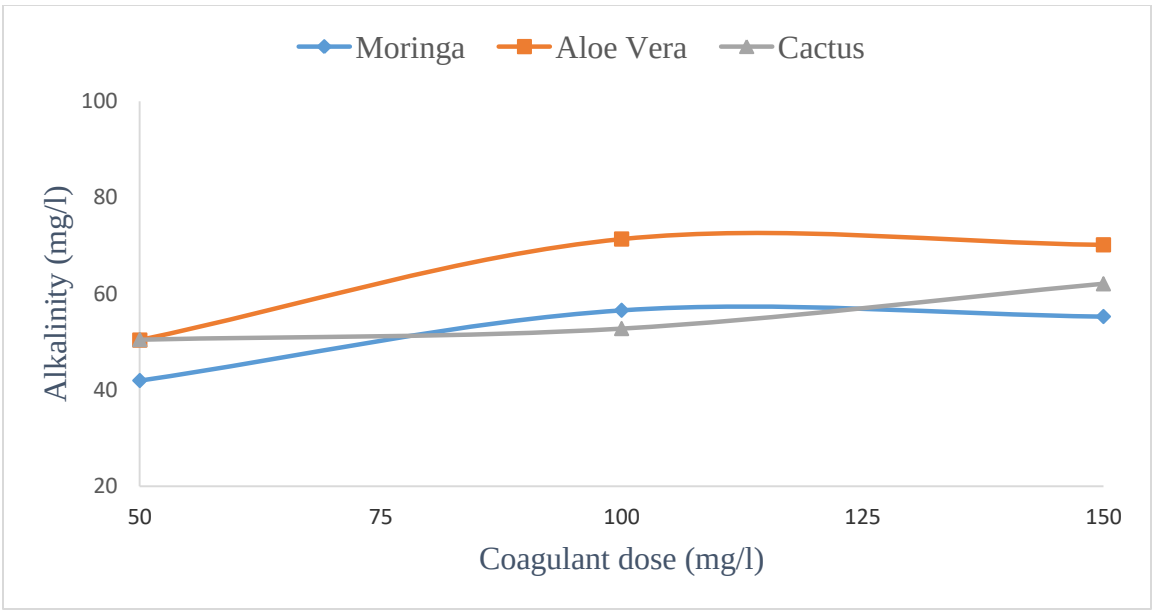
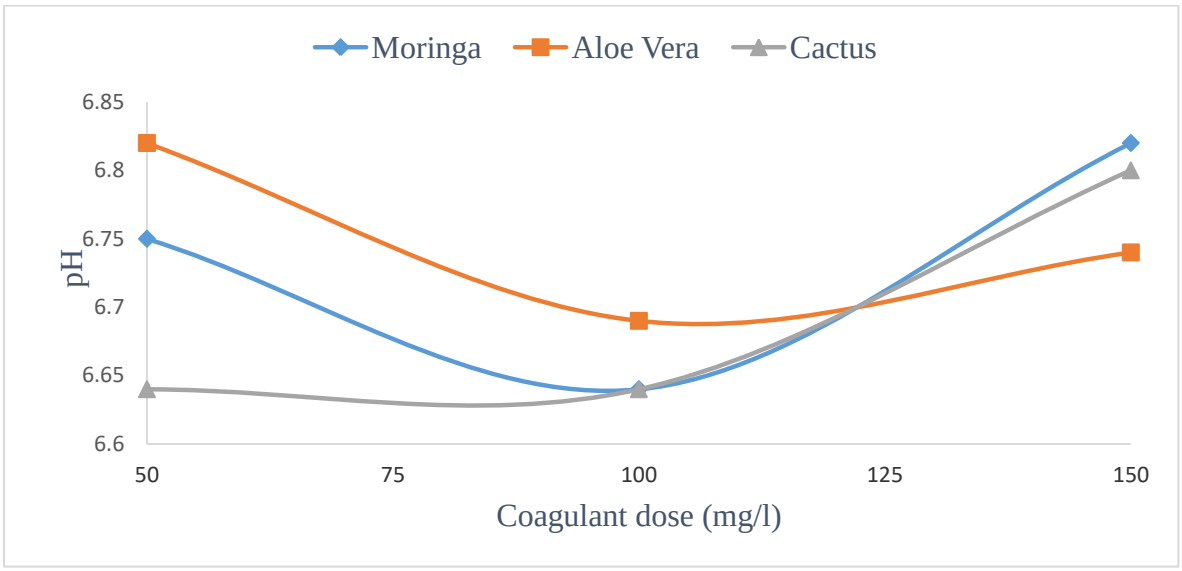


(c)

Figure 4:5 Effect of coagulant Dose on Alkalinity of the treated water (a) at pH of 6.5 (b) at pH of 7.5 & (c) at pH of 8.5



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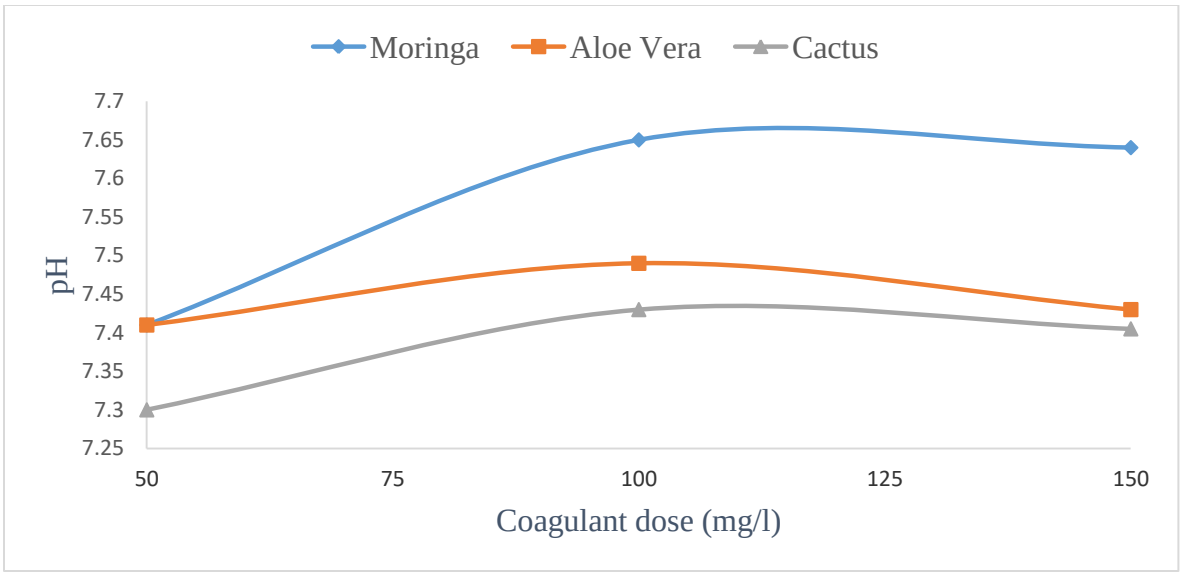

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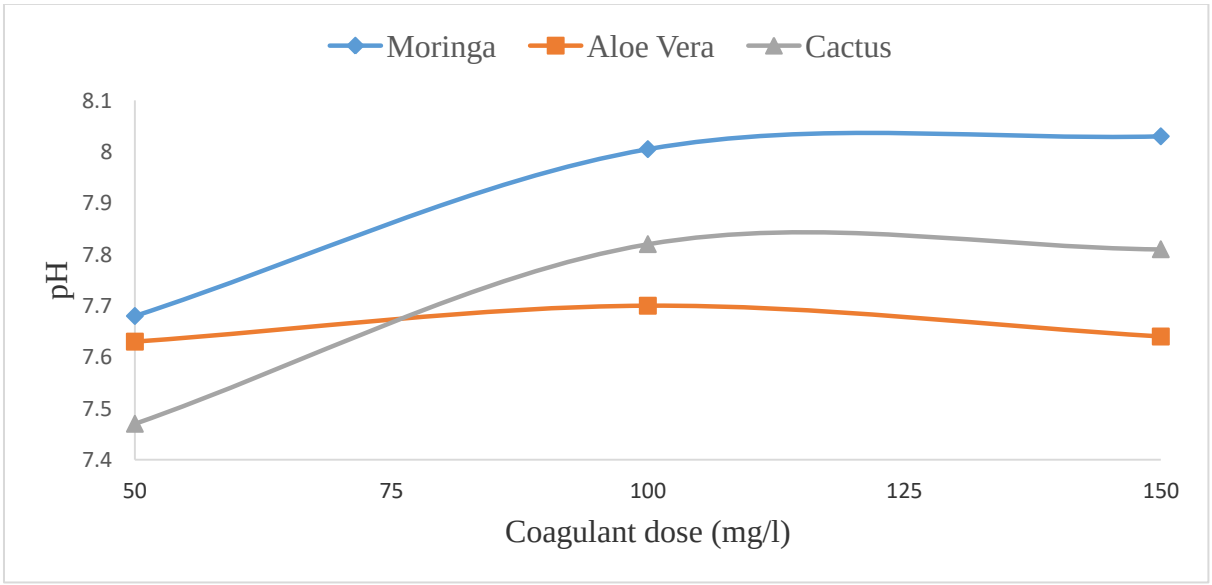


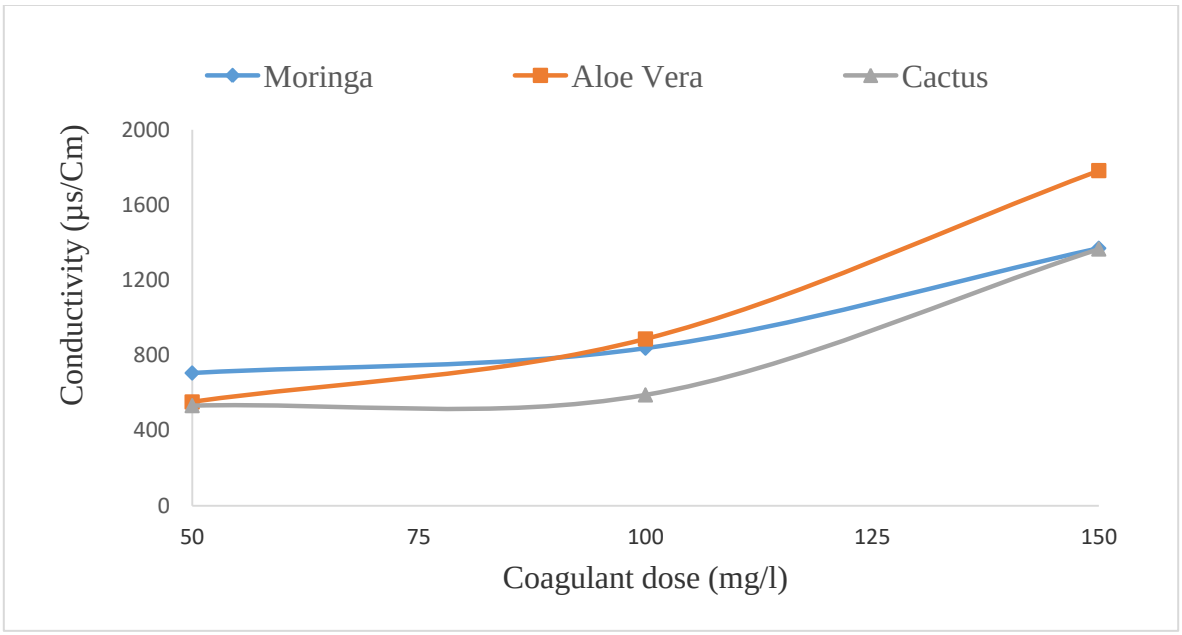
(c)

Figure 4:6 Dose of Coagulant versus response pH (a) at pH of 6.5 (b) at pH of 7.5 & (c) at pH of 8.5

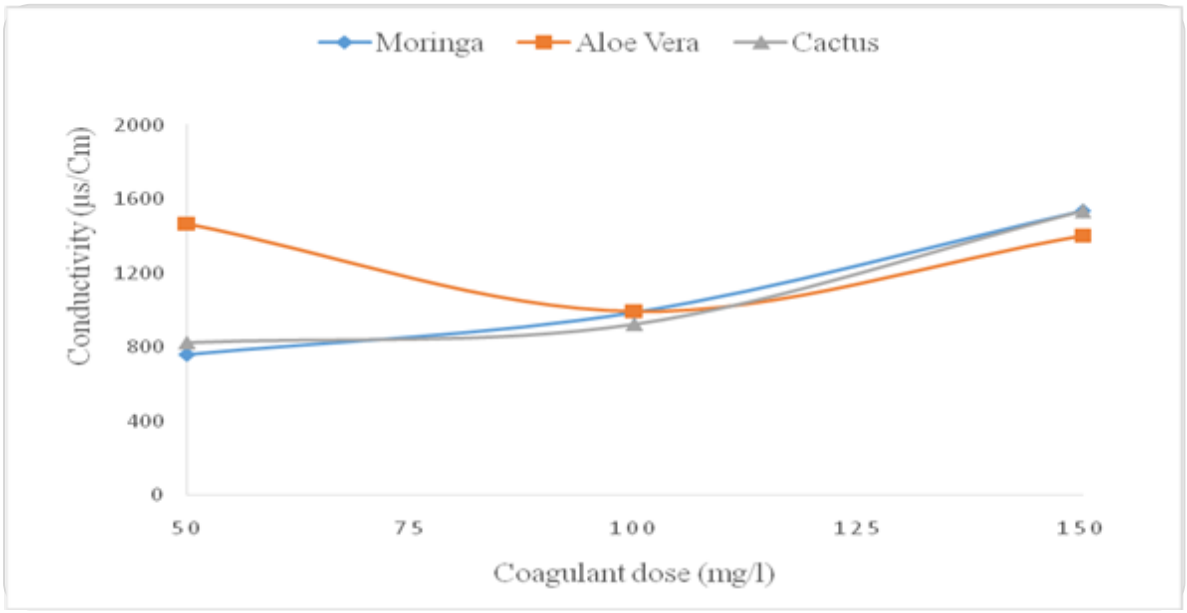
4.5.3. Effect of Bio-coagulants, dose, and pH on Conductivity

Conductivity was one of the vital parameters within the experiment similar to other studies (G.Muthuraman, 2013 Ramavandi, 2014 & VaraSaritha, 2019). Because the Conductivity of water is an important parameter to analyze in water treatment how much-dissolved

substances, chemicals, and minerals are present in water especially indicates the presence of phosphorus and nitrogen. A higher conductivity indicates higher impurities. Conductivity is a measure of the capacity of a watery solution to carry out an electric current. This ability depends on the presence of ions; their total concentration, mobility, and valency; and on the temperature of measurement. Conductivity was slightly increased for three bio-coagulants with an increased dose as presented in figure 4.7. At a pH of 6.5 as can be seen in figure 4.6, the amount of moringa, aloe Vera and cactus were increased from 50mg/l to 150 mg/l. the Conductivity (µs/cm) increased from 706 to 1369.5, 552 to 1782.5 & 532.5 to 1365.5 respectively as presented on table 4.3. As presented in figure 4.7.b. at a pH of 7.5, Conductivity was slightly increased for Moringa and Cactus bio-coagulant with increasing their dose. When the amount of Moringa and Cactus were increased from 50mg/l to 150 mg/l, Conductivity (µs/cm) was slightly increased from 757.5 to 1534 and 824.5 to 1536 respectively. Similarly, when the amount of Aloe Vera was increased from 50mg/l to 100mg/l, the Conductivity (µs/cm) decreased from 1467 to 994.5. But when the amount increased from 100mg/l to 150 mg/l, the Conductivity (µs/cm) increased from 994.5 to 1404.5. Conductivity was slightly increased for the Cactus bio-coagulant with increasing its dose is presented in figure 4.7.c. a pH of 8.5. Also, conductivity was slightly decreased for Moringa and aloe Vera bio-coagulants with increasing their dose from 50 mg/l to 100 mg/l. Be that as it may, when the dosage expanded from 100 mg/l to 150 mg/l, the Conductivity was slightly expanded. According to (G. Muthu Raman, 2013) Conductivity reported value was 1740 µs/cm studied on Moringa oleifera, S. potatorum, and P. Vulgaris. And in the United States, potable waters range from 50 to 1500 µs/cm in conductivity (APHA, 1998). Generally, the result of conductivity was increased when the pH of the water and the dose of bio-coagulants were increased this implies that those bio-coagulants did not affect the minimizing of Conductivity of water. According to (Franciele Pereira Camacho, 2016) this was due to the saline extraction of bio-coagulants, producing ions by dissociation of the coagulants (B.I. Gandiwa, 2020), and the sludge formation of the coagulants during the coagulation process (Hayelom Dargo Beyene, 2016).


Moringa
Aloe Vera
Cactus
Conductivity (µs/Cm)
2000
1600
1200
800
400
0
50
75
100
125
150
Coagulant dose (mg/l)

(a)


Moringa
Aloe Vera
Cactus
Conductivity (µs/Cm)
2000
1600
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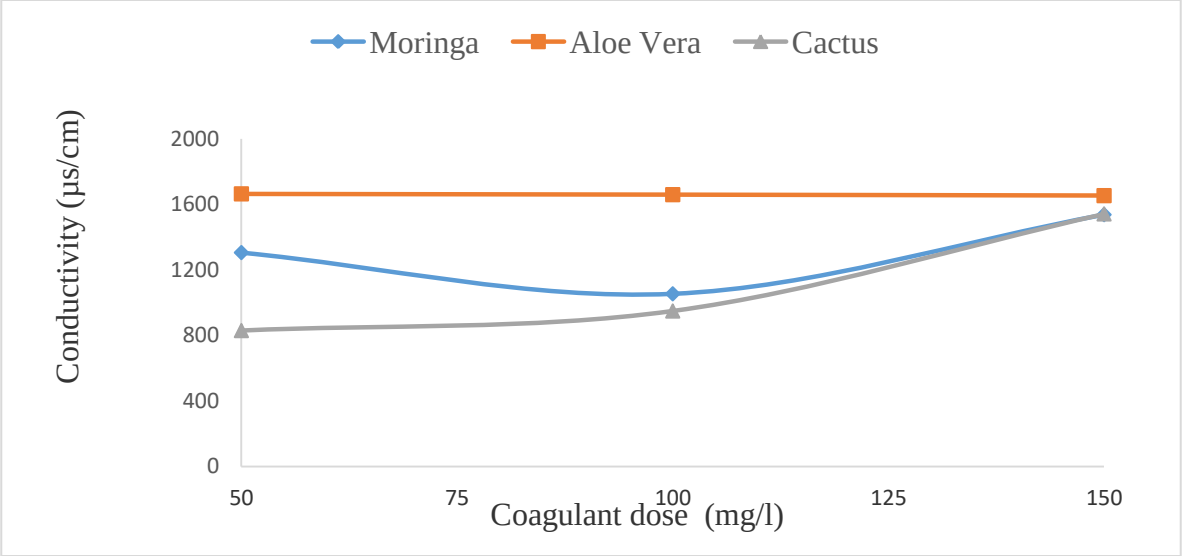
50
75
100
125
150
Coagulant dose (mg/l)

(b)



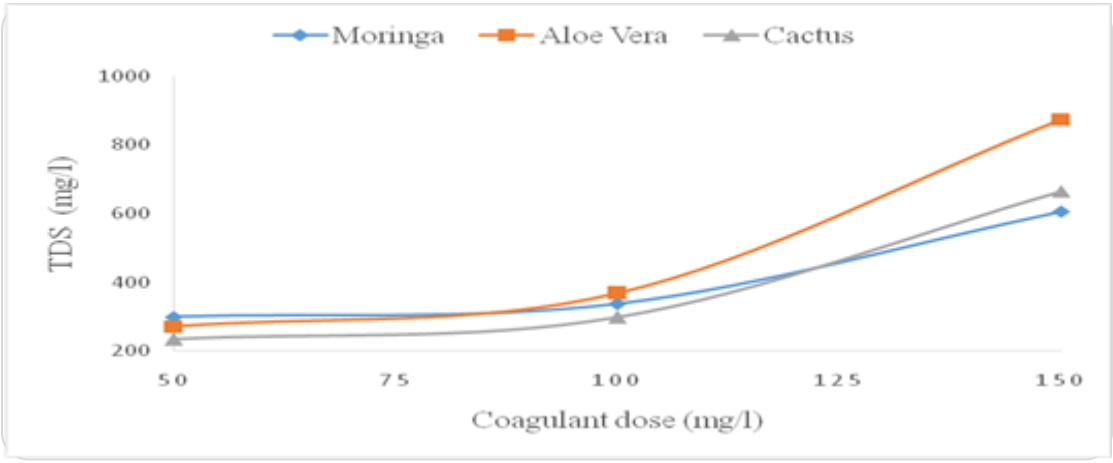
(c)

Figure 4:7: Dose of Coagulant versus Conductivity (a) at pH of 6.5 (b) at pH of 7.5 & (c) at pH of 8.5

4.5.4. Effect of Bio-coagulants, dose, and pH on Total dissolved solids

The concentration of dissolved minerals is utilized to express by Total dissolved solids and It includes organic matters, and inorganic salts and they contain carbonates, chlorides, sulfates, phosphates, and nitrates of calcium, magnesium, sodium, and potassium (A.J. Is sand O.M. Babiker, 2014). As figure 4.8 a. at a pH of 6.5, the Total dissolved solid was clearly increased for three bio-coagulants by increasing their dose. When the amount of moringa, aloe Vera and cactus bio-coagulants increased from 50mg/l to 150 mg/l, the Total dissolved solids (mg/l) increased to 299 to 606, 271.5 to 874, and 234 to 664.5 respectively. Total dissolved solids was slightly increased for Moringa and Cactus bio-coagulants with increasing their dose is presented in figure 4.8.b. at pH of 7.5. As it can be seen in figure 4.8, when the amount of Moringa and Cactus were increased from 50mg/l to 150 mg/l, the Total dissolved solids (mg/l) increased from 308.5 to 675 and 437 to 752 respectively. Similarly, when the amount of Aloe Vera was increased from 50mg/l to 100mg/l, the Total dissolved solids (mg/l) decreased from 712 to 478. But when the amount increased from 100mg/l to 150 mg/l, the Total dissolved solids (mg/l) increased from 478 to 724. As presented in figure

4.8.c. at pH of 8.5, when the amount of Aloe Vera and Cactus were increased from 50mg/l to 150 mg/l, the Total dissolved solids (mg/l) increased from 727.5 to 809 and 440 to 763 respectively. When the amount of Moringa was increased from 50 mg/l to 100mg/l, the Total dissolved solids (mg/l) decreased from 717 to 533.5. But when the amount increased 100mg/l to 150 mg/l, the Total dissolved solids (mg/l) increased from 533.5 to 757. In this study, when the pH and dosage of bio-coagulants were expanded, total dissolved solids also increased. According to (J. Sanchez-Martin, 2010) stated that excess protein and other organics of TDS are due to applying the high dosage of bio-coagulants.



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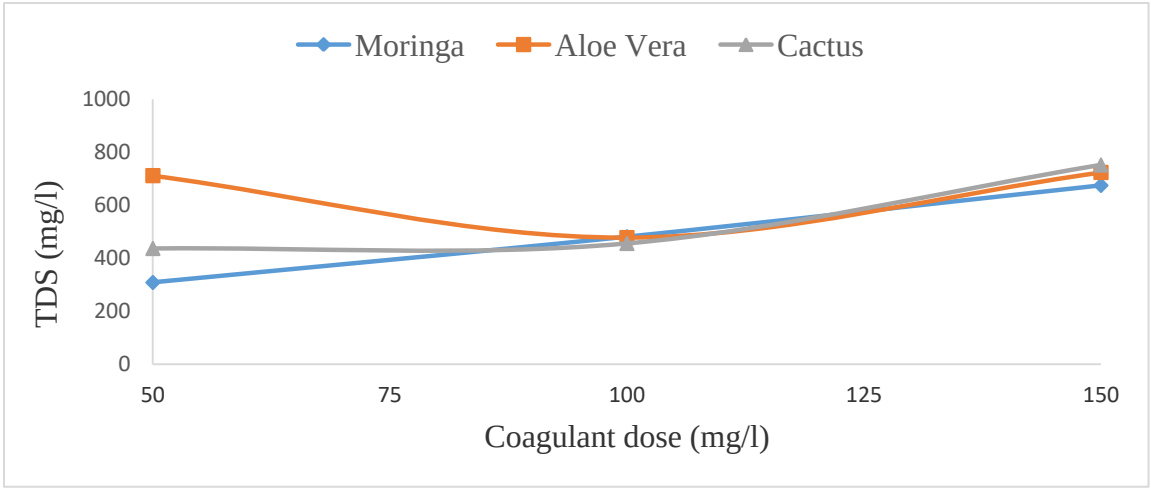

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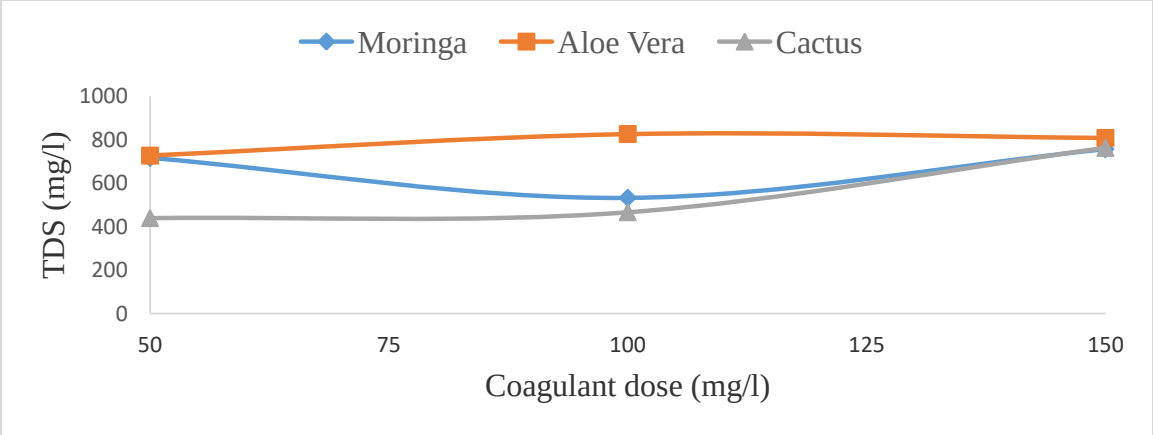


(c)

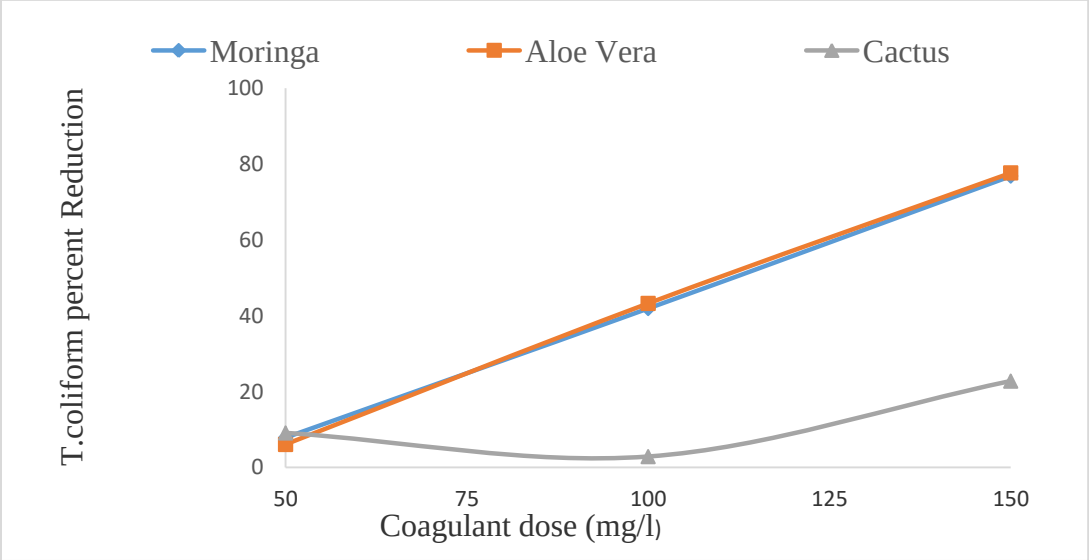
Figure 4:8 Dose of Coagulant versus TDS (a) at pH of 6.5 (b) at pH of 7.5 & (c) at pH of 8.5

4.6. Factors affecting Microbials

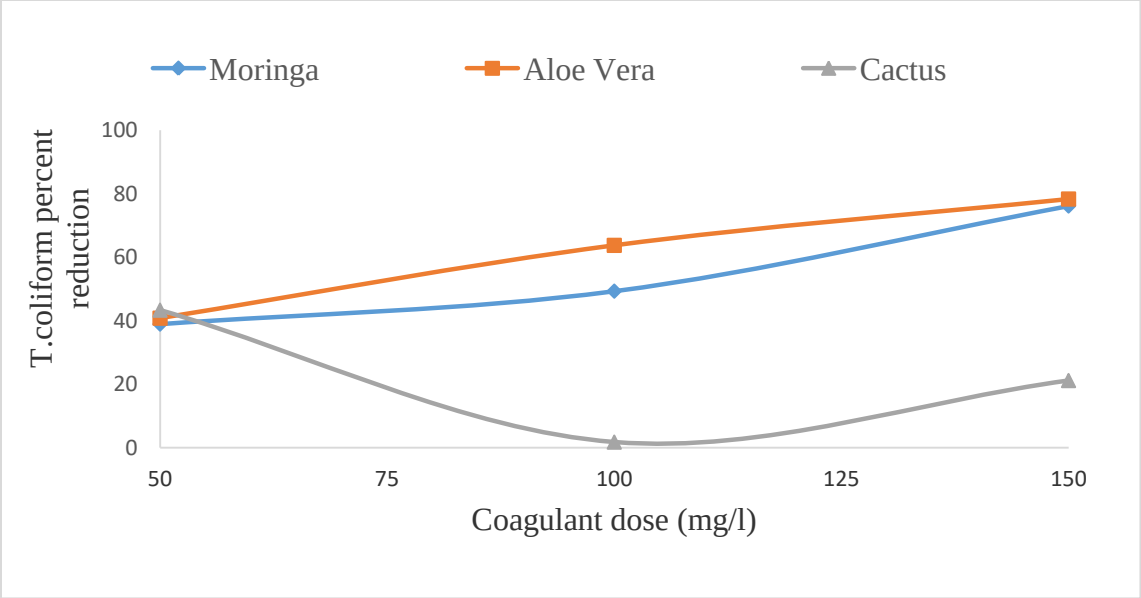
4.6.1. Effect of Bio-coagulants, dose, and pH on Total coliform reduction

Plate count agar solution was utilized to get ready the plate media for Total coliform. The plate media was inoculated by the three-serial dilution of samples and incubated for 24 hours at 37 ^{o}C within the incubator. The colony on the plate was numbered by colony counter and communicated by the colony-forming unit (CFU). The total coliform reduction was clearly increased for moringa and aloe Vera bio-coagulants with increasing their dose is presented in figure 4.9. As it can be seen in figure 4.8, when the amount of moringa and aloe Vera increased from 50mg/l to 150 mg/l, the Total coliform percent reduction increased from 7.84 to76.815 &6.07 to 77.655 respectively. In the same way, when the amount of cactus was increased from 50mg/l up to 100mg/l, the Total coliform reduction slightly decreased from 9.105 to 2.865 and when the amount increased from 100mg/l to 150 mg/l, the Total coliform reduction was increased from 2.865 to 22.765. The total coliform reduction was slightly increased for moringa and aloe Vera bio-coagulants with increasing their dose is presented in figure 4.9. b. at pH of 7.5. As it can be seen in the figure, when the amount of moringa and aloe Vera were increased from 50mg/l to 150 mg/l, the Total coliform reduction increased from 38.955 to 76.14 and 40.81 to 78.33 respectively. In the same way, when the amount of cactus was increased from 50mg/l up to 100mg/l, the Total coliform reduction decreased

from 43.335 to 1.77 and when the amount increased from 100mg/l to 150 mg/l, the Total coliform reduction was increased from 1.77 to 21.165. The total coliform reduction was clearly increased for moringa and aloe Vera bio-coagulants with increasing their dose is presented in figure 4.9. a. at pH of 6.5. As it can be seen in figure 4.9, when the amount of Moringa and Aloe Vera was increased from 50mg/l to 150 mg/l, the Total coliform reduction increased from 46.035 to 77.655 and 43.845 to 75.63 respectively. When the quantity of Cactus was increased from 50 mg/l to 100mg/l (figure 4.9), the Total coliform reduction decreased from 5.57 to 4.14 and when the amount increased from 100mg/l to 150 mg/l, the Total coliform reduction was increased from 4.14 to 22.85 (table 4.3). Table 4.3 showed that the result of the Total coliform reduction maximum value was 77.66 % achieved by using Aloe Vera bio-coagulant at pH of 6.5 and a dose of 150 mg/l. A similar study on moringa oleifera (Abatneh Y. et al 2014) and Plantago ovata reported (Ramavandi, 2014) highly eliminated microorganisms with loss of their structure.



(a)



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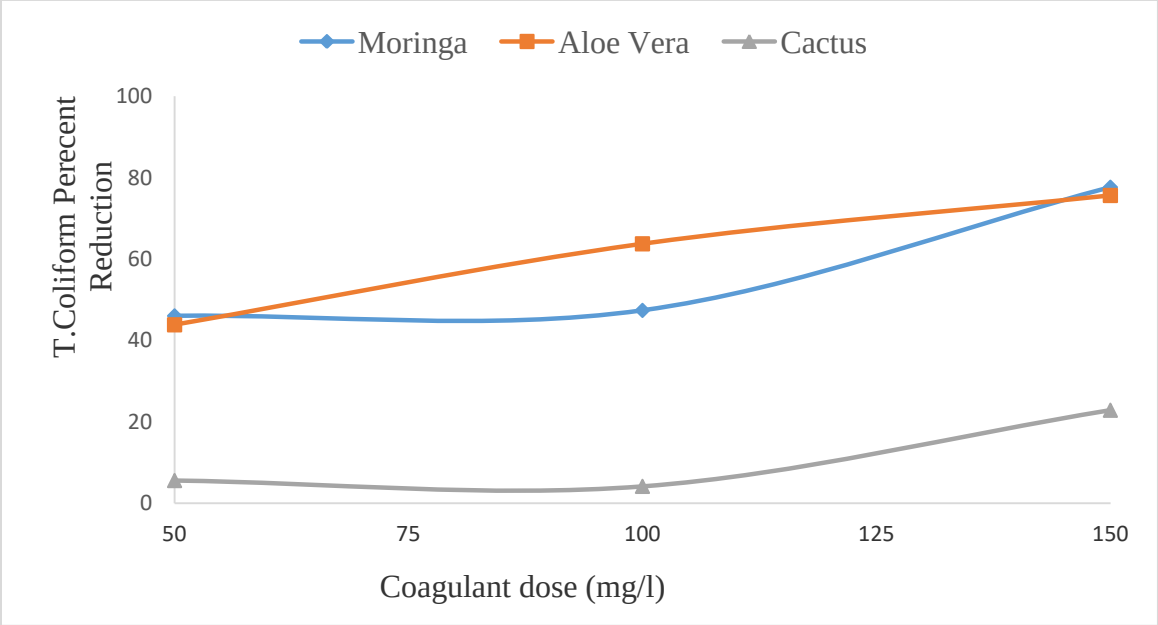


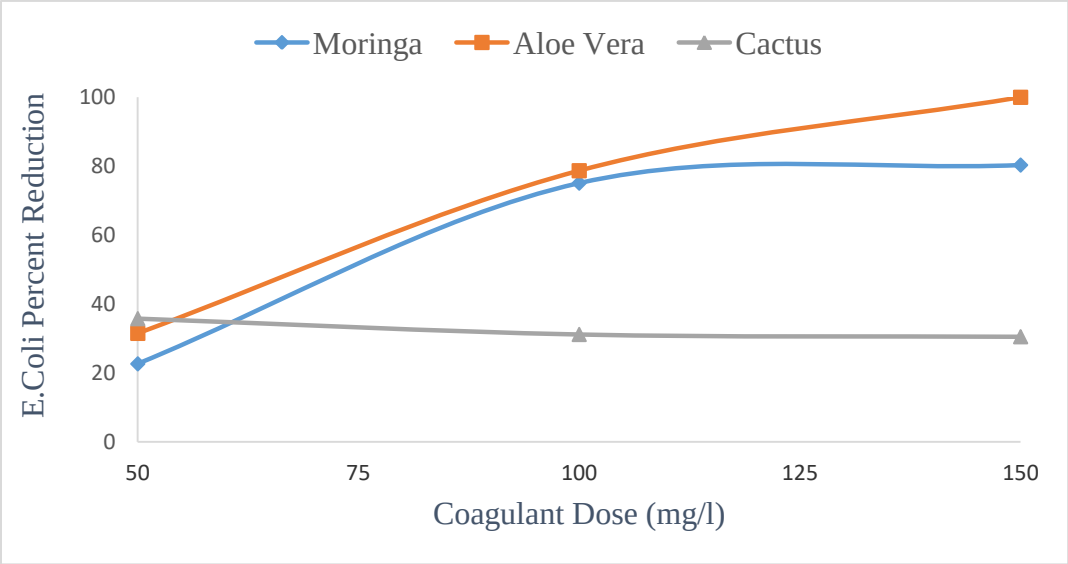
(c)

Figure 4:9 Plot Dose of Coagulant versus T. Coliform percent reduction (a) at pH of 6.5 (b) at pH of 7.5 & (c) at pH of 8.5

4.6.2. Effect of Bio-coagulants, dose, and pH on E. coli reduction

E. coli reduction was one of the critical parameters within the test. EMB agar solution was used to prepare the plate media of E. coli. The plate media was immunized by the serial dilution of samples and incubated for 24 hours at 37oC on the incubator. The colony on the plate was tallied by the colony counter and expressed by the colony-forming unit (CFU). The E. coli reduction was clearly increased for moringa and aloe Vera bio-coagulants with increasing their dose is presented in figure 4.10.a. pH of 6.5. When the amount of moringa and aloe Vera bio-coagulants were increased from 50mg/l to 150 mg/l (figure 4.10), the E. coli reduction increased from 22.625 to 80.325 and 31.475 to completely remove respectively. Although when the amount of cactus bio-coagulant was increased from 50mg/l to 150 mg/l, the E. coli reduction decreased from 35.735 to 30.495 as presented in table 4.3. The E. coli reduction was clearly increased for moringa bio-coagulant with increasing its dose is presented in figure 4.10.b. the pH of 7.5. When the quantity of moringa bio-coagulant was increased from 50mg/l to 150 mg/l, the E. coli reduction was increased from 40.655 to completely removed. when the amount of aloe Vera bio-coagulant was enhanced from 50mg/l to 100mg/l, the E. coli reduction increased from 38.685 to completely removed, and when the amount increased to 100mg/l up to 150 mg/l, the E. coli reduction decreased from not detected (N.D) to 72.95. When the amount of Cactus as a bio-coagulant was improved from 50mg/l to 150 mg/l, the E. coli reduction decreased from 44.92 to 28.36 (table 4.3). According to figure 4.10.c. at a pH of 8.5, The E. coli reduction was clearly increased for moringa bio-coagulant by increasing its dose. When the amount of moringa bio-coagulant was increased from 50mg/l to 150 mg/l, the E. coli reduction was increased from 42.13 to not detected (N.D). Even if the amount of aloe Vera bio-coagulant was increased from 50mg/l up to 100mg/l, the E. coli reduction increased from 40.655 to 78.035. And when the amount was increased from 100mg/l to 150 mg/l, the E. coli reduction was maximized from not detected to 73.28. When the quantity of Cactus bio-coagulant was increased from 50mg/l to 100mg/l, the E. coli reduction was minimized from 35.085 to 26.725. When the amount of Cactus bio-coagulant was increased from 100mg/l to 150 mg/l, the E. coli reduction was maximized from 26.725 to 27.54. Table 4.3 showed that result of E. coli reduction maximum value was completely removed by using Moringa and Aloe Vera bio-coagulants at pH of 6.5 and 7.5 on

the dose of 150 mg/l. According to (Charlotte Farrell, 2017) studied 86% of E. coli was removed by attached Fe (ll). The maximum E. coli reduction was completely removed by using Moringa and Aloe Vera bio-coagulants in the dose of 100mg/l and 150mg/l.



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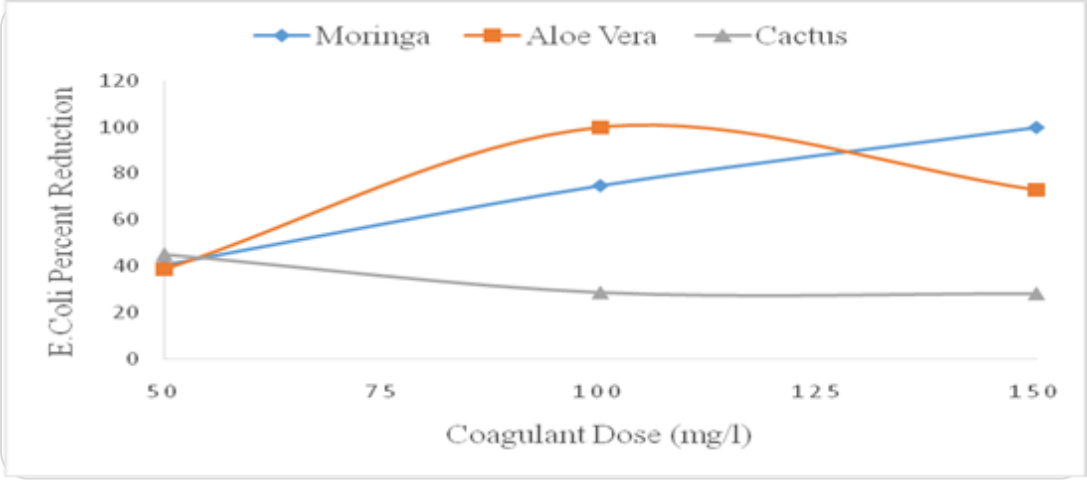

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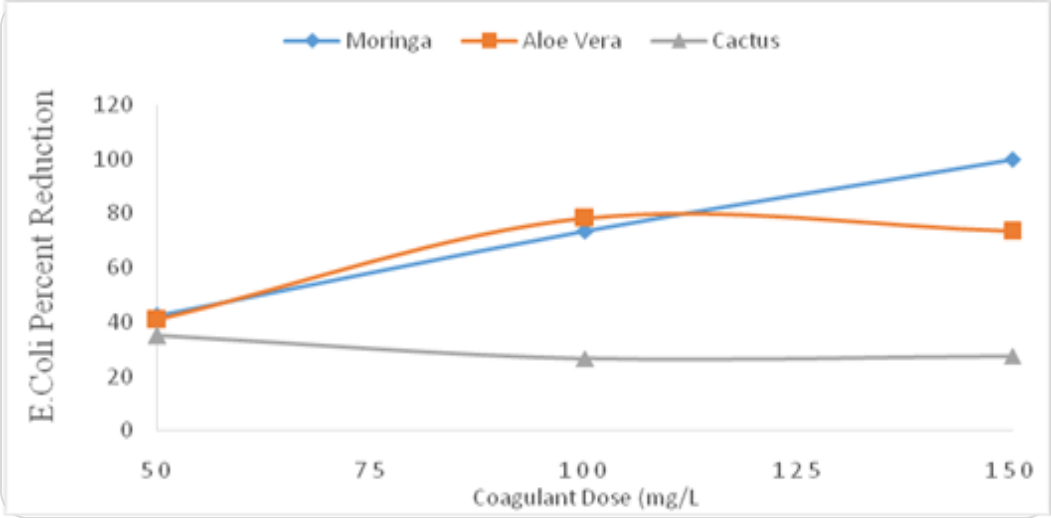


(c)

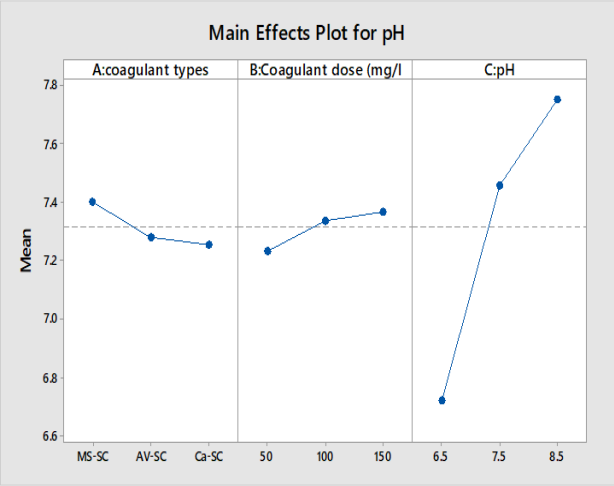
Figure 4:10 Dose of Coagulant versus E. coli percent reduction (a) at pH of 6.5 (b) at pH of 7.5 & (c) at pH of 8.5

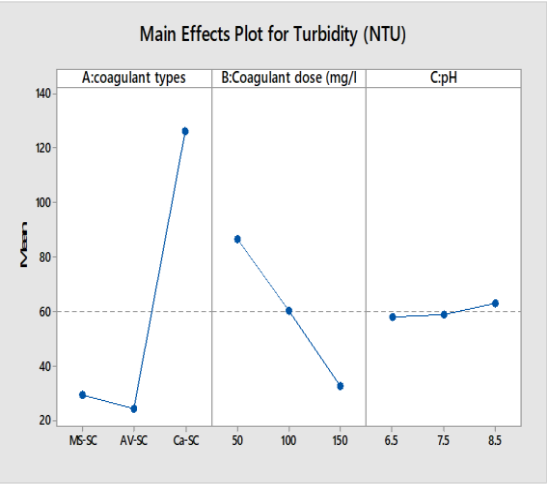
4.7. Statistical optimization of the treatment process

4.7.1. Main effects

According to the ANOVA table generated from SPSS and factorial method from design expert software the response of pH, significantly (([F (2,53)] =7.256; p= 0.002), ([F (2,53) = 89.801; P=000), ([F (2,53) = 28.837; P=000) ([F (2,53) = 9.542; P=000) and ([F (2,53) = 4.937; P=013)) affected by all individual factors and the interaction between (coagulant type and dose; pH and dose) of treated water respectively (see in figure 4.11 & annex table7.5). The main factors of Coagulant type and dose; the interaction between (coagulant type and pH) significantly ([F (2, 53)] =22.774; p= 0.000) and ([F (2, 53) = 80.591; P=000) and ([F (4, 53) = 3.081; P=028)) affected turbidity reduction efficiency respectively (see in figure 4.11& annex table7.5). The analysis of variance is shown in annex table7.7, the main effects of coagulant type, pH, coagulant dose and their interactions were significantly ([F (2,53)] = 13.701; P=000), ([F (2,53)] = 11.395; P=000) and ([F (1,53)] = 100.455; P=0.000) effect on the alkalinity of water respectively (see in figure 4.11). the main effects coagulant type, pH, coagulant dose and their interactions were significantly ([F (2,53)] = 11.486; P=000), ([F

(2,53)] = 23.925; P=000) and ([F (1,53)] = 105.270; P=0.000) affected conductivity of water respectively (see annex table7.8) The analysis of variance in the annex table 7.9, indicated that the main effects of coagulant type, pH, coagulant dose and the interactions between (dose and pH; coagulant type and pH) were significantly ([F (2,53)] = 5.623; P=007), ([F (2,53)] = 22.793; P=000), ([F (1,53)] = 83.975; P=000) and ([F (2,53)] = 8.765; P=001) & ([F (4,53)] = 4.859; P=003) affected total dissolved solids of water respectively (see in figure 4.11). the main effects of pH, coagulant dose and their interaction between (coagulant type and dose; pH and dose ;coagulant type and pH) were significantly ([F (2,53)] = 28.139; P=000), ([F (1,53)] = 163.039; P=000), ([F (2,53)] = 33.654; P=000), ([F (2,53)] = 16.320; P=000), ([F (4,53)] = 5.888; P=001)([F (2,53)] = 33.654; P=000), ([F (4,53)] = 5.888; P=001) & ([F (2,53)] = 16.320; P=000) affected on the total coliform percent reduction respectively (see annex table7.10). As shown in annex table 7.12, the main effect of coagulant dose and the interaction between (coagulant type and dose) were a significantly ([F (1, 53)] = 41.645; P=000) and ([F (2, 53)] = 18.834; P=000) affected on the E. coli percent reduction respectively (see figure 4.11).





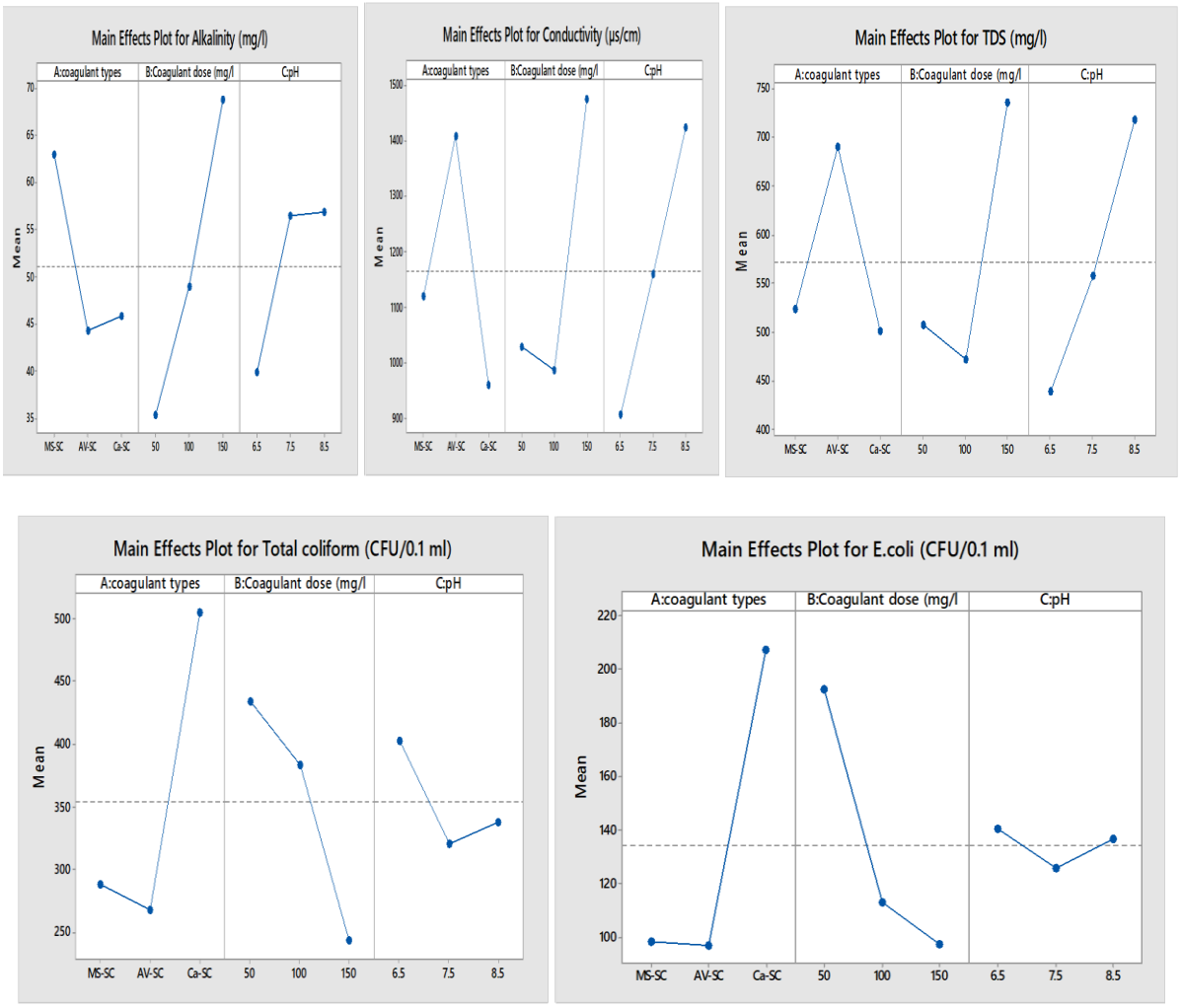


Figure 4.11: The main effect of the factors

4.7.2. Evaluation of factorial model

There are 54 sets of experimental runs obtained from the design as shown in annex table 7.1 which contains three factors with seven responses. The designed table was set up utilizing Design Expert v7.0.0 software with General factorial design. And the SPSS Software version 20 was utilized in this study to analyze the experimental data. The correlation result is attached in annex table 7.4.

Analysis of variance (ANOVA) with a 95% confidence interval was utilized to decide the statistical significance of the examination. ANOVA was utilized to compare the mean and evaluate the significance of the main impact and their interaction. The coefficient of R squared value was obtained from the ANOVA table 4.4, the value of pH, turbidity, alkalinity,

conductivity, TDS, T. coliform, and E. coli which are 0.9937, 0.9816, 0.906, 0.877, 0.9001, 0.9680, and 0.9565 respectively. The coefficient of R squared values shows that there's high dependence and relationship between the experimental variables and anticipated values of the dependent variable (P. Wan, 2003), and the created model is better to foresee the reaction when R^2 is closer to 1. And the R^2 value greater than 0.75 is considered worthy (Shak, 2014). Subsequently, the models are acknowledged for all dependent factors.

Table 4.4: ANOVA results for response models

Response	***pH***	***turbidity***	***alkalinity***	***conductivity***	***TDS***	***T. coliform***	***E. coli***
R-Squared	*0.9937*	*0.9879*	*0.906*	*0.877*	*0.9001*	*0.9680*	*0.9565*
AdjR-Squared	*0.9904*	*0.9816*	*0.861*	*0.819*	*0.8472*	*0.9511*	*0.9335*
Pred.R-Squared	*0.9842*	*0.9695*	*0.6754*	*0.5424*	*0.7480*	*0.9193*	*0.8902*
Adeq. Precision	*51.102*	*35.392*	*14.701*	*18.903*	*16.770*	*22.416*	*17.53*
C.V. %	*0.62*	*8.49*	*2.51*	*11.32*	*2.72*	*0.99*	*2.33*

As shown in Table 4.4, the R-squared for the dependent variable models is explained about 99.37%, 98.16%, 90.6%, 87.7%,90.01%, 96.8%and 95.65% percent of variation on the value of response pH, turbidity, alkalinity, conductivity, TDS, T. coliform, and E. coli respectively. This is often contributed by the main effect of coagulant type, pH, dosage, and their interaction. The remaining percent of the variety has not been clarified by the response model. The measure of Adequate Precision of the response models were 54.464, 30.373, 14.701, 18.903, 16.770, 22.416, and 17.53 for pH, turbidity, alkalinity, conductivity, TDS, T. coliform, and E. coli model respectively. These values represent the degree of signal-to-noise proportion and a proportion greater than 4 is desirable. Hence, in this study, the Adequate Precision value for all response models was more than four demonstrating that those models are desirable and give a satisfactory signal utilized for exploring within the design space. The coefficient variation is defined as the standard deviation divided by the mean. As it can be seen in table 4.4, the CV% results for pH, turbidity; alkalinity, conductivity, TDS, T. coliform, and E. coli are 0.49, 3.79, 2.51, 11.32, 2.72, 0.99, and 2.33%, respectively. This

indicates that all responses except conductivity were achieved CV less than 10%, which indicates that the experimental results are precise and reliable (Lanan Fabm, 2020).

4.7.3. Interaction effects

The interaction between all factors in the two-way form has a synergetic effect on the pH of treated water is presented in figure 4.12. As it can be seen in figure 4.12, the interaction between coagulant type and coagulant dose, coagulant type and pH, and also coagulant dose and pH have significantly impacted the pH of treated water. Hence, the pH of the treated water solution was slightly increased for MS-SC and Ca-SC when the dose increased from 50 mg/l to 100 mg/l. The interaction performance was varying greatly at the dose of 150 mg/l by using AV-SC. The pH of treated water was increased for all bio-coagulants and doses when the pH increased from 6.5 to 7.5 and the performance varies at the pH of 8.5.

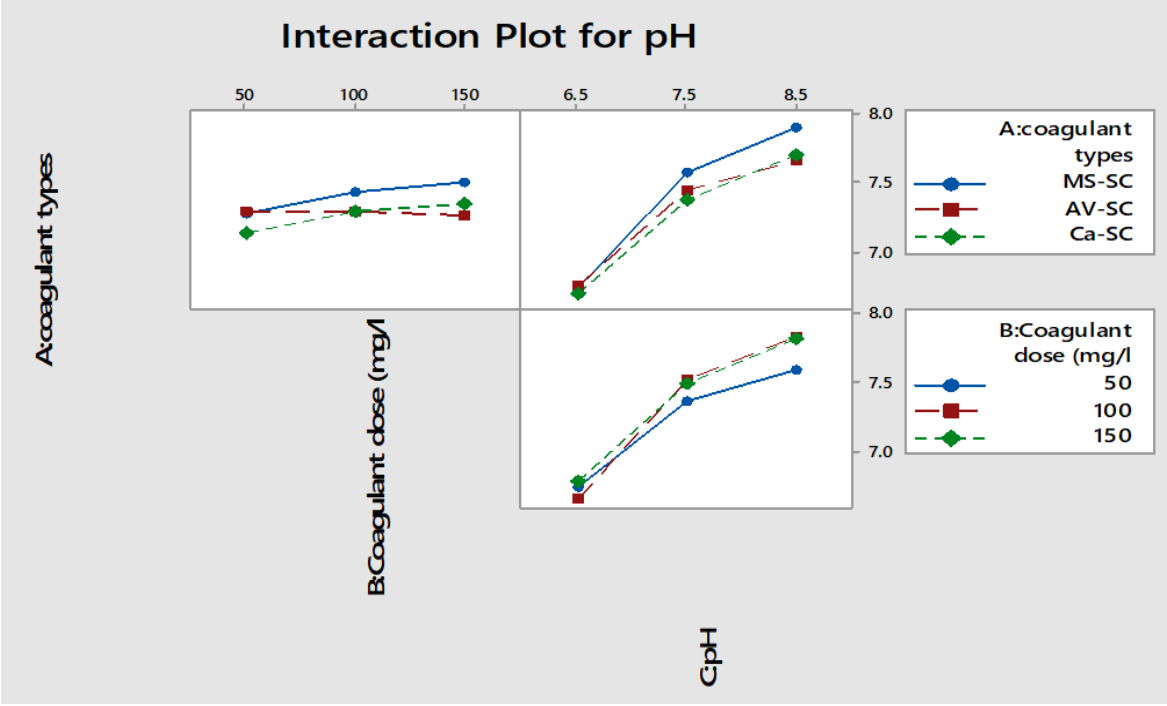


Figure 4.12. The interaction effect of factors on pH of treated water

The interaction between coagulant type and coagulant dose; coagulant type and pH (MS-SC and AV-SC) were significantly impacted with turbidity removal as presented in figure 4.12. As it can be seen in figure 4.13, turbidity removal was to some extent decreased for MS-SC and AV-SC with their dose increased from 50 mg/l to 150 mg/l. However, the Ca-SC did not

interact with other bio-coagulants. The turbidity removal was increased by using a Ca-SC bio-coagulant when the dose of bio-coagulants increased from 50 mg/l to 100 mg/l. but the turbidity was clearly decreased when its dose increased from 100mg/l to 150 mg/l. When the dose increased, the turbidity removal was reduced. All three types of bio-coagulants show much lower turbidity of water solution at a dose of 150 mg/l. However, the performance varies greatly at a dose of 100 mg/l of Ca-SC. MS-SC bio-coagulant seems to perform good turbidity removal at the dose of 150 mg/l and pH of 6.5. The turbidity removal was slightly decreased for MS-SC and AV-SC bio-coagulants when the pH increased from 6.5 to 8.5. The AV-SC bio-coagulant seems to perform good turbidity removal at a pH of 8.5. There was no interaction between pH and coagulant dosage since the line is parallel. But the 150 mg/l dose has good performance at a pH of 6.5.

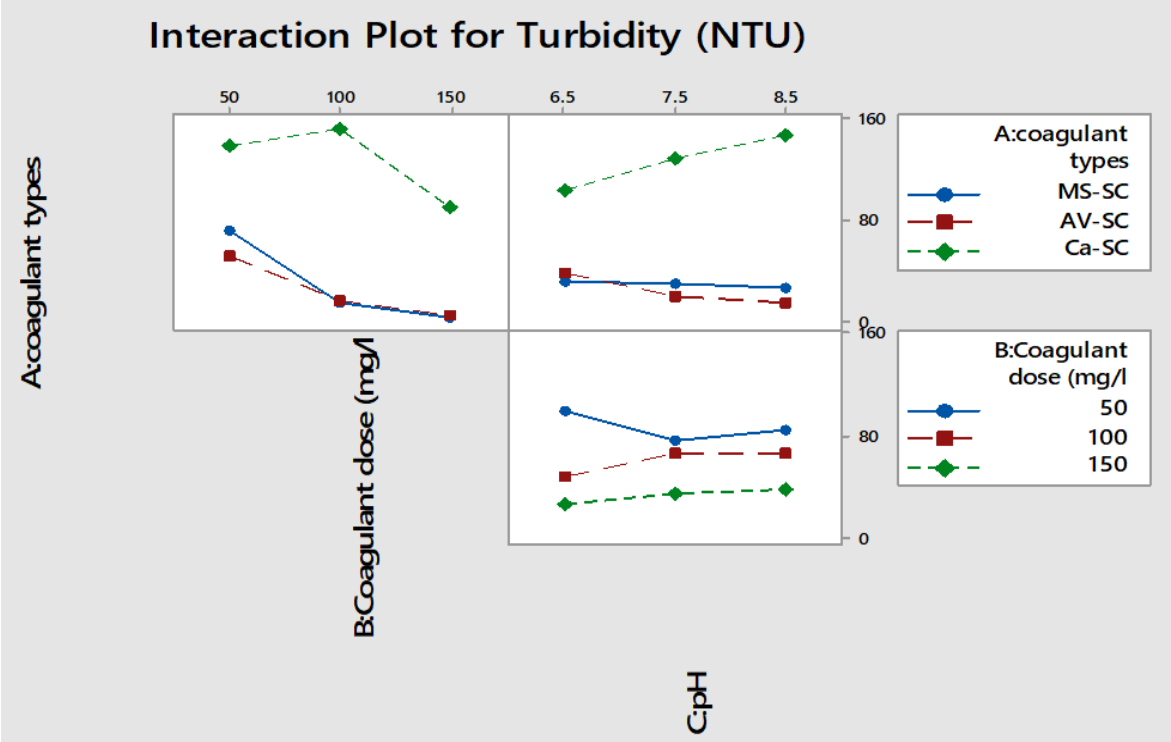


Figure 4.13. The interaction effect of factors on turbidity

The interaction between all factors in the two-way form has a synergetic effect on the alkalinity of treated water is presented in figure 4.14. The alkalinity of the water solution was to a few degrees expanded by utilizing MS-SC and AV-SC bio-coagulants when the measurements expanded from 50mg/l to 100 mg/l. But alkalinity was clearly increased

and there was no interaction when the dose increased from 100mg/l to 150 mg/l for MS-SC bio-coagulant. When the dose increases, MS-SC bio-coagulant is affected. MS-SC bio-coagulant shows a much higher alkalinity of water solution at a dose of 150 mg/l, and much lower alkalinity at a dose of 50 mg/l. However, AV-SC and Ca-SC bio-coagulants show much higher alkalinity of water solution at a dose of 100 mg/l, and much lower alkalinity at a dose of 50 mg/l. MS-SC bio-coagulant performance varies greatly at a dose of 150 mg/l and AV-SC bio-coagulant seems to perform low alkalinity of water at a dose of 50 mg/l. As it can be seen in figure 4.13, Alkalinity was clearly increased by using all bio-coagulants when the pH of water increased from 6.5 to 7.5. And the Alkalinity of a water solution was decreased when the pH of water increased from 7.5 to 8.5. When the pH of water increases, MS-SC bio-coagulant is affected by the pH of water increment. AV-SC and Ca-SC bio-coagulants show much higher Alkalinity of water solution at pH of 7.5 &8.5, and much lower alkalinity at pH of 6.5. However, MS-SC bio-coagulant shows higher Alkalinity at pH of 7.5, and much lower Alkalinity at pH of 8.5. The performance of MS-SC bio-coagulant varies greatly at a pH of 7.5 and Ca-SC bio-coagulant seems to perform well at a pH of 6.5. There was no interaction between pH and coagulant dose since the line is parallel. But the 50 mg/l dose has good performance at a pH of 6.5.

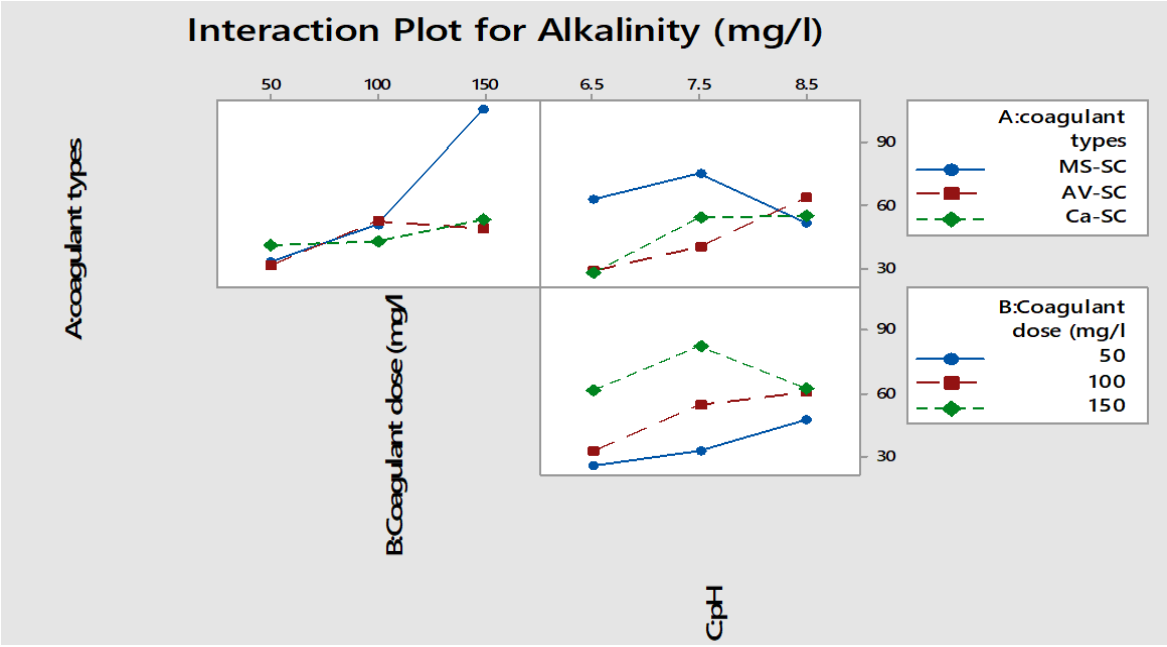


Figure 4.14. The interaction effect of factors on alkalinity

The interaction between all factors in the two-way form has a significant impact on the conductivity of treated water. As it can be seen in figure 4.15, the interaction between coagulant dose and pH has a synergetic effect on conductivity. So, as the graph indicated that conductivity of water solution was to some extent increased by using three bio-coagulants when the dose increased from 50 mg/l to 150 mg/l. But there was no interaction between coagulant type and dose, coagulant type, and pH. when the dose increases, AV-SC bio-coagulant is affected. MS-SC bio-coagulant shows much higher conductivity of water solution at a dose of 150 mg/l, and much lower conductivity at a dose of 50 mg/l. However, AV-SC and Ca-SC bio-coagulants show much higher conductivity in water solution at a dose of 150 mg/l, and much lower conductivity at a dose of 100 mg/l. AV-SC bio-coagulant performance varies greatly at a dose of 100 mg/l and Ca-SC bio-coagulant seems to perform low conductivity of water at a dose of 50 mg/l. As it can be seen in figure 4.14, conductivity was clearly increased by using all bio-coagulants when the pH of water increased from 6.5 to 8.5. when the pH of water increases, 50 mg/l of dose of bio-coagulant is affected by the pH of water increment. 100 mg/l and 150 mg/l of bio-coagulants show much higher conductivity of water solution at pH of 8.5, and much lower conductivity at pH of 6.5. However, 50 mg/l doses of bio-coagulant show higher conductivity at pH of 8.5, and much lower conductivity at pH of 6.5. The 50 mg/l dose of bio-coagulants seems to perform good conductivity of water.

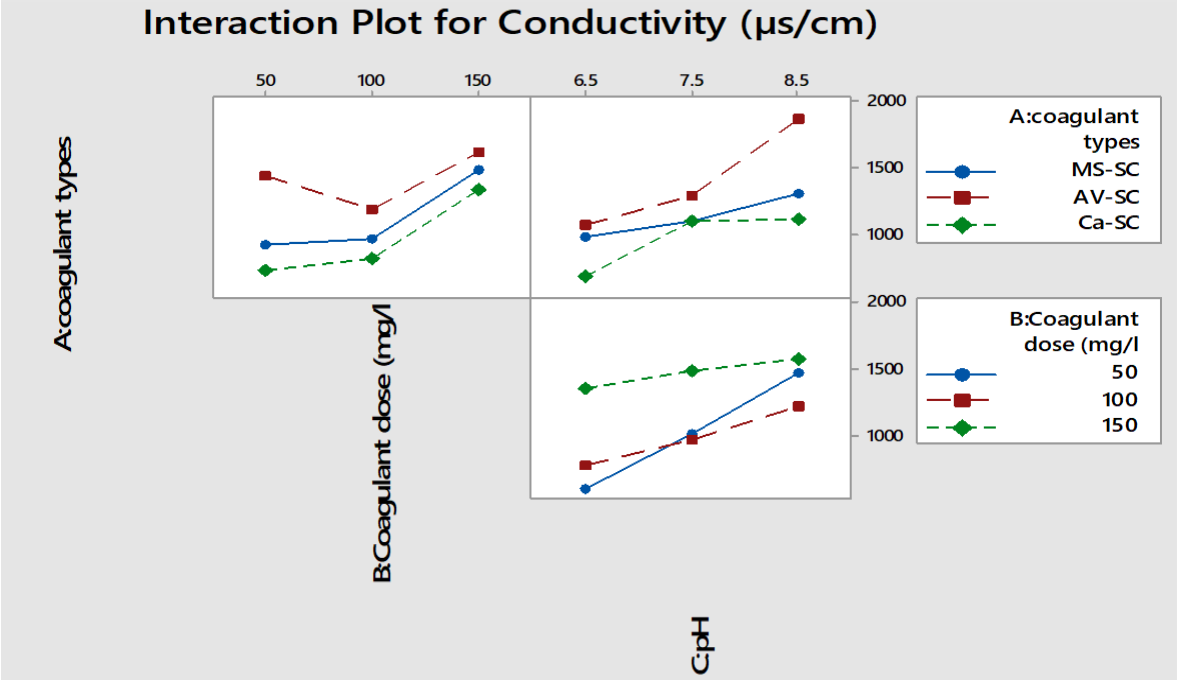


Figure 4.15. The interaction effect of factors on conductivity

The interaction between all factors in the two-way form has a significant effect on the TDS of treated water as presented in figure 4.16. As it can be seen in figure 4.16, the TDS of water solution was to some extent increased by using three bio-coagulants when the dose increased from 50mg/l to 150 mg/l. when the dose increases, AV-SC bio-coagulant is affected. MS-SC bio-coagulant shows much higher TDS of water solution at a dose of 150 mg/l, and much lower TDS at a dose of 50 mg/l. However, AV-SC and Ca-SC bio-coagulants show much higher alkalinity of water solution at a dose of 150 mg/l, and much lower TDS at a dose of 100 mg/l. AV-SC bio-coagulant performance varies greatly at a dose of 100 mg/l and Ca-SC bio-coagulant seems to perform low TDS of water at a dose of 50 mg/l. As it can be seen in figure 4.16, TDS was clearly increased by using all bio-coagulants when the pH of water increased from 6.5 to 8.5. when the pH of water increases, 50 mg/l of dose of bio-coagulant is affected by the pH of water increment. 100 mg/l and 150 mg/l of bio-coagulants show much higher TDS of water solution at pH of 8.5, and much lower alkalinity at pH of 6.5. However, 50 mg/l doses of bio-coagulant show higher TDS at a pH of 8.5, and much lower TDS at a pH of 6.5. The 50 mg/l dose of bio-coagulants seems to perform good TDS of treated water.

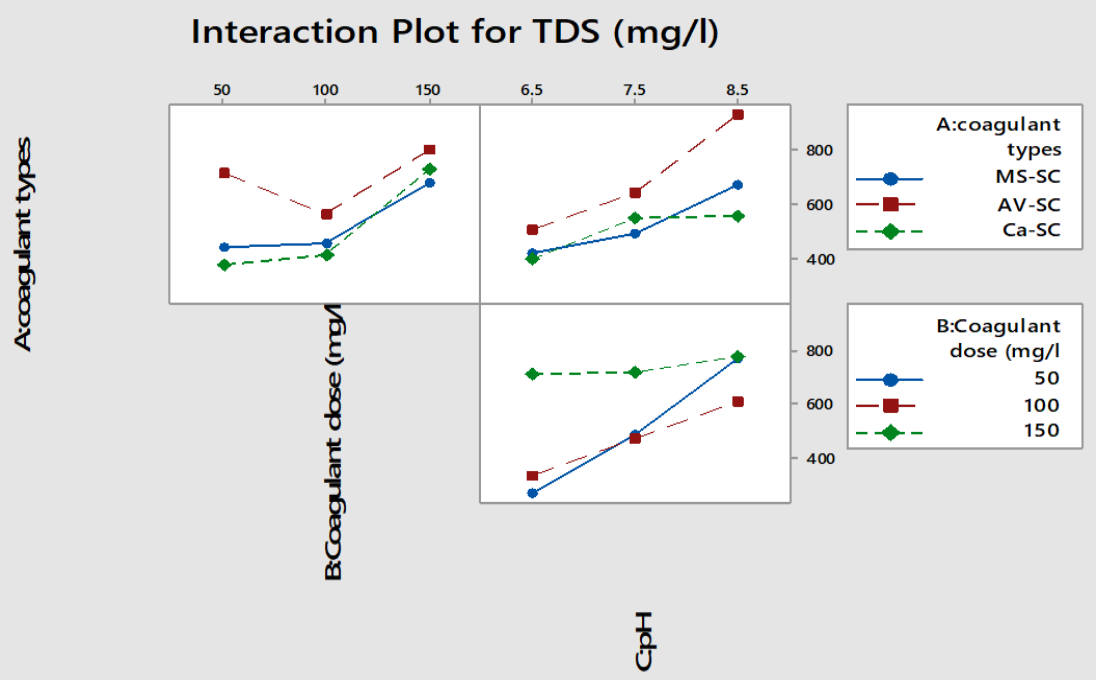


Figure 4.16. The interaction effect of factors on TDS

The interaction between factors had a significant effect on T. Coliform reduction of treated water (see figure 4.17). As it can be seen in figure 4.17, the T. Coliform reduction of water was to some extent decreased by using MS-SC and AV-SC bio-coagulants when the dose increased from 50mg/l to 150 mg/l. when the dose increases from 100mg/l to 150 mg/l, Ca-SC bio-coagulant is affected which means the T. Coliform reduction was clearly decreased. MS-SC and AV-SC bio-coagulants show much higher T. Coliform reduction of water solution at a dose of 150 mg/l, and much lower T. Coliform reduction at a dose of 50 mg/l. However, Ca-SC bio-coagulant shows a much higher T. Coliform reduction in treated water at a dose of 50 mg/l, a much lower T. Coliform reduction at a dose of 100 mg/l. Ca-SC bio-coagulant performance varies greatly at a dose of 100 mg/l and Ca-SC bio-coagulant seems to perform low T. Coliform reduction of treated water at a dose of 50 mg/l. when interaction between coagulant dose and pH, T. Coliform reduction of treated water was greatly varied at pH of 7.5 and dose of 50 mg/l. As it can be seen in figure 4.17, the best performance of T. Coliform reduction was at pH of 7.5 and 8.5 by using AV-SC. The 150 mg/l dose of bio-coagulants seems to perform good T. Coliform reduction in treated water.

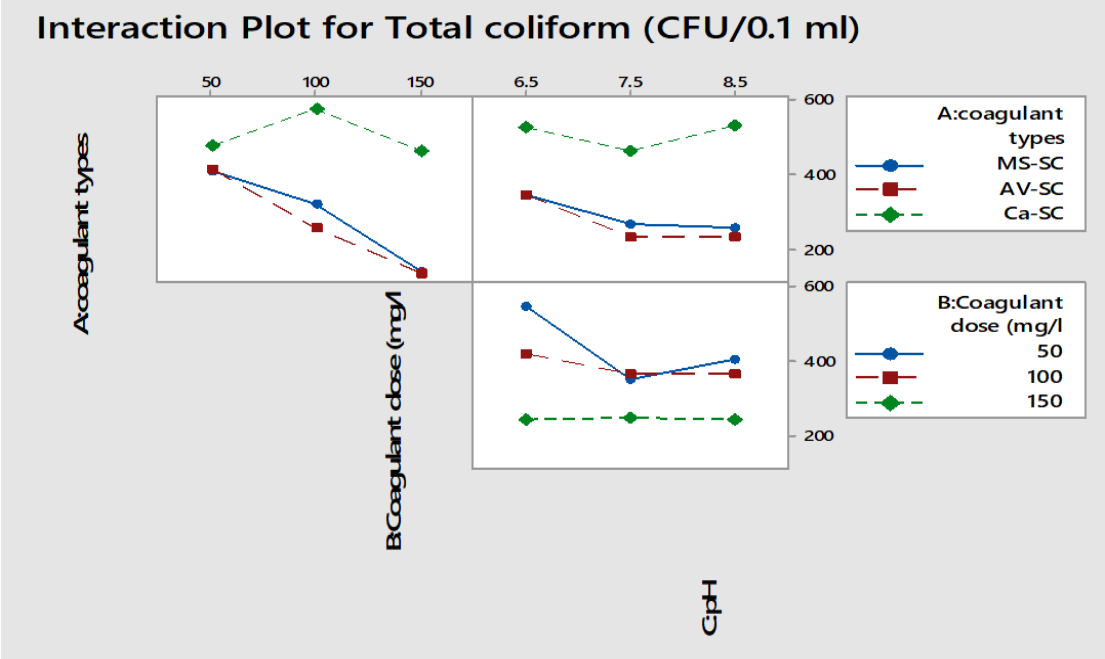


Figure 4.17. The interaction effect of factors on T. Coliform

The interaction between factors had a significant effect on E. coli reduction in treated water (see figure 4.18). As it can be seen in figure 4.18, the E. coli reduction of water was to some extent decreased by using MS-SC and AV-SC bio-coagulants when the dose increased from 50mg/l to 100 mg/l. when the dose increases from 100mg/l to 150 mg/l, AV-SC bio-coagulant is affected which means the E. coli reduction was slightly increased. MS-SC bio-coagulant shows much higher E. coli reduction of water solution at a dose of 150 mg/l, and much lower E. coli reduction at a dose of 50 mg/l. However, AV-SC bio-coagulant shows much higher E. coli reduction in treated water at a dose of 100 mg/l, and much lower E. coli reduction at a dose of 50 mg/l. AV-SC bio-coagulant performance varies greatly at a dose of 100 mg/l and Ca-SC bio-coagulant seems to perform low E. coli reduction of water at a dose of 50 mg/l. As it can be seen in figure 4.18, the best performance of E. coli reduction was at pH of 6.5 and 8.5 by using AV-SC and MS-SC respectively. The 150 mg/l dose of bio-coagulants seems to perform good E. coli reduction in treated water.

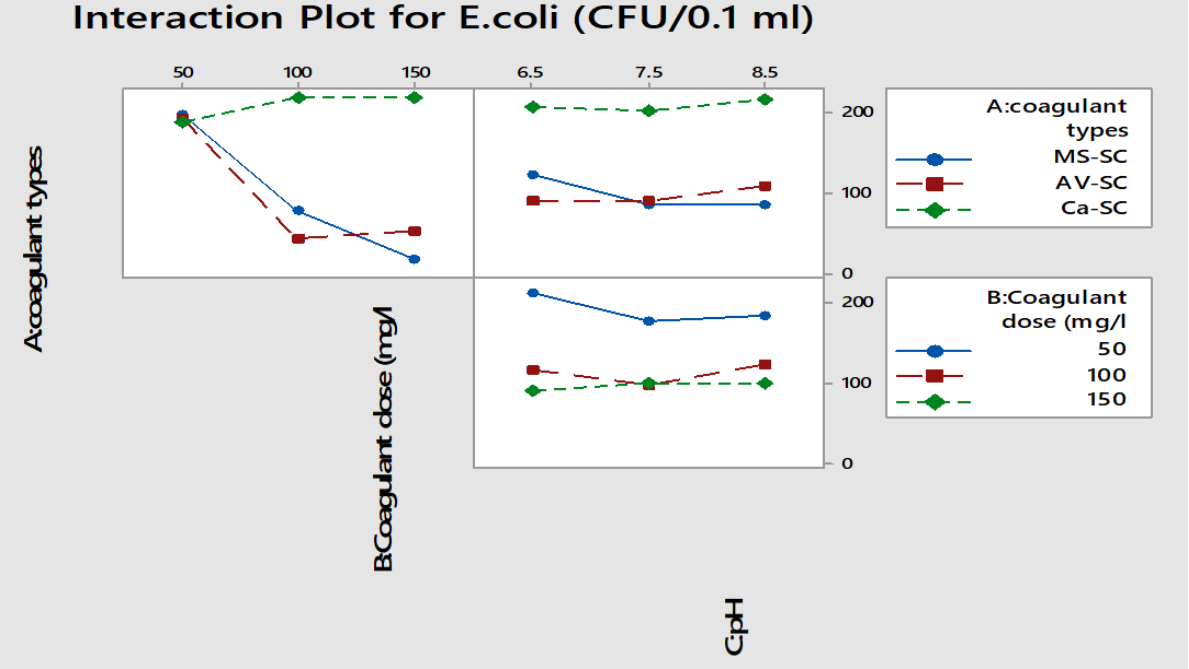


Figure 4.18. The interaction effect of factors on E. coli

4.7.4. Development of Empirical Model

In this study, to optimize the parametric and operational condition of the bio-coagulants in the water treatment by using the Response surface method through central composite design. The experimental data were analyzed by the Response surface method to interpret the interaction of the components and the dependent factors. According to the central composite design, the fitted response surface model for each response was Quadratic model for pH, 2FI mode for turbidity, 2FI model for alkalinity, Quadratic model for conductivity, Quadratic model for TDS, Quadratic model for T. coliform, and Quadratic model for E. coli. The final fitting regression model for each dependent variable in terms of coded levels is shown (Equations 4.1to 4.7). These equations have some statistically non-significant terms containing the lowest F value and P-value >0.05. Therefore, it is essential to remove these non-significant terms from the response equations as shown below:

pH $Y = +7.49 + 0.067\,A + 0.51\,B + 0.088\,C[1] - 0.031C[2] + 0.048\,A + 0.041AC[1] - 0.077\,AC[2] + 0.071\,BC[1] - 0.061BC[2] - 0.22\,B\text{^}2$……..(4.1)

Turbidity (NTU))

$$Y = +6.06 - 2.27A - 1.85C[1] - 2.73C[2] + 0.43AB - 1.00AC[1] - 0.13AC[2] - 0.095\,BC[1] - 0.92\,BC[2] + 0.64\,A\text{^}2 \quad (4.2)$$

Alkalinity (mg/l)

$$Y = +51.06 + 16.66\,A + 8.43\,B + 11.91\,C[1] - 6.72C[2] + 19.09\,AC[1] - 8.17\,AC[2] - 14.06\,BC[1] + 9.12\,BC[2] \quad (4.3)$$

Conductivity (µs/cm)

$$Y = +1005.43 + 257.50\,A + 223.58\,B - 19.57\,C[1] + 199.81\,C[2] - 112.04AB + 230.28\,A\text{^}2 \quad (4.4)$$

TDS (mg/l)

$$Y = +474.57 + 137.67\,A + 116.11B - 32.54\,C[1] + 87.69\,C[2] - 74.62\,AB + 126.22\,A\text{^}2 \quad (4.5)$$

Total coliform (CFU/0.1 ml)

$$Y = +350.04 - 94.75\,A - 32.53\,B - 65.30\,C[1] - 85.74\,C[2] + 36.25\,AB - 41.42AC[1] - 44.50AC[2] - 11.47\,BC[1] - 23.06BC[2] - 44.31A^2 + 50.03\,B\text{^}2 \quad (4.6)$$

E. coli (CFU/0.1 ml)

$$Y = +96.93 - 43.89\,A - 28.19\,C[1] - 44.69\,C[2] + 15.25\,AB - 33.86\,AC[1] - 24.94\,AC[2] + 43.11\,A\text{^}2 \quad (4.7)$$

Notes: A-Coagulant dose, B-pH, C-coagulant types

The created fitting regression model was assessed by the decided coefficient of R squared which elucidated the percent of variety on the value of dependent factors anticipated by the model. The coefficient of R squared values was obtained from ANOVA of each Response Surface Quadratic Model of pH, turbidity, alkalinity, conductivity, TDS, T. coliform, and E. coli which are 0.9803, 0.9445, 0.7191, 0.7871, 0.8658, 0.9150, and 0.8715 respectively.

4.7.5. Surface Response Optimization

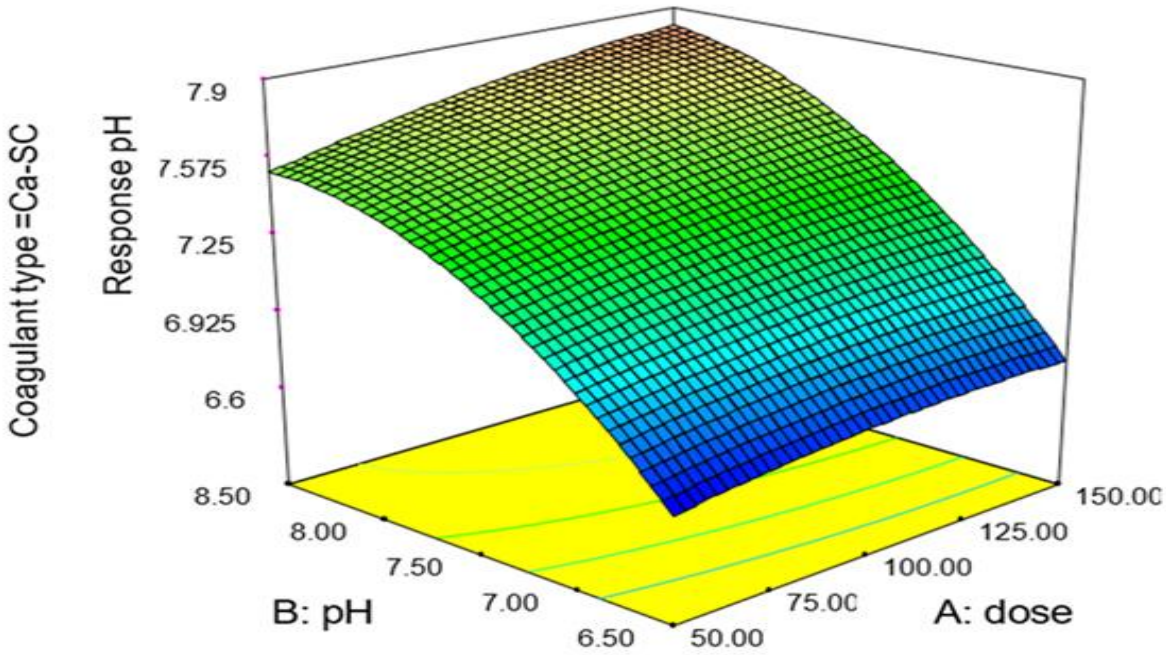
By utilizing the Response surface method from design expert software was displayed the 2D contour and 3D surface plot of the interaction of the responses model and two factors (dose and pH) are presented in figure 4.19. These graphs can be worked to explore the relations between those factors. And the graph of the curvatures, saddle, and convex parts are demonstrated the local lowest and highest values. So, in this study, the optimum value pH of treated water was 7.13 by using moringa stenopetala (MS-SC) bio- coagulant at the pH of 7.0 to 7.5 and the dose of 75mg/l to 100mg/l (figure 4.18). The optimum value pH of treated water was 7.02 by using aloe Vera (AV-SC) bio- coagulant at the pH of 7.5 to 8.5 and the dose of 100mg/l. And also, the optimum pH of treated water was 7.01 by using cactus (Ca-SC) bio-coagulant at the pH of 7.5 to 8.0 and the dose of 75mg/l.

Coagulant dosage and pH were continuous variables that have an interaction impact on turbidity reduction. According to figure 4.19, the 2D contour and 3D surface plot of the turbidity response model show two factors (X_1axis: dose andX_2 axis: pH) and the response of turbidity on the Y-axis. As the graph indicated the optimum turbidity reduction was 2.34 NTU by using moringa stenopetala (MS-SC) bio-coagulant at the pH of 7.5 and the dose of 100mg/l to 125mg/l. The optimum turbidity reduction was 11.3 NTU by using aloe Vera (AV-SC) bio- coagulant at the pH of 7.5 and the dose of approximately 125mg/l. The alkalinity of water was affected by the interaction of continuous factors is presented in figure 4.18. As it can be seen in figure 4.19, 2D contour and 3D surface plot of the alkalinity response model, when the dose increased from 50 mg/l to 150 mg/l alkalinities was clearly increased. The minimum alkalinity was 92.99 mg/l by using moringa stenopetala (MS-SC) bio- coagulant at the optimum pH of 7.5 and optimum dose of 75mg/l. Thus, the minimum and best performance of alkalinity in the study was 21.76 mg/l by using aloe Vera (AV-SC) bio- coagulant at the optimum pH of 7.00 and optimum dose of 50mg/l. And the minimum alkalinity was 27.92 mg/l by using cactus (Ca-SC) bio- coagulant at the optimum dose of approximately 50mg/l and

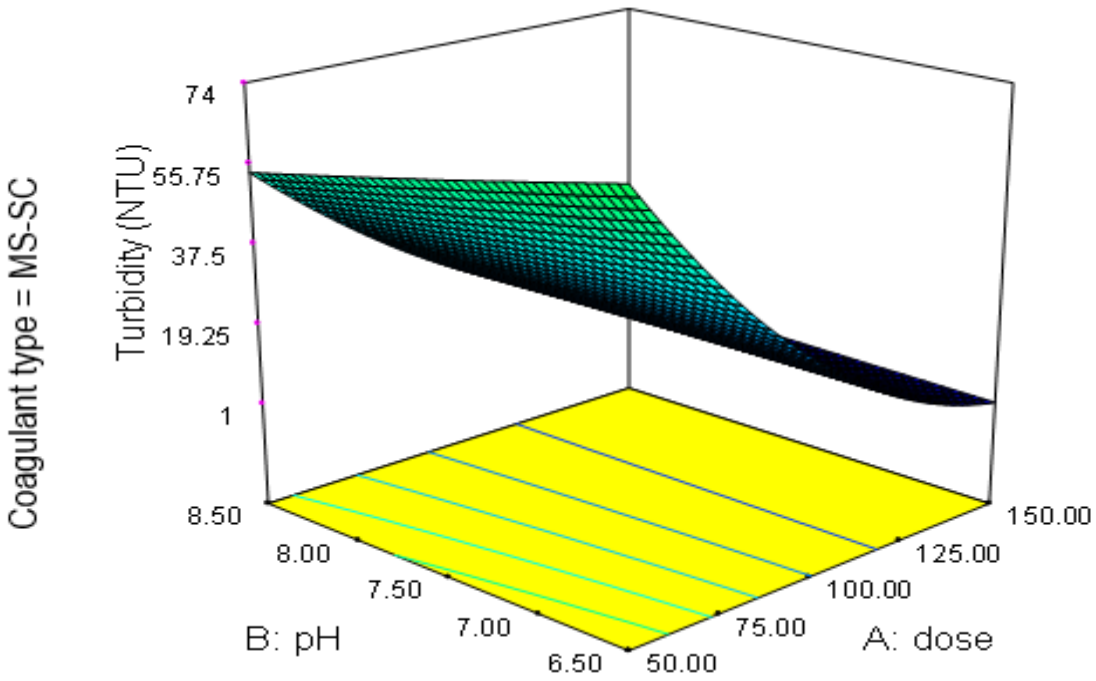
optimum pH of 7.5 to 8.00. According to figure 4.19, the 2D contour and 3D surface plot of the conductivity response model show two factors (X1axis: dose andx2 axis: pH) and the response of conductivity on the Y-axis. In the study, the dose on the quadratic effect on PH when the dose increased, pH also increased. As the graph indicated, the minimum conductivity was 777.258 µs/cm by using moringa stenopetala (MS-SC) bio- coagulant at the optimum pH of 7.5 and optimum dose of 100mg/l to 125 mg/l. The minimum conductivity was 950.605µs/cm by using aloe Vera (AV-SC) bio- coagulant at the optimum dose of 125mg/l and optimum pH of 7.5. And The minimum conductivity in the study was 570.693 µs/cm by using cactus (Ca-SC) bio- coagulant at the optimum pH of 7.5 to 8.00 and optimum dose of 125 mg/l. According to figure 4.19, 2D contour and 3D surface plot of the total dissolved solids response model indicated that the minimum total dissolved solids were 319.992 mg/l by using moringa stenopetala (MS-SC) bio-coagulant at the optimum pH of 7.0 to 7.5 and optimum dose of 100mg/l to 125 mg/l. The minimum total dissolved solids were 432.163 mg/l by using aloe Vera (AV-SC) bio- coagulant at the optimum dose of 125mg/l and optimum pH of 7.0 to 7.5. And The minimum total dissolved solids in the study was 296.056 mg/l by using cactus (Ca-SC) bio- coagulant at the optimum pH of 7.5 and optimum dose of 100 mg/l.

As figure 4.19, 2D contour and 3D surface plot showed a function of the interaction between the designed dependent variable (Y) and two independent variables. The plot saddle part is the optimum point of design to desired parameters. The optimum condition for T. coliform reduction was determined by the response model found from the experimental data. The optimum T. coliform reduction was 171.141CFU/0.1ml by using moringa stenopetala (MS-SC) bio- coagulant at the optimum pH of 7.5 to 8.5 and optimum dose of 150mg/l. The optimum T. coliform reduction in the study was 149.246 CFU/0.1ml by using aloe Vera (AV-SC) bio- coagulant at the optimum pH of 7.5 and optimum dose of 125mg/l. And the optimum T. coliform reduction was 460.628 CFU/0.1ml by using cactus (Ca-SC) bio- coagulant at the optimum dose of 150 mg/l and optimum pH of 7.5 to 8.0. The interaction impact between

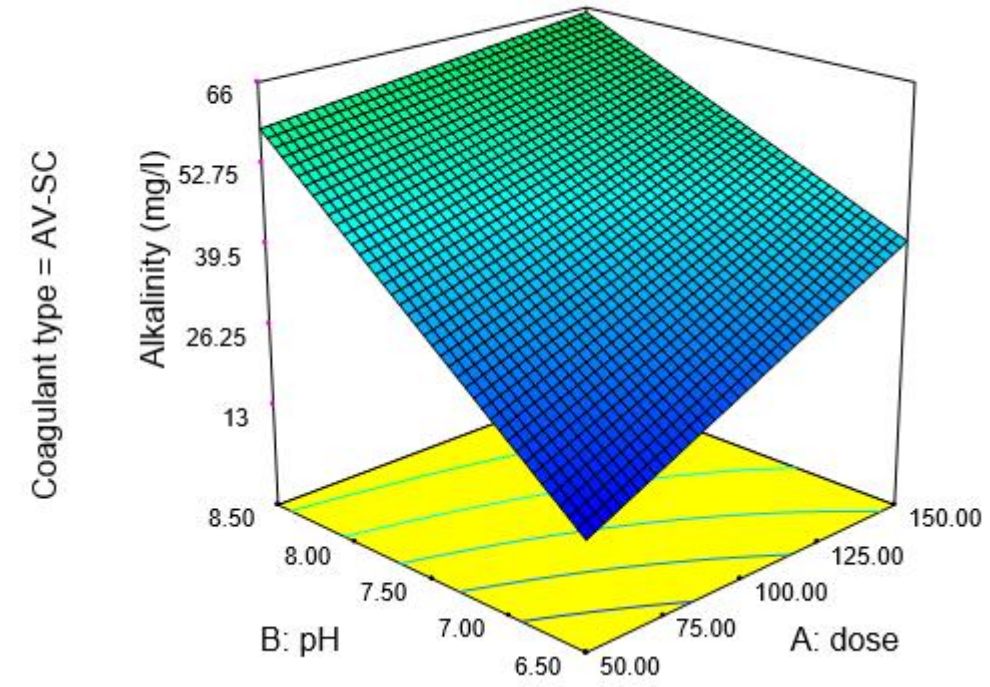
coagulant dosage and pH has a synergistic impact on the E. coli reduction of treated water. The optimum condition for E. coli reduction was determined by the response model found from the experimental data. As figure 4.19, the optimum E. coli reduction in the study was 46.909 CFU/0.1ml by using moringa stenopetala (MS-SC) bio-coagulant at the optimum pH of 7.5 and the optimum dose of 100mg/l to 125 mg/l. The optimum E. coli reduction was 51.12 CFU/0.1ml by using aloe Vera (AV-SC) bio- coagulant at the optimum pH of 7.5 to 8.0 and optimum dose of 100mg/l to 125 mg/l. And the optimum E. coli reduction was 183.73 CFU/0.1ml by using cactus (Ca-SC) bio- coagulant at the optimum pH of 6.5 to 8.5 and optimum dose of 100 mg/l. For the most part, we conclude from the response surface method design the 2D form and the 3D surface plot showed that a function of the interaction between two independent variables and designed dependent variable (Y) is presented in figure 4.19. As it can be seen in figure 4.19, the plots are showed curvature, convex, and saddle parts by using the response surface method through central composite design which is the minimum point of design to desired parameters. The optimum condition and value of parameters pH, turbidity, alkalinity, conductivity, TDS, T.coliform, E.coli were 7.0133, 2.34 NTU, 21.76 mg/l, 570.69 µs/cm, 296.01 mg/l, 149.15 CFU/0.1 ml and 46.909 CFU/0.1 ml by using 100 mg/l dosage of Cactus (Ca-SC) at pH of 7.5 to 8.0; 125 mg/l to 150 mg/l dosage of Moringa (MS-SC) bio-coagulant at pH of 7.5; 75 mg/l dosage of Aloe Vera (AV-SC) bio-coagulant at pH of 7.0; 100 mg/l dosage of Cactus (Ca-SC) bio-coagulant at pH of 7.0; 100 mg/l dosage of Cactus (Ca-SC) bio-coagulant at pH of 7.0; 125 mg/l dosage of Aloe Vera (AV-SC) bio-coagulant at pH of 7.5 and 125 mg/l to 150 mg/l dosage of Moringa (MS-SC) bio-coagulant at pH of 7.5 respectively.


Coagulant type =Ca-SC
Response pH
7.9
7.575
7.25
6.925
6.6
8.50
8.00
7.50
7.00
6.50
B: pH
50.00
75.00
100.00
125.00
150.00
A: dose

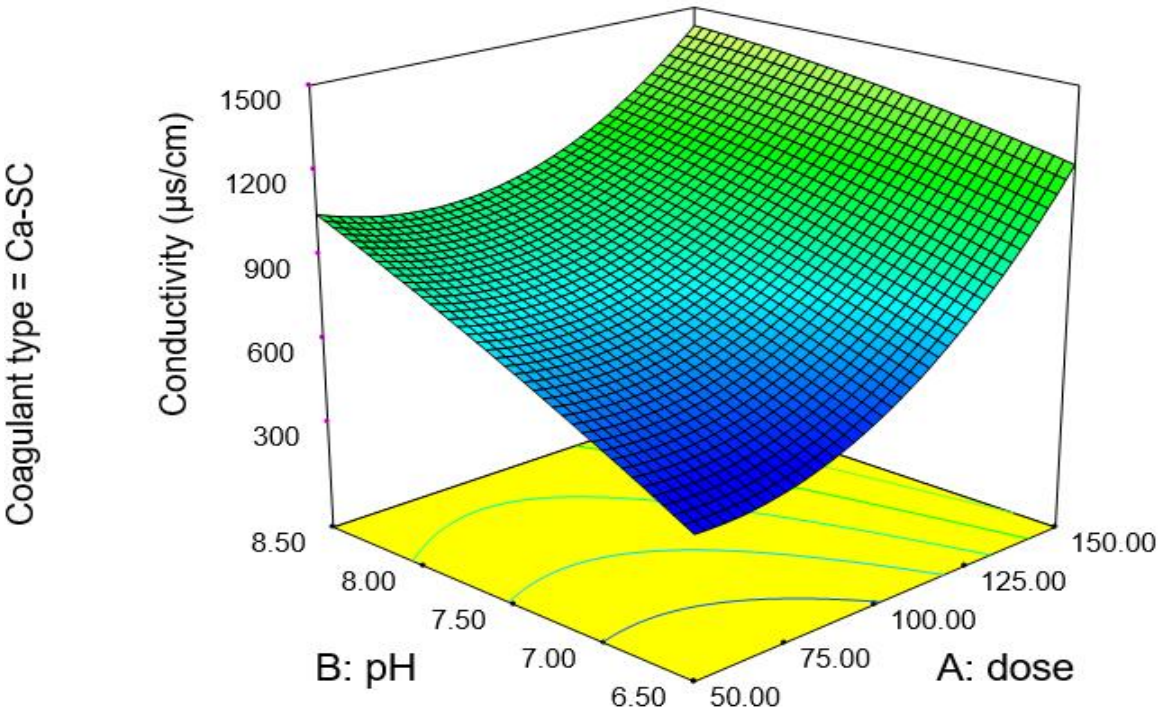
a)


Coagulant type = MS-SC
Turbidity (NTU)
74
55.75
37.5
19.25
1
8.50
8.00
7.50
7.00
6.50
B: pH
50.00
75.00
100.00
125.00
150.00
A: dose

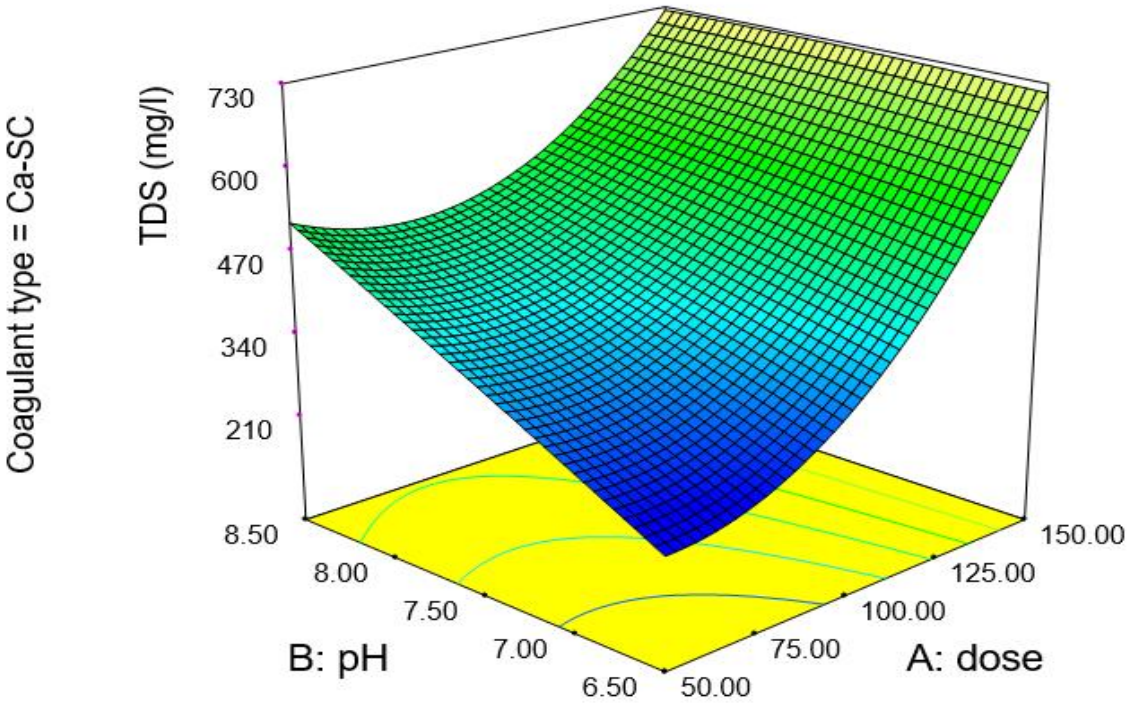
b)


Coagulant type = AV-SC
Alkalinity (mg/l)
66
52.75
39.5
26.25
13
8.50
8.00
7.50
7.00
6.50
B: pH
50.00
75.00
100.00
125.00
150.00
A: dose

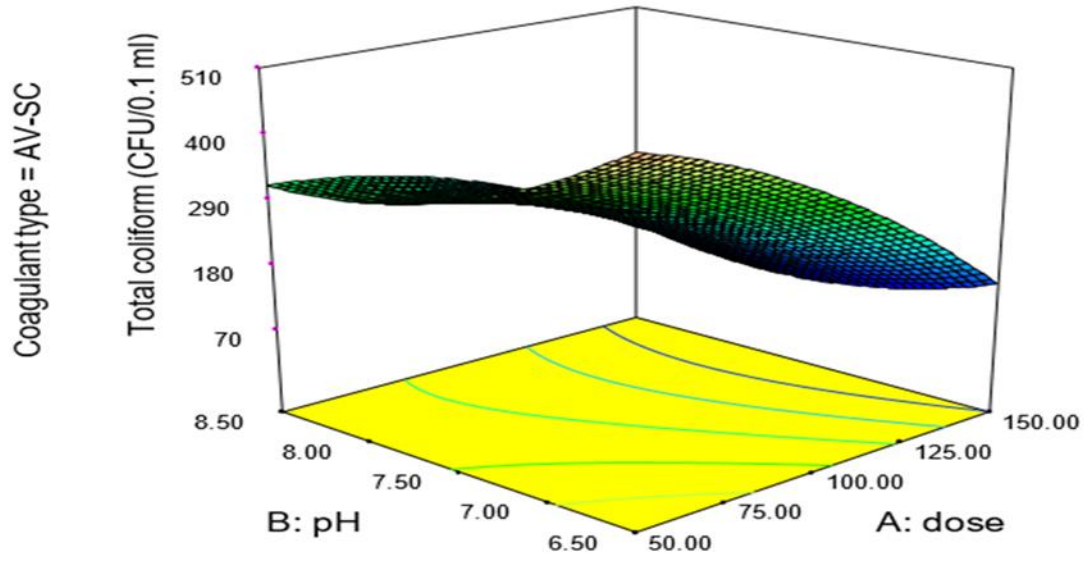
c)


Coagulant type = Ca-SC
Conductivity (µs/cm)
1500
1200
900
600
300
8.50
8.00
7.50
7.00
6.50
B: pH
50.00
75.00
100.00
125.00
150.00
A: dose

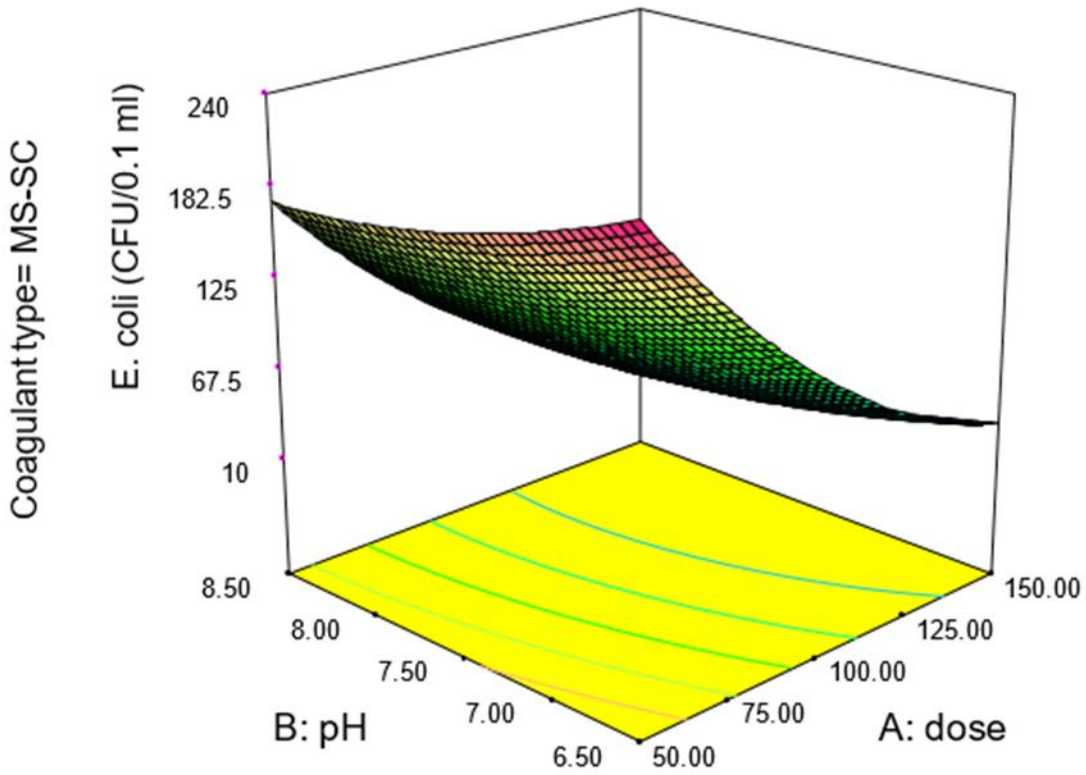
d)


Coagulant type = Ca-SC
TDS (mg/l)
730
600
470
340
210
8.50
8.00
7.50
7.00
6.50
B: pH
50.00
75.00
100.00
125.00
150.00
A: dose

e)


Coagulant type = AV-SC
Total coliform (CFU/0.1 ml)
510
400
290
180
70
8.50
8.00
7.50
7.00
6.50
B: pH
50.00
75.00
100.00
125.00
150.00
A: dose

f)



g)

Figures 4.19: 3D surface response results; a) Response pH, b) turbidity, c) alkalinity, d) conductivity e) TDS, f) T. coliform & g) E. coli.

5. Conclusions and Recommendation

5.1. Conclusion

This study intends to test the use moringa stenopetala seed, Aloe Vera leaves skin, and cactus leaves as bio coagulants to remove turbidity and microbial from the surface water of Legedadie Dam with 239.67 NTU turbidity. The bio coagulants dose of 50 mg/l, 100mg/l & 150 mg/l with the pH of 6.5, 7.5 & 8.5 was investigated for the water quality by analyzing the parameters of pH, Turbidity, Alkalinity, Conductivity, TDS, Total coliform, and E. coli. The study also shows that the turbidity of water is significantly reduced with bio-coagulants of MS-SC, AV-SC, and Ca-SC. So, the turbidity removal efficiency (67-98.83%)/ (76 to 2.83 NTU); (59.61-98.73 %) / (91.75 to 2.9275 NTU) and (27.4-69.83 %) / (174 to 72.87 NTU) were recorded by moringa stenopetala seed powder, Aloe Vera leaves skin and cactus leaves as bio coagulant extracted by 1M NaCl lab graded salt respectively.

When 150 mg/l doses of moringa (MS-SC) and aloe Vera (AV-SC) bio- coagulants were used, the turbidity removal was achieved at the lowest value of 2.81 NTU at pH 6.5 and 2.89 NTU at pH 8.5 respectively. The better performance of alkalinity was 14.2 mg/l of total alkalinity when using the 50mg/l dose of aloe Vera (AV-SC) bio-coagulant at the pH of 7.5. However, the highest value was recorded on the dosage of 150mg/l of moringa (MS-SC) bio-coagulant at pH of 6.5 and 7.5. Complete removal of E. coli was achieved with MS-SC and AV-SC bio coagulants at a pH of 7.5 and 6.5 on the dose of 150 mg/l respectively. The interaction between all factors in the two-way form has a synergetic effect on the response pH of treated water. Based on the interaction effect the performance varies greatly at a dose of 150 mg/l of Ca-SC bio coagulant and MS-SC bio-coagulant seems to perform a good turbidity removal at the dose of 150 mg/l and pH of 6.5 and also AV-SC bio-coagulant seems to perform a good turbidity removal efficiency at the pH of 8.5. According to the Numerical optimization, the maximum value of parameters of pH, Turbidity, Alkalinity, Conductivity, TDS, Total coliform, and E. coli was found to be 6.66389, 5.86537 NTU, 44.8778 mg/l, 849.759 µs/cm, 335.519 mg/l, 18.284 CFU/0.1 ml, 10.433 CFU/0.1 ml

with100 mg/l dose of moringa bio-coagulant and pH of 6.5 respectively. Generally, it was found that the bio coagulants extracted from moringa stenopetala seed and aloe Vera leave to have a better performance to treat water with the required drinking water turbidity limit set by WHO recommended standards. Such low-cost bio-coagulant can be applied in the rural community for the removal of turbidity and microbial contaminants from surface water which is the most water supply source for smallholder farmers.

5.2. Recommendation

- The Bio coagulants should be further investigated in the toxicity level and residual effects before using for the treated water.
- Future research should be done to compare the effect of Bio-coagulants by using different methods of extraction.
- The study was focused only on one settling time even though future researcher should be done to compare the effect of Bio-coagulants by using interval settling time.
- Future research should be done to identify the effect of Bio-coagulants by using the different polarities of lab-graded NaCl solution & by using increasing valency number of salts.
- A future investigation should be done to identify the capacity of those bio coagulants on the pathogenic protozoa.

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7. Appendices

Table 7:1 Experimental design involving three experimental factors with seven experimental responses.

Factors						**Responses**						
			Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5	Response 6	Response 7
Std	Run	Replications	A: coagulant types	B: Coagulant dose (mg/l)	C: pH	pH	Turbidity (NTU)	Alkalinity (mg/l)	Conductivity (µs/cm)	TDS (mg/l)	Total coliform (CFU/0.1 ml)	E. coli (CFU/0.1 ml)
43	1	R 1	MS-SC	100	8.5	8.01	13.6	56.2	1100	531	313	83
17	2	R 1	Ca-SC	150	6.5	6.81	72.3	37.4	1371	667	456	210
27	3	R 1	AV-SC	100	7.5	7.46	4.59	53.4	998	485	212	N. D
35	4	R 1	Ca-SC	150	7.5	7.35	89	59.8	1537	753	465	218
23	5	R 1	Ca-SC	50	7.5	7.28	124	50.2	820	435	340	172
5	6	R 1	Ca-SC	50	6.5	6.61	132	23.2	485	233	540	202
25	7	R 1	MS-SC	100	7.5	7.66	12.3	58.2	990	482	300	75
37	8	R 1	MS-SC	50	8.5	7.66	62.4	42.2	1321	721	322	175
21	9	R 1	AV-SC	50	7.5	7.39	37.9	14.2	1461	707	354	190
51	10	R 1	AV-SC	150	8.5	7.62	2.98	70.6	1656	810	142	80
41	11	R 1	Ca-SC	50	8.5	7.41	164	50.2	820	435	564	201
11	12	R 1	Ca-SC	100	6.5	6.62	109	24.2	605	293	578	209
49	13	R 1	MS-SC	150	8.5	8.05	4.48	55.4	1538	758	130	N. D
9	14	R 1	AV-SC	100	6.5	6.67	18.59	33.4	884	365	333	62
7	15	R 1	MS-SC	100	6.5	6.66	17.4	38.2	835	335	347	78
3	16	R 1	AV-SC	50	6.5	6.84	96.8	25.4	503	249	560	210
31	17	R 1	MS-SC	150	7.5	7.67	3.26	138	1536	677	140	N. D
19	18	R 1	MS-SC	50	7.5	7.4	73.5	29.6	764	310	360	179
33	19	R 1	AV-SC	150	7.5	7.4	8.2	49.2	1406	726	127	80
15	20	R 1	AV-SC	150	6.5	6.71	4.28	26.6	1784	876	130	N. D

1	21	R 1	MS-SC	50	6.5	6.71	75.3	28.8	744	302	543	235
29	22	R 1	Ca-SC	100	7.5	7.4	172	51.8	920	455	583	215
13	23	R 1	MS-SC	150	6.5	6.84	2.85	120.8	1368	605	136	62
39	24	R 1	AV-SC	50	8.5	7.64	27.7	51	1670	730	330	180
53	25	R 1	Ca-SC	150	8.5	7.85	106	60.2	1541	761	459	222
47	26	R 1	Ca-SC	100	8.5	7.81	174	52.6	945	465	570	224
45	27	R 1	AV-SC	100	8.5	7.69	13.92	71.2	1658	825	213	65
52	28	R 2	AV-SC	150	8.5	7.65	2.89	69.8	1651	808	147	83
40	29	R 2	AV-SC	50	8.5	7.61	30.4	49.8	1659	725	336	182
38	30	R 2	MS-SC	50	8.5	7.69	63.4	41.8	1291	713	318	178
12	31	R 2	Ca-SC	100	6.5	6.65	104	24.8	574	301	574	211
34	32	R 2	AV-SC	150	7.5	7.45	8.26	48.4	1403	722	130	85
28	33	R 2	AV-SC	100	7.5	7.52	4.61	53.2	991	481	218	N. D
10	34	R 2	AV-SC	100	6.5	6.71	18.71	33.8	890	371	340	68
36	35	R 2	Ca-SC	150	7.5	7.46	91.1	59	1535	751	470	219
42	36	R 2	Ca-SC	50	8.5	7.52	159	50.8	840	445	556	195
2	37	R 2	MS-SC	50	6.5	6.79	77.04	27.6	668	296	550	237
44	38	R 2	MS-SC	100	8.5	8	13.4	57	1009	536	311	79
24	39	R 2	Ca-SC	50	7.5	7.31	117	50.8	829	439	332	164
26	40	R 2	MS-SC	100	7.5	7.63	12.4	57.8	985	480	301	79
30	41	R 2	Ca-SC	100	7.5	7.45	173	52.8	930	457	582	219
46	42	R 2	AV-SC	100	8.5	7.71	14.01	71.6	1661	829	217	69
8	43	R 2	MS-SC	100	6.5	6.61	16.8	38.6	841	339	342	74
4	44	R 2	AV-SC	50	6.5	6.8	86.7	27.2	601	294	554	208
48	45	R 2	Ca-SC	100	8.5	7.83	173.2	53	954	469	567	223
18	46	R 2	Ca-SC	150	6.5	6.79	74.4	37.2	460	662	460	214
6	47	R 2	Ca-SC	50	6.5	6.67	129	23.6	580	235	538	190
14	48	R 2	MS-SC	150	6.5	6.79	2.81	121.4	1371	607	139	58
16	49	R 2	AV-SC	150	6.5	6.76	4.32	27	1781	872	135	N. D
22	50	R 2	AV-SC	50	7.5	7.43	30.6	22.2	1473	717	348	184

50	51	R 2	MS-SC	150	8.5	8.01	4.56	55.2	1537	756	135	N. D
20	52	R 2	MS-SC	50	7.5	7.42	74.01	29.2	751	307	364	183
32	53	R 2	MS-SC	150	7.5	7.61	3.31	137.4	1532	673	143	N. D
54	54	R 2	Ca-SC	150	8.5	7.77	104	64	1543	765	454	220

Table 7:2 Correlations of protein, moisture, and ash content of proximate analysis.

		Protein (%)	Moisture (%)	Ash (%)
protein (%) +	Pearson Correlation	1	.495	.463
	Sig. (2-tailed)		.505	.537
	Sum of Squares and Cross-products	.880	3.452	13.370
	Covariance	.293	1.151	4.457
	N	4	4	4
moisture (%)	Pearson Correlation	.495	1	.988*
	Sig. (2-tailed)	.505		.012
	Sum of Squares and Cross-products	3.452	55.128	225.960
	Covariance	1.151	18.376	75.320
	N	4	4	4
Ash (%) ++++	Pearson Correlation	.463	.988*	1
	Sig. (2-tailed)	.537	.012	
	Sum of Squares and Cross-products	13.370	225.960	948.800
	Covariance	4.457	75.320	316.267
	N	4	4	4

*. Correlation is significant at the 0.05 level (2-tailed).

Table 7:3 Correlations of protein content, factors, and turbidity

		Coagulant type	pH	Coagulant dose (mg/l)	protein content	Turbidity removal (%)
Coagulant type	Pearson Correlation	1	.000	.000	-.904**	-.707**
	Sig. (2-tailed)		1.000	1.000	.000	.000
	N	54	54	54	54	54
pH	Pearson Correlation	.000	1	.000	.000	-.037
	Sig. (2-tailed)	1.000		1.000	1.000	.788
	N	54	54	54	54	54
Coagulant dose (mg/l)	Pearson Correlation	.000	.000	1	.000	.396**
	Sig. (2-tailed)	1.000	1.000		1.000	.003
	N	54	54	54	54	54
protein content	Pearson Correlation	-.904**	.000	.000	1	.444**
	Sig. (2-tailed)	.000	1.000	1.000		.001
	N	54	54	54	54	54
Turbidity removal (%)	Pearson Correlation	-.707**	-.037	.396**	.444**	1
	Sig. (2-tailed)	.000	.788	.003	.001	
	N	54	54	54	54	54

**. Correlation is significant at the 0.01 level (2-tailed).

Table 7:4 ANOVA for significance test between factors and pH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	10.773[a]	17	.634	113.271	.000
Intercept	397.505	1	397.505	71050.645	.000
Coa	.081	2	.041	7.256	.002
pH	1.005	2	.502	89.801	.000
Dos	.161	1	.161	28.837	.000
coa * Dos	.107	2	.053	9.542	.000
pH * Dos	.055	2	.028	4.937	.013
coa * pH	.007	4	.002	.298	.877
coa * pH * Dos	.024	4	.006	1.076	.383
Error	.201	36	.006		
Total	2898.279	54			
Corrected Total	10.975	53			

Table 7:5 ANOVA for significance test between factors and Turbidity removal (%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	27094.897[a]	17	1593.817	28.141	.000
Intercept	21246.153	1	21246.153	375.134	.000
Cao	2579.615	2	1289.808	22.774	.000
PH	203.731	2	101.865	1.799	.180
Dos	4564.354	1	4564.354	80.591	.000
Cao * PH	697.875	4	174.469	3.081	.028
PH * Dos	278.704	2	139.352	2.460	.100
Cao * Dos	141.835	2	70.917	1.252	.298
Cao * PH * Dos	262.040	4	65.510	1.157	.346
Error	2038.903	36	56.636		
Total	332882.300	54			
Corrected Total	29133.800	53			

Table 7:6 Correlations on Turbidity removal (%

		Coagulant type	pH	Coagulant dose (mg/l)	Turbidity removal (%)
Coagulant type	Pearson Correlation	1	.000	.000	-.694**
	Sig. (2-tailed)		1.000	1.000	.000
	Sum of Squares and Cross-products	36.000	.000	.000	-723.590
	Covariance	.679	.000	.000	-13.653
	N	54	54	54	54
pH	Pearson Correlation	.000	1	.000	-.036
	Sig. (2-tailed)	1.000		1.000	.796
	Sum of Squares and Cross-products	.000	36.000	.000	-37.600
	Covariance	.000	.679	.000	-.709
	N	54	54	54	54
Coagulant dose (mg/l)	Pearson Correlation	.000	.000	1	.390**
	Sig. (2-tailed)	1.000	1.000		.004
	Sum of Squares and Cross-products	.000	.000	90000.000	20305.500

	Covariance	.000	.000	1698.113	383.123
	N	54	54	54	54
	Pearson Correlation	-.694**	-.036	.390**	1
	Sig. (2-tailed)	.000	.796	.004	
Turbidity removal (%)	Sum of Squares and Cross-products	-723.590	-37.600	20305.500	30185.891
	Covariance	-13.653	-.709	383.123	569.545
	N	54	54	54	54

Table 7:7 ANOVA for significance test between factors and Alkalinity

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	34417.780[a]	17	2024.575	20.365	.000
Intercept	2425.920	1	2425.920	24.402	.000
Coa	2724.189	2	1362.094	13.701	.000
pH	2265.646	2	1132.823	11.395	.000
Dos	9986.671	1	9986.671	100.455	.000
coa * Dos	6608.136	2	3304.068	33.235	.000
pH * Dos	1798.736	2	899.368	9.047	.001
coa * pH	1547.935	4	386.984	3.893	.010
coa * pH * Dos	3878.278	4	969.569	9.753	.000
Error	3578.913	36	99.414		
Total	178695.600	54			
Corrected Total	37996.693	53			

Table 7:8 ANOVA for significance test between factors and Conductivity

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	7016556.870[a]	17	412738.639	15.123	.000
Intercept	2707301.852	1	2707301.852	99.198	.000
coa	626927.894	2	313463.947	11.486	.000
pH	1305943.815	2	652971.907	23.925	.000
Dos	2873025.000	1	2873025.000	105.270	.000
coa * Dos	201759.500	2	100879.750	3.696	.035
pH * Dos	573020.167	2	286510.083	10.498	.000
coa * pH	741074.772	4	185268.693	6.788	.000
coa * pH * Dos	674194.833	4	168548.708	6.176	.001
Error	982508.167	36	27291.894		
Total	80337028.000	54			
Corrected Total	7999065.037	53			

Table 7:9 ANOVA for significance test between factors and TDS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1742209.926[a]	17	102482.937	12.614	.000
Intercept	608244.892	1	608244.892	74.863	.000
Coa	91378.481	2	45689.241	5.623	.007
pH	370376.259	2	185188.130	22.793	.000
Dos	682276.000	1	682276.000	83.975	.000
coa * Dos	29454.167	2	14727.083	1.813	.178
pH * Dos	142431.500	2	71215.750	8.765	.001
coa * pH	157907.058	4	39476.765	4.859	.003
coa * pH * Dos	134686.333	4	33671.583	4.144	.007
Error	292490.167	36	8124.727		
Total	18735829.000	54			
Corrected Total	2034700.093	53			

Table 7:10 ANOVA for significance test between factors and Total coliform percent reduction (%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	35938.444[a]	17	2114.026	37.545	.000
Intercept	542.320	1	542.320	9.632	.004
Coa	55.609	2	27.805	.494	.614
pH	3168.825	2	1584.413	28.139	.000
Dos	9180.099	1	9180.099	163.039	.000
coa * Dos	3789.838	2	1894.919	33.654	.000
pH * Dos	1837.783	2	918.892	16.320	.000
coa * pH	1326.086	4	331.521	5.888	.001
coa * pH * Dos	851.418	4	212.855	3.780	.011
Error	2027.022	36	56.306		
Total	125762.832	54			
Corrected Total	37965.467	53			

Table 7:11 Correlations on Total coliform percent reduction (%)

		coagulant type	PH	Coagulant dosage(mg/L)	Total coliform percent reduction (%)
coagulant type	Pearson Correlation	1	.000	.000	-.562**
	Sig. (2-tailed)		1.000	1.000	.000
	Sum of Squares and Cross-products	36.000	.000	.000	-656.980
	Covariance	.679	.000	.000	-12.396
	N	54	54	54	54
PH	Pearson Correlation	.000	1	.000	.169
	Sig. (2-tailed)	1.000		1.000	.223
	Sum of Squares and Cross-products	.000	36.000	.000	197.170
	Covariance	.000	.679	.000	3.720
	N	54	54	54	54
Coagulant dosage(mg/L)	Pearson Correlation	.000	.000	1	.492**
	Sig. (2-tailed)	1.000	1.000		.000
	Sum of Squares and Cross-products	.000	.000	90000.000	28743.850
	Covariance	.000	.000	1698.113	542.337
	N	54	54	54	54
Total coliform percent reduction (%)	Pearson Correlation	-.562**	.169	.492**	1
	Sig. (2-tailed)	.000	.223	.000	
	Sum of Squares and Cross-products	-656.980	197.170	28743.850	37965.467
	Covariance	-12.396	3.720	542.337	716.330
	N	54	54	54	54

Table 7:12 ANOVA for significance test between factors and E. coli percent reduction (%)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	33745.511[a]	17	1985.030	16.479	.000
Intercept	4731.778	1	4731.778	39.283	.000
Coa	1370.458	2	685.229	5.689	.007
pH	518.504	2	259.252	2.152	.131
Dos	8772.508	1	8772.508	72.828	.000
coa * pH	263.392	4	65.848	.547	.703
pH * Dos	379.084	2	189.542	1.574	.221
coa * Dos	7825.362	2	3912.681	32.483	.000
coa * pH * Dos	515.877	4	128.969	1.071	.385
Error	4336.364	36	120.455		
Total	207347.484	54			
Corrected Total	38081.875	53			

Table 7:13 Correlations of E. coli percent reduction (%)

		coagulant type	PH	Coagulant dosage(mg/L)	E. coli percent reduction (%)
coagulant type	Pearson Correlation	1	.000	.000	-.349**
	Sig. (2-tailed)		1.000	1.000	.010
	Sum of Squares and Cross-products	36.000	.000	.000	-396.380
	Covariance	.679	.000	.000	-7.479
	N	54	54	54	54
PH	Pearson Correlation	.000	1	.000	.020
	Sig. (2-tailed)	1.000		1.000	.886
	Sum of Squares and Cross-products	.000	36.000	.000	22.640
	Covariance	.000	.679	.000	.427
	N	54	54	54	54
Coagulant dosage(mg/L)	Pearson Correlation	.000	.000	1	.104
	Sig. (2-tailed)	1.000	1.000		.454
	Sum of Squares and Cross-products	.000	.000	90000.000	5902.000
	Covariance	.000	.000	1698.113	111.358
	N	54	54	54	54
E. coli percent reduction (%)	Pearson Correlation	-.349**	.020	.104	1
	Sig. (2-tailed)	.010	.886	.454	
	Sum of Squares and Cross-products	-396.380	22.640	5902.000	35809.283
	Covariance	-7.479	.427	111.358	675.647
	N	54	54	54	54

**. Correlation is significant at the 0.01 level (2-tailed).

Figure 7:1 Bio-coagulant extraction process from moringa stenopetalla plant(A-F) *(source from photo).*

Figure 7:2 Bio-coagulant extraction process from Aloe Vera plant (A-I) *(source from photo).*

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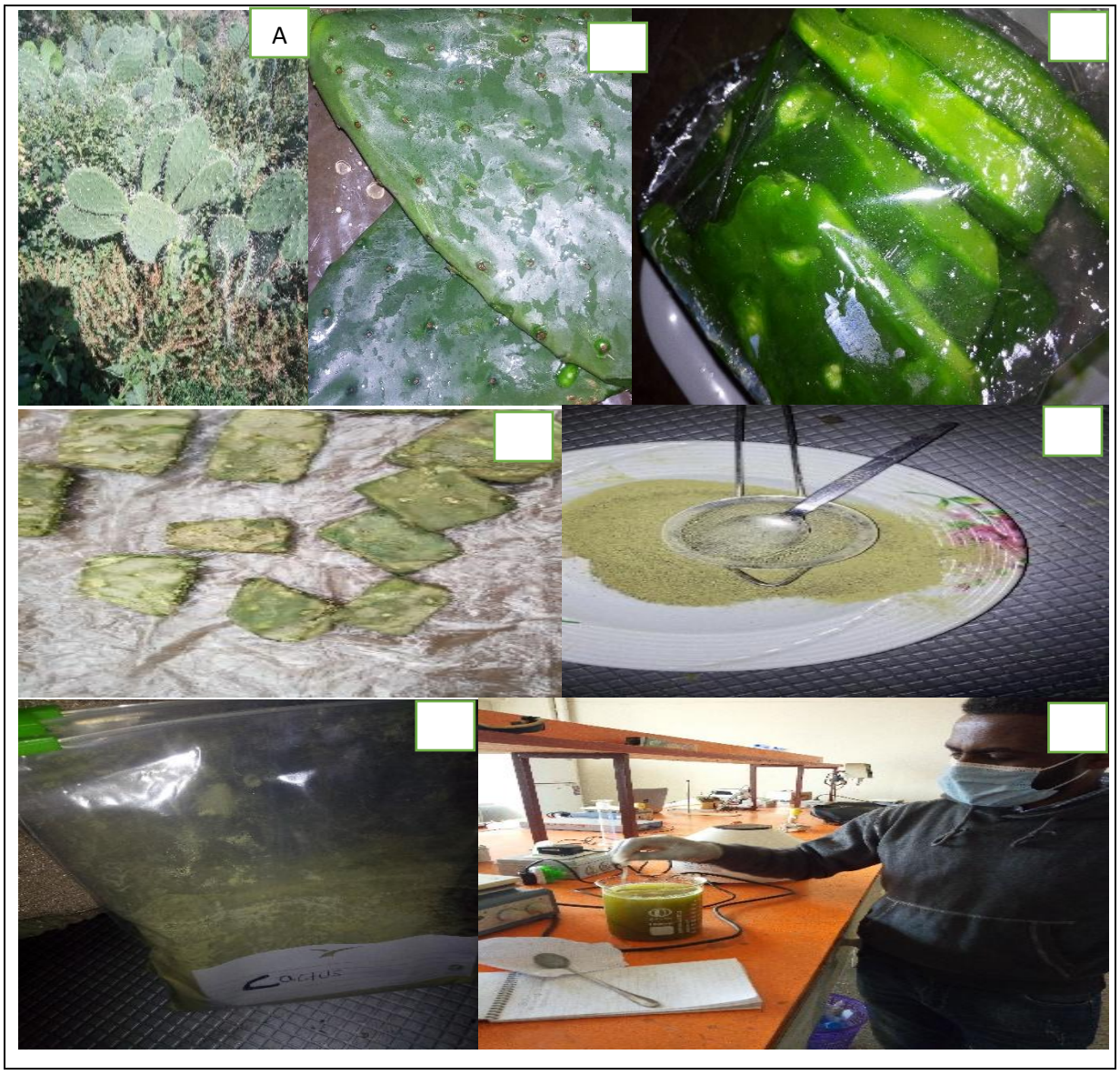

Figure 7:3 Bio-coagulant extraction process from Cactus plant (A-G) *(source from photo).*

Figure 7: 4 Total coliforms (white plates) and E. coli (blue plates) inoculated process (sourced from photo)

Figure 7:5 Total coliforms, E. coli bacteria colony, and not detected indicator bacteria (sourced from photo)

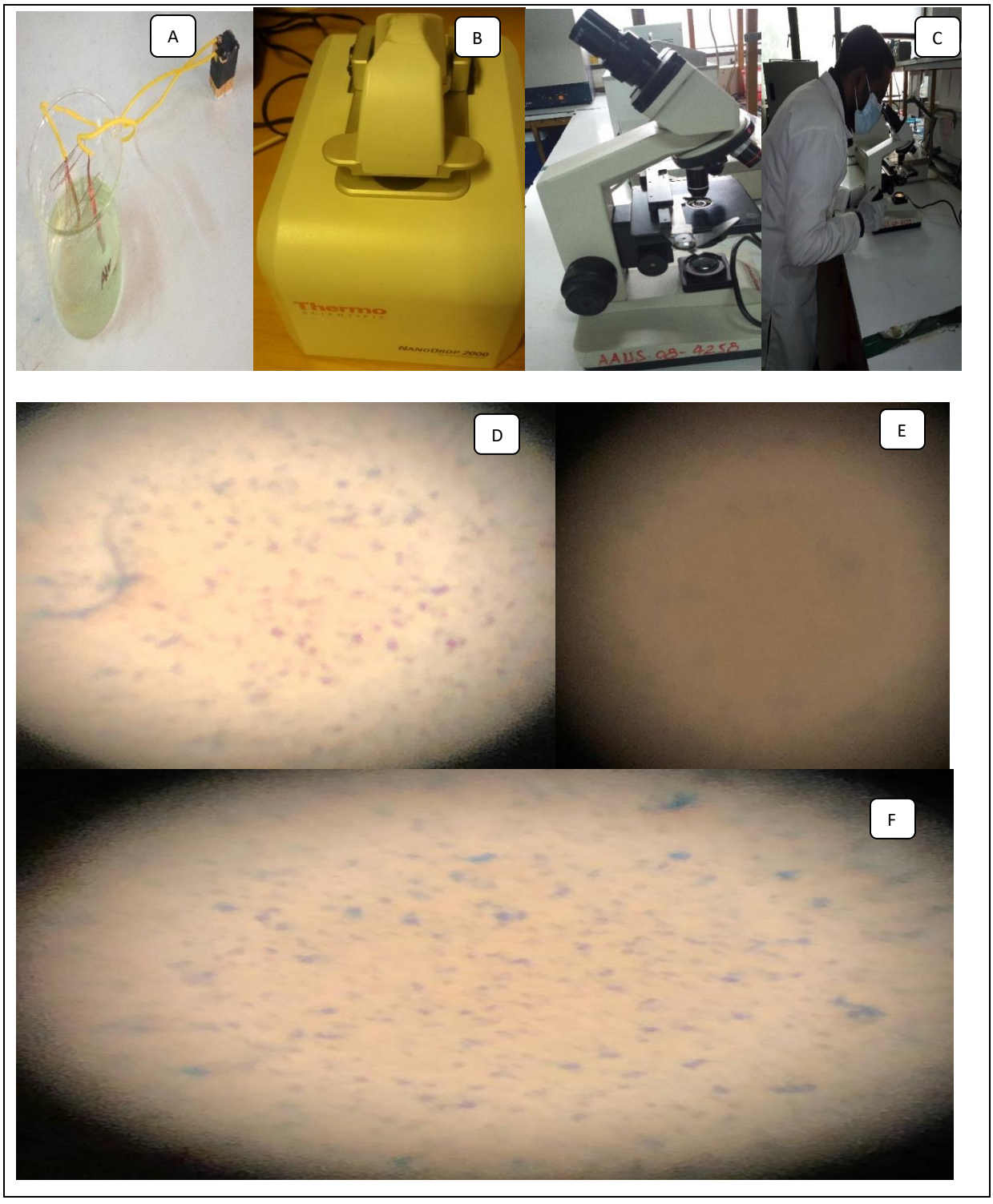

Figure 7.6. a) electrophoresis Setup &materials, b) Nanodrop 2000, c) microscope and image of colloidal particles [d) moringa, e) cactus, f) aloe Vera].